Effect of fatty acid(s) extracted from marine topse (Polynemus paradiseus) fish oil on obesity and obesity related metabolic disorders

Thesis Submitted to Vidyasagar University

for the Partial Fulfilment of the Doctor of Philosophy in Science

Submitted by

Riya Kar

Research Scholar

Biodiversity & Environmental Studies

Midnapore City College

Vidyasagar University

Registration No with Date & year: **963/Ph.D. (Sc.); 09. 12. 2022**

Under supervision of

Dr. Shrabani Pradhan

Assistant Professor in Nutrition, Dept. of Paramedical and Allied Health Sciences



MIDNAPORE CITY COLLEGE

Kuturiya, P.O. Bhadutala, Pin-721129

Paschim Medinipur

West Bengal, India

2026

Certificate

This is certified that the thesis entitled, **"Effect of fatty acid(s) extracted from marine topse (*Polynemus paradiseus*) fish oil on obesity and obesity related metabolic disorders"** submitted by Ms. Riya Kar (Reg. No.: 963/Ph.D./Sc. of 09.12.2022), who got her name registered on 24.02.2023 for the award of Ph.D. (Science) degree of Vidyasagar University, is absolutely based upon her own work under the supervision of Dr. Shrabani Pradhan, Assistant Professor, at Midnapore City College, Department of Paramedical and Allied Health Sciences and that neither this thesis nor any part of it has been submitted for either any degree/diploma or any other academic award anywhere before.

Shrabani Pradhan

Dr. Shrabani Pradhan

Supervisor

Dept. Of Paramedical and Allied Health Sciences,

Midnapore City College

MIDNAPORE CITY COLLEGE Estd.-2017 Paschim Medinipur

Dr. Shrabani Pradhan
Assistant Professor & Coordinator in Nutrition
Department of Paramedical & Allied Health Sciences
Midnapore City College

Vidyasagar University

Plagiarism Testing Report

Name of Research Scholar: ***Riya Kar***

Title of the thesis: ***Effect of fatty acid(s) extracted from marine topse (Polynemus paradiseus) fish oil on obesity and obesity related metabolic disorders***

Name of Supervisor : ***Dr. Shrabani Pradhan***

~~Department~~ /Research Centre Name : ***Biodiversity & Environmental Studies***

Institute Name: ***Midnapore City College***

This is the report that the above thesis was scanned for similarity detection excluding bibliography and references. Result of testing report is given below:

Software used: ***DrillBit***

Similarity Indexed: **8 %**

The similarity index is within the accepted range.

Date: ***26 May 2026***

Place: ***Midnapore***

Sr. Information Scientist
Vidyasagar University

Director, IQAC
Vidyasagar University

26/05/26
Registrar
Vidyasagar University

Sr. Information Scientist
Vidyasagar University
Midnapore-721102
infsc@mail.vidyasagar.ac.in

Director
Internal Quality Assurance Cell
Vidyasagar University
Midnapore 721102

Registrar
VIDYASAGAR UNIVERSITY
Midnapore-721102, W.B.

Effect of fatty acid(s) extracted from marine topse (*Polynemus paradiseus*) fish oil on obesity and obesity related metabolic disorders

Abstract

Obesity is a major public health concern, and its prevalence is growing alarmingly on a global level. Obesity is determined by genetic, neurological, metabolic, gastrointestinal, and behavioural factors which are connected with the number of comorbid abnormalities, such as cancer, diabetes mellitus, and cardiovascular disease. Consumption of fish has become popular in regular meals. Current research has demonstrated that marine fish oil (FO) may offer an alternative method because of their significant health benefits for lowering obesity and its related issues. Marine FO enrich in long- chain omega- 3 polyunsaturated fatty acids (LC n- 3 PUFA), long- chain omega- 6 polyunsaturated fatty acids (LC n- 6 PUFA) and monounsaturated fatty acids (MUFAs) performs a crucial role in decreasing metabolic syndrome abnormalities and has a number of disease combating properties, including cardioprotective, anti-atherosclerotic, anti-obesity, anti-cancer, and anti-inflammatory activities. Considering the adverse effects of marketed drugs (orlistat, lorcaserin, liraglutide, semaglutide etc), to minimize the risk of obesity, we extracted, isolated and characterized marine *Polynemus paradiseus* FO in this investigation for the first time. Marine *P. paradiseus* fish widely found in the Eastern Indian Ocean and Western Pacific region including India, Thailand, Myanmar, Bangladesh and Pakistan. This fish locally named as topse fish are indigenous source which highly thrives in West Bengal's marine areas in India also. Due to limitation of biological activities on *P. paradiseus* FO, we design *in vivo* model experiment to investigate the efficacy of marine *P. paradiseus* FO in treating obesity-related metabolic diseases. In our experiment, compared to the high fat diet (HFD)-induced obese control (OC) group, the *P. paradiseus* FO supplemented obese mice group (Treatment-I and Treatment-II) represented a remarkable reduction in body weight, body mass index (BMI), and serum lipid profiles. The FO-supplemented HFD-induced obese mice group demonstrated an average diminution in obesity and inflammatory-related adipocytokines in FO supplemented groups compared to the OC group. *P. paradiseus* is a reasonable choice since it contains a significant number of essential fatty acids such as 51.15 % of PUFAs including methyl ganma-linolenate, methyl linolelaidate, DPA, methyl arachidonate, DHA etc, and 25.04% of MUFAs such as methyl cis- vaccenate, gondoic acid, methyl cis-11-icosenoate, and methyl cis-12-octadecenoate etc and its physicochemical features confirm that it is harmless for human

ingestion. Among all the MUFAs present in *P. paradiseus* FO, Gondoic acid (GA), has been considered as a possible substitute for fish-derived fatty acids, its function in molecular level and adipocyte proliferation remains unclear. Present research examines the anti-obesity efficacy and mechanisms of GA treatment in HFD-induced obese mice and adipocyte development in 3T3-L1 cells, presenting an innovative research viewpoint in a current topic of research. Our findings, explored that administering GA reduced body weight, serum lipid profile levels, modified inflammatory responses and adipogenesis in diet-induced obese mice. From *in vitro* study, cellular viability assay was performed to investigate possible cytotoxicity of GA. In a culture of matured 3T3-L1 adipocytes, lipid buildup was evaluated using Oil Red O (ORO) staining and triglyceride accumulation (TG). Adipogenic transcription parameters were used to assess adipocyte differentiation, and semi-qPCR was performed to examine the regulation of lipid metabolizing genes. GA's anti-adipogenic impact was caused by the reduction of adipogenic transcription factors such as sterol-regulatory element binding protein-1c (SREBP-1c) and peroxisome proliferator-activated receptor gamma (PPAR-γ) and also reduced the expression of lipid accumulation gene, fatty acid synthase (FAS). In HFD-induced mice, GA treatment enhanced glucose sensitivity, lowered fasting insulin levels, and altered adipocytokine levels, enhancing thermogenesis and β-oxidation in mitochondria via the AMPK mechanism.

In conclusion, our overall study explored that *P. paradiseus* FO enriched with a significant number of essential fatty acids (FAs), it may be employed as a nutritional supplement to combat obesity. Secondly, *P. paradiseus* FO derived GA showed inhibition of adipocyte development, lipid build up in 3T3-L1 cells. Our research also explored that the treatment of 4 mg GA supplementation with the duration of 35-42 days may serve as an effective strategy to mitigate obesity associated metabolic dysfunction by enhancing browning, reducing lipogenesis, improving some of the metabolic and insulin signalling abnormalities linked with HFD feeding.

Keywords: obesity, essential fatty acids, gondoic acid, preadipocyte cell, metabolic imbalance, TG accumulation, mitochondrial biogenesis

DECLARATION

I do here by declare that the present Ph.D. thesis entitled, "**Effect of fatty acid(s) extracted from marine topse (*Polynemus paradiseus*) fish oil on obesity and obesity related metabolic disorders**" is the original research work carried out by me in the Department of Paramedical and Allied Health Sciences, Midnapore City College, Kuturiya, Bhadutala, Paschim Medinipur, West Bengal, India under the supervision of Dr. Shrabani Pradhan, Assistant Professor in Nutrition, Department of Paramedical and Allied Health Sciences, Midnapore City College, Kuturiya, Bhadutala, West Bengal, India. No part thereof has been submitted for any degree of diploma in any university.

Date: ...20.05.2026.....

Place: Midnapore

Riya Kar

.....

Riya Kar

Research Scholar

Acknowledgement

First and foremost, I convey my deepest sense of reverence and gratitude to my respected guide **Dr. Shrabani Pradhan**, Assistant Professor in Nutrition, Department of Paramedical and Allied Health Sciences, Midnapore City College, Paschim Medinipur, 721129, West Bengal for encouraging me in to this work as well as her invaluable guidance, helpful suggestions, understanding and patience, necessary assistance and endless effort throughout this research study, as my supervisor despite her many other academic and professional commitments and without her this study wouldn't have been completed. I am very fortunate for having her as my guide.

My sincere thanks to all esteemed members, of my department of Paramedical and Allied Health Sciences, Midnapore City College. I am thankful to **Dr. Suchismita Roy**, Assistant Professor in Nutrition, Department of Paramedical and Allied Health Sciences, Midnapore City College, for her guidance, encouragement and support throughout the research work. A special thanks to my lab **mates Ms. Pipika Das, Mrs. Titli Panchali, Mrs. Ananya Dutta, Ms. Amina Khatun, Mrs. Manisha Phoujdar, Mr. Sahadeb Jana, Mr. Palash Mitra, Ms. Sibani Rout** for their co-operation.

I sincerely acknowledge my deepest sense of gratitude to **Professor Pradip Ghosh**, Founder, Director, Midnapore City College for his constant support, invaluable guidance, and necessary assistance moreover he gave me a big opportunity and a platform for execute my research work, also for giving many well maintenances well performed instrument in our lab.

I gratefully acknowledge the funding received from the **Indian Council of Medical Research (ICMR),** Government of India for providing financial support as Extramural Adhoch Research Grant **File no. 5/9/1453/ 22-Nut. dated 02.01.2023** and for providing a scholarship to me.

I desire to express my heartily expression and indebtedness to **Dr. Sudipta Chakrabarti**, Principal, Midnapore City College, West Bengal for extending the necessary Institutional facilities for Ph.D. work.

I expressed my deepest sense of gratitude to **Dr. Kuntal Ghosh**, Vice Principal, Midnapore City College, West Bengal for encouraging me in to this work as well as his helpful suggestions and necessary assistance throughout this research study.

I wish to convey my gratefully acknowledgement to the Hon'ble Vice Chancellor**, Prof. Dipak Kumar Kar** and Respected Registrar of **Dr. Jayanta Kishore Nandi**, Vidyasagar University for giving the necessary facilities from University for this Investigation.

I sincerely acknowledge my deepest thanks to the help rendered by all research Scholar, faculty and technical staffs, Midnapore City College for their constant support.

I expressed my deepest sense of gratitude to **Sandor Speciality Diagnostics** Pvt. Ltd, 8-2-326/5, Road No.3, Banjara Hills, Hyderabad - 500 034, Telangana, INDIA for conduct some experiment and Instrumental facilities.

I do express my thanks and regards to good number of scientist and researchers in my country and abroad who have taken me their valuable research publication in response to my request.

I particularly want to express my deepest gratitude to the Editorial Board of different National and International Journals for publication my original research work related to the Ph.D. and their valuable comments and criticism to develop my research knowledge.

I owe my deepest gratitude to the entire Staffs of Midnapore City College especially **Mr. Subho Mal and Mr. Suman Dey** for help me to fulfil my research work.

Last but not least, I must pay my deepest regards to my parents and all members of the family for their great sacrifice, constant inspiration and generous help. I acknowledge the sacrifice of my father **Mr. Dulal Chandra Kar**, mother **Mrs. Mina Kar**, sister **Ms. Raima Kar**, for their absolute love, constant encouragement, endless co-operation, inspiration and tolerance during the study. They were always with me in both good and bad times of this journey and made me believe in myself.

Chapter No.	Contents	Page No.
I	**General Introduction**	1-5
II	**Review of Literature**	6-11
III	**Aim & Objectives**	12
IV	**Objective (i): Extraction, isolation, and characterization of fatty acids (FAs) from *P. paradiseus* FO.** Introduction Materials & Methods Results Discussion Conclusion	13-26
V	**Objective (ii): To search out the anti-obesity potentiality of *P. paradiseus* FO *in vivo* model.** **Experimental Design I** Introduction Materials & Methods Results Discussion Conclusion **Experimental Design II** Materials & Methods Results Discussion Conclusion	27-62
VI	**Objective (iii): To establish the anti- obesity potentiality of isolated FA both *in vitro* and *in vivo* model.** **Experimental Design I** Introduction Materials & Methods Results Discussion Conclusion **Experimental Design II** Materials & Methods Results	63-104

	Discussion Conclusion	
VII	**Objective (iv): To explore the role of isolated FA in alleviating obesity related metabolic disorder *in vivo* model.** Introduction Materials & Methods Results Discussion Conclusion	105-129
VIII	**Summary & Conclusion**	130-133
IX	**Future Direction**	134
X	**Bibliography**	135-161
XI	**Publications**	162-163
XII	**Conference Proceedings**	164-165

List of Tables

Chapter IV

Table 1a. Total percentage of fatty acids composition from *P. paradiseus* FO measured using GC-FID.

Table 1b. List of PUFAs from *P. paradiseus* FO with their RT value, molecular weight and biological significance listed after GC-MS analysis

Table 1c. List of MUFAs from *P. paradiseus* FO with their RT value, molecular weight and biological significance listed after GC-MS analysis

Table 2. Physicochemical Properties of *P. paradiseus* FO

Chapter V

Experimental Design I

Table 1. Following primer sequences used in semi q PCR analysis.

Experimental Design II

Table 1. Following primer sequences used in semi q PCR analysis.

Chapter VI

Experimental Design I

Table 1. Utilizing primer list for semi-quantitative PCR.

Experimental Design II

Table 1. Listed primer sequences used in semi q PCR analysis.

Chapter VII

Table 1. List of primers used in this experiment for RT- PCR

List of Figures

Chapter I

Figure 1. Overview of alterations in AT with obesity associated adipogenesis that responsible for release of some adipocytokines, free fatty acid, triglycerol and raised the risk of several metabolic disorders in an obese individual.

Figure 2. Mechanisms mediating effects of n-3 PUFA on liver and AT metabolism. LC n-3 PUFA increase fatty acid oxidation in the liver and adipose tissue, thus limiting fat storage in these tissues. LC n-3 PUFA also decrease the production and release of pro-inflammatory adipokines by two major pathways way one is PPAR- γ and AMPK pathway. All mechanisms depicted here contribute to an improved metabolic profile.

Chapter III

Figure 1. Distribution range colors indicate degree of suitability of habitat of ***P. paradiseus*** fish (**Eastern Indian Ocean and Western Pacific) (Source: FishBase)**

Chapter IV

Figure 1. Flow chart of oil extraction process using FAME method, followed by a variety of analyses and assessments.

Figure 2. GC-MS chromatograms analysis of *P. paradiseus* FO. This chromatogram showing the presence of nine SFA, eleven PUFAs and nine MUFAs.

Chapter V

Experimental Design I

Figure 1. FO supplementation reduces body weight increase and fat formation in HFD-fed mice. (a) measurements of weekly food consumption (b) variation in the body weight (c) BMI (gm/cm^2) (d) weight of visceral WAT (gm) in different experimental group (n=6) values are expressed as mean ±SEM. Statistical analysis evaluated via one way ANOVA followed by Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05.

Figure 2. Assessments of total lipid profiles such as a) TC, b) TG, c) HDL, d) LDL, e) VLDL and f) total serum glucose level in different experimental group (n=6) values are expressed as mean ±SEM. Followed by one way ANOVA and Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05

Figure 3. Effects of FO on hepatic lipid profile of HFD-fed mice groups. Groups are separated as a) control; b) obese control; c) T-I (HFD feeding and received 200mg/kg body weight / day of *P. paradiseus* oil) and d) T-II (HFD feeding and received 400mg/ kg body weight / day of *P. paradiseus* crude oil). Tissue sections were stained with hematoxylin and eosin. (e) Estimation of liver TG in different experimental groups. Results are expressed as mean ± SEM for each group (n=6). The observation was made under 10 × magnification and Scale bar was 100 μm. Arrows (→) indicate the enlargement of cytoplasm in different experimental groups.

Figure 4. The impact of *P. paradiseus* oil on skin and vWAT histology. Groups are separated as control group (feed with normal diet), OC group (received HFD), T-I group (HFD feeding and received 200mg/kg body weight / day of *P. paradiseus* oil) and T-II group (HFD feeding and received 400mg/kg body weight / day of *P. paradiseus* crude oil). (A) sWAT and (B) vWAT sections were stained with hematoxylin and eosin (HE) staining. The observation was made under 10 × magnification and Scale bar was 100 μm. Arrows (→) indicate the size of vWAT and accumulation of sWAT in different experimental groups.

Figure 5. Expression of obesity, inflammatory and anti-inflammatory related adipocytokines by semi q-PCR. (a) Relative gene expression level of (b) Leptin, (c) Adiponectin, (d) SREBP-1c, (e) FAS, (f) LPL (g) CPT-1 (h) IL-1Ra, (i) TNF-α, (j) IL-10, and (k) IL-6 in adipose tissue of male albino BALB/c mice in different experimental groups (n=6) were adjusted to control gene expression level (GAPDH; b-k). Values are expressed as mean ±SEM. Statistical analysis evaluated via one way ANOVA followed by Tukey's multiple comparison test. ****$p<0.0001$, ***$p<0.001$, **$p<0.01$, *$p< 0.05$.

Figure 6. ELISA was used to investigate the impact of *P. paradiseus* oil on the synthesis of a) leptin b) IL-10 and c) TNF-α in different experimental groups (C, OC, T-I, and T-II). Values are expressed as mean± SEM. Statistical analysis evaluated via one way ANOVA followed by Tukey's multiple comparison test. ****$p<0.0001$, ***$p<0.001$, **$p<0.01$, *$p< 0.05$.

Figure 7. Protein expression analysis of Ob (H-5), PPAR-α, PPAR-γ and FAS in WAT. Groups are separated as control group (feed with normal diet), OC group (received HFD), T-I group (HFD feeding and received 200mg/kg body weight / day of *P. paradiseus* oil) and T-II group (HFD feeding and received 400mg/kg body weight / day of *P. paradiseus* crude oil). Results were normalized with respect to β-actin. Representative Images of western blot WAT (a) Relative protein expression, (b) ob (H-5), (c) PPAR-γ, (d)PPAR-α and (e) FAS. Values are

expressed as mean ±SEM. Statistical analysis evaluated via one-way ANOVA followed by Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05.

Experimental Design II

Figure 1. FO supplementation reduce weight gain and fat buildup in HFD-fed mice (a) fat accumulation in different experimental groups, (b) evaluations of dietary intake each week (c) variations in the body weight (d) BMI (gm/cm^2) (e) represent the weight of the kidney (g), liver (g), and WAT (g) in each experimental group (n=6) values are expressed as mean ±SEM. Statistical analysis evaluated via one way ANOVA followed by Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05.

Figure 2. Analysis of total biochemical parameters such as serum a) cholesterol, b) triglycerides, c) HDL, d) LDL, e) VLDL, f) glucose, g) protein, h) uric acid, i) urea, j) SGOT, and k) SGPT in each of the six experimental groups. The values are given as mean ±SEM. Tukey's multiple comparison test and one-way ANOVA followed next. **p<0.01, *p<0.05, ***p<0.001, ****p<0.0001.

Figure 3. Efficacy of FO on hepatic lipid buildup in HFD-fed mice groups. Groups are segregated as control; obese control; T-I (14 days), T-II (21 days), T-III (28 days), T-IV (35 days) and treated with selective dose (400 mg/ kg body weight/ day) of FO. (A) Represent the image of liver, HE staining of (B) liver tissue, (C) sWAT, (D) vWAT. The results are presented as mean ± SEM for each group (n=6). The observation was made under 10 × magnification and scale bar was 100 μm. In liver section arrows (→) reveal the restoration of cytoplasm and in WATs section arrows (→) indicate the fat accumulation layer as well as adipocyte size in different experimental groups.

Figure 4A. (a) Relative mRNA expression of some obesity and inflammatory related gene markers level in AT of male albino BALB/c mice in different FO treated groups (n=6) were adjusted to compare with house-keeping gene GAPDH.

Figure 4B. Expression of obesity related adipocytokines by semi q-PCR. (b) Adiponectin, (c) Leptin, (d) SREBP-1c, (e) FAS, (f) LPL, (g) CPT-1, (h) PPAR-α, (i) IL-6, (j)TNF-α, (k) iNOS, (l) IL-10 and (m) IL-1Ra in AT of male albino BALB/c mice in several experimental groups (n=6) was altered to adjusted gene expression levels (GAPDH; b-m). One-way ANOVA and Tukey's multiple comparison test are used to evaluate statistical analysis. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05.

Figure 5. Protein expression analysis of CPT-1, PPAR-ɣ and PPAR-α in WAT. Experimental groups are segregated as control; obese control; T-I (14 days), T-II (21 days), T-III (28 days) and T-IV (35 days). β-actin was used to normalise the results. Western blot images of WAT adipose tissue: (a) relative protein expression, (b) CPT-1, (c) PPAR-γ, and (d) PPAR-α. Values are expressed as mean ±SEM. Statistical analysis evaluated via one way ANOVA followed by Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05.

Figure 6. FO and its PUFAs and MUFAs are the suitable agents to prevent obesity.

Chapter VI

Experimental Design I

Figure 1. Efficacy of GA on 3T3-L1 adipocyte viability. (a) Chemical structure of GA used in this experiment, (b) Cell viability of 3T3-L1 adipocytes after treated with various doses of GA for 24h, respectively, by using the MTT assay. Values are represented as the mean ± SEM (n = 3). Statistical analysis assessed via one way ANOVA followed by Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05.

Figure 2. Effects of GA on Lipid deposition and progression in 3T-3L1 adipocyte. In the presence of GA (0.25μM, 0.5μM, 0.75μM and 1μM), two-day post-confluency preadipocytes were cultured with differentiation media to become complete adipocytes compared with control group. (A) ORO staining was used to quantify lipid accumulation, (B) TG accumulation in 3T3-L1 adipocytes, (C) Immunofluorescent visualization by DAPI staining. Values are represented as the mean ± SEM (n = 3). Statistical analysis assessed via one way ANOVA followed by Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05.

Figure 3. Semi quantitative PCR analysis of obesity related cytokines such as (b) leptin, (c) adiponectin, (d) AMPK, (e) PPAR-ɣ, (f) PPAR-α, (g) CPT-1, (h) FAS, (i) insulin, (j) GLUT-4, (k) SREBP-1c, (l) LPL, (m) IL-10, (n) IL-1Ra, (o) IL-6 by treating with different doses (0.25,0.5, 0.75 and 1 μM) of GA on 3T3-L1 cell line (1×10^5/ml). The expression profiles of each gene are shown on the y-axis, while the effect of three doses in comparison to the control is represented on the x-axis. Values are represented as the mean ± SEM (n = 3). Statistical analysis assessed via one way ANOVA followed by Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05.

Figure 4. Quantitative evaluation of (a) IL-1ra (b) IL-10 & (c) TNF-α by treating GA with different doses (0.25, 0.5, 0.75 and 1 μM) on 3T3-L1 cell line. Values are represented as the mean ± SEM (n = 3). Statistical analysis assessed via one way ANOVA followed by Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05.

Figure 5. Effects of GA on adipogenic transcription factors and AMPK phosphorylation. (a) Relative protein expression, (b) AMPK, (c) SREBP-1c, (d)PPAR-α, (e) PPAR-ɣ, (f) CEBP-α and (g) adiponectin. Confluent 3T3-L1 cells were differentiated in the presence or absence of different concentrations of GA (0.25, 0.5, 0.75 and 1 μM). After differentiation, 3T3-L1 adipocytes were lysed and measured using SDS-PAGE and Immunoblotting. β-actin protein level served as an internal reference. Values are represented as the mean ± SEM (n = 3). Statistical analysis assessed via one way ANOVA followed by Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05.

Figure 6. Gondoic acid suppresses adipocyte differentiation and lipogenesis-related protein expression in 3T-3L1 cell line.

Experimental Design II

Figure 1. Impacts of GA on body and peripheral organ weight in mice consuming a high-fat diet. (a) Advancement of fat build up; (b) measurements of weekly food consumption; (c) body weight measurements taken over the study duration; (d) BMI (g/cm^2); (e) weight of WAT; (f) weight of liver; (g) weight of kidney in different experimental groups. Values are means ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA accompanied by Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05.

Figure 2. Efficacy of GA administration on lipid metabolism in obese mice. After the development of obesity, obese mice were segregated in to six groups, normal chow feeding, control group (C); normal chow feeding+ olive oil, vehicle control group (VC), HFD feeding, obese control group (OC); HFD+ 1mg GA/day (T-I group); HFD+ 2mg GA/ day (T-II group); HFD+ 4 mg GA/ day (T-III group). Blood samples from every mouse were taken on the final day of the experiment. Blood samples were used to separate serum, and its concentrations were determined of (a) glucose; (b) cholesterol; (c) triglyceride; (d) HDL; (e) VLDL; (f) LDL; (g) protein; (h) urea; (i) uric acid; (j) SGOT and k) SGPT level in all the experimental group (n=6). Values represent the mean ±SEM. Statistical analysis was performed using one-way ANOVA

accompanied by Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05.

Figure 3. Histological analysis of liver tissues. Groups are separated as normal chow feeding, control group (C); normal chow feeding+ olive oil, vehicle control group (VC), HFD feeding, obese control group (OC); HFD+ 1mg GA/day (T-I group); HFD+ 2mg GA/ day (T-II group); HFD+ 4mg GA/ day (T-III group). (a) Represent the image of liver, tissue sections were stained with (b) HE & (c) Assessment of liver TG. The results for all groups (n=6) are presented as mean ± SEM. Statistical analysis was performed using one-way ANOVA accompanied by Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05. In the staining part arrows (→) represent the way of cytoplasm enlargement in several experimental groups. The scale bar correspondence to 100 μm.

Figure 4. Effect of GA supplementation on adipose tissue in obese mice. Groups are segregated as normal chow feeding, control group (C); normal chow feeding+ olive oil, vehicle control group (VC), HFD feeding, obese control group (OC); HFD+ 1mg GA/day (T-I group); HFD+ 2mg GA/ day (T-II group); HFD+ 4 mg GA/ day (T-III group). Mass of sWAT and vWAT sections were stained with hematoxylin and eosin (HE) staining (a, b). (c) Mean adipocyte size diameter. The results for all groups (n=6) are presented as mean ± SEM. Statistical analysis was performed using one-way ANOVA accompanied by Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05. In the staining part arrows (→) represent the fat cell size (visceral AT) and fat accumulation layer (subcutaneous AT) in several experimental groups. The scale bar correspondence to 100 μm.

Figure 5. Impact of GA supplementation on obesity and inflammatory-related adipocytokines using semi-q-PCR (a) Relative gene expression level of (b) *AMPK*; (c) *Adiponectin*; (d) *Leptin*; (e) *SREBP-1c*; (f) *GLUT 4*; (g) *FAS*; (h) *LPL*; (i) *CPT-1*; (j)*insulin*; (k) *PPAR-α*; (l) *PPAR-γ*; (m) *TNF-α*; (n) *IL-10* and (o) *IL-1ra* in AT of male BALB/c obese mice in different experimental groups (n=6) were adjusted to control gene expression level (*GAPDH*; b-o). The results for all groups (n=6) are presented as mean ± SEM. Statistical analysis was performed using one-way ANOVA accompanied by Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05.

Figure 6. Efficacy of GA intervention on protein in the WAT of nutritionally obese mice. Groups are segregated as normal chow feeding, control group (C); normal chow feeding+ olive oil, vehicle control group (VC), HFD feeding, obese control group (OC); HFD+ 1mg GA/day

(T-I group); HFD+ 2mg GA/ day (T-II group); HFD+ 4mg GA/ day (T-III group). β-actin was used in order to normalise the results. (a) AMPK; (b) CEBP-α; (c) SREBP-1c; (d) FAS; (e) PPAR-γ; (f) Ob (H-5). The results for all groups (n=6) are presented as mean ± SEM. Statistical analysis was performed using one-way ANOVA accompanied by Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05

Chapter VII

Figure 1. GA decreases fat mass and body weight *in vivo*. (a) Representative morphological images of experimental group; (b) measures of dietary intake each week; (c) assessments of body weight; (d) BMI (gm/cm^2); (e) tissue weight; (f) tissue index; (g) lee's index of different experimental groups (n=6). Data are represented as mean ±SEM. Tukey's multiple comparison test is used following one-way ANOVA to assess statistical analysis. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05.

Figure 2. GA reduces insulin resistance and enhances glucose and fat metabolism. Lipid profile such as (a) TC; (b) TG; (c) HDL; (d) VLDL; (e) LDL; (f) fasting blood glucose; (g) fasting blood insulin; (h) Homeostatic model assessment of insulin resistance (HOMA-IR) values (i, j) Glucose sensitivity tests were performed in mice, determined the area under the curve (AUC); serum (k) Insulin; (l) TNF-α; (m) IL-6 levels were estimated using ELISA kits in different experimental groups (n=6). Data are represented as mean ±SEM. Statistical analysis assessed via one way ANOVA followed by Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05.

Figure 3. GA improve hepatic health and reduces adipocyte size in subcutaneous fat. (a) Represent the morphological images of liver; (b) HE staining of liver tissue; (c) Estimation of liver TG; (d) Cholesterol; (e) fat accumulation in experimental groups; stained by HE in (f) sWAT; (g) vWAT; and (h) adipocyte diameter. Results are represented as mean ± SEM for each group (n=6). The scale bar is corresponding to 100 μm.

Figure 4. Representative immunofluorescence images showing the expression and localization of (A) PPAR-ɣ (green fluorescence) and cell nuclei (DAPI, blue fluorescence) in liver tissue sections. The bottom row displays merged images of DAPI and PPAR-ɣ staining, (B) PPAR-α (green fluorescence) and cell nuclei (DAPI, blue fluorescence) in liver tissue sections. The bottom row displays merged images of DAPI and PPAR-α staining.

Figure 5. Effects of GA intervention on obesogenic markers (a) *AMPK*; (b) *leptin*; (c) *Adiponectin*; (d) *SREBP-1c*; (e) *PPAR-γ*; (f) *PPAR-α*; (g) *CEBP-α*; (h) *FAS*; (i) *GLUT-4*; (j) *GLUT-3*; (k) *GLUT-1*; (l) *MCP-1*; (m) *CRP*; (n) *IL-6*; (o) *TNF-α* in different experimental groups (n=6). Data are represented as mean ±SEM. Statistical analysis assessed via one way ANOVA followed by Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05.

Figure 6. GA enhances mitochondrial biogenesis *in vivo* model. (a) Relative protein expression, (b) PGC1-α; (c) AMPK; (d) *Ob* (H-5); (e) adiponectin; (f) SREBP-1c; (g) PPAR-γ; (h) PPAR-α; (i) CEBP-α; (j) FAS; (k) GLUT-4; (l) GLUT-3; (m) GLUT-1 in different experimental groups (n=6). Data are represented as mean ±SEM. Statistical analysis assessed via one way ANOVA followed by Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05.

Figure 7. Effects of n-9 GA on obesity and associated disorders during *in vivo* studies. Numerous molecular and inflammatory processes that affect metabolism are influenced by obesity and its related conditions, including insulin resistance, dyslipidaemia, metabolic inflammation, dysbiosis, and non-alcoholic hepatic steatosis. GA increase β-oxidation in the liver and adipose tissue, thus limiting hypertrophy and hyperplasia via activating AMPK in these tissues. GA also decrease the production and release of lipogenic genes and proinflammatory adipokines. AMPK is one of the possible mechanisms that is shown here that could help with a better metabolic profile.

Chapter VIII

Figure 1. Exploration of antiobesity efficacy of marine *P. paradiseus* oil and its outcomes via underlying biological mechanism.

Abbreviations

The following abbreviations are used in this manuscript

ACC Acetyl Co-A carboxylase

AMPK AMP-activated protein kinase

AT Adipose tissue

AUC area under curve

BAT Brown adipose tissue

BMI Body mass index

BSA Bovine serum albumin

C/EBP-α CCAT/enhancer-binding protein alpha

C/EBP-β CCAT/enhancer-binding protein beta

CFAs Conjugated fatty acids

ChREBP Carbohydrate- responsive element binding protein

CLA Conjugated linoleic acid

COX-3 cyclooxygenase-3

CPT-1 Carnitine Palmitoyl transferase 1

CRP C-reactive protein

D9D Delta-9 desaturase

DAB 3,3',4,4'- tetra aminobiphenyl tetrahydrochloride

DHA Docosahexaenoic acid

DMEM Dulbecco's modified Eagle's medium

DMSO Dimethyl sulfoxide

EPA Eicosapentaenoic acid

FABP Fatty acid binding protein

FAME Fatty acid methyl ester

FAS Fatty acid synthase

FBS Foetal bovine serum

FFAs Free fatty acids

FGF 21 Fibroblast growth factor 21

FO Fish oil

GA Gondoic acid

GAPDH Glyceraldehyde 3-phosphate dehydrogenase

GGE03 Ginger ethanolic extract

GLUT-1 Glucose transporter type 1

GLUT-2 Glucose transporter type 2

GLUT-4 Glucose transporter type 4

GTT Glucose tolerance test

HDL-c High-density lipoprotein cholesterol

HE Hematoxylin and eosin

HFD High fat diet

HOMA-IR Homeostasis model assessment of insulin resistance

HRP Horseradish peroxidase

HSL Hormone sensitive lipase

IBMX Isobutyl-3-methyl-Xanthine

ICMR Indian Council of Medical Research

IL-10 Interleukin 10

IL-1Ra Interleukin 1 Receptor antagonist

IL-1β Interleukin 1 beta

IL-6 Interleukin 6

iNOS Nitric oxide synthase

IR Insulin resistance

ITT Insulin tolerance test

LC n-3 PUFAs Long chain omega 3 PUFAs

LDL-c Low-density lipoprotein cholesterol

LPL Lipoprotein lipase

LxRα Liver X receptor alpha

MCP-1 Monocyte chemoattractant protein-1

mRNA messenger RNA

MTT 3-(4.5- dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide

MUFAs Monounsaturated fatty acids

Myd88 Myeloid Differentiation primary response 88

NCCS National Centre for Cell Science

NF-κB Nuclear transcription factor kappa B

NIDDM non-insulin dependent diabetes mellitus

PGC-1α Peroxisome proliferator activated receptor coactivator- 1α

PPAR-α Peroxisome proliferator- activated receptors

PRDM16 PR domain containing 16

PUFAs Polyunsaturated fatty acids

RIPA Radioimmuno precipitation assay

ROS Reactive oxygen species

RT-PCR Real time Polymerase Chain Reaction

SCD1 Stearoyl Co-A desaturase 1

SDS-PAGE Sodium dodecyl- Sulfate- Polyacrylamide gel electrophoresis

Semi qPCR Semi Quantitative Polymerase Chain Reaction

SFA Saturated fatty acid

SREBP-1c Sterol regulatory element binding protein- 1c

SVF Stromal vascular fraction

TC Total cholesterol

TG Triglycerides

TNF-α Tumor necrosis factor-alpha

UCP1 Uncoupling Protein 1

UCP2 Uncoupling Protein 2

VLDL-c Very low-density lipoprotein cholesterol

WAT White adipose tissue

WHO World Health Organization

Chapter I: General Introduction

Introduction

In the present scenario, obesity is a challenging health problem and its prevalence along with comorbidities are on the rise around the world. Life has gotten faster and more convenient as a result of technological advancements, but it has also brought with it a variety of health risks. Some reasons which generating health hazards include lack of physical activity, work stress, disturbed biological cycle and other variables such as consumption of junk foods rich in saturated fat, nutritional inadequacies that influence human beings of all generation **(Balwan & Saba 2021)**. Over 1 billion people worldwide suffer from obesity, according to the **World Obesity Atlas 2026.** Recently the prevalence rate of obesity in India is 40.3% **(Venkatrao et al., 2021).** According to projections, the percentage of children and adolescents aged 5 to 19 who are overweight or obese has increased from 14.6% in 2010 to 20.7%. According to the report, 4 billion people, or half of the world's population, might be impacted by 2035 if prompt action is not taken. According to **Ferreira et al. (2024)**, it should come as no surprise that obesity has gained prominence on the global public health agenda and has drawn funding from a variety of societal sectors in an effort to slow down this grave health issue. The World Health Organization (WHO) defines obesity as a chronic, recurrent disorder that results from complex interactions between genetics and neurobiology. Weight and height measurements are used to determine a person's body mass index (BMI), which is calculated as weight (kg)/height² (m^2). In addition to the BMI, other parameters like the waist circumference can aid in the diagnosis of obesity. Adults with a BMI between 18 and 24.9 kg/m^2 are considered normal; those with a BMI over 25 kg/m^2 are overweight, and those with a BMI over 30 kg/m^2 are obese. Class 1 (BMI of 30 to <35), Class 2 (BMI of 35 to <40), and Class 3 (BMI of >40) are further subcategories of obesity **(Ray et al., 2019)**. The metabolism of fat and glucose is greatly influenced by adipose tissue (AT), which is a primary location of energy storage. Adipokines, such as leptin, factor-alpha, interleukin-6, and monocyte chemoattractant protein-1, are known to be expressed and secreted by AT **(Antuna-Puente et al., 2008)**. Due to the disruption of adipokine secretion from white adipose tissue (WAT), an excessive amount of WAT contributes to obesity and obesity-related disorders like diabetes mellitus, hypertension, dyslipidemia, and cardiovascular disease in the current nutritionally abundant environment.

Fish is regarded as a budget-friendly source of a wide range of beneficial elements, mostly high in fat and protein, both of which are important components of a human diet. Fish protein is particularly vital for muscle tissue growth and repair, as well as immune system enhancement **(Pal et al., 2018)**. The oil obtained from fish contain triglycerides, phospholipids, fatty acids, wax esters, sterols, other minor compounds like glyceryl esters, glycolipids, hydrocarbons like

squalene, sulpholipids etc. While phospholipids and sterols are structural elements of the cell membrane, other lipids function as energy reserves. Marine fish oil (FO) contains a more diverse range of fatty acids, with a particular emphasis on polyunsaturated and monounsaturated fatty acids (PUFAs and MUFAs) **(Das et al., 2024)**. Dietary sources of long chain omega -3 PUFAs (LC n-3 PUFAs), such as eicosa pentaenoic acid (EPA) and docosahexaenoic acid (DHA), from marine FO have been shown to have hypotriglyceridemic, anti-inflammatory, and cardioprotective effects **(Carpentier et al., 2006)**. Thus, it is particularly interesting to see how marine FO (LC n-3 PUFAs) affects body weight and body composition. We provide an update on the impact of marine FO (LC n-3 PUFAs) on obesity and related disorders in this review, emphasizing the possible mechanisms for LC n-3 PUFAs in improving body composition, lowering body weight, and mitigating the negative metabolic effects of obesity. The association between n-3 PUFAs and cardiovascular disease was established when it was discovered that the Greenland Inuit had a low death rate from coronary heart disease while consuming a diet rich in fat. Compared to n-3 PUFAs found in other plant sources, the marine source of n-3 PUFAs has greater nutritional benefits. Numerous value-added food compounds and health-food capsules contain fish oils as nutritious ingredients and additions **(Das et al., 2024; Ruxton et al., 2004)**. In accordance with Mahapatra et al. **(2014)**, numerous kinds of marine fish found widely in various Indian states, including Pomfret, Hilsa, Prawn, Surmai, Indian Mackerel, Pedvey, Snail, Crab, Indian Salmon, Bombay duck, Tapra, Topse, Ruli, Ribon, various types of Croker, and Pabda, those are nutrition rich and cost wise affordable for all community people. Numerous studies explored that health beneficial effects of sea FO containing PUFAs such as curing blood pressure, arthritis, diabetes, heart attack, cancer, thyroid, asthma, ulcer, acidity, constipation, gynecological problems and several other diseases **(Das et al., 2024)**.

Basic pathomechanism of obesity

The basic pathogenesis of obesity involves controlling cellular processes, physical activity, and other factors to either up-regulate appetite or down-regulate calorie utilization. This dysregulation causes an abundance of adipocytes to form, which boosts the release of cytokines and causes the development of vascular complications. The excess blood level of fatty acids and triglycerol causes accumulation of adipocytes which leads to an increase in oxidative stress, hypertriglyceridemia, lipotoxicity, diabetes, and various metabolic syndromes. Thus, lowering oxidative stress, which is a common etiological factor in many pathological conditions, may be advantageous in minimizing the risky effects of obesity and other complications **(Karri et**

al., 2019). The adipocytes are responsible for stimulating the release of adipocytokines such as leptin, adiponectin and vastatin where adiponectin may responsible for cytotoxic autophagy in breast, colon, prostate and female specific carcinogenesis **(Muppala 2017)**. These hormones regulate hunger, satiety, and body fat, and their dysregulation can lead to obesity **(Karri et al., 2019) (Figure 1)**.

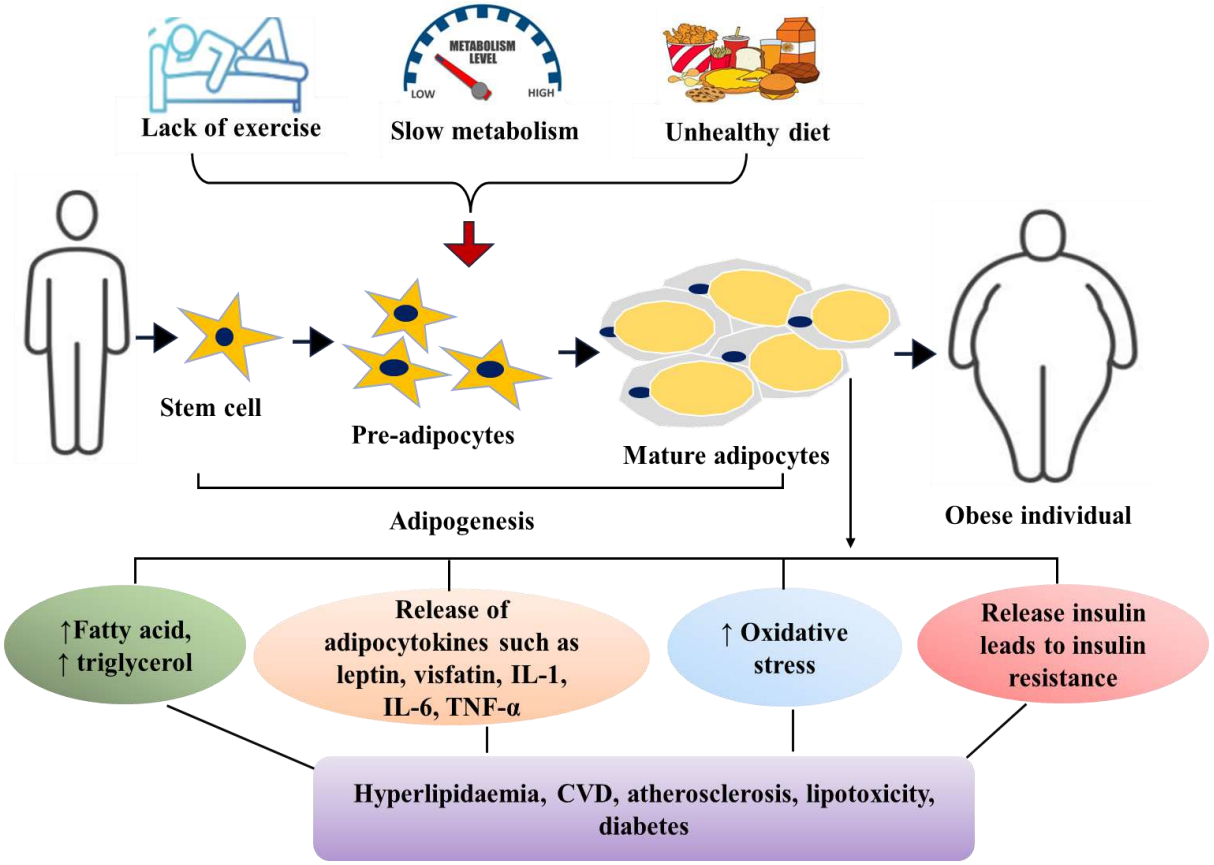


Figure 1. Overview of alterations in AT with obesity associated adipogenesis that responsible for release of some adipocytokines, free fatty acid, triglycerol and raised the risk of several metabolic disorders in an obese individual.

Role of adipocytokines in obesity and obesity induced metabolic disorders

Leptin play important role to regulates hepatic lipogenesis by suppressing the expression of key enzymes in the fatty acid synthesis pathway **(Cohen et al., 2002)**. **AMP- activated protein kinase** (AMPK) is a crucial cellular energy sensor that is activated by leptin, which enhances muscle fatty acid oxidation **(Minokoshi et al., 2002)**. In obesity, metabolic stress is diminished by protective role of **adiponectin** through its anti-inflammatory effects. **Tumor necrosis factor -alpha** (TNF- α) production in obese mice is inhibits by adiponectin. Low levels of plasma adiponectin are associated with increased **c-reactive protein** (CRP) in humans **(Ouchi et al.,2003)**. Adiponectin levels are negatively correlated with the adiposity and fasting glucose.

The main role of TNF-α is promotes insulin resistance of obesity and non-insulin dependent diabetes mellitus (NIDDM). Specifically, in muscle and fat tissues insulin sensitivity improves through neutralization of TNF-α by enhancing the activity of the insulin receptor tyrosine kinase **(Habib et al., 2024).** Numerous studies stated that increasing circulating level of **resistin** increases the risk of type 2 diabetes, inflammatory markers, atherosclerosis **(Chen et al., 2009). Interleukin 6** (IL-6) is one of the major pro-inflammatory cytokines as well as an anti-inflammatory myokine, in obese patients IL-6 serum level was found to be increased that resulting the development of chronic inflammatory conditions and serum lipid concentrations abnormalities **(Galcheva et al.,2011)**. According to **Bluher et al. (2005)**, **Interleukin 10 (IL-10)** is inversely linked with body mass index (BMI) and fasting blood sugar levels. Low levels of IL-10 are associated with both the metabolic syndrome and diabetes. **Fatty acid synthase (FAS)** is a central enzyme in lipogenesis which identified as a candidate gene for determining body fat. **Sterol regulatory element binding protein-1c (SREBP-1c)** is a member of a family of transcription factors that was initially engaged in the control of genes by the cellular availability of cholesterol **(Sewter et al., 2002)**. Main function of **Lipoprotein lipase (LPL)** is hydrolysis of triglyceride (TG) in chylomicron and very low-density lipoprotein cholesterol (VLDL) and clearance of plasma TG rich particles those are used for metabolic energy or re-esterified into triglyceride which stored in AT. Moreover, **peroxisome proliferator- activated receptors** alpha (PPAR-α) reduces endogenous fatty acid synthesis by blocking enzymes like acetyl-CoA carboxylase (ACC) and FASN. **Ghrelin** has various physiological functions, such as the stimulation of growth hormone release and of appetite, and fat accumulation. Ghrelin is the only peripheral hormone to transmit satiety signal **(Sato et al., 2014)**.

Mechanism of n-3 PUFAs improve adiposity and obesity through lipid metabolism

There are several proposed mechanisms by which n-3 PUFA could work in reducing body weight and improving the metabolic profile. AT expansion in obesity occurs via adipocyte hypertrophy (enlargement of adipocytes) and hyperplasia (increase in adipocyte number due to adipogenesis). LC n-3 PUFAs alter expression and nuclear localization of both the transcription factor SREBP-1c and the Carbohydrate-responsive element-binding protein (ChREBP), which control several lipogeneic genes, including those regulating cholesterol and fatty acid synthesis. Furthermore, n-3 PUFAs suppress hepatic lipogenesis by reducing both messenger RNA (mRNA) and active protein expression of SREBP-1c, which results in reduced expression of many genes involved in lipogenesis, including FAS and acetyl-coA carboxylase. PPARs are transcription factors that form heterodimers with retinoid X receptors in the promoter regions

of several genes involved in lipid and glucose metabolism. For example, LC n-3 PUFA activation of PPAR-α decreases lipogenesis by suppressing FAS activity **(Kaur et al., 2014)**. It has been suggested that PPAR-γ plays a significant role in the ability of n-3 PUFA, specifically DHA, to stimulate M2 macrophage polarization and thereby reduce inflammation. LC n-3 PUFAs have been shown to increase mitochondrial biogenesis and fatty acid oxidation in the liver, AT and small intestine of rodents, possibly through PPAR-α and cyclooxygenase (COX3) induction **(Hensler et al., 2011)**. PUFA-controlled genes involved in lipid oxidation and thermogenesis include mitochondrial HMG-CoA synthase, peroxisomal acyl-CoA oxidase, hepatic Carnitine Palmitoyl transferase 1 (CPT-1), Fatty acid- binding protein (FABP) and fatty acid transporter proteins. Activation of PPARα can also increase fatty acid oxidation. Increases in fatty acid oxidation by n-3 PUFA may also be mediated by AMPK, a known regulator of cellular energy metabolism. AMPK upregulation by n-3 PUFA has been proved in both AT and cultured adipocytes. As a central energy sensor, the AMPK has now been explored to play a paramount role in fat synthesis and catabolism, especially in regulating the energy expenditure of brown/beige adipose tissue and the browning of WAT **(Kasbi et al., 2012) (Figure 2)**.

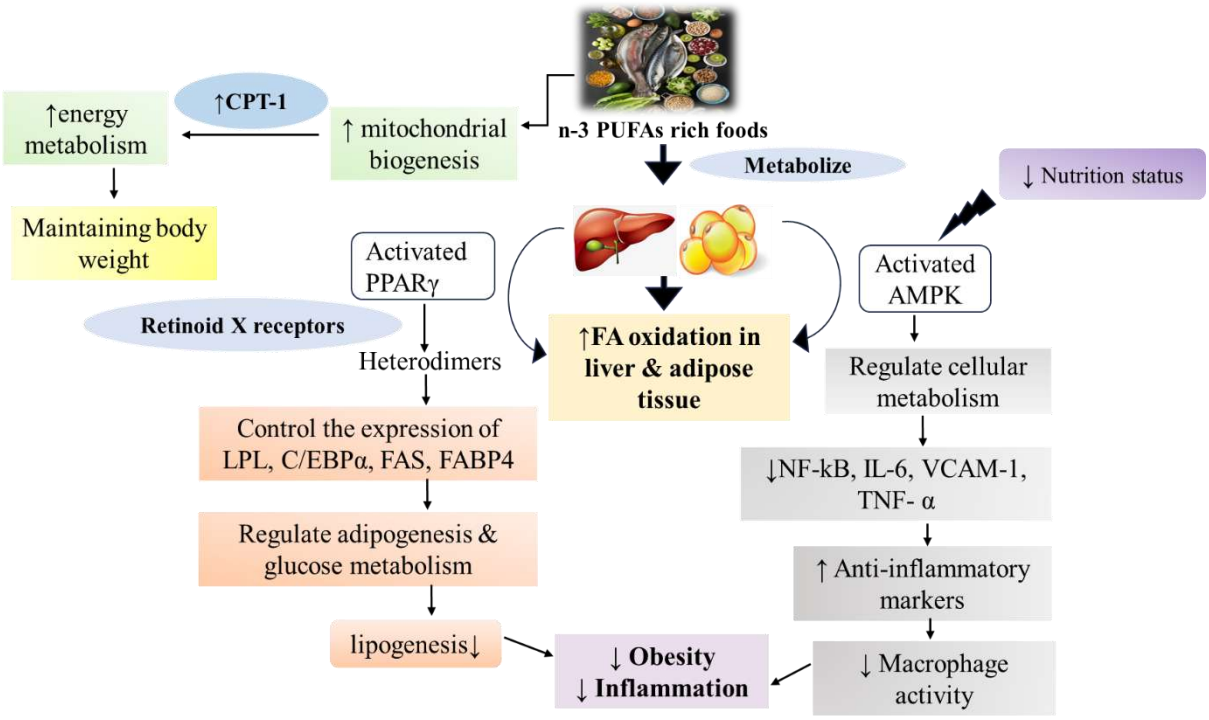


Figure 2. Mechanisms mediating effects of n-3 PUFA on liver and AT metabolism. LC n-3 PUFA increase fatty acid oxidation in the liver and adipose tissue, thus limiting fat storage in these tissues. LC n-3 PUFA also decrease the production and release of pro-inflammatory adipokines by two major pathways way one is PPAR- γ and AMPK pathway. All mechanisms depicted here contribute to an improved metabolic profile.

Chapter II: Review of Literature

Review of Literature

National Status:

Over the next ten years, the prevalence of obesity is predicted to develop Worldwide, according to the 2023 World Obesity Atlas **(Peng et al., 2023)**. As per the Ministry of Statistics and Programme Implementation in 2021, there are around 1.39 billion people living in India, where almost 135 million people in India suffering from obesity **(Chakraborty et al., 2021)**. In accordance to the 2015 ICMR-INDIAB survey, the prevalence rates of obesity and central obesity vary from 11.8% to 31.3% and 16.9% to 36.3%, correspondingly **(Ahirwar & Mondal, 2019)**. According to Banerjee et al. **(2020)**, the prevalence of obesity was 44.6% (35.4% in men and 55.6% in women). However, **Banerjee et al. (2020)** also explored that the prevalence of obesity was 44.6% (35.4 % in males and 55.6 % in females). In accordance to World Health Organization (WHO), 2019 India ranks 3rd in Global Obesity Index. As per WHO, over 2.8 million individuals die each year due to being overweight or obesity-related disorders **(World Health Organization).** In a current study **Das et al. (2025)** explored how the ω-6/ω-3 PUFA mixture prevented adipogenesis in preadipocytes by diminishing the transcription of PPAR-γ and CEBP-α. The study provides scientific evidence that the LA (linoleic acid) /DPA (docosa pentaenoic acid) ratio can inhibit adipose-derived inflammation and lipogenesis through AMPK (adenosine monophosphate-activated protein kinase) activation while promoting β-oxidation and lipolysis. These results showed that LA and DPA together can stop 3T3-L1 preadipocytes differentiation, suggesting that they could be utilised therapeutically and defeat obesity. In line with **Panchali et al. (2024),** Phasa FO's physicochemical properties were satisfactory and included a variety of health-promoting MUFAs and PUFAs. By activating β-oxidation and lipolysis, *Setipinna phasa* oil treatment may reduce BMI, lipid profile levels, and lipid accumulation in HFD-induced obese mice and decrease the expression of inflammatory markers linked to obesity. According to **Das et al. (2024)**, FO has given medical research a lot of attention in recent decades; some doctors recommend consuming fish oil due to its functional component. Fish is essential for maintaining our mental and bodily well-being. The review concentrated on the health benefits of marine fish found in various coastal regions of India. FO supplements are generally harmless for ingestion by humans. They also discussed how nutritional supplements made from fish can alter the gut microbiota and encourage the colonisation of helpful bacteria. Therefore, the results of this analysis may help future research on FO and the investigation of its nutritional value. In another investigation **Pradhan et al. (2020)** established that the Tapra FO had good physicochemical quality and was enhanced with fatty acids that are favourable for health. In obese mice, tapra FO supplementation may reduce

body weight, BMI, serum TC, TG, LDL-C, and VLDL-C. The obese mice supplemented with Tapra FO also demonstrated a decrease in TNF-α and leptin.

International Status:

Adult obesity is expected to impact over 1 billion individuals globally by 2025, with a prevalence rate of 18% in men and over 21% in women. The rates vary widely by nation; for example, the United States has seen a slight decline recently, whereas China and India have a high absolute number of affected individuals **(Kerr et al., 2025)**. Overweight and obesity were predicted to have contributed to 94 million disability-adjusted life years and 3.4 million deaths globally in 2010 **(Lim et al., 2012)**. The Global Burden of Disease study's systematic analysis of data on the prevalence of overweight and obesity in children and adults from 1980 to 2013 revealed that the number of people who were overweight or obese (defined as having a BMI) of more than 25 kg/m2) had increased by 27.5% for adults and 47.1% for children, or from 921 million in 1980 to 2.1 billion in 2013. The frequency and the possibilities of developing obesity differ between and within populations, despite the fact that this tendency is monotonic in communities worldwide **(Ng et al., 2013)**. Both genetic (including ethnic) differences between populations and variations in the degree to which local circumstances are "obesogenic" are expected to contribute to the variation in obesity prevalence and risk **(Murphy et al., 2017)**. Adult obesity has been linked to type 2 diabetes, heart disease, cancer, osteoarthritis, chronic renal disease, late-onset Alzheimer's disease, and pregnancy complications. It is associated with a reduced lifespan and a shorter healthy life expectancy **(Marchi et al., 2015)**. According to **Zhang et al. (2025)** in HFD-induced mice, combined berberine and evodiamine administration resulted in decreased body weight, smaller adipocyte size, and relieved hepatic steatosis. Additionally, it increased HDL-C concentrations while lowering circulating TC, LDL-C, and TG levels. Furthermore, BBE promoted the expression of browning genes and proteins, enhanced glucose sensitivity, decreased fasting insulin levels, and altered adipocytokine levels. Overall, by promoting browning and triggering the FGF21/PGC-1α (Fibroblast Growth Factor 21 / Peroxisome Proliferator-Activated Receptor Coactivator-1 alpha) signalling pathway, berberine and evodiamine reduce obesity. In accordance with **Choi et al. (2025)**, steamed ginger ethanolic extract (GGE03) has drawn interest lately because of its improved bioactive qualities, especially its contain high amount of 6-shogaol, a powerful phytochemical with metabolic and anti-inflammatory advantages. In HFD-induced mice, GGE03 alleviated obesity-related metabolic disorders, with positive effects linked to lipid metabolism and AMPK signalling. These results point to GGE03's potential as a useful food

additive for managing and preventing obesity. As we all know that metabolism and general health are greatly impacted by ageing. Obesity and metabolic dysfunction are major public health concerns because their risk and prevalence increase with age. Menopause causes physical and hormonal changes that significantly affect metabolic health and increase the risk of metabolic problems in middle-aged or older people. This study showed that by preventing inflammation and modifying the gut microbiota in the ovariectomized mouse model, FO supplementation and daily heat treatment enhanced metabolic and insulin sensitivity. These results imply that FO and hypothermia treatment might be helpful for metabolic dysregulations and obesity linked to menopause **(Fan et al., 2025)**. In response to another study, it is crucial to look at how different amounts and kinds of dietary lipids affect adipocyte function because AT plays a crucial role in metabolic regulation. Thus, this study's goal was to investigate how lipogenesis is affected by a PUFA to SFA ratio of 5 and different MUFA levels (MUFA = 30% or 60%). This study concluded that a lower concentration of an M60 fatty acid (P/S ratio = 5, MUFA 60%) initially induces apoptosis by increasing the expression of hormone-sensitive lipase (HSL) and adiponectin and decreasing the expression of genes linked to adipogenesis, such as PPAR-γ, LPL, GLUT-4, and leptin **(Lim et al., 2024).** According to **Rahman et al. (2023)** the efficacy of four extraction techniques—Soxhlet extraction, wet rendering, acid silage, and microwave-assisted extraction—on the oil yield, physicochemical characteristics, fatty acid profiling, and nutritional quality assessment of pangus FO were assessed**.** Therefore, taking into account both technical viability and quality indicators such as extraction yield, optimal physical qualities, oxidative stability, and fatty acid concentrations, microwave-assisted extraction would be the most effective way to extract pangus FO.

There is growing evidence that the aetiology of type 2 diabetes and obesity might be significantly influenced by disruptions in the gut flora. One of the main byproducts of the bacterial fermentation of indigestible carbohydrates is the short chain fatty acid butyrate, which is said to have anti-inflammatory qualities as well as benefits for insulin sensitivity and body weight control **(Deuren et al., 2022)**. According to **Wang et al. (2021)** beneficial effects of LC n-3 PUFAs vary between different sources. It has been established that LC n-3 PUFAs improve vascular health. By blocking TLR4 signalling in a way dependent on MyD88 (Myeloid Differentiation primary response 88), FO and perilla oil—a vegetable source—show comparable protective effects on metabolic diseases and inflammation. These results demonstrate that both marine and plant n-3 PUFA are appealing alternative sources that offer a mechanistic understanding for reducing obesity and associated metabolic disorders. Based to

another research, it is no longer true that obesity is a lifestyle issue among wealthy, educated, and urban people in emerging nations. People in middle socioeconomic levels in rural regions are starting to exhibit increased vulnerability to the burden of overnutrition as rural areas become more developed along the rural-urban gradient. The problem is most severe for people with less varied diets. This supports the claim that the inability to purchase a variety of nutrient-dense foods raises the risk of obesity. Therefore, their findings highlight the significance of eating a variety of foods to prevent a possible overnutrition epidemic **(Aiyar et al., 2021)**. Krill oil supplementation is now regarded as safe and well-tolerated. Bloating, diarrhoea, and flatulence are among the few or non-existent side effects. Nevertheless, more research is required to prove this nutraceutical's safety and effectiveness because the evidence now available is scant. In conclusion, early clinical research indicates that krill oil might be a helpful supplement for treating a number of ailments, such as dysmenorrhea, premenstrual syndrome, osteoarthritis, and cardiovascular diseases. The cost-efficacy ratio of krill oil could be increased by using cutting-edge technologies to increase its concentration and purification (**Colletti et al., 2021).** According to **Esteves et al. (2020),** an increasing number of individuals worldwide suffer from obesity and related comorbidities. In this trial, they discovered that consuming fructose through water enhanced adiposity, weight gain, and blood triglycerides without producing steatosis or liver damage. FO reversed these changes and further reduced fat tissue and serum cholesterol. As a result, they demonstrated that FO and its PUFAs might be useful agents to stop the metabolic effects of fructose consumption. However, in order to avoid harmful modifications while maintaining a therapeutic impact, an appropriate dosage needs to be investigated in additional research and clinical trials. In **2020**, one researcher **Vard** stated that, it may be possible to employ biological indicators of obesity as early cardiovascular risk factors. The criteria appear to be particularly useful in the obese population with high blood pressure. This study suggested using ghrelin to assess cardiovascular risk as it was shown to be a reliable marker for every group under investigation. Additionally, it has been established that biological indicators of obesity have a negative correlation with BMI, ghrelin, and adiponectin. According to a different study, n-3 PUFAs have been used to modulate the lipid and insulin profile in obesity and inflammation in a number of clinical and experimental research. In PCOS rats, the three sources of n-3 PUFAs effectively improved the lipid and hormonal profile and decreased blood glucose, weight gain, and histological damage. However, FO may be a novel way to treat PCOS by lowering weight and metabolic abnormalities brought on by EPA and DHA (**Komal et al., 2020)**. However, **Kwok et al. (2020)** found that changed sWAT transcriptome is linked with long-term body weight change in adult women. Weight loss

is correlated with the expression of genes related to inflammation, insulin response, adipogenesis, and lipogenesis. Nevertheless, some processes, like autophagy, not only correlate with but also forecast future weight growth, indicating that intrinsic mechanisms in sWAT influence tissue expansion. On the other hand, eight weeks of periodic nutritional supplementation with conjugated linoleic acid (CLA) and FO (EPA/DHA) increased body metabolism and changed mitochondrial energy metabolism, according to **Rossignoli et al. (2018)**. EPA/DHA stimulates biogenesis as an adaptive response to energy supply, whereas CLA directly uncouples oxidative phosphorylation. Together, they cause uncoupling linked to UCP-5 mRNA expression levels, lower the generation of reactive oxygen species (ROS), and enhance mitochondrial dynamics in the hippocampus, all of which support bodily wellness. In contrast to a lower consumption of n-3 PUFAs and a shorter feeding time (6 week), several studies showed that raising the concentration of n-3 PUFAs by using dietary FO rich in EPA and DHA and a longer intake (8 week) greatly improved insulin sensitivity. Similarly, regardless of the amount and duration of n-3 PUFA consumption, the anti-hypertensive impact was comparable. These effects may be caused by oxidation of n-3 PUFAs, alterations in the lipid composition of adipocyte plasma membranes, or changes in the substrates available for the synthesis of active lipid metabolites that affect insulin resistance and hypertension **(Oliart Ros et al., 2018)**. According to **Gao et al. (2017)** recommended that short-term fish oil administration was more advantageous to insulin tolerance than prolonged supplementation. The relationship between FO supplements and insulin sensitivity may be explained by various mechanisms. Nutraceuticals and functional dietary ingredients help reduce insulin resistance and dyslipidaemia by lowering serum TC, VLDL. Additionally, they proposed that dietary supplements based on PUFA could enhance metabolic syndrome-related indicators. **Chiu et al. (2017)** found for the very first time, supplementing chitosan or FO in the diet had a negative impact on hepatic lipid metabolism in obese rats induced by the HFD. This was achieved through liver X receptor α (LXRα) inhibition and PPAR-α promotion-mediated lipogenesis inhibition. According to some research, maintaining healthy adipose functions and reducing ectopic fat deposition by lowering hepatic lipogenesis/adipogenesis are key goals in reducing the symptoms of metabolic syndrome. A crucial factor in the management of metabolic syndrome is the interaction between adipose and hepatic tissues, which is controlled by n-3 PUFA. However, by avoiding lipotoxicity and inflammation, n-3 PUFA also control the metabolism of fats and carbohydrates in muscles and pancreatic islets (**Huang et al., 2016)**.

Significant correlations between AT and FAS expression and human indicators of obesity, fat distribution, ATs function (macrophage infiltration, adipocyte size, adiponectin serum concentration), adipocyte apoptosis, and insulin sensitivity (fasting plasma insulin) have not been thoroughly studied. FAS expression was considerably elevated in omental compared to subcutaneous, in obese compared to lean people, and in patients with type II diabetes compared to those with normal glucose tolerance in AT overall, as well as in isolated adipocytes and SVF (stromal vascular fraction) cells. These findings imply that the observed group variations and correlations linked to adipose tissue FAS expression are caused by alter in both adipocyte and SVF cell FAS expression **(Bluher et al., 2014)**. According to research by **Yang et al. (2011)**, consuming MUFA generated from FO lowers insulin resistance as measured by the insulin tolerance test and lessens metabolic syndrome by reducing hyperglycemia, hyperinsulinemia, and hyperlipidaemia. When MUFA was added to the diet, the positive shift in adipocytokines may be correlated with better metabolic risk factors linked to insulin resistance. Another study investigated how FO prevented female KK mice from gaining weight and demonstrated an anti-obesity effect by inhibiting lipid formation. Furthermore, by inducing fatty acid oxidation, fenofibrate therapy significantly reduced body weight increase. WAT weight considerably decreased in mice fed DHA-rich FO plus fenofibrate, but plasma adiponectin levels did not rise. They concluded that mice given DHA-rich FO and fenofibrate showed a remarkable increase in adiponectin sensitivity than mice given only DHA-rich FO **(Arai et al., 2009)**. Fucoxanthin and FO together have a beneficial effect on the reduction of WAT weight gain in diabetic/obese KK-Ay mice, but not in a synergistic way. The BAT (Brown Adipose Tissue) weight, on the other hand, normalised body weight and is associated with energy expenditure. Additionally, fucoxanthin in the meal reduced plasma insulin levels in a dose-dependent manner. According to this research, fucoxanthin improves insulin resistance and reduces serum glucose levels, at least partially, via down-regulating TNF-α mRNA in KK-Ay mice's WAT **(Maeda et al., 2007)**.

Chapter III: Aim & Objectives

Aim & Objectives

The Topse fish, scientifically known as ***Polynemus paradiseus***, has an elongated, slightly compressed golden body and small eyes with filamentous pectoral fin rays and a sharply forked tail. This species can be found throughout the Eastern Indian Ocean and Western Pacific. It is also observed widely at the Bay of Bengal's as well as West Bengal's maritime areas. **The main aim of this study was to established the anti-obesity potentiality of *P. paradiseus* FO.**

Objectives:

i. Extraction, isolation, and characterization of fatty acids (FAs) from *Polynemus paradiseus* fish oil.

ii. To search out the anti-obesity potentiality of *Polynemus paradiseus* fish oil *in vivo* model.

iii. To establish the anti- obesity potentiality of isolated fatty acid both *in vitro* and *in vivo* model.

iv. To explore the role of fatty acid in alleviating obesity related metabolic disorder *in vivo* model.



Figure 1. Distribution range colors indicate degree of suitability of habitat of ***P. paradiseus*** fish **(Eastern Indian Ocean and Western Pacific) (Source: FishBase)**

Chapter IV: Objective (i)

Objective (i): Extraction, isolation, and characterization of fatty acids (FAs) from *P. paradiseus* FO.

This experiment was conducted to extract, isolate and characterize the different fatty acid(s) from marine *P. paradiseus* FO and as well as checked the edibility of this oil for human consumption.

Introduction

Encouragement of increased fish consumption is necessary, particularly in developing nations where food security is a major issue. Additionally, more people need to know about the quality of the proteins, essential fatty acids and other substances found in fish, fish-based foods, and nutraceuticals **(Dongho Dongmo et al., 2023)**. Several studies reported that marine FO contains plenty amount of PUFAs (EPA, DHA, arachidonic acid, etc.), MUFAs (nervonic acid, palmitoleic acid, mead acid, oleic acid, etc.), and least amount of saturated fatty acids (SFAs) (palmitic acid, stearic acid, etc.) **(Pradhan et al., 2020)**. Since ancient times, fish has been a significant component of the diets of the people in our nation, India, particularly in Bengal **(Majumder et al. 2016)**. FO has been utilized for centuries as a dietary supplement for improving insulin tolerance and has strong anti-inflammatory, hypolipidemic, cardiovascular protection, and body weight-reduction outcomes since it contains a high level of n-3 PUFAs, basically EPA (20:5 n-3) and DHA (22:6 n-3) **(Zhang et al. 2020)**. Although local fish are highly productive and wide-ranging, not much has been researched about FOs from marine *Polynemus paradiseus*, particularly regarding their physicochemical traits, nutritional value, and biological qualities. Systematic position of this species is as follows Phylum: Chordata, Class: Actinopterygii (Ray-finned fishes), Order: Perciformes (Perches), Family: Polynemidae (Threadfins), Genus: *Polynemus* and Species: *P. paradiseus* (Linnaeus, 1758). The Topse fish (*P. paradiseus*), commonly known as the *paradise threadfin*, has an elongated, slightly compressed body and small eyes with filamentous pectoral fin rays and a sharply forked tail. The fish is typically golden with grayish fins, and it has a complete lateral line. In this species pectoral fins are separated into two parts: the upper section has the longest three unbranched rays, while the bottom part has seven free filamentous rays. This species can be found throughout the Indian subcontinent, including Bangladesh, India, Pakistan, and Sri Lanka. It has also been observed at the Bay of Bengal's Indo-Pacific Ocean confluence **(Chaklader et al., 2016)**. The local names of this fish are muni, rishi, bairagi, topse, ramsos, tapasi, riksha, and tapasi **(Majumder et al.,2016)**. Thus, studies of fatty acid composition and lipid nutritional profile of marine fish species from Bay of Bengal region ensure good choices for the consumers, improving their health. So, it's necessary to establish a simple, rapid simultaneous determination of unsaturated fatty acids content in the *P. paradiseus* FO. Therefore, this study aimed at evaluating the total lipid content, fatty acid composition and lipid nutritional profiles of *P. paradiseus* FO as well as its authentication analysis.

Materials and Methods

Reagents & Chemicals

The following chemicals and reagents were acquired for completing the experiments in this study. Hexane, isopropanol, chloroform and the solvents used in estimation of physicochemical properties, purchased from Merck, Darmstadt, Germany.

Sample collection & characterization

Firstly, marine *P. paradiseus* was collected with their overall length (12–15 cm) and weight (25–30 g) from the Digha Mohana fish landing center, these are approximately 21°63' N and 87°54' E. The altitude ranges of coastal plains of Purba Medinipur from 5 to 7 meters above mean sea level. The species were collected with the help of fishermen during October-December 2023 and January-February 2024. After that fish dust will be prepare by using solvent extraction (*n*-hexane /isopropanol by 3:2 *v/v* per 10 g fish dust) followed by shaker at 140 rpm (3.286×g) for 24 hours, Soxhlet apparatus for 12 hours, centrifugation for 20 minutes at 2,500 rpm (1048.12×g). The rotary evaporator is employed as a last processing step to separate the oil from the more volatile solvent, after which fish oil was collected for further examination **(Panchali et al., 2024)**.

GC–MS instrumentation and conditions of *P. paradiseus* FO

FO was analysed using a GC ultra-gas chromatograph (Thermo Fisher Scientific S.P.A., Milan, Italy, Model: Trace 1300 series, S/N − 717100576), and a Mass Spectrometer was used for fatty acid analysis in FO (Model: ISQ QD Single Quadrupole Mass Spectrometer). The material was passed through a stationary phase and a TG-WAXMS column containing 5% phenyl polysilphenylene siloxane (30 m × 0.25 mm ID × 0.25 m film thickness). Helium gas was flowed at a rate of 1.5 ml/min during the experiment. The column temperature was 50°C for the first 10 min. It was then elevated to 220°C (5°C/min) and maintained there for another 10 min. The MS was carried out in electron ionization mode (EI) at 70 eV with a temperature of 250°C and a detector setup of 40–600 D. The mass spectrum from GC-MS was evaluated using the National Institute of Standards and Technology (NIST) database. Molecular weights of the compounds were determined using the XCALIBUR program. The absolute quantification of FAMEs was carried out by internal standardization, using methyl ester of tricosanoic acid (23:0) (Sigma, USA). Theoretical correction factors for GC-FID (Gas chromatography – flame

ionization detection) determine fatty acid concentration on samples in mg g-1 of total lipids, according to equation given below $FA = A_X W_{IS} CF_X / A_{IS} W_X CF_{AE} \times 100$

FA the fatty acids concentration in mg per g of total lipids, A_X the peak area (fatty acids), A_{IS} the peak area of internal pattern methyl ester of tricosanoic acid (23:0), W_{IS} the mass of the internal pattern (in mg) added to the sample, W_X the sample mass (in mg), CF_X the theoretical correction factor and CF_{AE} the conversion factor required to express the results in mg of fatty acids **(Gonçalves et al., 2021).**

Determination of fish oil chemical quality

Quality Indexes of FO such as acid value, saponification value, peroxide value, totox value p-anisidine value and iodine values were evaluated according to standard methods of AOAC **(1980)** and AOCS **(1989)**. The acid value was a measurement of the free fatty acids (FFA) in the fat or oil and indicates how many milligrams of KOH are needed to neutralise the organic acids in 1g of fat. The saponification value was the weight of KOH needed to saponify 1g of fat under particular circumstances. The average molecular weight (or chain length) of all the fatty acids that are present in the sample as triglycerides was measured by this. Iodine value was the mass of iodine used by 100g of oil and was a measure of the unsaturation of oil. The peroxide value was defined as the reactive oxygen content expressed in mEq of free iodine per kg of oil. It quantifies the amount of primary oxidation in an oil sample; the amount of secondary oxidation can be evaluated using the p anisidine test. Totox value was measured as following formula.

TOTOX=2 Peroxide value+ Anisidine value.

All the analyses were done in triplicate.

Statistical Analysis

The results were expressed as mean ±standard error. The means were compared by the ANOVAI test to the 5%probability threshold using Graph Pad InStat software version2000 **(Noutsa et al., 2022)**.

Results

Sample collection and oil extraction by FAME method

The FAME (Fatty Acid Methyl Ester) method for fish oil extraction involves a multi-step transesterification process includes preparing the fish sample, sun drying, moisture elimination fish dust preparation, Soxhlet extraction, centrifugation, purification and final product filtration. Following the extraction procedure, 100 g of raw fish yielded approximately 2.75 ± 1.03 ml of crude fish oil (FO), corresponding to 2.53 ± 0.95 g of oil (**Figure 1**). Oil yields of 4.9–9.0 g/100 g for whole fish and 4.6–9.5 g/100 g for byproducts are reported by Aitta et al. **(2021)**, with recovery percentages in relation to solvent extraction from the Baltic herring (*Clupea harengus membras*), one of the most prevalent commercially fished fish species from the Baltic Sea.

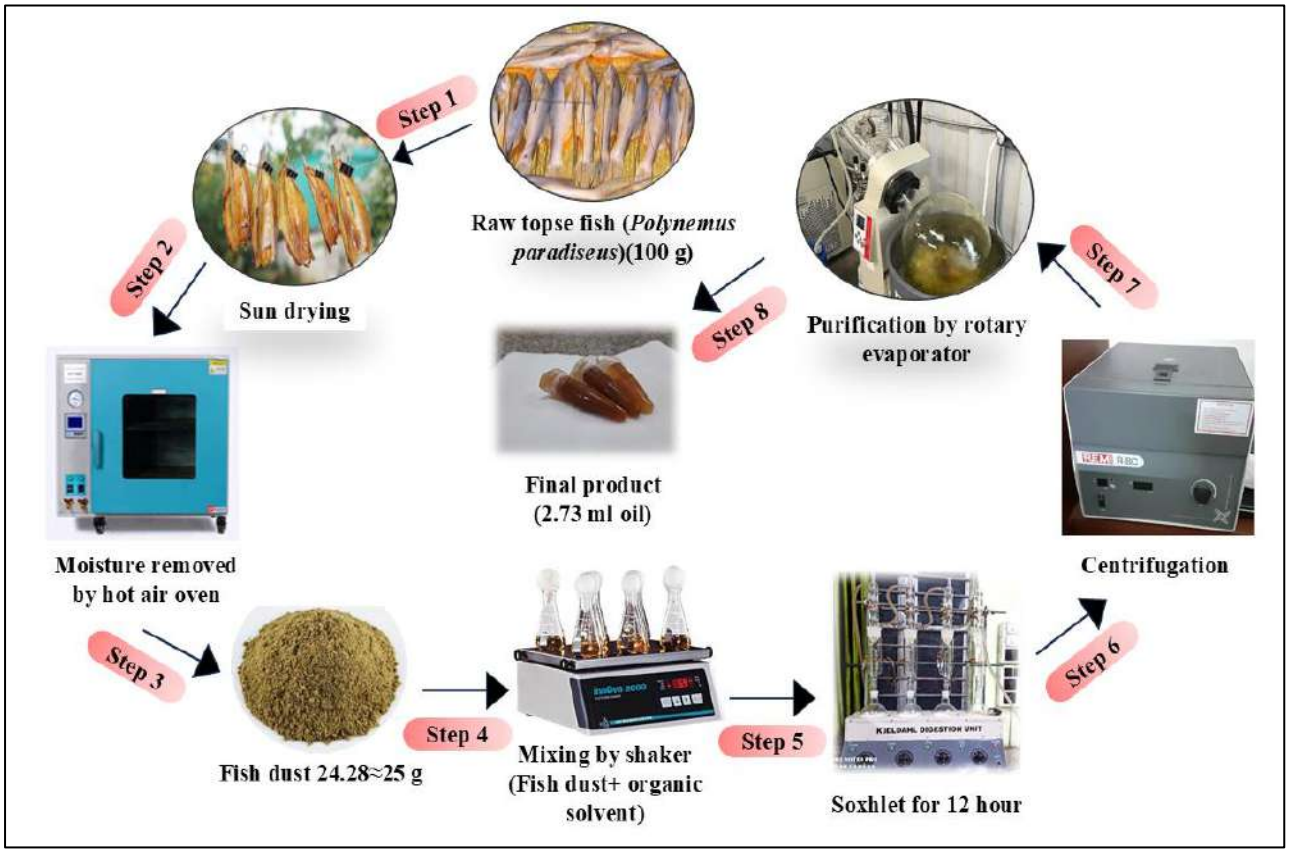


Figure 1. Flow chart of oil extraction process using FAME method, followed by a variety of analyses and assessments.

Chromatographic analysis and GC-MS characterization of *P. paradiseus* FO

GC-MS analysis clearly identified the presence of 9 SFA,11 PUFAs, and 9 MUFAs (**Figure 2 and Table 1a**) in this oil. GC-FID was used in this investigation to determine the overall

proportion of FAs from marine *P. paradiseus* FO. SFAs include Methyl myristate, Tetradecanoic acid, Pentadecanoic acid, Methyl palmitate, Phosphoric acid-3TMS, Methyl margarate, Nonadecanoic acid, Tetracosanoic acid, Methyl pentacosanoate or Pentacosanoic acid. However, PUFAs include methyl ganma-linolenate, methyl linolenate, methyl linoleate, methyl linolelaidate, (9E,12E)-octadeca-9,12-dienoic acid, methyl arachidonate, methyl cis-5,8,11,14,17-eicosapentaenoate, methyl cis-4,7,10,13,16,19-docosahexaenoate, methyl cis-7,10,13,16,19-docosapentaenoate, (7Z,10E,13E,16E,19E)-docosa-7,10,13,16,19-pentaenoic acid, docosa-4,7,10,13,16,19-hexaenoic acid (DHA), octadeca-6,9,12-trienoic acid, and (5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraenoic acid. The MUFAs identified were methyl cis-vaccenate, methyl cis-10-heptadecenoate, heptadec-10-enoic acid, gondoic acid, methyl cis-12-Octadecenoate; (Z)-octadec-12-enoic acid, methyl cis-11-icosenoate, and methyl cis-12-octadecenoate. Additionally, Table 1 b & 1 c highlight the presence of total PUFAs and MUFAs in *P. paradiseus* FO, along with their RT values, molecular weight, and biological relevance.

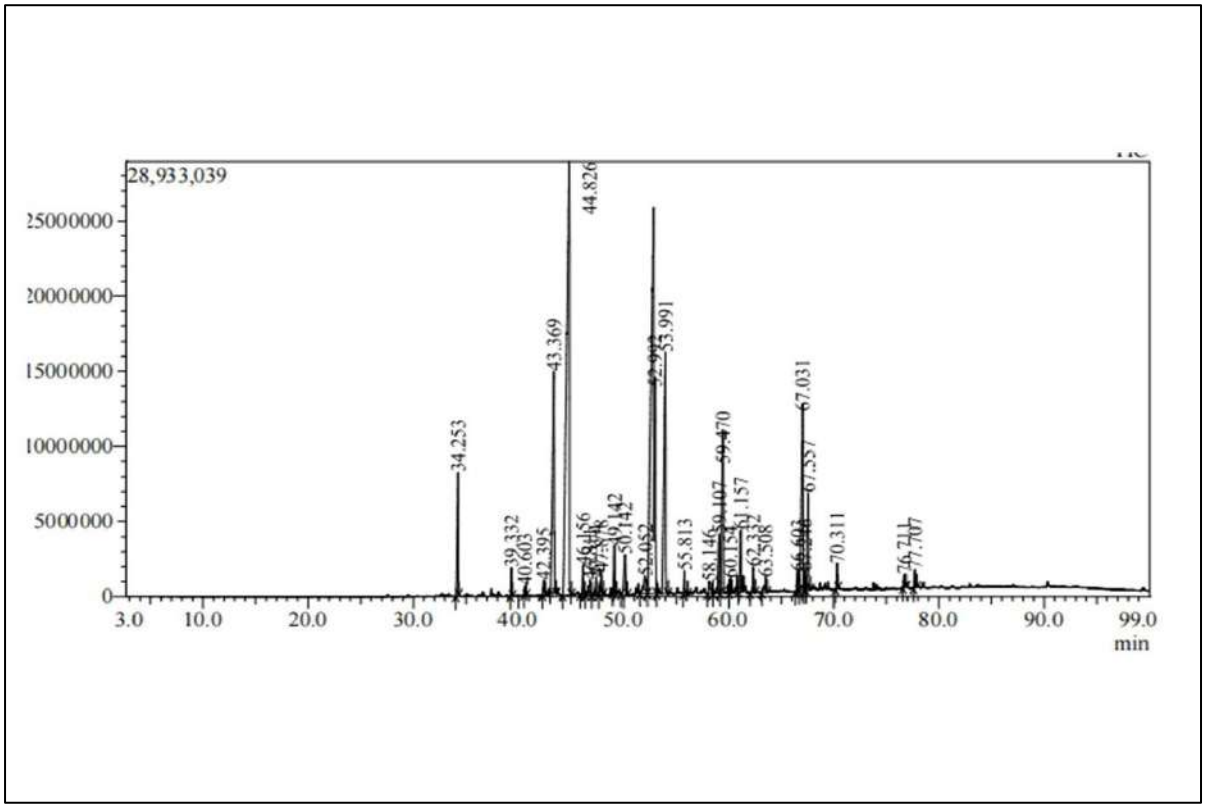


Figure 2. GC-MS chromatograms (Model: ISQ QD Single Quadrupole Mass Spectrometer) analysis of *P. paradiseus* FO. This chromatogram showing the presence of nine SFA, eleven PUFAs and nine MUFAs.

Table 1a. Total percentage of fatty acids composition from *P. paradiseus* FO measured using GC-FID

Sl.no	FA (%)	Types of fatty acid	*P. paradiseus*
1.	Methyl myristate	SFA	2.91
2.	Tetradecanoic acid	SFA	2.32
3.	Pentadecanoic acid	SFA	1.02
4.	Methyl palmitate	SFA	11.28
5.	Phosphoric acid-3TMS	SFA	0.42
6.	Methyl margarate	SFA	2.32
7.	Nonadecanoic acid	SFA	0.38
8.	Tetracosanoic acid	SFA	1.11
9.	Methyl pentacosanoate or Pentacosanoic acid	SFA	2.05
			SFA 23.81%
10.	Methyl ganma-linolenate; Methyl linolenate or (9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid (Linolenic acid)	n-6 PUFA	1.2
11.	Methyl linoleate (linoleic acids); Methyl linolelaidate	n-6 PUFA	2.05
12.	(9E,12E)-octadeca-9,12-dienoic acid (Linoeladic acid)	n-6 PUFA	4.12
13.	Octadeca-6,9,12-trienoic acid	n-6 PUFA	0.44
14.	(5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraenoic acid	n-6 PUFA	0.82
			n-6 PUFA 8.63%
15.	Methyl arachidonate	n-3 PUFA	6.48
16.	Methyl cis-5,8,11,14,17-Eicosapentaenoate or Icosa-5,8,11,14,17-pentaenoic acid	n-3 PUFA	7.22
17.	Methyl cis-4,7,10,13,16,19-Docosahexaenoate	n-3 PUFA	8.03
18.	Methyl cis-7,10,13,16,19-docosapentaenoate	n-3 PUFA	6.52

19.	(7Z,10E,13E,16E,19E)-docosa-7,10,13,16,19-pentaenoic acid (Clupanodonic acid)	n-3 PUFA	2.38
20.	Docosa-4,7,10,13,16,19-hexaenoic acid (DHA)	n-3 PUFA	11.89
			n-3 PUFA 42.52%
21.	Methyl palmitoleate (Palmetoleic acid); Hexadec-9-enoic acid	MUFA	4.56
22.	Methyl cis-vaccenate	MUFA	1.84
23.	(Z)-octadec-11-enoic acid; Methyl cis-10-heptadecenoate	MUFA	2.02
24.	Heptadec-10-enoic acid	MUFA	1.56
25.	Icos-11-enoic acid or gondoic acid	MUFA	6.38
26.	Methyl cis-12-Octadecenoate	MUFA	0.28
27.	(Z)-octadec-12-enoic acid (Petroselinic acid)	MUFA	4.31
28.	Methyl cis-11-icosenoate	MUFA	0.89
29.	Methyl cis-12-Octadecenoate	MUFA	3.2
			MUFA 25.04%

Table 1b. List of PUFAs from *P. paradiseus* FO with their RT value, molecular weight and biological significance listed after GC-MS analysis

RT Value	PUFAs	Molecular weight (g/mol)	Formula	Biological significance
42.395	Methyl ganma-linolenate; Methyl linolenate or (9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid (Linolenic acid)	292.46	C19H32O2	The prevention of hepatic fat buildup and diminished insulin signaling can be achieved independently by either alpha-linolenic acid supplementation or exercising training **(Miotto et al., 2017).**

52.053	Methyl linoleate (linoleic acids); Methyl linolelaidate	294.5	C19H34O2	In the present investigation, meals supplemented with tea tree oil had lowered SFA (lauric acid and palmitic acid) and raised PUFA (primarily methyl linolelaidate) levels in meat **(Yang et al., 2022).**
52.053	(9E,12E)-octadeca-9,12-dienoic acid (Linoeladic acid)	280.45	C18H32O2	Overall, research on humans, animals, and *in vitro* suggests that Trans fatty acids (Trans vaccenic acid, elaidic acid, rumenic acid, trans 10, cis 12, conjugated linoleic acid, and linoelaidic acid) have little or minimally positive effects on metabolic parameters, inflammatory markers, and cardiovascular health **(Valenzuela et al. 2019)**.
59.103	Methyl arachidonate	318.5	C21H34O2	In the current study, it was examined whether consuming pomegranate juice, which is high in polyphenols, for six weeks may reduce the risk of overall metabolic syndromes. Additionally, the plasma's SFA level increased while arachidonic acid levels considerably reduced **(Kojadinovic et al., 2007)**.
59.470	Methyl cis-5,8,11,14,17-Eicosapentaenoate or Icosa-5,8,11,14,17-pentaenoic acid	316.5	C21H32O2	Diet-induced weight loss increases the number of unsaturated FAs such as cis-5,8,11,14,17-Eicosapentaenoate (C20:5 n-3) and linolenate (C18:3 n-3) in the femoral muscle, suggesting that it lessens the symptoms of obesity and may enhance the quality of rabbit meat **(Li et al., 2021)**.

60.153	Methyl cis-4,7,10,13,16,19-Docosahexaenoate	342.5	C23H34O2	Not reported clearly.
63.512	Methyl cis-7,10,13,16,19-docosapentaenoate	344.53	C23H36O2	Not reported clearly.
66.603	(7Z,10E,13E,16E,19E)-docosa-7,10,13,16,19-pentaenoic acid (Clupanodonic acid)	330.50	C22H34O2	Not reported clearly.
67.031	Docosa-4,7,10,13,16,19-hexaenoic acid (DHA)	328.48	C22H32O2	Additionally, 1% DHA and 1% EPA reduce GPR120, which inhibits adipogenesis; via upregulating PPARγ, 4% DHA promotes browning of WAT and enhances IR and inflammatory infiltration **(Wei et al., 2021)**.
67.245	Octadeca-6,9,12-trienoic acid	278.4	C18H30O2	A study reported that bee bread was characterized in terms of its physicochemical composition where the most abundant fatty acids were C18:3 (all-cis-9,12,15) octadeca-6,9,15-trienoic acid, followed by C16:1 (9Z)-hexadec-9-enoic acid, C21:0 heneicosanoic acid and C18:2 (all-cis-9,12) (9Z,12Z)-octadeca-9,12-dienoic acid, respectively **(Dranca et al., 2020)**.
67.553	(5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraenoic acid	304.47	C20H32O2	Not reported clearly.

Table 1c. List of MUFAs from *P. paradiseus* FO with their RT value, molecular weight and biological significance listed after GC-MS analysis

RT Value	MUFAs	Molecular weight (g/mol)	Formula	Biological significance
43.370	Methyl palmitoleate (Palmetoleic acid); Hexadec-9-enoic acid	268.4	C17H32O2	Palmitoleic acid (POA) has demonstrated anti-inflammatory and lipid-lowering benefits linked to prevention of metabolic syndrome. This finding implies that POA may be activating AMPK to produce at least part of its positive benefits **(Waehler 2023)**.
43.370	Methyl cis-vaccenate	296.5	C19H36O2	According to the findings of this study, Canadians who are at risk for diabetes found that higher levels of saturated and n-6 fatty acids and lower levels of cis-vaccenic acid consistently correlated with lower insulin sensitivity and cell function, with the majority of correlations occurring in the PL (Phospoloipid) fraction **(Johnston et al., 2016).**
43.370	(Z)-octadec-11-enoic acid; Methyl cis-10-heptadecenoate	282.46	C18H34O2	The most prevalent TFAs in butter and margarines, which are (E)-octadec-11-enoic acid (C18:1 t11; trans vaccenic acid) and (E)-octadec-9-enoic acid (C18:1 t9; elaidic acid), were reflected in the significantly different serum levels of TFAs measured after the dietary interventions **(Guggisberg et al., 2022)**.
46.812	Heptadec-10-enoic acid	268.4	C17H32O2	Not reported.

47.878	Icos-11-enoic acid or gondoic acid	310.5	C20H38O2	Oleic acid (OA), gondoic acid (GA), and nervonic acid (NA) all showed inverse correlations with LDL-C, HDL-C, and eGFR in a partial correlation analysis that was controlled for age and gender but direct connections with markers of inflammation and endothelial activation as well as heart failure **(Delgado et al., 2017)**.
52.995	Methyl cis-12-Octadecenoate	296.5	C19H36O2	Not reported.
52.995	(Z)-octadec-12-enoic acid (Petroselinic acid)	282.46	C18H34O2	The medium- and long-chain NEFA receptor FFA4 (free fatty acid receptor 4, formerly known as GPR120) has been associated with insulin-sensitizing and anti-inflammatory effects. The effects of oleic acid (18: 1n-9), petroselinic acid (18: 1n-12), and cis-vaccenic acid (18: 1n-7) on FFA4 were reduced **(Christiansen et al., 2015)**.
61.153	Methyl cis-11-icosenoate	324.54	C21H40O2	Not reported.
76.712	Methyl cis-12-Octadecenoate	296.5	C19H36O2	Not reported.

Chemical quality index of *P. paradiseus* FO

As part of chemical characterisation, the following values are assessed: acid value, saponification value, iodine value, peroxide value, p-anisidine value, and total oxidation value (Totox value). The results are shown in **Table 2**, where all of the parameters remained within the range defined by the Codex Alimentarius International Food Standards **(Panchali et al., 2024; Pena-Muniz et al., 2015)**. The oil's acid value was 4.01±0.12 mg KOH/g, saponification value was 145.98±2.23 mg KOH/g, peroxide value was 3.95±0.15 mEq/kg, p-anisidine value was 0.46 ± 0.19, totox value was 8.36±0.27 and iodine value was 131.33± 3.89 mg.

Table 2. Physicochemical Properties of *P. paradiseus* FO

Parameters	Value (Mean ±SE)	Codex Alimentarius standard values
Acid value (mg KOH/g)	4.01±0.12	4-5 mg KOH/g
Saponification value (mg KOH/g)	145.98±2.23	140-260 mg KOH/g
Peroxide value (mEq/kg) (PV)	3.95±0.15	10 mEq/kg
P- anisidine value (AV)	0.46 ± 0.19	<10
Totox value (mg)(2*PV+AV)	8.36±0.27	-
Iodine value (mg)	131.33± 3.89	120-139 mg

Discussion

In this study, we isolated and analysed *P. paradiseus* FO and assessed its edibility parameters. FO is extracted using the FAME method because it is a quick, accurate, and efficient way to identify the different fatty acids in the oil and the FO extraction process was carried out using Das et al. **(2025)**. Marine FOs are generally acknowledged as wholesome and extremely nutrient-dense food additives. In general, the quantities of PUFAs in marine fish samples were very high, with alpha-linolenic acid, EPA, and DHA having the most positive impacts on human health **(Nadia et al., 2023)**. As per the statement our present study explored the presence of diverse range of PUFAs (51.15%) and MUFAs (25.04%) in *P. paradiseus* FO (**Table 1a**) was determined using GC-MS (**Figure 1**) as well as the biological significance of the characterized fatty acids were explained in **Table 1 b & 1 c**. In our study **Table 2** shows the results of the physicochemical investigation of *P. paradiseus* FO. The acid value 4.01 ± 0.12 mg KOH/g, an indicator of the oils quality and freshness where our result implies good stability of oil and this result was comparable to that of earlier research **(Panchali et al., 2024)** on *Setipinna phasa* oil. The *P. paradiseus* oil has a high percentage of long chain fatty acids, as indicated by the saponification number of 145.98 ± 2.23 mg KOH/g. Similarly, Pradhan et al. **(2020)** explored that the saponification number of *Opisthopterus tardoore* FO was 129.8 ± 5.09 mg KOH/g indicating that the oil contained PUFAs in a large proportion. Peroxide value and lower totox value indicate the good shelf-life and nutritional value of the experimental oil side

by side a lower p-anisidine value indicates better quality and less oxidation of oil also and this outcome was similar to previous studies on *Rastrelliger kanagurta* oil **(Agnesia et al., 2023)**. We observed that iodine value was 131.33± 3.89 mg, signifies that the FO was more unsaturated in nature and this result is consistent with few previous investigations **(Dongho Dongmo et al., 2023; Rahman et al., 2023)**. The total quality control assessment results showed the *P. paradiseus* FO's advantageous physicochemical properties and bioactivity, laying the groundwork for future purification and possible uses. The present findings explore the possibilities of utilizing *P. paradiseus* FO in human diet as a healthy source of essential fatty acids including PUFAs and MUFAs.

Conclusion

In conclusion, the primary goal of this study was to characterized the fatty acid composition of marine fish (*P. paradiseus*) with significant commercial importance. In the West Bengal coastal region, this fish is extremely plentiful. *P. paradiseus* is a viable choice because it contains a large number of conjugated fatty acids (CFAs), and its physicochemical characteristics demonstrate that it is suitable for human ingestion.

Chapter V: Objective (ii)

Objective (ii): To search out the anti-obesity potentiality of *P. paradiseus* FO *in vivo* model

This experiment was performed to evaluate the antiobesity potency of crude oil via identifying the effective dose and duration of *P. paradiseus* oil *in vivo* model.

Introduction

Obesity is a metabolic syndrome caused by an energy imbalance, including excessive calorie consumption and reduced energy utilization **(WHO, 2016).** More than 2.1 billion people globally, including more than half a billion without regard to age or gender, having a BMI between 25.0 to 30.0, according to a survey. Obesity, which is defined as persistent inflammation of fat or decreased satiety signalling, is associated with a number of chronic illnesses, including cardiovascular conditions, type II diabetes, hypertension, and multiple cancer types **(Lee et al., 2013)**. Adipocyte size and quantity expand with obesity, contributing to an increase of WAT composition **(Serra et al., 2013)**. Overabundance of WAT causes inflammation and hypertrophy, which in turn triggers the secretion of many pro-inflammatory cytokines, including TNF-α, IL-6 , IL-1β (Interleukin 1 beta), leptin (which causes a low-grade infection in the body, which lowers adiponectin secretion), and anti-inflammatory cytokines like IL-10 and IL-1Ra (Interleukin 1 Receptor antagonist) **(Maury et al., 2007; McLaughlin et al., 2014)**. Recent research has connected the obesity pandemic to the excessive consumption of high-fat diets (HFD) **(Hu et al., 2018)**. Dietary supplements and functional foods may prevent tissue dysfunction and the improvement of metabolic disorders by decreasing oxidative stress and inflammation **(Miralles-Pérez et al., 2021)**. Dietary fatty acids are either saturated (palmitic acid, stearic acid etc) or unsaturated (PUFAs and MUFAs) in nature. Given the different fatty acid's components in HFD, it is of interest to determine whether and how fatty acid composition in HFD influences obesity and obesity-associated immune regulation and tumour progression **(Liu et al., 2020)**. One of the most significant sources of animal protein, fish, contributes to 16% of all animal protein ingested by people worldwide and is regarded as a superior supply of essential elements for maintaining a healthy body **(Ondo-Azi et al., 2013).** Thus, it was affordable for both the wealthy and poor to eat fish on a regular basis. Additionally, FO protects against inflammation induced bone loss in chronic inflammatory diseases like lupus, rheumatoid arthritis, periodontitis, and osteoporosis **(Sun et al., 2003; Halade et al., 2010)**. Our experimental species *P. paradiseus* is the second-most significant fishery after the Hilsha in the West Bengal Estuary **(Majumder 2016)**. However, scientists have not paid much attention to the biological activities of *P. paradiseus* fish up until now. The Indian Council of Medical Research (ICMR) has only established proximate analysis reports (Energy 343 kcal, Moisture 16g, Protein 16g, Fat 12g, Mineral 17g, Calcium 1597mg, Phosphorus 595mg, Iron 41mg) for this fish **(Gopalan 2004)**; there is a shortage of knowledge related to biological activity so we concentrated on that gap and wished to establish some biological activities of *P.*

paradiseus FO. The present study provides the novel insight for the first time to investigate the therapeutic role of *P. paradiseus* FO in lipid metabolism of HFD-fed mice via the regulation of lipid transport-related signals in blood, liver and adipose tissues and to explore its putative key mediators in this mode of action.

Experimental Design I: Dose-Dependent investigation of *P. paradiseus* oil to determine the ideal therapeutic dosage for the management of obesity

Materials & Methods

Reagents & Chemicals

The following chemicals and reagents were acquired for completing the experiments in this study. Hexane, iso propanol, chloroform was purchased from Merck, Darmstadt, Germany; RNA-Xpress Reagent, Hi-cDNA synthesis kit, 2X PCR mixture, and 3,3′,4,4′- tetra aminobiphenyl tetrahydrochloride (DAB) were obtained from Himedia, HiGenoMB, Maharashtra, India. All primers used for PCR were purchased from Bioserve (India). The primary antibodies: Ob (H-5) mouse monoclonal IgG (Sc-393,043), PPAR-γ mouse monoclonal IgG (Sc-7273), PPAR-α mouse monoclonal IgG (Sc-398,394) and FAS mouse monoclonal IgG (Sc-74,540) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA-USA). The secondary antibody, Goat Anti-mouse IgG-HRP (cat# 11–301) was purchased from Abgenex (Odisha, India). Precision Plus Protein Dual Color Standards (cat# 1610374) were acquired from Bio-RAD, Hercules, California, United States.

Collection of fish samples

Samples were collected from the coastline region of Purba Medinipur in the Indian state of West Bengal. The collected specimens were then assessed for their overall length (15–16 cm) and weight (28–30 g) before being placed immediately in an icebox. The samples were then transported to the laboratory for further examination. Before being processed into fish dust, the samples were cleaned with water and dried out for 24 h at 60°C in a hot air oven.

Extraction of *P. paradiseus* FO

Briefly, fish dust was mixed with hexane/isopropanol (3:2 by volume) and shaken at 140 rpm (3.286×g) for 24 h before being-extraction using the Soxhlet method for 12 h. The leftover material was cooled and then centrifuged for 20 min at 2,500 rpm (1048.12×g) to separate the supernatants, which were then filtered through Whatman No. 1 filter paper. The filtrate product

was placed in a rotary evaporator to evaporate or remove volatile compounds, and the FO was retained for additional analysis **(Pradhan et al., 2020)**.

Ethical approval

All the experimental protocols of *in vivo* model were approved fromthe Institutional Animal Ethical Committee (IAEC), Midnapore City College (2256/PO/Re/S/23/CCSEA, MCC/IAEC-SP/10/23-003). The animal data has been submitted in accordance with the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) criteria.

***In vivo* experimental design**

Twenty-four inbreed male BALB/c mice (5–6-week-old) were randomly separated into four groups (six mice per group housed in four different cage) after a week of acclimatisation to a normal chow diet (Hindustan Lever, Mumbai, India) at an average weight of 18.2 ± 2.1 g. After that, each group was then placed in a controlled environment (12 h light/dark cycle, 32 ± 2°C temperature, 50 ± 5% humidity) with free availability of food and water for 6 weeks. The four groups were as follows: a. C group: normal chow feeding (protein with 20% kcal, carbohydrate with 70% kcal, fat with 10% kcal) with water ad libitum; b. Obese control (OC) group, high fat diet (HFD) feeding (protein with 20% kcal, carbohydrate with 35% kcal, fat from lard with 45% kcal) with water ad libitum; c. Treatment-I (T-I) group, HFD feeding and received 200mg/kg body weight / day of *P. paradiseus* FO by gavage; d. Treatment-II (T-II) group, HFD feeding and received 400mg/kg body weight/ day of *P. paradiseus* FO through oral gavage. The mice's body weight monitored every seven days and food consumption were monitored for daily basis. After 12 weeks, the mice were anesthetized with ether and euthanized by cervical dislocation. Blood samples were collected by puncturing the orbital plexus, centrifuged at 3000 rpm for 15 min at 4°C, and serum was stored at –20°C for biochemical analysis. The liver, subcutaneous white adipose tissue (sWAT), and visceral white adipose tissue (vWAT) were removed, weighed, and immediately submerged in liquid nitrogen, and stored at −20°C for further examination.

Assessment of somatic body weight index

Changes in body weight were recorded once per week for up to 12 weeks according to the standard protocol. A caliper (Central; Model no. 6420) was used to measure body length from the nasal to anal distance, to the nearest 0.1 mm. BMI was assessed using the standard procedure, and the results are represented as gm/cm^2.

Examination of total lipid profile and serum glucose level

The kit assay methods (Enzopak, India) were used to analyze the serum levels of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), triglycerides (TG), and total serum glucose levels **(Panchali et al., 2024)**.

Histological Examination

Liver tissues and ATs were fixed in 10% formalin and then were embedded in paraffin. Tissue sections of 5µm thickness were stained with Oil Red O and- hematoxylin and eosin (HE) for histological analysis. Histological images were captured using a compound microscope (Model No. CX21i) **(Arai et al., 2009)**.

RNA isolation and semi qPCR for the expression of genes related to obesity and obesity associated inflammation

Complete RNA was extracted from ATs samples using the HiPurA Total RNA Miniprep purification methodology (Hi-Media Hi-GenoMB, Maharashtra, India), the complete RNA was extracted from adipose tissue samples. The isolated RNA was quantified using a NanoDrop-2000 (Eppendorf) spectrophotometer, and cDNA was synthesized using reverse transcriptase. The reverse transcription reaction mixture was prepared using a Hi-cDNA synthesis kit (Himedia, HiGenoMB, Maharashtra, India). mRNA expression of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the housekeeping primer set (sense: GGTGAAGGTCGGAGTCAACG, antisense: GTGAAGACGCCAGTGGACTC). The genes used in this experiment are listed below:

Table 1. Following primer sequences used in semi q PCR analysis.

Genes	Primary Sequences	
	Forward Primer	**Reverse Primer**
Leptin	5’-CAAGCAGTGCCTATCCAGA-3’	5’-AAGCCCAGGAATGAAGTCCA-3’
Adiponectin	5’-GTCAGTGGATCTGACGACACCAA-3’	5’-ATGCCTGCCATCCAACCTG-3’
SREBP-1c	5’-ACGGAGCCATGGATTGCACA-3’	5’-AAGGGTGCAGGTGTCACCTT-3’
FAS	5’-TGCTCCCAGCTGCAGGC-3’	5’-GCCCGGTAGCTCTGGGTGTA-3’
LPL	5’-CCACAGCAGCAAGACCTTC-3’	5’-AGGGCGGCCACAAGTTTG-3’

CPT-1	5’-GTGACTGGTGGGAGGAATAC-3’	5’-GAGCATCTCCATGGCGTAG-3’
IL-1Ra	5’-GCAGCACAGGCTGGTGAATGAC-3’	5’-TGCCCCCGTGGATGCCCAAG-3’
TNF-α	5’-TTCTGTCTACTGAACTTCGGGGTGATCGGTCC-3’	5’-GTATGAGATAGCAAATCGGCTGACGGTGTGG-3’
IL-10	5’-TCTCCGAGATGCCTTCAGCAGA-3’	5’-TCAGACAAGGCTTGGCAACCCA-3’
IL-6	5’-TCAACTTCTCCAGCGTGATG-3’	5’-TCTTTCCCTCTTTTCCTCCTG-3’

ELISA assay

Following sacrifice of the mice, plasma was extracted from the blood and subjected to an ELISA kit (Wuhan Fine Biotech Co. Ltd, China) to measure the levels of several inflammatory cytokines in accordance with the manufacturer’s instructions. Complete blots of 32- cytokine arrays were imaged using a FluorChem E System (Protein Simple, Santa Clara, CA, USA). The concentrations of leptin, IL-10, TNF-α and granulocyte macrophage colony- stimulating factor (GM- CSF) in the mouse plasma were determined using the Mouse Inflammatory 4-Plex Magnetic Bead Panel (Invitrogen, Carlsbad, CA, USA) on a Luminex 200 system (Luminex, Austin, TX, USA).

Protein analysis by Immunoblotting assay

Using radioimmunoprecipitation assay (RIPA) buffer, AT samples were lysed, and the Lowry method was used to measure the protein content. Sodium dodecyl-sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to electrophorese 50 µg of protein samples after they had been boiled for three minutes in loading buffer and then moved to a nitrocellulose membrane (Bio-Rad, Model No. 165.8033FC). After blocking the membrane with 4% bovine serum albumin (BSA), the primary antibody was incubated at 4°C for the whole night. In this investigation, β-actin, Ob (H-5), PPAR-γ, PPAR-α, and FAS were employed as the main antibodies in a 1:1000 ratio. Next, the membrane was incubated for one hour at 4 °C with horseradish peroxidase (HRP)-labeled anti-mouse secondary antibody 1:7500 (Abgenex) after being cleaned three times with phosphate buffer saline containing tween-20 (PBST). Individual protein bands were identified via DAB.

Statistical Analysis

The data were evaluated using one-way ANOVA, followed by Tukey's multiple-comparison tests to compare each treatment group to the control group. The results (triplicate values) are expressed as mean ± SEM. Statistical analysis was performed using GraphPad Prism software. Values were considered significant at $p<0.0001$, $p<0.001$, $p<0.01$, and $p< 0.05$ **(Zhang et al., 2020).**

Results

Assessment of food intake, somatic body weight, BMI and weight of WATs in HFD induced BALB-c obese mice treated with *P. paradiseus* FO

To assess the anti-obesity benefits of *P. paradiseus* FO, we examined alterations in body weight and serum lipid profile of HFD-induced obese mice administered varying doses of *P. paradiseus* FO for 12 weeks. The preliminary body weights of the mice in each group were almost similar (C, 16.88 ± 1.05 g; OC, 16.86 ± 0.98 g; T-I, 15.69 ± 0.85 g; T-II, 16.01± 1.28 g). As shown in **Figure 1b**, mice fed a HFD acquired greater weight than the other groups after 12 weeks. The final mean body weights in C, OC, T-I, and T-II were 32 ± 2.87 g, 45.13 ± 2.66 g, 38.14 ± 3.54 g and 34.32±2.35 g after 4 weeks of the FO treatment (T-I, 200mg/kg body weight / day; T-II, 400mg/kg body weight / day). Food intake was also monitored every week, and the variation in food intake in the different experimental groups is shown in **Figure 1a**. Among the four groups, the OC group had a substantially higher energy intake than the other groups. BMI was computed by applying the standard procedure and was expressed as g/cm^2. On the basis of **Figure 1c** BMI was significantly higher in the OC group than in the C group. Conversely, although BMI was reduced after treatment with different doses of FO. After sacrificing the mice, the weight of visceral WAT was also measured, where the weight of WAT was higher in the OC group (2.41g ±0.10) than in the C group (0.843g ±0.01). In addition, HFD with FO showed significant efficacy in decreasing the weight of WAT in the treatment groups (T-I and, T-II) **(Figure 1d)**.

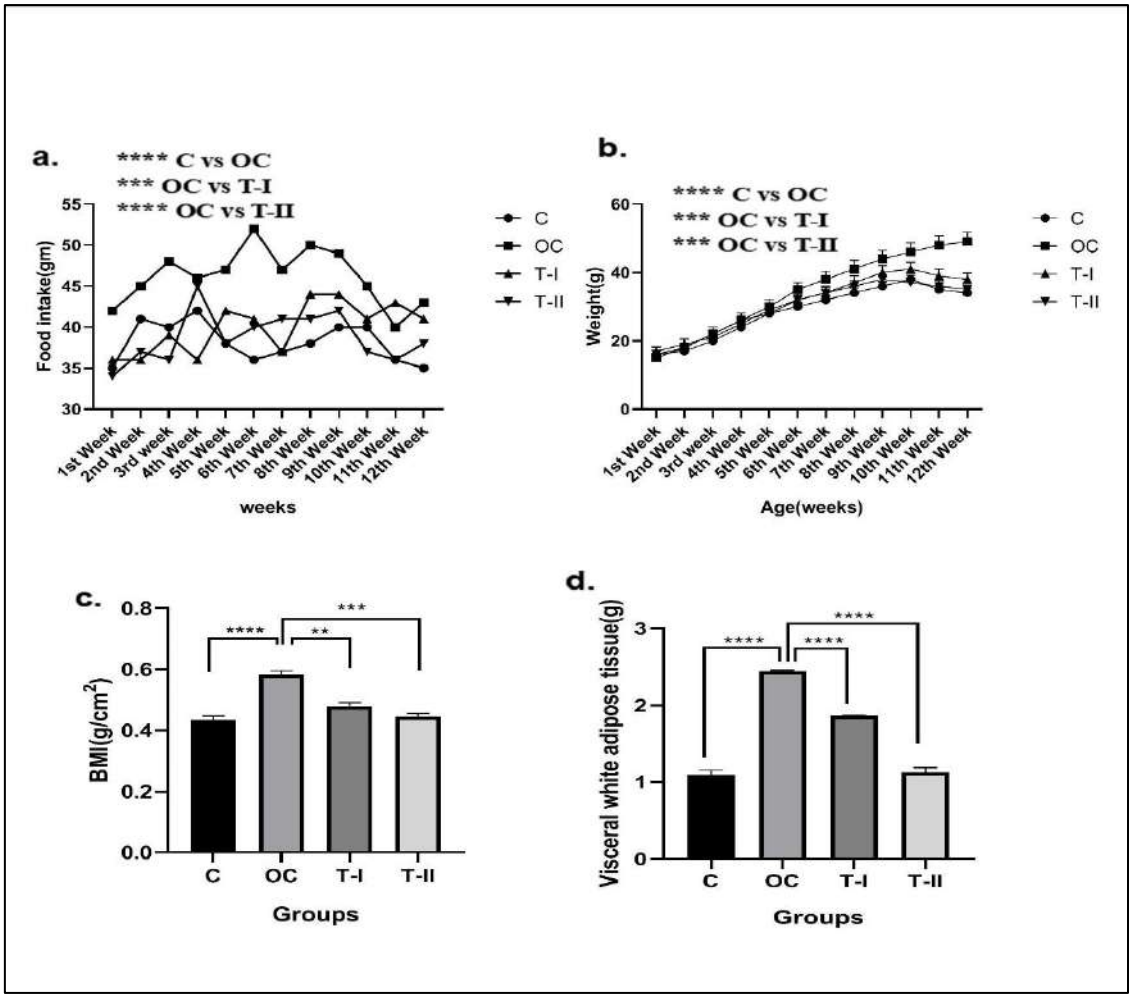


Figure 1. FO supplementation reduces body weight increase and fat formation in HFD-fed mice. (a) measurements of weekly food consumption (b) variation in the body weight (c) BMI (gm/cm^2) (d) weight of visceral WAT (gm) in different experimental group (n=6) values are expressed as mean ±SEM. Statistical analysis evaluated via one way ANOVA followed by Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05.

Assessment of lipid profile and total serum glucose level of HFD induced obese mice treated with *P. paradiseus* FO

Tested serum lipid profiles such as TC, TG, LDL, VLDL were 15–30% higher and HDL levels were 50% lower in OC group **(Figure 2a-e)**. Similarly, after the administration of topse FO, TC levels **(Figure 2a)**, and TG **(Figure 2b)** in the liver were decreased up to 25% in the treatment group. Furthermore, the level of serum LDL cholesterol was diminished (20–30%) due to FO supplementation, and $p < 0.05$ compared with the OC groups **(Figure 2d)**. Additionally, a decrease (18%) in VLDL cholesterol was also observed in the both T-I and T-II groups, in contrast to OC **(Figure 2e)**, though there was a deliberate increase in HDL cholesterol levels (55%) after *P. paradiseus* FO feeding **(Figure 2c)**. Total serum glucose

level was 51.19% higher in OC group compared to the C group **(Figure 2f)**. These findings demonstrate that FO therapy has a more pronounced effect on blood and hepatic metabolic parameters in obese mice.

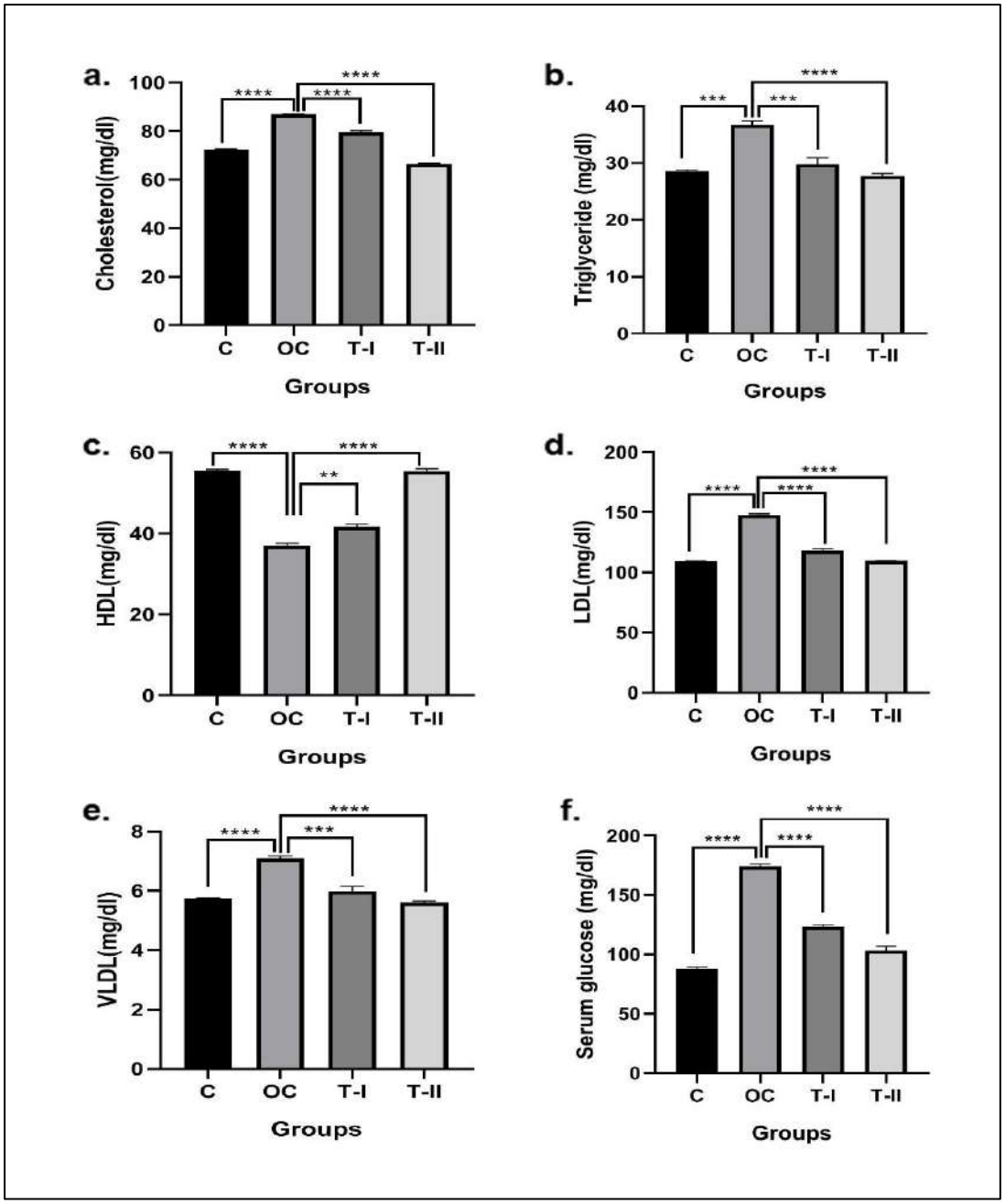


Figure 2. Assessments of total lipid profiles such as a) TC, b) TG, c) HDL, d) LDL, e) VLDL and f) total serum glucose level in different experimental group (n=6) values are expressed as mean ±SEM. Followed by one way ANOVA and Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05

Fat droplet accumulation in liver

In this study Figure. **3(a, b, c, d)**. demonstrates the impacts of FO on liver tissue, as determined by HE staining. Lipid metabolism is mostly regulated by the liver. The expanded cytoplasm of the HFD-fed group was packed with numerous fat droplets, although receiving FO therapy, accumulation of fat droplets was reduced in both the T-I and T-II groups. Between the two doses of FO, the T-II group showed a significant difference compared with the other groups. In the T-II group, sheets of hepatocytes were arranged in a uniform manner, and the central vein was proper in shape, similar to the control group. As anticipated, mice fed a high-fat diet (HFD) and supplemented with 400 mg/kg body weight/day FO showed a significant 38–45% decrease in TG as compared to the OC group **(Figure 3e)**.

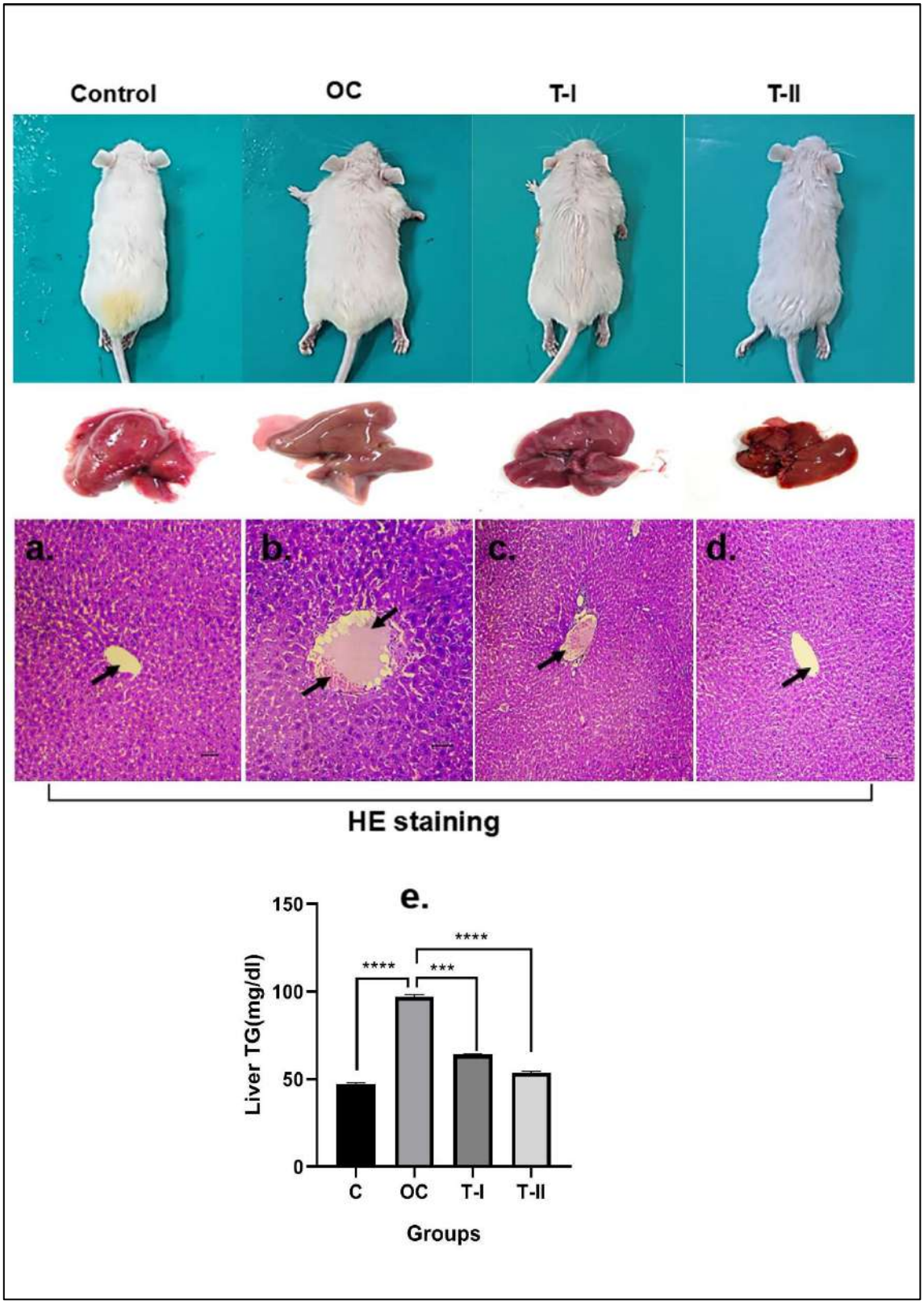


Figure. 3. Effects of FO on hepatic lipid profile of HFD-fed mice groups. Groups are separated as a) control; b) obese control; c) T-I (HFD feeding and received 200mg/kg body weight / day of *P. paradiseus* oil) and d) T-II (HFD feeding and received 400mg/ kg body weight / day of *P. paradiseus* crude oil). Tissue sections were stained with hematoxylin and eosin. (e) Estimation

of liver TG in different experimental groups. Results are expressed as mean ± SEM for each group (n=6). The observation was made under 10 × magnification and Scale bar was 100 µm. Arrows (→) indicate the enlargement of cytoplasm in different experimental groups.

Effect of *P. paradiseus* oil on abdominal fat accumulation in HFD induced mice

We used HE staining for histopathology to examine the impact of *P. paradiseus* oil on the abdominal section of the skin and visceral AT **(Figure 4a, b)**. The adipocytes in the subcutaneous and visceral AT of the OC group were not uniform in size, but they were generally large and contained fat vacuoles with large inner diameters. On the other hand, the adipocytes in the C group were arranged properly and neatly, and their vacuole inner diameter was smaller. Following FO treatment, the T-II group showed a greater reduction in abdominal fat, which was equivalent to the C group.

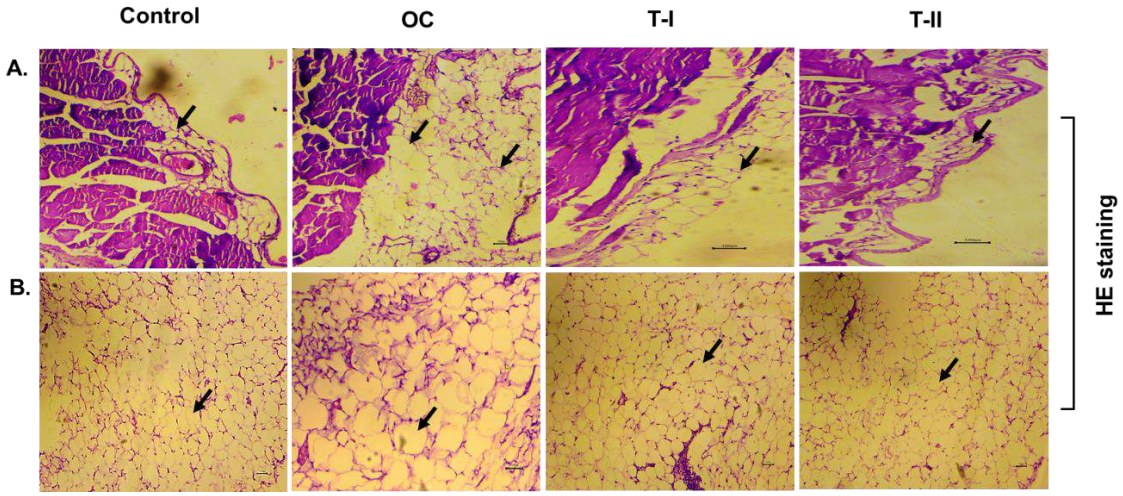


Figure 4. The impact of *P. paradiseus* oil on skin and vWAT histology. Groups are separated as control group (feed with normal diet), OC group (received HFD), T-I group (HFD feeding and received 200mg/kg body weight / day of *P. paradiseus* oil) and T-II group (HFD feeding and received 400mg/kg body weight / day of *P. paradiseus* crude oil). (A) sWAT and (B) vWAT sections were stained with hematoxylin and eosin (HE) staining. The observation was made under 10 × magnification and Scale bar was 100 µm. Arrows (→) indicate the size of vWAT and accumulation of sWAT in different experimental groups.

Impact of dietary *P. paradiseus* oil on mRNA expression in AT

Therefore, we quantified the degree of expression levels of some obesity- and inflammation-related adipocytokines mRNA, such as leptin, adiponectin, SREBP-1c, FAS, LPL, CPT-1, TNF-α, IL- 1ra, IL-10, and IL-6 in the AT of the mice groups using semi- quantitative PCR

(Figure 5). In this study, leptin mRNA and adiponectin mRNA levels were decreased by 1.45-fold and 1.27-fold, respectively, in the OC group compared to the control group. Leptin mRNA and adiponectin mRNA levels in AT increased significantly in mice fed 200 mg/kg body weight/day of *P. paradiseus* oil and 400 mg/kg body weight/day of *P. paradiseus* oil **(Figure 5b, c)**. The HFD diet, combined with FO, reduced mRNA expression of the lipogenic gene SREBP-1c in AT and liver by 1.36-fold (T-I) and 1.57- fold (T-II), respectively, compared to the HFD group **(Figure 5d)**. The HFD increased FAS expression by 1.06-fold in the OC group compared to that in the control group. In contrast, topse FO decreased FAS level by 1.13-fold and 1.48-fold, respectively, in the T-I and T-II groups compared to the obese group **(Figure 5e)**. Inconsistently, the expression of CPT-1 and LPL decreased in the OC group by 0.54-fold and 1.07-fold, respectively. After treatment with crude oil, the mRNA expression levels of CPT-1 and LPL increased by 1.14- and 1.83-fold, respectively, in the treated groups **(Figure 5f, g)**. Additionally, the mRNA expression of associated anti-inflammatory markers, including IL-1ra and IL-10, was increased by 1.48 - fold and 1.68 -fold in the T-II group after treatment **(Figure 5h, j)**. The pro-inflammatory markers, TNF-α and IL-6 were upregulated in the OC group by 1.02- fold, whereas *P. paradiseus* FO treatment significantly reduced the expression of TNF-α and IL-6 by 1.05- fold and 1.11- fold **(Figure 5i, k).**

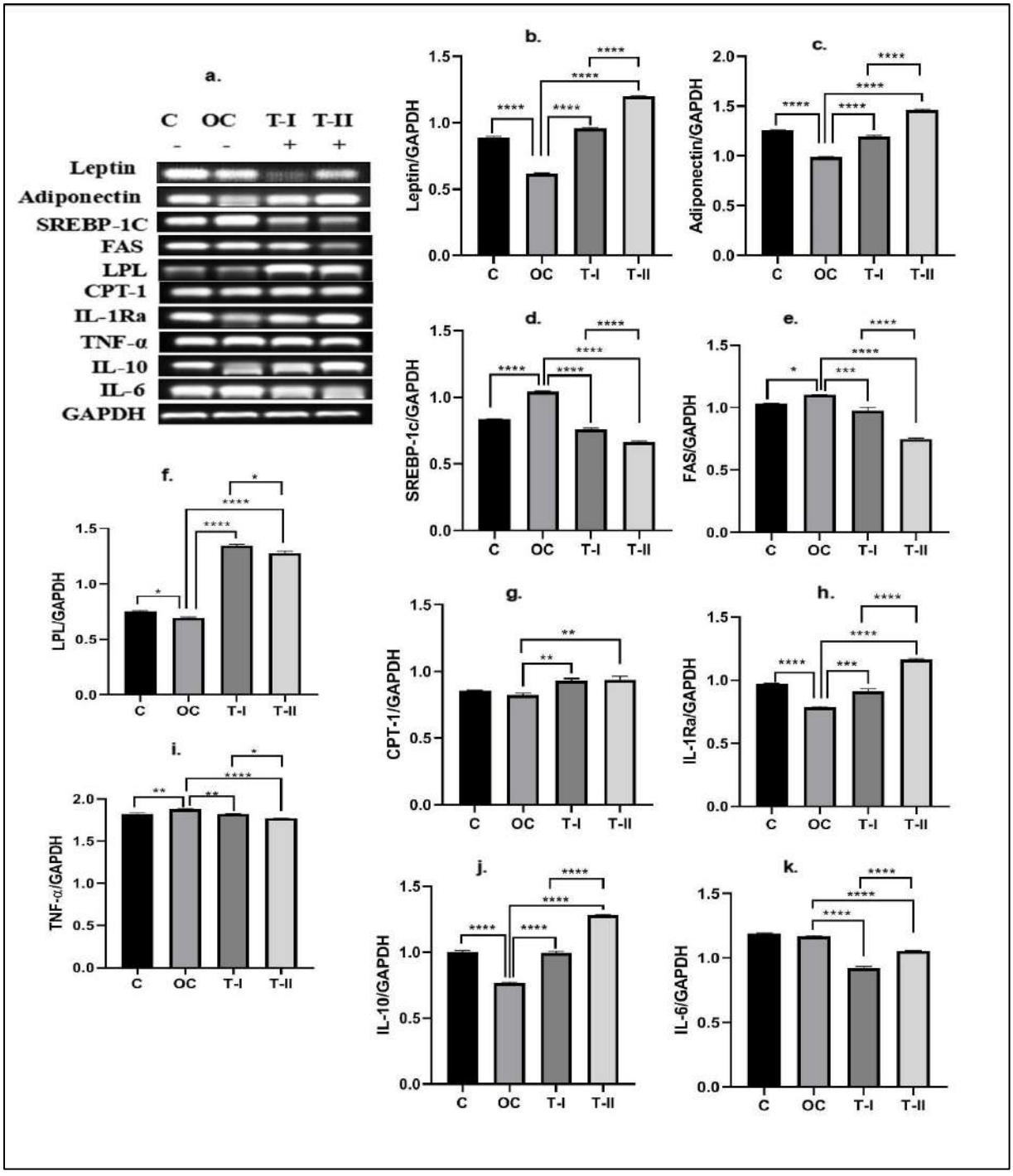


Figure 5. Expression of obesity, inflammatory and anti-inflammatory related adipocytokines by semi q-PCR. (a) Relative gene expression level of (b) Leptin, (c) Adiponectin, (d) SREBP-1c, (e) FAS, (f) LPL (g) CPT-1 (h) IL-1Ra, (i) TNF-α, (j) IL-10, and (k) IL-6 in adipose tissue of male albino BALB/c mice in different experimental groups (n=6) were adjusted to control gene expression level (GAPDH; b-k). Values are expressed as mean ±SEM. Statistical analysis evaluated via one way ANOVA followed by Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05.

Expression of leptin, IL-10 and TNF-α level in HFD-induced obese mice by ELISA assay

We assessed the levels of an important metabolic regulators leptin that plays important role in regulation of appetite, here leptin levels were down regulated by 43.28% in OC group compared to control group where as in FO treated group leptin levels were up regulated gradually by 30-52.6% due to proper regulation of leptin **(Figure 6a)**. IL-10 which is known to be an anti-inflammatory cytokine decreased the generation of pro-inflammatory cytokines, that interrupt proper function of insulin. As expected, IL-10 was reduced by 60.27% in the OC group compared with control. Compared to OC group, IL-10 levels were elevated by 67.65% after treatment with *P. paradiseus* oil **(Figure 6b).** Conversely, we assessed the TNF-α levels, which are known to be generated during the initial stages of inflammation. TNF-α was, as assumed, higher in the OC group than in the control group by 35.86%, whereas supplementation of FO reduced the inflammation by 20.39% **(Figure 6c)**.

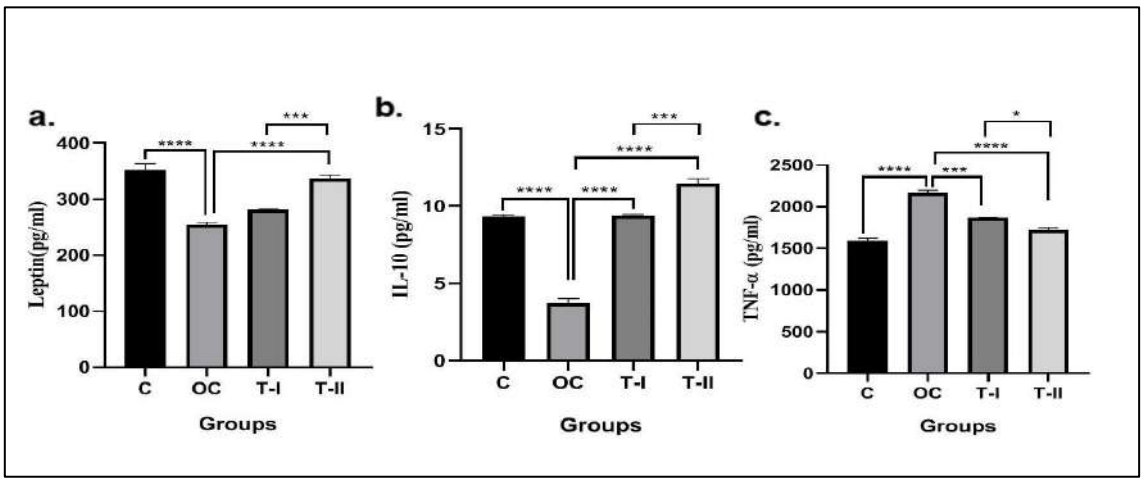


Figure 6. ELISA was used to investigate the impact of *P. paradiseus* oil on the synthesis of a) leptin b) IL-10 and c) TNF-α in different experimental groups (C, OC, T-I, and T-II). Values are expressed as mean± SEM. Statistical analysis evaluated via one way ANOVA followed by Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05.

Protein analysis for Ob (H-5), PPAR-γ, PPAR-α and FAS

To explore the effect of FO supplementation on energy expenditure, we analysed the protein expression of Ob (H-5), PPAR-γ, PPAR-α, and FAS genes in AT. In contrast to the C group, in the HFD group, the ratio of Ob (H-5)/β-actin and PPAR-α/β- actin was diminished in WAT by 1.29-fold and1.16-fold. FO supplementation in the HFD + FO groups (T-I, T-II) significantly reversed the HFD effects by increasing the Ob (H-5)/β- actin and PPAR-α/β-actin ratio by 1.56-fold to 1.67- fold and 1.48-fold to 1.51-fold in WAT, respectively. However, the ratio of PPAR-

γ/β- actin and FAS/β- actin was raised in the OC group by approximately 2-fold whereas FO administration decreased the protein expression of PPAR-γ and FAS serially in the T-I group by 1.15- fold,1.56- fold and in the T-II group by 1.29- fold,1.67- fold **(Figure 7)**.

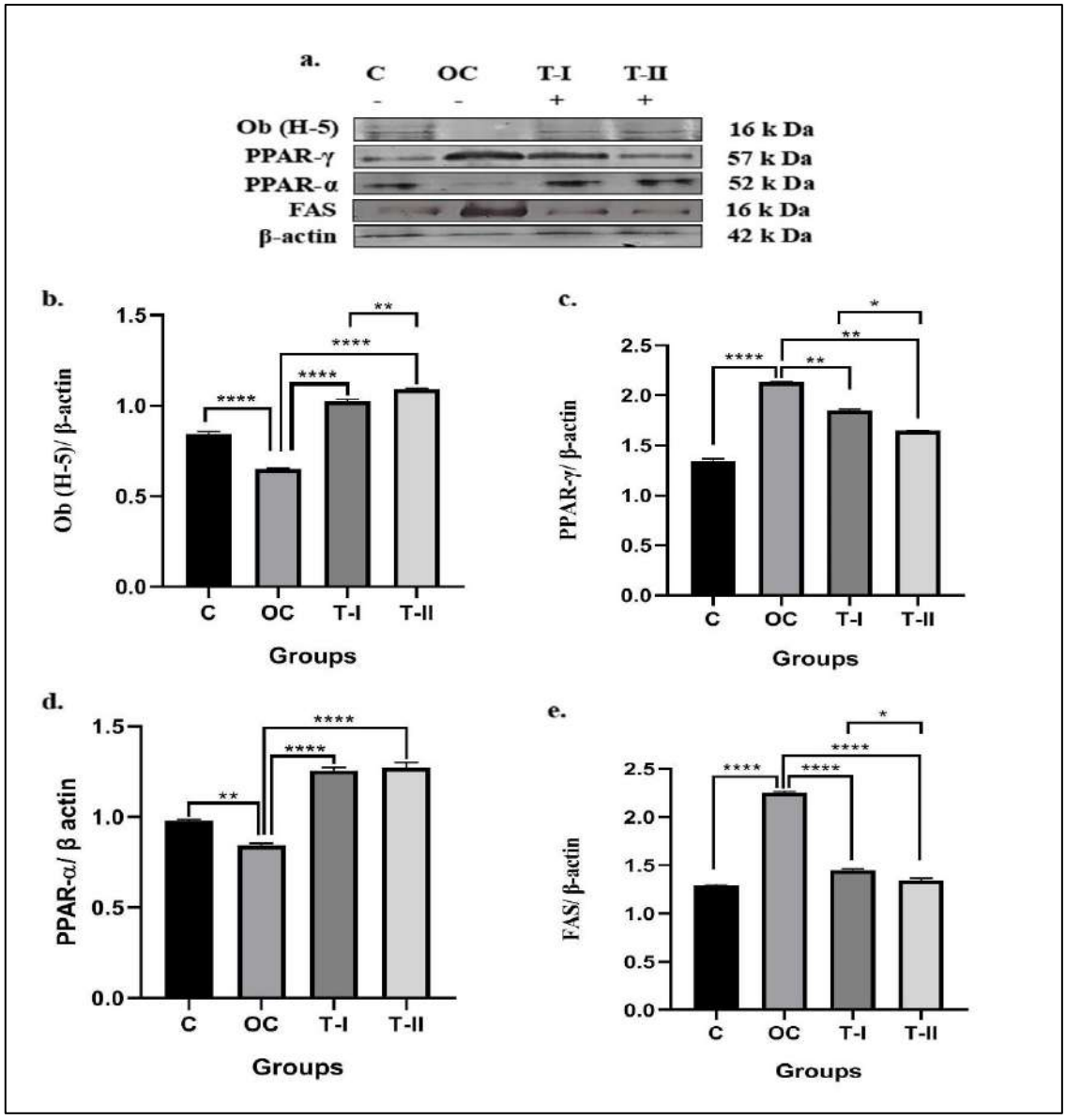


Figure 7. Protein expression analysis of Ob (H-5), PPAR-α, PPAR-γ and FAS in WAT. Groups are separated as control group (feed with normal diet), OC group (received HFD), T-I group (HFD feeding and received 200mg/kg body weight / day of *P. paradiseus* oil) and T-II group (HFD feeding and received 400mg/kg body weight / day of *P. paradiseus* crude oil). Results were normalized with respect to β-actin. Representative Images of western blot WAT (a) Relative protein expression, (b) ob (H-5), (c) PPAR-γ, (d)PPAR-α and (e) FAS. Values are expressed as mean ±SEM. Statistical analysis evaluated via one-way ANOVA followed by Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05.

Discussion

In the current study, we investigated the effects of *P. paradiseus* FO on lipid metabolism in male BALB/c mouse model. Although the bioactive substances and health benefits of various marine FOs have been well documented **(Pradhan et al., 2020),** but the biological activity of *P. paradiseus* oil has not been fully investigated. The HFD-fed mice (OC group) showed the greatest weight increase at the completion of the 12-week experimental period **(Figure. 1b)**, and they eventually achieved the highest BMI in comparison to the other groups **(Figure. 1c)**. After *P. paradiseus* FO feeding, mice in the HFD-induced treatment groups (T-I and, T-II) showed noticeable weight loss and decreased BMI. The weight of WAT changed for body weight and was considerably lower in the mice groups that were fed two different dosages of FO compared to the OC group **(Figure. 1d)**. This could be because *P. paradiseus* FO contains various anti-obesity ingredients, particularly fatty acids that are involved in fat metabolism. To investigate the anti-obesity potential of *P. paradiseus* FO, we analysed the total serum lipid profile and total serum glucose levels of each experimental group. According to our findings, a HFD causes insulin resistance and promotes the onset of lipid metabolism. Furthermore, insulin resistance is a major factor in fat degradation in AT, which can lead to the transport of excess free fatty acids (FFAs) and increase the threshold of lipotoxicity. Following this statement in our study, total serum glucose level was increased in OC group up to 51.19% compared to control group, and it was diminished 28.57%- 38.09% in both the FO treated groups. Additionally, dyslipidemia is highly correlated with the levels of TC, TG, LDL-C, VLDL-C, and HDL-C **(Lan et al., 2019)**. Our findings demonstrated that in the FO treated group in compare to the OC group, liver TC, TG, serum LDL-C, VLDL-C levels were significantly lower **(Figure. 2)**. In contrast, blood HDL-C concentrations increased dramatically following *P. paradiseus* FO administration, indicating that *P. paradiseus* FO could reduce the occurrence of lipotoxicity. Both doses of FO markedly lowered the total serum glucose levels between the treated groups compared to the OC group. This finding suggests that FO ingestion can reduce the hyper-insulinemic state of BALB/c mice **(Arai et al., 2009)**.

The liver is the primary organ that controls lipid metabolized **(Zhou et al., 2023)**. However, figure. 3**(a, b, c, d)** demonstrates the effect of FO on liver properties, as revealed by HE staining. HFD-fed mice exhibited a qualitative increase in lipid accumulation in the liver. The aberrant buildup of fat in the liver may be significantly reduced through daily intake of 200mg and 400mg of crude oil/kg of body weight **(Figure. 3e)**. Adipocyte hypertrophy and hyperplasia are both considered symptoms of obesity, which is defined as an excessive buildup

of lipids **(Schwartz et al., 2017)**. We used HE **(Figure. 4A and 4B)** for histological sections analysis of sWAT and vWAT. Adipocytes in the sWAT and vWAT of the OC group contained fat vacuoles with wide inner diameters and were not homogeneous in size. The T-II group showed a substantial difference in fat deposition in the epidermis between the two treatment groups.

Semi-quantitative PCR was used to analysed the mRNA expression of genes associated with glucose and lipid metabolism to determine how FO consumption modifies metabolic syndrome risk factors. Obesity is frequently associated with lipid metabolism problems **(Zhang et al., 2020)**. Higher levels of circulating leptin act to suppress hunger and promote energy utilisation in normal homeostasis when fat storage rise. Although a lower energy intake should result from higher levels of leptin in the blood, this isn't always the case for people who are overweight or obese. It is recognised that leptin central resistance in obese people may be the cause of this **(Feng et al., 2013). Figure 5 (b, c)** shows that after FO treatment, leptin and adiponectin mRNA levels steadily increased in the BALB/c obese mice group. The alteration in SREBP-1c expression disrupts the transcription of the SREBP-1c target gene FAS, which controls the synthesis of fatty acids **(Edwards et al., 2000)**. Our findings showed that FO supplementation drastically lowered SREBP-1c and FAS mRNA levels in HFD- induced obese BALB/c mice **(Figure 5d, e)**. Both LPL and CPT- 1 are rate-limiting enzymes, responsible for the con version of circulating triglycerides within chylomicrons and VLDL, to FFAs **(Chang et al., 2019)** and induce the beta- oxidation pathway **(Bonnefont et al., 2004)**. Accordingly, we observed a considerable elevation in the mRNA expression of LPL and CPT-1 in both FO- treated groups **(Figure 5f, g)**. IL-10 is an anti-inflammatory cytokine that increases the release of IL-1RA while attenuating inflammation induced by TNF-α, IL-6, and IL-1 **(Juge-Aubry et al., 2005)**. According to a recent study, TNF-α and IL-6 levels are predictive of the onset of type 2 diabetes and are associated with insulin resistance and obesity **(Pradhan et al., 2001)**. The reduction in TNF-α and IL-6 mRNA in the WAT of treated BALB/c obese mice suggests that FO reduces insulin resistance and lowers blood glucose levels, at least in part **(Figure. 5i, k)**. However, the expression of IL-1Ra and IL-10 was increased in both the T-I and T-II groups after treatment with *P. paradiseus* oil **(Figure 5h, j)**. Treatment with FO tended to decrease the expression of pro- inflammatory cytokines (TNF-α) and increase the expression of anti-inflammatory cytokines (IL-10) and metabolic regulators (leptin) in WAT **(Figure. 6)**. Next, we examined the effects of FO on the expression of proteins linked to hepatic lipogenesis in HFD- fed mice given a HFD. Here, **figure 7** illustrates how dietary supplementation with two distinct dosages

of FO markedly increase hepatic PPAR-α protein expression **(Figure. 7d)** and upregulated the expression of the Ob (H-5) hormone **(Figure. 7b)**. However, the reduced expression of PPAR-γ **(Figure. 7c)** and FAS protein levels in the liver **(Figure. 7e) (Chiu et al., 2017)**. CFAs not only offer anti-obesity potential but they also have anti-cancer qualities that can lower inflammation and promote the death of cancer cells. Recent research indicates that novel CFAs called linoelaidic acid, which were extracted from the marine *Opisthopterus tardoore* (Tapra fish), effectively inhibited the growth of human breast cancer cells **(Dutta et al., 2023)**. Additionally, FO therapy significantly reduces body weight by promoting fatty acid oxidation **(Arai et al., 2009)**. Studies have shown that consuming *P. paradiseus* FO reduces body weight and has an anti-obesity effect by preventing the synthesis of lipids in male BALB/C mice. In this study, the results indicated *P. paradiseus* FO had a protective effect against obesity, but between the two doses of FO, 400 mg/kg body weight/day (T-II group), *P. paradiseus* oil was more effective in reducing adiposity.

Experimental Design II: Investigation of *P. paradiseus* oil's duration-dependence to identify the ideal duration for managing obesity utilising the selective dose (400 mg/kg body weight/day)

Materials & Methods

Reagents & Chemicals

The following chemicals and reagents were acquired for completing the experiments in this study. Hexane, isopropanol, chloroform and the solvents used in estimation of physicochemical properties, purchased from Merck, Darmstadt, Germany; RNA-Xpress Reagent, Hi-cDNA synthesis kit, 2X PCR mixture, 3,3′,4,4′-Tetraaminobiphenyl tetrahydrochloride (DAB) obtained from Himedia, HiGenoMB, Maharashtra, India. All the primers used for PCR purchased from Bioserve, India. The primary antibodies: CPT1-C Antibody (B-1) (sc-390059), PPAR-α (H-2) (Sc-398394), PPAR-γ mouse monoclonal IgG (Sc-7273) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA-USA). The secondary antibody: Goat Anti-mouse IgG-HRP (cat# 11-301) was purchased from Abgenex (Odisha, India).

Animals and Diets

The present study was approved by the Institutional Ethics Committee, Midnapore City College (2256/PO/RE/S/23/CCSE, MCC/IAEC-SP/10/23-003) and all experimental protocol management was in accordance with the guidelines for the care and use of laboratory animals. Thirty-six inbreed male albino BALB/c mice with six weeks of age, weighing approximately 20 ± 2.1 g were acquired from Saha Enterprise, IEC No: BAEPS7949M (Habra, North 24 Pargana, West Bengal- 743271) and were acclimatized with a chow diet (Hindustan Lever, Mumbai, India) for one week. Six groups (n=6 of each group) were randomly divided from these acclimatized mice: (1) Normal chow feeding (protein with 20% kcal, carbohydrate with 70% kcal, fat with 10% kcal) with water ad libitum, Control group (C); (2) HFD feeding (protein with 20% kcal, carbohydrate with 35% kcal, fat from lard with 45% kcal) with water ad libitum, obese control group (OC); (3) HFD-fed mice groups supplemented with selected dose of 400 mg/kg body weight/ day of *P. paradiseus* FO by gavage for last two weeks of total experiment, T-I (14 days); (4) HFD-fed mice groups supplemented with selective dose/ day of *P. paradiseus* FO by gavage from 14th week of total experiment, T-II (21 days); (5) HFD-fed mice groups supplemented with selective dose/ day of *P. paradiseus* FO by gavage from 13th week of total experiment, T-III (28 days); (6) HFD-fed mice groups supplemented with selective dose/ day of *P. paradiseus* FO by gavage from 12th week of total experiment, T-IV

(35 days). All experiment animals were kept in individual stainless-steel cage offering a temperature (22 ±1°C), humidity (40-60% relative humidity) and lighting (12 h light/dark cycle) controlled environment **(Samane et al. 2009)**.

Collection of blood and tissue samples

After 16th weeks of the experiment, mice were anesthetized by ether and euthanized by cervical dislocation. Blood samples were collected by puncturing the orbital plexus and centrifuged at 3000 rpm for 15 min at 4°C and serum was stored at –20°C for biochemical analysis. The liver, sWAT, and vWAT were removed, weighed, and immediately submerged in liquid nitrogen, and being stored at −20°C for additional examination. Adipose fat depots were harvested, weighed, and processed as described below for protein expression and gene expression analysis **(Chiu et al., 2018)**.

Assessment of daily food intake, somatic body weight index, BMI (gm/cm^2) in different experimental group

For sixteen weeks, daily food consumption and weekly body weight were recorded. Body length was measured to the closest 0.1 mm using a calliper (Central; Model no. 6420) from nasal to anal distance. Using a food weight machine (ASIN, Model no. KS33), the amount of food consumed each day was measured. According to Pradhan et al. **(2020)**, BMI was measured using the conventional approach and the findings were expressed as gm/cm2.

Biochemical analysis

The Colorimetric methods (Enzopak, India) were used to analyse the serum TC, HDL-C, LDL-C, VLDL-C, triglycerides, total serum glucose, SGOT, SGPT, uric acid and urea level **(Panchali et al., 2024).**

Histopathological Analysis

The liver sample, sWAT, and vWAT were preserved with 10% formalin, embedded in paraffin wax, and then sliced in accordance with standard procedure. For histological examination, 5 µm thick tissue slices were stained with HE. The compound microscope (Olympus, Model No. CX21i) was used to take histological pictures **(Dasilva et al. 2017)**.

Gene expression study using semi qPCR

According to the HiPurA Total RNA Miniprep purification kit methodology (Hi-Media Hi-GenoMB, Maharashtra, India), the complete RNA was extracted from AT samples. Isolated

RNA was quantified using the NanoDrop-2000 (Eppendorf) spectrophotometer and cDNA was synthesized using reverse transcriptase. Reverse transcription reaction mixture will be prepared according to Hi-cDNA synthesis kit (Himedia, HiGenoMB, Maharashtra, India). The mRNA expression of GAPDH was used as the house-keeping primer set (sense: GGTGAAGGTCGGAGTCAACG, antisense: GTGAAGACGCCAGTGGACTC). PCR will perform as follow: Initial denaturation of DNA at 95˚c for 1min, denaturation at 95˚c for 30sec, DNA annealing at 65˚c for 45sec and extension of DNA at 72˚c for 2 min, final extension of DNA at 72˚c for 10min. the second and third step were repeated for total of 42 thermal cycles. Lastly the PCR tube with sample was hold at 4˚c. Same process will be followed for another primer.

Table 1. Following primer sequences used in semi q PCR analysis.

Genes	**Primary Sequences**	
	Forward Primer	**Reverse Primer**
Adiponectin	5'-GTCAGTGGATCTGACGACACCAA-3'	5'-ATGCCTGCCATCCAACCTG-3'
Leptin	5'-CAAGCAGTGCCTATCCAGA-3'	5'-AAGCCCAGGAATGAAGTCCA-3'
SREBP-1c	5'-ACGGAGCCATGGATTGCACA-3'	5'-AAGGGTGCAGGTGTCACCTT-3'
FAS	5'-TGCTCCCAGCTGCAGGC-3'	5'-GCCCGGTAGCTCTGGGTGTA-3'
LPL	5'-CCACAGCAGCAAGACCTTC-3'	5'-AGGGCGGCCACAAGTTTG-3'
CPT-1	5'-GTGACTGGTGGGAGGAATAC-3'	5'-GAGCATCTCCATGGCGTAG-3'
PPAR-α	5'-CCTGAACATCGAGTGTCGAATAT-3'	5'-GTTCTTCTTCTGAATCTTGCAGCT-3'
IL-6	5'-TCAACTTCTCCAGCGTGATG-3'	5'-TCTTTCCCTCTTTTCCTCCTG-3'
TNF-α	5'-TTCTGTCTACTGAACTTCGGGGTGATCGGTCC-3'	5'-GTATGAGATAGCAAATCGGCTGACGGTGTGG-3'
iNOS	5'-CCCTTCCGAAGTTTCTGGCAGCAGC-3'	5'-GGCTGTCAGAGCCTCGTGGCTTTGG-3'
IL-10	5'-TCTCCGAGATGCCTTCAGCAGA-3'	5'-TCAGACAAGGCTTGGCAACCCA-3'

IL-1Ra	5’-GCAGCACAGGCTGGTGAATGAC-3’	5’-TGCCCCCGTGGATGCCCAAG-3’

Immunoblotting test for protein analysis

RIPA buffer was used to lyse AT samples, and the Lowry technique was used to measure the protein level. After boiling 50 µg protein samples in loading buffer for five minutes, they were electrophoresed using SDS-PAGE and transferred to a nitrocellulose membrane (Bio-Rad, Model No. 165.8033FC). After blocking the membrane with 4% BSA, the primary antibody was incubated at 4°C for the entire night. CPT-1, PPAR-γ, and PPAR-α at a 1:1000 ratio were the main antibodies employed in this investigation. Following three rounds of washing with phosphate buffer saline with tween-20 (PBST), the membrane was incubated for one hour at 4 °C with horseradish peroxidase (HRP)-labeled anti-mouse secondary antibody 1:7500 (Abgenex). Using DAB, specific protein bands were found. Densitometric analysis was used to quantify the bands using Image J densitometry software.

Statistical Analysis

All data (triplicate values) are presented as mean± SEM. Differences among the groups were assessed by one-way ANOVA followed by Tukey's multiple-comparison tests to comparing each treatment group to the control group, with the level of significance set at $P<0.0001$, $P<0.001$, $P<0.01$ and $P < 0.05$. Analyses were performed with Graph Pad Prism **(Samane et al., 2009)**.

Results

Fish oil supplementation ameliorated HFD-induced overweight, decreased BMI(g/cm^2) and maintained peripheral organs weight

According to our previous study we co-administered a selective dose of *P. paradiseus* FO (400 mg/kg body weight /day) along with HFD among the several experimental groups. The regular calorie consumption of all group shows almost equal, as indicated in **Figure 1b**; on the other hand, OC group consumed slightly more calories than the other FO supplemented groups. Initially, body weight was the same in all FO treated groups **(Figure 1c)**. However, the HFD-fed OC group's body weight growth was 9.54±2.75 g greater than that of the mice fed a lean control diet. Interestingly, the OC and T-I groups gained nearly the same amount of total body

weight. Animals from the FO treated groups had significantly lower body weights than the OC after 12 weeks in the T-II (6.23 ±1.80 g), T-III (6.70±1.93 g), and T-IV (6.65± 1.92 g) groups. **Figure 1d** presents data regarding BMI (g/cm^2) of different experimental groups. Retroperitoneal fat elevated considerably in the OC group, and similar between control and FO treated T-II group (duration of 21 days treatment), while epididymal fat was reduced only in the FO supplemented groups (mostly in T-II & T-III group). Therefore, the mice group's BMI (g/cm^2) was as follows for C: 0.45±0.01, OC: 0.61±0.03, T-I: 0.56±0.03, T-II: 0.49±0.02; T-III: 0.52±0.02; T-IV: 0.52±0.02. When comparing the water consumption of each group, there were no noticeable variations. Additionally, the weights of adipose tissue (1.79± 0.05 g), liver (2.14± 0.08 g) and kidney (0.81±0.03 g) of OC group fed HFD were greater than the C group **(Figure e, f, g)** and supplementation of 400 mg/day FO could considerably counteract this negative impact by a 0.83±0.09 g, 1.04±0.02 g, 1.14 ±0.02 g decrease in relative AT weight and side by side weight of liver diminished by 1.31±0.02 g, 1.48±0.03 g, 1.65 ±0.02 g; however, there was little noticeable variation in kidney tissue weight between the HF+FO group.

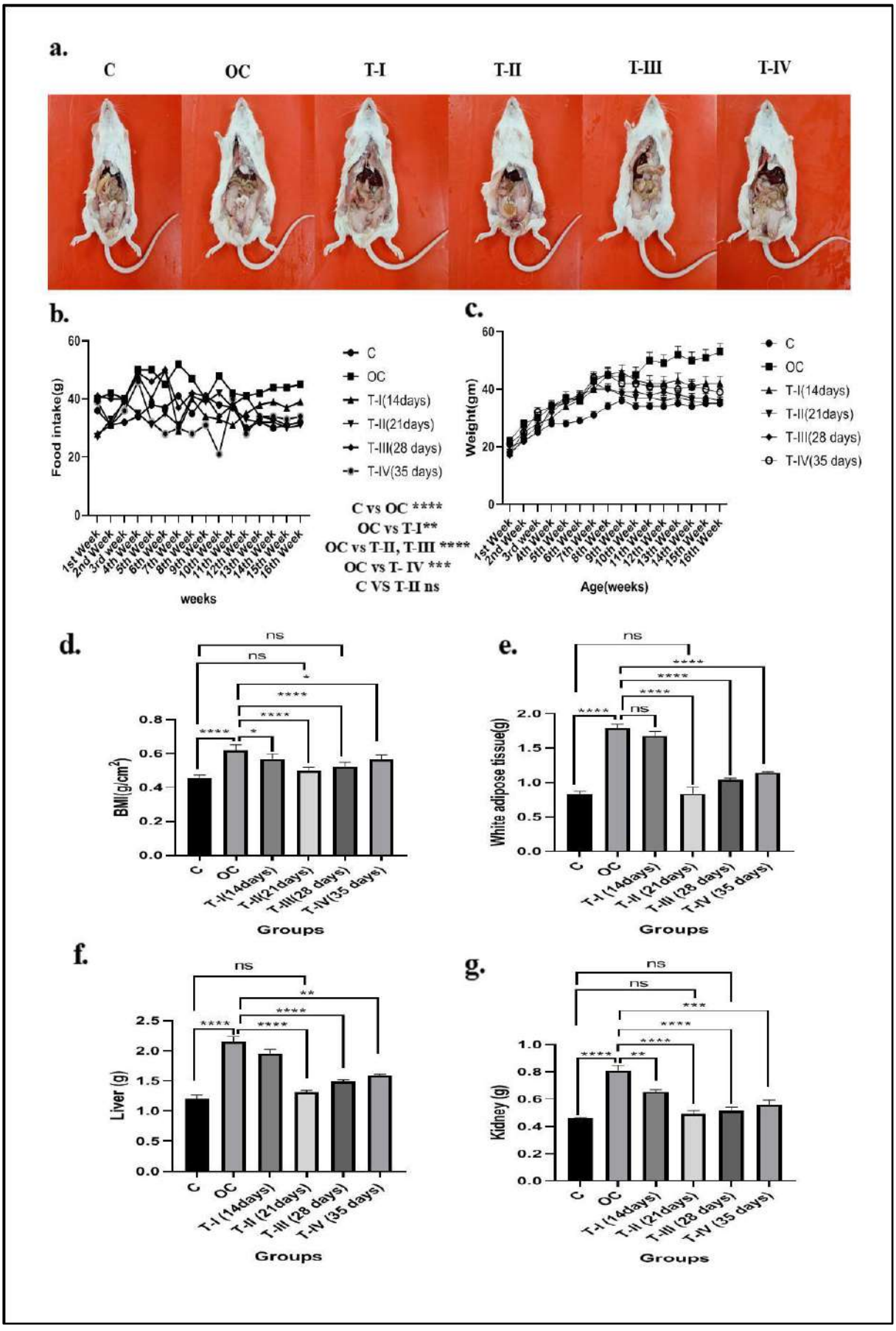

a.
C
OC
T-I
T-II
T-III
T-IV
b.
Food intake(g)
weeks
C
OC
T-I(14days)
T-II(21days)
T-III(28 days)
T-IV(35 days)
C vs OC ****
OC vs T-I**
OC vs T-II, T-III ****
OC vs T- IV ***
C VS T-II ns
c.
Weight(gm)
Age(weeks)
d.
BMI(g/cm2)
e.
White adipose tissue(g)
f.
Liver (g)
g.
Kidney (g)
Groups

Figure 1. FO supplementation reduce weight gain and fat buildup in HFD-fed mice (a) fat accumulation in different experimental groups, (b) evaluations of dietary intake each week (c) variations in the body weight (d) BMI (gm/cm^2) (e) represent the weight of the kidney (g), liver (g), and WAT (g) in each experimental group (n=6) values are represented as mean ±SEM. Statistical analysis was performed using one-way ANOVA accompanied by Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05.

Assessment of total lipid profile, serum glucose, protein and toxicity markers level in HFD induced obese mice treated with selective dose of *P. paradiseus* oil in different duration

In this study, **Figure 2** illustrates lipid profiles alterations in the blood after the fifth week of FO dietary supplementation. When assessing variations among multiple groups for blood lipid profiles and features of liver and kidney injury, the OC group reported dramatically increased the circulating TC (a 17.47% elevate; **Figure 2a**), TG (a 42.61% elevate; **Figure 2b**), LDL (a 30.84% elevate; **Figure 2d**),VLDL (a 44.80% elevate; **Figure 2e**) and serum glucose level(a 29.65% elevate; **Figure 2f**) in contrast to the C group; side by side, the OC group had markedly lower the plasma HDL level (a 37.92% reduce; **Figure 2c**) compared to the C group. As anticipated, HFD-fed mice groups administered with 400mg/kg body weight/day FO demonstrated a considerable diminution in the HFD-induced mentioned rise of lipid profiles, including TC (a 10-16.12 % elevate), TG (a 32-38 % elevate), LDL (a 16.94-27.38 % elevate; Figure 2d), VLDL (a 44.80% elevate; Figure 2e) and serum glucose level (a 29.65% elevate; Figure 2f) compared to the OC group. Next, we examined the levels of serum protein levels and certain toxicity indicators in mice's livers and AT in each dietary class. The OC group had considerably greater levels of uric acid, urea, SGOT, and SGPT (about 40-50%) than the C group. Whereas, serum protein level **(Figure 2g)** was decreased (48.08%) in OC group. Mice groups fed HFD combined with 400 mg FO had a lower serum uric acid (a 44.6% decrease; **Figure 2h**), urea (a 35.04% decrease; **Figure 2i**), SGOT (a 45.87% decrease; **Figure 2j**), SGPT (a 69.31% decrease; **Figure 2k**) levels compared to the OC group. Moreover, although there were no obvious differences observed between the OC and the T-I group (treated with FO only 14 days).

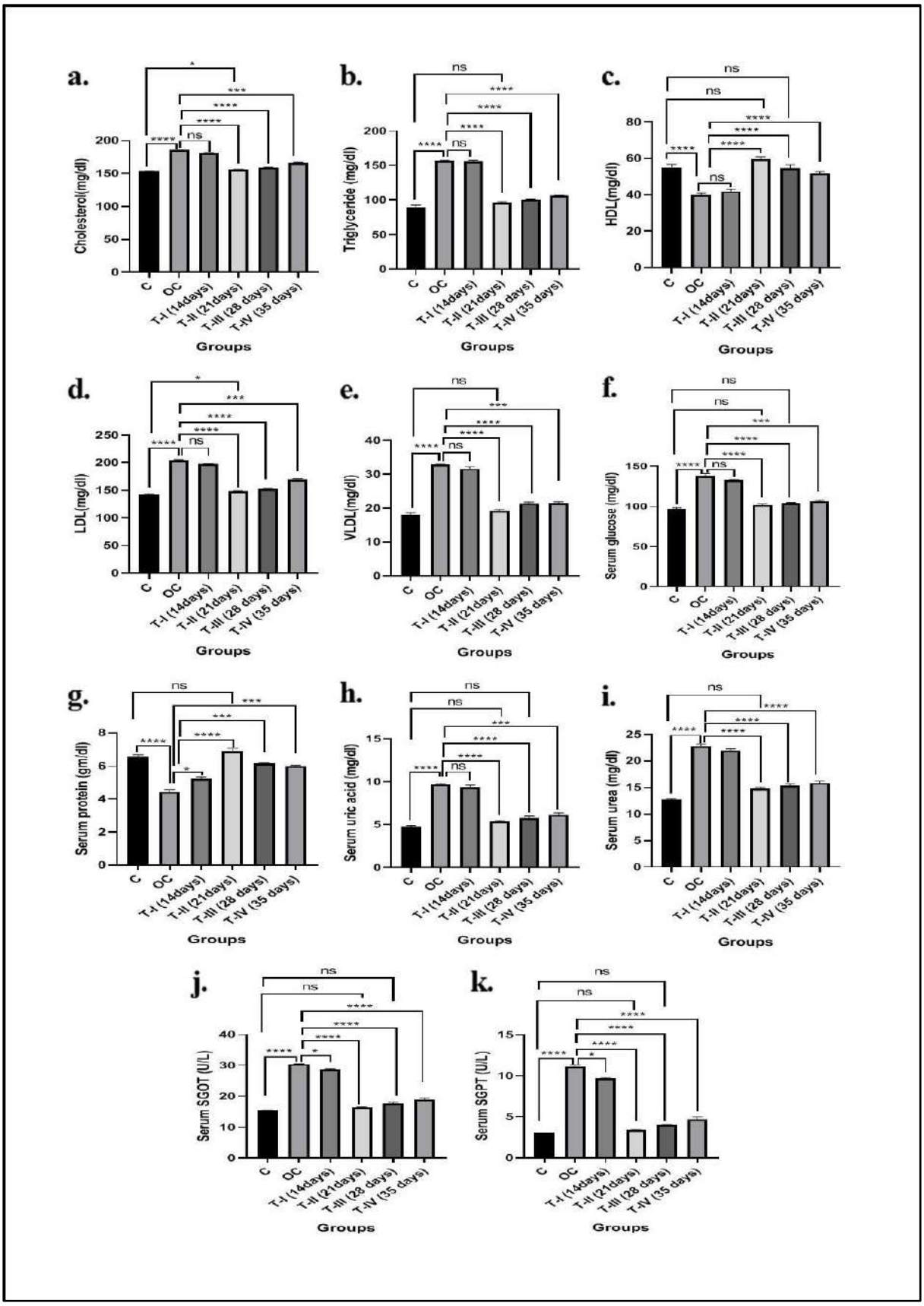


Figure 2. Analysis of total biochemical parameters such as serum a) cholesterol, b) triglycerides, c) HDL, d) LDL, e) VLDL, f) glucose, g) protein, h) uric acid, i) urea, j) SGOT,

and k) SGPT in each of the six experimental groups. The values are given as mean ±SEM. Tukey's multiple comparison test and one-way ANOVA followed next. **p<0.01, *p<0.05, ***p<0.001, ****p<0.0001.

Histopathological alterations in fatty liver caused by HFD+FO

In this study, the HFD-fed animals displayed primarily big lipid-filled vacuoles (macrovesicular steatosis) in histological sections from liver tissue stained with HE (**Figure 3A-B)**. After receiving therapy for 21 days, lipid buildup in the form of lipid droplets or even small lipid droplets (microvesicular steatosis) was reduced in liver sections from the HFD+FO group.

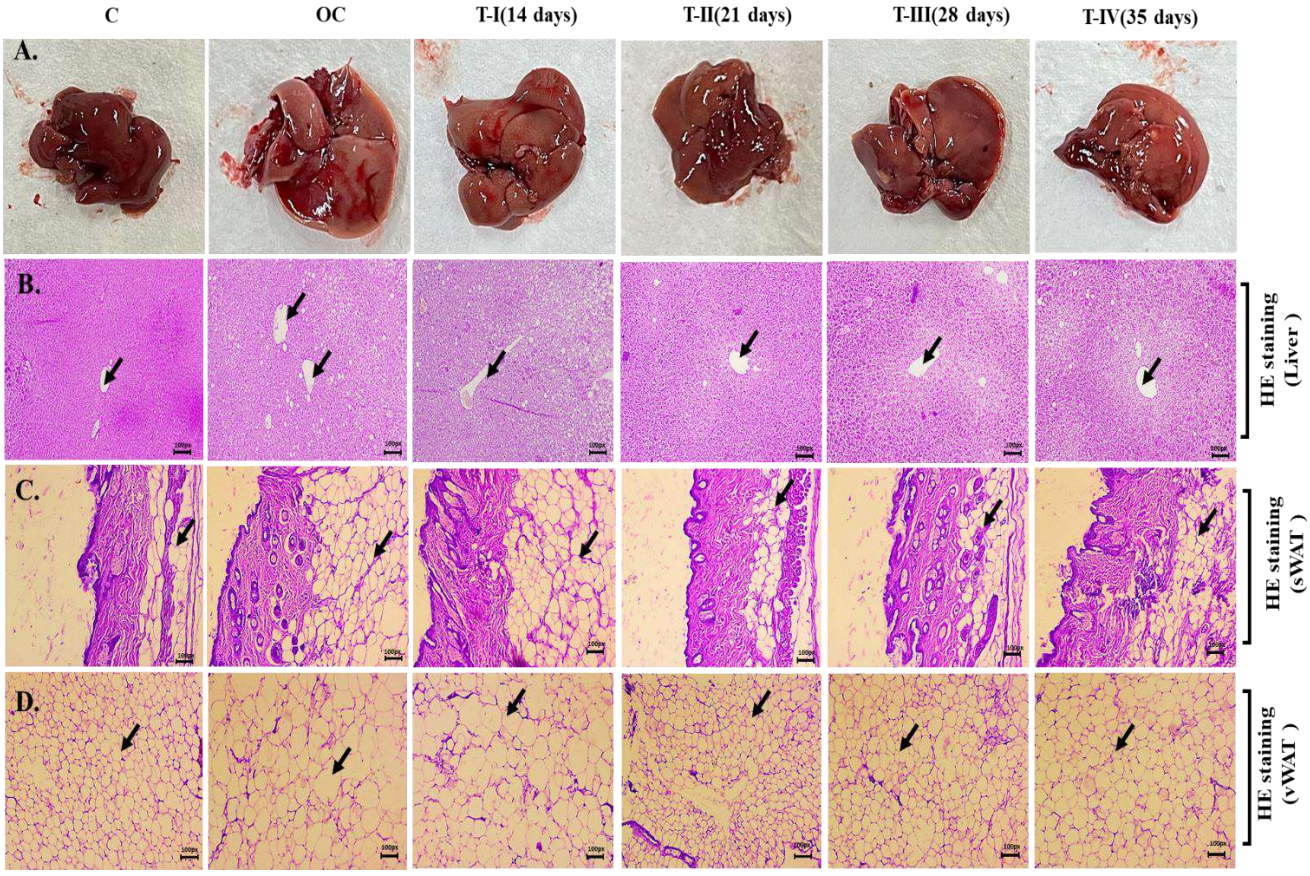


Figure 3. Efficacy of FO on hepatic lipid buildup in HFD-fed mice groups. Groups are segregated as control; obese control; T-I (14 days), T-II (21 days), T-III (28 days), T-IV (35 days) and treated with selective dose (400 mg/ kg body weight/ day) of FO. (A) Represent the image of liver, HE staining of (B) liver tissue, (C) sWAT, (D) vWAT. The observation was conducted under 10 × magnification and scale bar was 100 µm. In liver section arrows (→) reveal the restoration of cytoplasm and in WATs section arrows (→) indicate the fat accumulation layer as well as adipocyte size in different experimental groups.

Dietary FO reduced inflammation and adipocyte expansion in AT

The distribution of white fat is associated with obesity. The HFD-induced OC group had the highest sWAT and vWAT fat weight, according to pathological investigation using HE staining (**Figure 3C-D**). The fat cells in vWAT were larger than those in sWAT, according to an analysis of the tissue structure. This has to do with how various ATs have different cell structures. Low-grade chronic inflammation in AT is typically associated with obesity and is prevalent in visceral fat. There are more inflammatory cells and larger fat cells in vWAT than in sWAT. In obese mice, FO increased total fat, and the duration-dependent treatment groups had lower total fat mass than the OC group.

Efficacy of FO on lipometabolic in HFD-fed mice groups via gene expression study

We assessed the gene expressions of lipometabolic mediators and lipid transport-related genes, including adiponectin, leptin, SREBP-1c, FAS, LPL, CPT-1, and PPAR-α, in order to obtain additional understanding of the critical role of FO via uptake and excretion of lipids involved in the in peripheral organs for modified lipid homeostasis. Therefore, we also quantified the expression of some inflammation related adipocytokines such as IL-6, TNF-α, inducible nitric oxide synthase (iNOS), IL-10 and IL-1ra in AT of the mice groups using semi quantitative PCR (**Figure 4A**). Leptin mRNA and adiponectin mRNA in AT was greatly elevated by 1.26-fold and 1.4- fold in the mice (T-II group) fed with a selective dose of *P. paradiseus* oil with the duration of 21 days (**Figure 4B b & c)**. In this investigation, the SREBP-1c and FAS expression in the HFD-induced obese group increased by 1.38 and 1.21 times, correspondingly, while the SREBP-1C and FAS expression in the *P. paradiseus* oil-treated group reduced by 1.35 and 1.24 times, respectively **(Figure 4B d & e)**. LPL and CPT-1 expression was serially raised in FO supplemented T-II, T-III group by 2.07 to 2.15 -fold and 1.06 to1.16- fold individually **(Figure 4B f & g)**, on the other hand PPAR-α expression have elevated 1.28-fold in T-III group compared with HFD induced OC group **(Figure 4B h)**. However, treatment with *P. paradiseus* FO, the T-II group's mRNA expression of related anti-inflammatory markers, such as IL-1ra and IL-10, raised by 1.13 and 1.42 times, respectively **(Figure 4B m & l)**. Proinflammatory genes such as TNF-α, IL-6, and iNOS were found to be more elevated in the OC group than in the C group, while *P. paradiseus* FO treatment dramatically decreased their expression in the T-II and T-III groups by 1.16, 1.56, and 1.31 times, respectively **(Figure. 4B j, i & k)**.

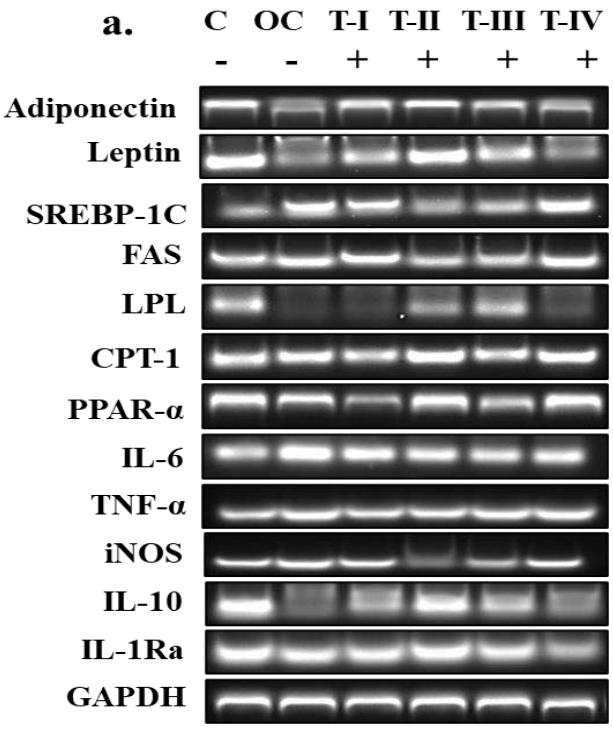


Figure 4A. (a) Relative mRNA expression of some obesity and inflammatory related gene markers level in AT of male albino BALB/c mice in different FO treated groups (n=6) were adjusted to compare with house-keeping gene GAPDH.

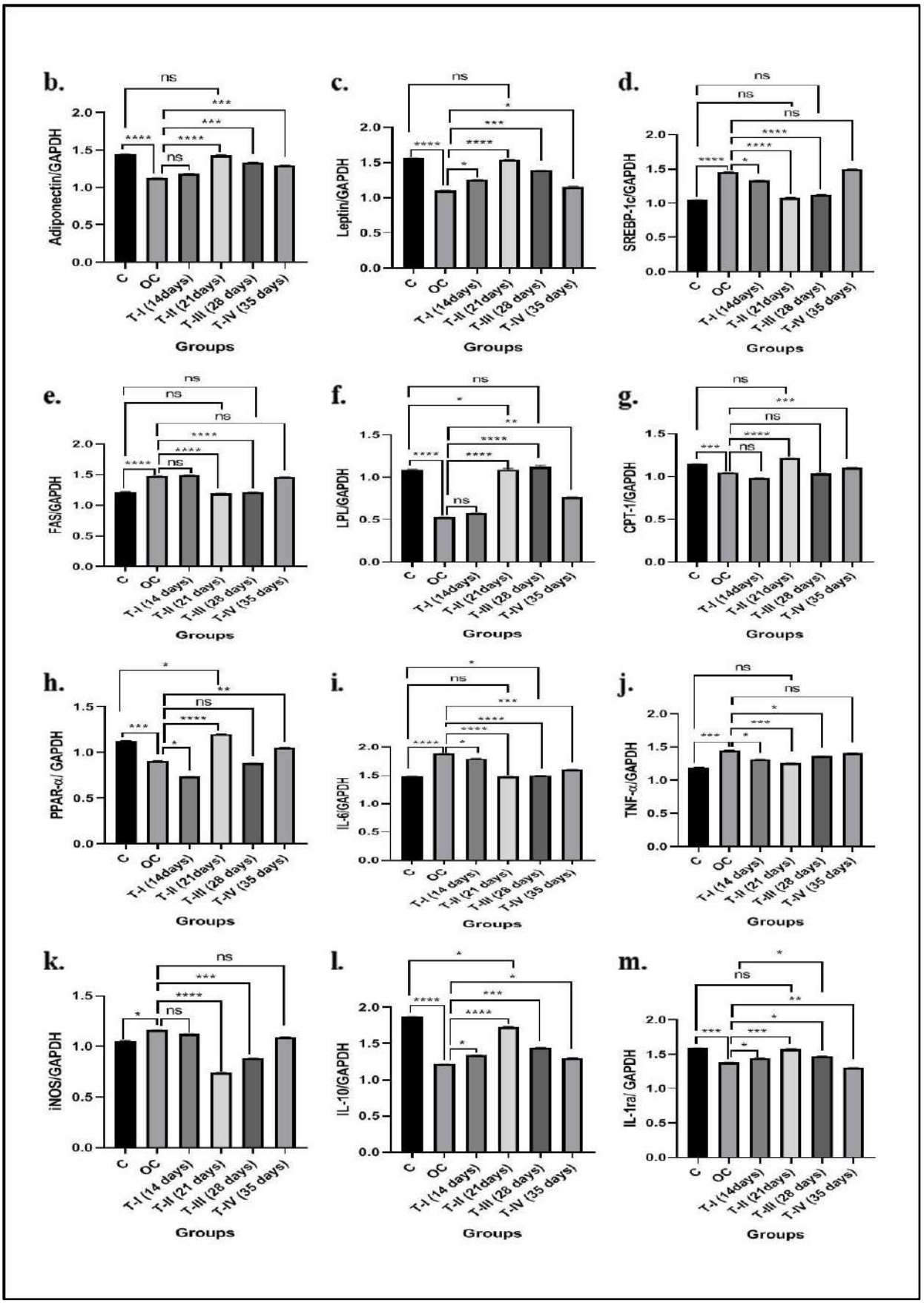

b.
Adiponectin/GAPDH
c.
Leptin/GAPDH
d.
SREBP-1c/GAPDH
e.
FAS/GAPDH
f.
LPL/GAPDH
g.
CPT-1/GAPDH
h.
PPAR-α/ GAPDH
i.
IL-6/GAPDH
j.
TNF-α/GAPDH
k.
iNOS/GAPDH
l.
IL-10/GAPDH
m.
IL-1ra/ GAPDH
C
OC
T-I (14days)
T-II (21days)
T-III (28 days)
T-IV (35 days)
Groups

Figure 4B. Expression of obesity related adipocytokines by semi q-PCR. Experimental genes were as follows (b) Adiponectin, (c) Leptin, (d) SREBP-1c, (e) FAS, (f) LPL, (g) CPT-1, (h) PPAR-α, (i) IL-6, (j)TNF-α, (k) iNOS, (l) IL-10 and (m) IL-1Ra in AT of male albino BALB/c mice in several experimental groups (n=6). One-way ANOVA and Tukey's multiple comparison test are used to evaluate statistical analysis. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05.

Protein expression in AT

The HFD-fed mice's protein expressions of CPT-1 (**Figure 5b**) and PPAR-α (**Figure 5d**) were markedly reduce than those of the C group, while the T-II group's protein expressions of CPT-1 and PPAR-α were up to 1.07- fold higher after *P. paradiseus* FO supplementation. HFD-up regulated protein expressions of PPAR-γ in OC group; however, PPAR-γ expression were significantly down regulated in T-II (1.39-fold) and T-III (1.21-fold) group, which could be also reduced by taking supplements of *P. paradiseus* FO **(Figure 5d)**.

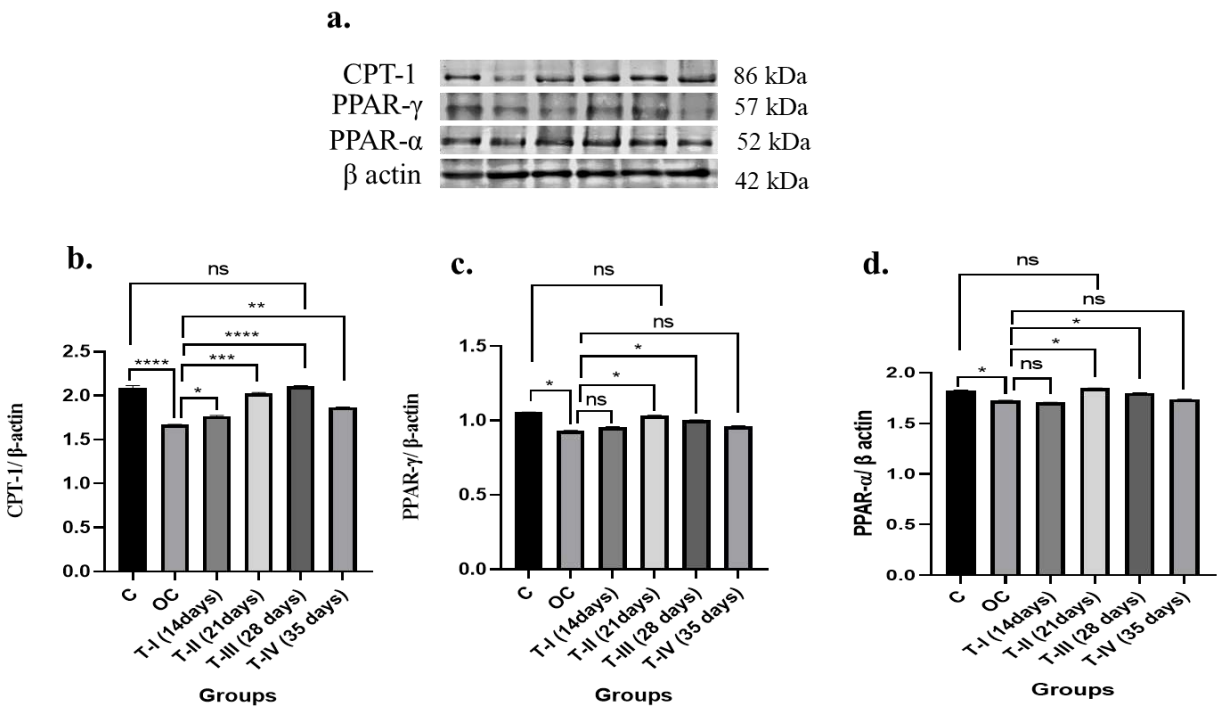


Figure 5. Protein expression analysis of CPT-1, PPAR-ɣ and PPAR-α in WAT. Experimental groups are segregated as control; obese control; T-I (14 days), T-II (21 days), T-III (28 days) and T-IV (35 days). β-actin was used to normalise the results. Western blot images of WAT adipose tissue: (a) relative protein expression, (b) CPT-1, (c) PPAR-γ, and (d) PPAR-α. Values are represented as mean ±SEM. Statistical analysis evaluated via one way ANOVA followed by Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05.

Discussion

This study aimed to determine whether PUFAs and MUFAs enriched *P. paradiseus* FO supplementation reduces obesity in mice model with in a duration dependent (T-I, supplemented for 14 days; T-II, treated for 21 days; T-III, treated for 28 days; T-IV, treated for 35 days) manner using a selective dose (400 mg/kg body weight/ day) of *P. paradiseus* FO when compared with a normal fed C group. Our research indicated that the consumption of dietary FO can modulate certain characteristics of the metabolic abnormalities in the HFD induced BALB/C mice groups, where the BALB/c is more attuned to fat formation, gaining weight, and glucose uptake disturbances when fed an HFD, as shown by considerable alterations in abdominal fat weight and adipocyte volume **(Samane et al., 2009)**. Numerous investigations have demonstrated that FO has positive effects on the regulation of FFAs in the liver and AT of HFD-fed animals **(Chiu et al., 2018)**. Moreover, our previous investigations also found that *P. paradiseus* FO explored as a major modulator of lipometabolic dysfunction in liver and AT in HFD-fed mice groups. In this duration dependent study all the mice fed with HFD except C group where all the BALB/c male mice exerted a remarkable body weight gains from an early stage. *P. paradiseus* FO+ HFD fed mice exerted moderate body weight gain and after the completion of 5 weeks treatment, weight gain supressed in treated group mostly in T-II (treated for 21 days) and T-III (treated for 28 days) group without affecting daily food intake. BMI variations were comparable to somatic body weight index variations. We observed significant variation in the peripheral organs (WAT, liver, kidney) of different experimental treated groups **(Figure 1)**. Hyperlipidaemia and other metabolic diseases associated with obesity are caused by significant weight gain and fat buildup **(Hollie & Hui 2011)**. Therefore, supplementing with ω-3 PUFA may decrease fat buildup in the liver by raising the level of ω-3 PUFA **(Dasilva et al., 2017)**, which may promote β-oxidation and the absorption of cholesterol from plasma, bile acid production, and its extraction. Furthermore, supplementing with ω-3 PUFA may reduce TG production and cholesterol level **(Miralles-Pérez B et al., 2021)**. We found that the HFD diet slightly increased the buildup of TC, TG, LDL, and VLDL in the liver and decreased the concentrations of HDL in plasma compared to the normal standard diet, however in the FO supplemented groups the parameters were diminished mainly in the duration of 21 days of HFD+FO treatment (T-II group). However, the plasma and liver total lipid profile concentrations were unaltered between OC and T-I group (treated with HFD+FO for 14 days). Moreover, the fatty acid overflow causes adaptive reactions such

enhanced β-oxidation and fatty acid desaturation **(Feillet-Coudray et al., 2013)**, which could account for the unchanged quantities of TG, TC, LDL, and VLDL in the liver and plasma. In comparison to the normal control group, the HFD-induced obese mice in the study had significantly higher levels of urea and uric acid that impaired kidney function, which may be brought on by elevated oxidative stress and hyperglycemia. Serum urea and uric acid levels significantly decreased after the administration of *P. paradiseus* oil (400 mg/kg body weight/day dosage), indicating the oil's protective impact on the kidneys. The liver is the primary organ for metabolism; in a diabetic state, the concentration of hepatic enzymes is altered. Increased concentrations of SGOT and SGPT are found in current study, indicating histoarchitectural irregularities that could have been caused by tissue ischemia and oxidative damage as a result of this enzyme leaking into the bloodstream. After the CO administration (400 mg/kg body wt/day) with different duration period normalization in these parameters were obtained with signifies the reduced stress level, remodelling of tissue architecture, and hence reduced concentration of SGOT and SGPT **(Das et al., 2026).** Following FO treatment, the hepatic histopathology transformed (Figure 3A-B). The HFD+FO-fed mice group had higher hepatic mRNA expression of genes related to fatty acid oxidation, such as CPT1, a rate-limiting enzyme that transfers fatty acids to mitochondria for oxidation, and PPARα and PGC1α, transcription factors (or coactivators) that regulate hepatic lipid metabolism. We also looked at adipocyte morphology to obtain further understanding of the underlying mechanisms of FO supplementation on insulin sensitivity against HF diet-fed animals. HFD+FO-fed mice exhibit variations in adipocyte size in contrast to the OC group **(Figure 3C-D)**. The AT is an endocrine organ that plays a crucial part in controlling metabolism and homeostasis. A molecular connection between elevated body weight and the onset of diabetes mellitus, metabolic syndromes, and cardiovascular diseases may be found in the production and secretion of either too many or too few adipokines, which have a significant impact on insulin sensitivity, glucose metabolism, inflammation, and atherosclerosis **(Arçari et al., 2009)**. In the present study the expression of adiponectin, leptin, SREBP-1c, FAS, LPL, CPT-1, PPAR-α, IL-6, TNF-α, iNOS, IL-10, IL-1ra, in AT were directly regulated by HFD. Furthermore, we reported that treatment with 400 mg/100g body weight/day of *P. paradiseus* oil was able to restore these genes' expression **(Figure 4A, B)**. Increased circulating leptin levels in normal homeostasis work to reduce appetite and promote energy utilisation when fat storage rise. Although lower energy intake should result from increased circulating levels of leptin, this isn't always the case in overweight or obese people. This may result from leptin central resistance in obese people, as is known, but it may also be linked to daily fluctuations in the leptin system **(Bates & Myers**

2003). In accordance with the previous statement, our research revealed a decrease in leptin levels in the OC group, which was reversed by FO treatment (400 mg/kg body weight/day) **(Figure 4B c)**. Numerous researchers have demonstrated that FO supplementation raises adiponectin levels **(Duda et al., 2007)** and upregulates PPARα, an important gene that regulates the control of hepatic peroxisomal β-oxidation of fatty acids **(Martin et al., 2007) (Figure 4B b)**. As shown in **Figure 4B d**, the mRNA level of SREBP-1c, a key transcription factor associated with the activation of lipogenic genes and its one representative target gene, FAS, was significantly lowered in WATs from FO-supplemented mice **(Figure 4B e)**. Interestingly, visceral WAT showed minimal changes in the mRNA level of FAS **(Choi et al., 2007)**. According to these findings, oral FO treatment may prevent body fat storage by reducing the mRNA expression of lipogenic genes. According to this study, obesity increases the production of a number of inflammatory cytokines, which are essential for metabolic disorders and inflammation associated with obesity. TNF-α is a powerful cytokine that affects insulin action, glucose transport, and lipid metabolism in addition to inducing the synthesis of IL-6, the primary determinant of the acute phase response **(Kern et al., 2001).** In this experiment, TNF-α and IL-6 levels are consistently elevated in the OC group, and following treatment with *P. paradiseus* FO, a decrease in adipose mass results in a decrease in these expression levels in the treatment groups **(Figure 4B i & j)**. Conversely, anti-inflammatory indicators like IL-10 and IL-1ra genes lower inflammation by inhibiting macrophage activity. In this case, FO-treated groups had higher IL-10 and IL-1ra expressions than OC groups **(Figure 4B l & m).** The majority of factors that cause obesity, hyperglycemia, and insulin resistance, such as free fatty acids, endotoxins, and oxidative stress, increase the production of iNOS. Mice given an HFD showed a high iNOS signal in the AT, which was mitigated by FO intake **(Figure 4B k).** Additionally, it has been observed that omega-3 PUFAs reduce hepatic steatosis and insulin resistance brought on by obesity by phosphorylating AMPK **(Bates et al., 2018).** In the present investigation, we noticed that FO could modulate hepatic and plasma lipid profiles via activating protein expressions of hepatic CPT-1 and PPAR-α in HFD-fed mice group, while the hepatic PPAR-γ protein expressions were upregulated **(Figure 5)**. The biological activity of *P. paradiseus* oil at a dose of 400 mg/kg body weight/day found in this study clearly demonstrated the oil's enormous potential as an anti-obesity and anti-hyperlipidemic treatment. *P. paradiseus* oil taken orally for 21–28 days decreased both the quantity of ATs and the amount of circulating lipids, which led to a drop in body weight. FO appears to exert such effects by altering the metabolism of lipids by decreasing lipogenesis activity while increasing fatty acid oxidation.

Conclusion

Based on our research, eating a HFD increased serum trans-fat levels, body mass index, and adiposity. The addition of FO to the diet alters the metabolism of lipids via altering adipocyte cell size, lipolytic rates, and response to fatty acids and hormones. Dietary *P. paradiseus* FO with a selective dose (400 mg/kg body weight/day) alleviated HFD-induced obesity and obesity-related complications such as hyperlipidaemic changes that induce cardiovascular disease and NAFLD with the duration of 21-28 days. Furthermore, we found that the therapy improves the expression of many genes associated with the obesity management.

Efficacy of *P. paradiseus* oil on adipocytokines regulation

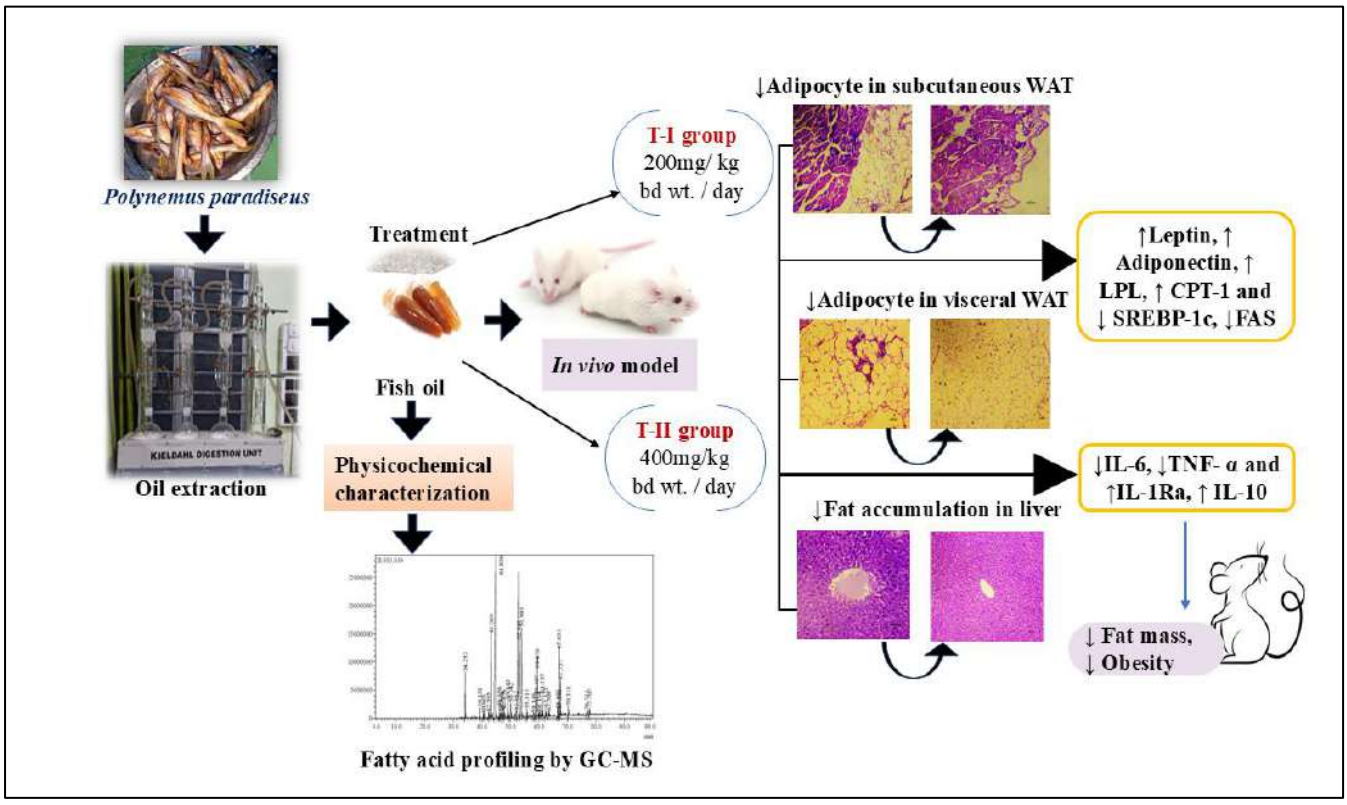


Figure 6. FO and its PUFAs and MUFAs are the suitable agents to prevent obesity.

Chapter VI: Objective (iii)

Objective (iii): To establish the anti- obesity potentiality of isolated FA both *in vitro* and *in vivo* model.

To determine the antiobesity efficacy of newly isolated FA from marine *P. paradiseus* oil via molecular approach on *in vitro* & *in vivo* model as well as to understand the exact molecular mechanism for antiobesity potentiality.

Introduction

Obesity is acknowledged by the scientific community as the primary health issue associated with the development of metabolic syndrome. Indeed, according to estimates from the WHO, there are 2.5 billion overweight persons and 890 million obese individuals in Globally. These projections emphasise how vital it is to find a solution for this massive global issue **(Lim et al., 2024)**. Nowadays, most public health organizations recommend raising physical exercise and eating a balanced, healthful diet in order to reduce body weight. However, a lot of People are struggling sticking to these lifestyle adjustments, particularly over a long-time duration. Therefore, there is a desire to investigate dietary habits that may aid in managing body weight. One such option may be use of maritime FO includes a more diversified blend of fatty acids, with a special emphasis by some bioactive lipids, such as medium-chain fatty acids, n-3 and n-6 PUFAs, and MUFAs, have an impact in both lipid storage and lipid breakdown physiology **(Izuddin et al., 2023)**. According to studies, n-3 PUFA's ability to prevent obesity is often mediated by the alteration of adipocytokines linked to lipid metabolism (adipogenesis and lipogenesis), which increase resting energy expenditure, increase fat oxidation, and decrease hunger **(Vaidya & Cheema 2014; Goel et al., 2018)**. At the molecular state, obesity is identified by a development of adipogenesis **(Stephens 2012)**. A reliable and well characterized *in vitro* model for evaluating adipogenesis and adipocyte differentiation is the preadipocyte mouse embryo fibroblasts or preadipocyte cell line (3T-3L1 cell line) **(Ruiz-Ojeda et al., 2016)**. Numerous transcriptional regulators are associated in lipid accumulation during differentiation of 3T3-L1 adipocytes. There are multiple stages associated to obesity involved in the regulated development of preadipocytes into adipocytes **(Tang et al., 2003)**. For those kinds of reasons, numerous research projects have been carried out in 3T3-L1 cells to look for novel compounds or anti-obesity medications for weight management. Recently, scientists have observed that the fatty acid composition of the fat particles affects the growth of adipocytes **(Yao-Borengasser et al., 2008)**. A series of transcription factors are triggered during 3T3-L1 differentiation to change the effectivity of genes responsible for adipocyte formation. Two essential transcriptional regulators of adipocyte development, CEBP-α and PPAR-γ are essential for stimulating a significant number of downstream target genes that determine the traits of developed lipocytes or fat cells **(Moseti et al., 2016)**. The primary functions of these target adipogenic genes include TG hydrolysis, lipogenesis, and cellular absorption of glucose and fatty acids. The formation and breakdown of fatty acids are modulated by AMPK, a regulatory sensor of cellular metabolic pathways that is essential for

preserving the body's energy homeostasis **(Kim & Kong 2010)**. As a mediator of cellular metabolic pathways, AMPK controls the synthesis and breakdown of fatty acids and serves an important role in preserving the body's energy equilibrium. AMPK inhibits ACC, a downstream enzyme of AMPK and a crucial molecule for lipid production **(Xi et al., 2015)**. Elevated AMPK reduces lipid buildup in the process of adipogenesis by decreasing the production of SREBP-1c, CEBP-α, PPAR-γ, and FAS **(Lee et al., 2018)**. In light of this, AMPK may be a target for obesity treatment. Numerous studies found that in mouse hepatocytes, FO administration enhanced the production of uncoupling protein 2 (UCP-2), which may account for the defence against obesity **(Pedersen et al., 2011)** due to its essential fatty acids content. The potential antiobesity pathways engaged in FO has been suggested in various experimental investigation, incorporating higher levels of circulating adiponectin and adipocyte apoptosis **(Wakutsu et al., 2012)**, side by side changed energy usage and lipid oxidation **(Pérez-Echarri et al., 2009; Haugaard et al., 2006)**. For reliable, prolonged treatment throughout diet therapy, anti-obese dietary supplements made from natural components are typically preferred over medications with potential adverse effects. LC n-3 and LC n-6 FAs are widely documented in the context of their health advantages **(D'Angelo et al., 2020)**. However, LC n-9 FAs have lately attracted widespread interest owing to new research and findings concerning their biological benefits. LC n-9 FAs are a kind of unsaturated FA with a double bond at the 9th position from the methyl end. The most common well known LC n-9 FAs are hypogeic acid, oleic acid, elaidic acid, gondoic acid, mead acid, erucic acid and nervonic acid. In comparison to the other MUFAs mentioned, oleic acid earned the greatest interest in study **(Johnson et al., 2014)**. Gondoic acid (GA, cis-11-Eicosenoic acid, molecular formula $C_{20}H_{38}O_2$, molecular weight 310.51 g/mol), a 20-Carbon MUFA, is contained in a variety of plant oils such as jojoba oil camelina seeds oil and nuts **(Farag& Gad 2022)**. Additionally, GA can be generated *in vivo* through the fatty acid metabolism pathway. Although kupffer cells are well known for their role in the liver's metabolic and immunological defence systems, GA has the ability to activate them and lower inflammation **(Fan et al., 2022)**. Recently Kar et al. (**2025**) explored a novel source of GA, they found that marine *Polynemus paradiseus* FO which have antiobesity potentiality contain 6.38 % of GA. This is the initial investigation to show an anti-obesity efficacy of n-9, GA characterized from marine *P. paradiseus* FO *in vitro* and *in vivo* model. The results exert that by triggering the AMPK signalling pathways, GA suppresses the expression of genes linked to adipogenesis and the generation of inflammatory cytokines.

Experimental Design I: Antiobesity efficacy of GA *in vitro* model to supress the adipocyte differentiation

Materials & methods

Materials

To complete the experiments in this study, the mentioned chemicals and reagents were purchased. Gondoic acid or cis-11- Eicosenoic acid (E3635-100MG) which used as treatment compound, purchased from Merck, Darmstadt, Germany; Dulbecco's modified Eagle's medium (DMEM), 10% foetal bovine serum (FBS), 1.5% penicillin-streptomycin, cell viability kit for 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay, Hi-cDNA synthesis kit, 2X PCR mixture, DAB, dimethyl sulfoxide (DMSO), Oil Red O cell culture tested solution (ORO) collected from Himedia, HiGenoMB, Maharashtra, India. Isobutyl-3-methylxanthine (IBMX), dexamethasone, and bovine insulin purchased from Sigma Chemical Co. (St. Louis, MO, USA); Triglyceride Quantification Colorimetric/Fluorometric kit purchased from Histosec, Merck, Darmstadt, Germany; DAPI (4',6-Diamidino-2-Phenylindole) (Cat no. 62248) from Invitrogen; fluorescent mounting medium purchased from Dako, Hamburg, Germany. Mouse ELISA kit for IL-10, TNF-α and IL-1ra was obtained from Wuhan Fine Biotech (Wuhan, China) All the primers used for PCR purchased from Bioserve, India. The primary antibodies: AMPK mouse McAb (Cat No.: 66536-1-Ig), SREBP-1c Mouse McAb (Cat No.: 66875-1-Ig), CEBP-α Mouse McAb (Cat No.: 66649-I-Ig/), , β- actin Mouse McAb (Cat No.:66009-1-Ig) were purchased from Proteintech (ChromoTek GmbH & Proteintech Germany) and PPAR-α (H-2) (Sc-398394), PPAR-γ mouse monoclonal IgG (Sc-7273), Ob (H-5) mouse monoclonal IgG (Sc-393043), were purchased from Santa Cruz Biotechnology (Santa Cruz, CA-USA). The secondary antibody: Goat Anti-mouse IgG-HRP (cat# 11-301) was purchased from Abgenex (Odisha, India).

Cell culture and maintenance

The National Center for Cell Science (NCCS) in Pune provided 3T3L1 cell lines, which were cultivated at 37°C in a humidified atmosphere with 5% CO2 and 95% air. The media used to culture the cells contained 1.5% penicillin-streptomycin, 10% FBS, and DMEM with high glucose. To develop into pre adipocytes to mature adipocytes, these cells were seeded at a density of 1×10^5 cells/well in 35-mm dishes and grown to confluency in the above-mentioned medium. Differentiation was started two days after the cells attained confluence by adding 0.5 mM IBMX, 1 μM dexamethasone, and 10 μg/mL bovine insulin to differentiation media

(DMEM with high glucose, 10% FBS, and 1% penicillin streptomycin). Fresh differentiation medium containing only insulin was administered for an additional three days (Day 3) until sample collection **(Li et al., 2017)**.

Fatty acids treatments

Using treatment compound named Gondoic acid (GA) or cis-11- Eicosenoic acid (E3635-100MG), purchased from Merck (Darmstadt, Germany) and dissolved in DMSO. To explore the effects of *P. paradiseus* FO derived n-9, GA on preadipocyte maturation into adipocytes, cells were cultured in differentiation media at a density of 1×10^5 cells/well with varying dosages (0.25 µM, 0.5 µM, 0.75 µM and 1 µM) of GA.

Cell viability assessment

The quantity of viable cells in the culture was determined using an MTT assay. In short, GA at different doses (0.25 µM, 0.5 µM, 0.75 µM, and 1 µM) was added to 3T3-L1 preadipocytes at a density of 1×10^5 cells/well in 96-well plates for 24 hours. Following the addition of 10 µl of MTT reagent (5 mg/ml) to the medium, the cells were incubated at 37 °C for two to four hours. After the purple formazan crystals were dissolved in DMSO, the ELISA plate reader (LDxR1, Labsystems diagnostics) measured the absorbance at 570 nm.

The percentage of proliferation was calculated using the following formula: % of viable cells = (OD sample – OD blank)/ (OD treated – OD blank) × 100 **(Dutta et al., 2023)**.

Triglyceride accumulation assay

TG concentrations were measured employing a Triglyceride Quantification Colorimetric/Fluorometric kit (Merck, MAK266) on 3T3-L1 cells that had been cultured for six days in the occurrence or absence of fatty acids. In general, heated lipid extraction solution was used to remove entire cellular lipids. After that, the TG concentration was measured by adding lipase, which changed TG into glycerol. Glycerol interacted with the probe to produce colour, it can be determined using spectrophotometry in an ELISA plate reader at 570 nm (LDxR1, Labsystems diagnostics). A standard curve created from TG standards was used to compute TG concentrations, which were then standardised to the total cellular protein content **(Li et al., 2017)**.

Adipocyte cell staining by Oil Red O (ORO)

Preadipocytes cells were cultured on a six-well plate at a density of 1×10^5 cells/well/0.5 ml culture media. The separation of preadipocytes to adipocytes were assessed employing the ORO staining method. Conditioned media were collected after receiving the required treatment. Next, adipocytes cells were fixed using a 10% formaldehyde solution in PBS for 30 minutes, followed by a cold phosphate-buffered saline wash (PBS; pH 7.4). After being rinsed two times with PBS, cells were stained approximately 30 minutes in ORO working solution (stock: water, 3:2). Cells are subjected to water washes after ORO elimination. Following that, coloured lipid droplets in cells were seen and captured on camera using a fluorescent microscope (Olympus, Model No. BX43F) **(Kinkel et al., 2004)**.

Fluorescent Microscopy of Lipid Bodies by DAPI staining

3T-3L1 cell lines will be cultured at six well plates at a density of 1×10^5 cells/well/0.5 ml of DMEM culture media and treated with selected compound for 24 hours with different doses. Cells were then cultivated and coloured with DAPI (5mg/ml) in PBS (1000:1 v/v) before being examined under a fluorescent microscope (Olympus, Model No. BX43F) using the standardized technique of Dutta et al **2023**.

RNA isolation and semi qPCR

3T3-L1 cells were seeded and rinsed with PBS. Thereafter, total RNA was quantified with a NanoDrop-2000 (eppendrof). First-strand cDNA synthesis was performed using a thermal cycler (BioRad) with a Hi-cDNA Synthesis Kit (Hi Media) and RNA (2 μg) as a template in accordance with the directions provided by the manufacturer. Freshly produced cDNA was amplified from 3T3-L1 control cells and GA-administered cells by specific PCR primers (Bioserve Biotechnologies (India) Pvt Ltd) and the 2X PCR Taq Mixture (HiMedia). The mRNA expression of GAPDH was utilised as a housekeeping gene. The following primers were utilized in this study:

Table 1. Utilizing primer list for semi-quantitative PCR.

Genes	Primary Sequences	
	Forward Primer	**Reverse Primer**
Leptin	5’-CAAGCAGTGCCTATCCAGA-3’	5’-AAGCCCAGGAATGAAGTCCA-3’
Adiponectin	5’-GTCAGTGGATCTGACGACACCAA-3’	5’-ATGCCTGCCATCCAACCTG-3’
AMPK	5’-AGGAAGAATCCTGTGACAAGCAC-3’	5’-CCGATCTCTGTGGAGTAGCAGT-3’

PPAR-γ	5’-AGGCCGAGAAGGAGAAGCTGTTG-3’	5’-TGGCCACCTCTTTGCTCTGCTC-3’
PPAR-α	5’-CCTGAACATCGAGTGTCGAATAT-3’	5’-GTTCTTCTTCTGAATCTTGCAGCT-3’
CPT-1	5’-GTGACTGGTGGGAGGAATAC-3’	5’-GAGCATCTCCATGGCGTAG-3’
FAS	5’-TGCTCCCAGCTGCAGGC-3’	5’-GCCCGGTAGCTCTGGGTGTA-3’
Insulin	5’-CCCCAGAAAAACCTCTTCAGG-3’	5’-GTCACATTCCCAACATCGCC-3’
GLUT-4	5’-GAGCCTGAATGCTAATGGAG-3’	5’-GAGAGAGAGCGTCCAATGTC-3’
SREBP-1c	5’-ACGGAGCCATGGATTGCACA-3’	5’-AAGGGTGCAGGTGTCACCTT-3’
LPL	5’-CCACAGCAGCAAGACCTTC-3’	5’-AGGGCGGCCACAAGTTTG-3’
IL-10	5’-TCTCCGAGATGCCTTCAGCAGA-3’	5’-TCAGACAAGGCTTGGCAACCCA-3’
IL-1Ra	5’-GCAGCACAGGCTGGTGAATGAC-3’	5’-TGCCCCCGTGGATGCCCAAG-3’
IL-6	5’-TCAACTTCTCCAGCGTGATG-3’	5’-TCTTTCCCTCTTTTCCTCCTG-3’

Cytokines Quantification by ELISA assay

During a 24-hour incubation of preadipocytes cells with GA, certain cytokines such as IL-1Ra, IL-10 and TNF-α in the growth medium were assessed using a sandwich ELISA, as directed by the manufacturer (Fine Test). In a short, capture antibodies were used to 96-well plates and left on them at 4°C overnight. Test solvent was then used to stop non-specific protein binding, and excess antibodies were discarded. Before applying the standard and samples, quickly wash the plate twice. After adding 100µl of purified cytokine standards and cell culture supernatants to each well, incubate for about an hour and thirty minutes at 37°C. After that, aspirate and wash the plates twice. Fill each well with 100 microliters of the biotin-labeled antibody working solution, then incubate for 60 minutes at 37°C. During three washing procedures, fill all wells with 100µl of SABC Working Solution and incubate at 37°C for 30 minutes. Following that, wash the plates five times. After washing, add 90µl of TMB substrate solution and incubate for 15 to 30 minutes at 37°C in the dark. A 50µl stop solution was then introduced, and a Bio Rad microplate reader worked to evaluate the absorbance at 450 nm **(Ramanjaneya et al., 2010)**.

Protein analysis by Immunoblotting assay

The 3T-3L1 cells treated with the different concentration of GA were ruptured with RIPA buffer, and the protein concentration was measured using the Lowry technique. Protein samples (50 µg) were boiled in loading buffer for 5 minutes, electrophoresed using SDS-PAGE, and transferred to a nitrocellulose membrane (Bio-Rad, Model No. 165.8033FC). The membrane was followed by blocking with 4% BSA before incubating with the primary antibody overnight at 4°C. The primary antibody used in this study included β-actin, AMPK, SREBP-1c, PPAR-α, PPAR-γ, CEBP-α and Ob (H-5) at a 1:1000 ratio. The membrane was then rinsed three times with phosphate buffer saline with tween-20 (PBST) before being incubated with HRP-labeled anti-mouse secondary antibody 1:7500 µl at 4 °C for one hour. Particular protein bands were identified using DAB. Bands were quantified using Image J densitometry software **(Dayarathne et al., 2025)**.

Statistical Analysis

The results were analysed using one-way ANOVA and Tukey's multiple-comparison tests to compare each treatment group to the control group. The results (triplicate values) were given as mean ± SEM (Rocke, 2004). The statistical analysis was conducted out with GraphPad Prism programme. Values were deemed significant at $P < 0.05$.

Results

Dose dependent efficacy of GA on cell viability and adipogenesis

In order to examine the impact of GA on preadipocyte proliferation, 3T3-L1 preadipocytes were grown for 24 hours in DMEM high glucose media supplemented with several amounts of GA (0.25µM, 0.5µM, 0.75µM, and 1µM). Within 24 hours, the cell viability of mature adipocytes was unaffected by GA treatments at the three concentrations (0.25µM, 0.5µM, and 0.75µM). However, cell viability was considerably reduced by 89.98% at GA (1 µM) doses greater than 0.75 µM. Furthermore, during a 24-hour period, 3T3-L1 pre-adipocytes showed constant cell survival of 98.97% at these concentrations in comparison to the group treated with DMSO. This result suggesting that the *P. paradiseus* derived GA had no toxic effects **(Figure 1b)**.

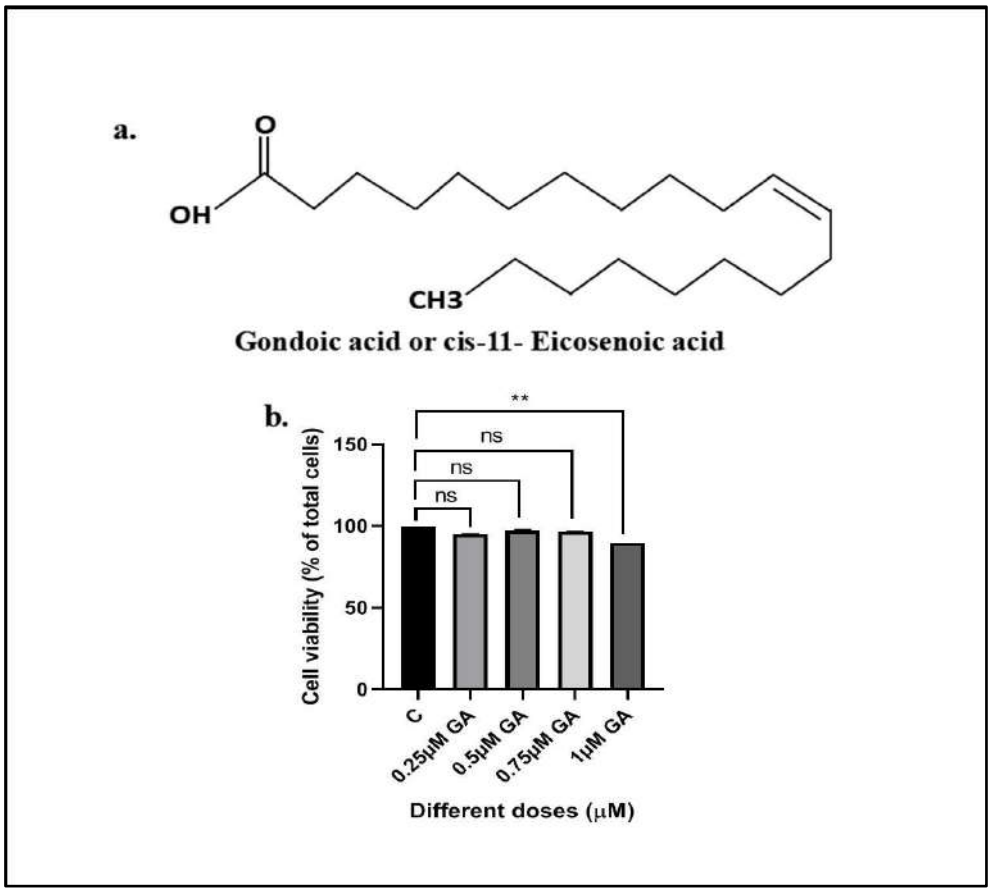


Figure 1. Efficacy of GA on 3T3-L1 adipocyte viability. (a) Chemical structure of GA used in this experiment, (b) Cell viability of 3T3-L1 adipocytes after cultured with various doses of GA for 24h, respectively, by using the MTT assay. Values are represented as the mean ± SEM (n = 3). Statistical analysis assessed via one way ANOVA followed by Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05.

GA inhibited lipid builds up in preadipocytes cell

In this present experiment, ORO staining was used to visualise how GA affected the intracellular lipid accumulation in mature adipocytes. The measured drop in fat buildup was validated by the TG quantification assay. In comparison to the control and other groups, the representative ORO staining images showed that 0.5μM and 0.75μM doses of GA could suppress lipid accumulation more **(Figure 2A)**. Different GA doses, such as 0.25μM, 0.5μM, 0.75μM, and 1μM significantly reduced the TG concentration in 3T3-L1 adipocytes by 6.09%, 18.82%, 18.28%, and 12.39%, respectively, in comparison to the differentiated control cells, as illustrated in **Figure 2B**. These findings suggest that GA markedly inhibited 3T3-L1 adipocyte development.

Fluorescent Microscopy of Lipid Bodies by DAPI

We stimulated the differentiation of 3T3-L1 preadipocytes supplied with different concentrations of GA (0.25μM, 0.5μM, 0.75μM, and 1μM) in the presence of insulin and IBMX in order to examine the obesogenic qualities of GA. This present study performed DAPI staining to assess cell differentiation and lipid accumulation where control group shows significantly increased adipocytes compare to treated group. Among the four dosages, 0.5μM and 0.75 μM of GA displayed the greatest efficacy, as shown in **Figure 2C**.

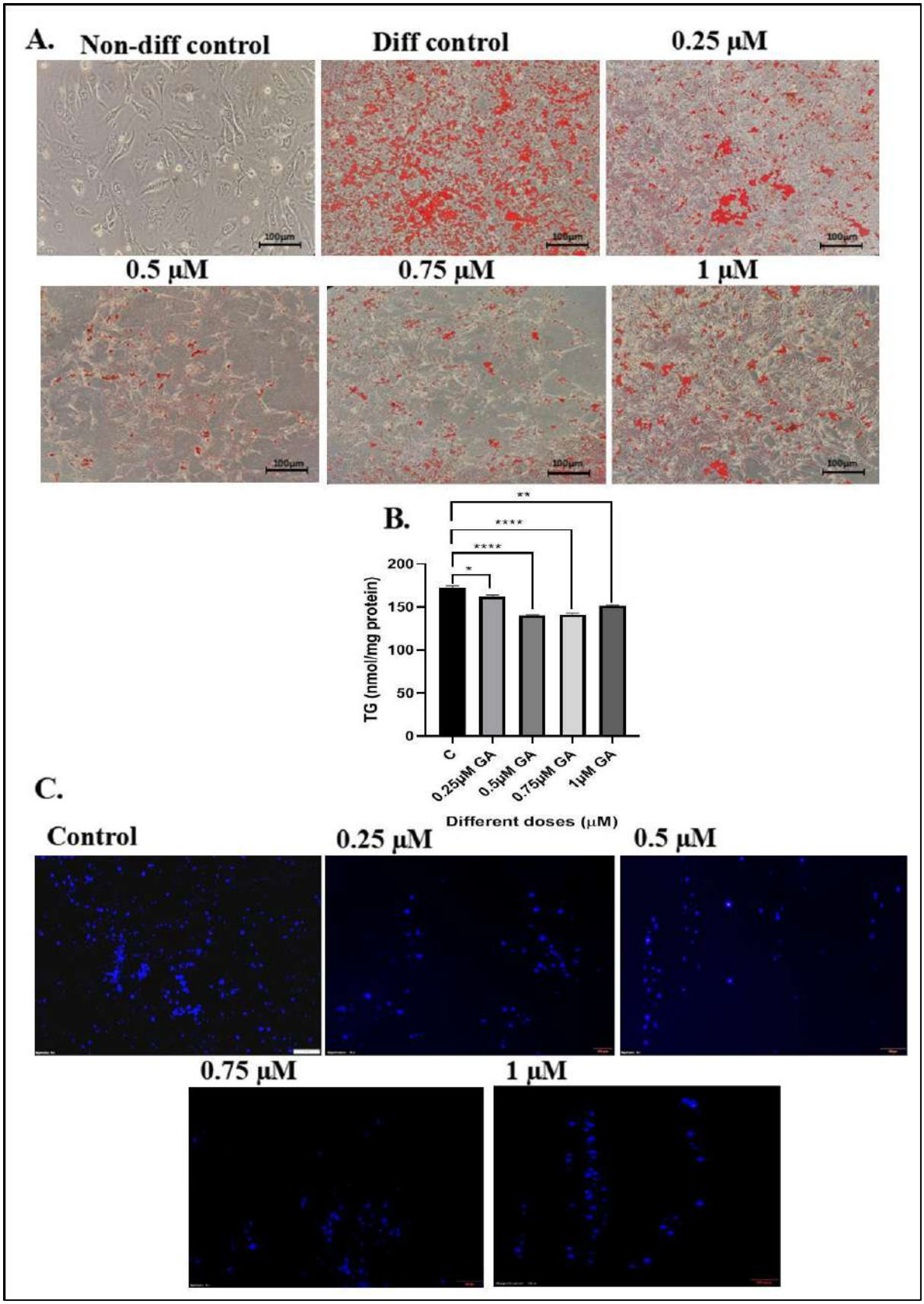


Figure 2. Effectivity of GA on Lipid deposition and progression in 3T-3L1 adipocyte. In the presence of GA (0.25µM, 0.5µM, 0.75µM and 1µM), two-day post-confluency preadipocytes

were cultured with differentiation media to become complete adipocytes compared with control group. (A) ORO staining was used to quantify lipid accumulation, (B) TG accumulation in 3T3-L1 adipocytes, (C) Immunofluorescent visualization by DAPI staining. Values are represented as the mean ± SEM (n = 3). Statistical analysis assessed via one way ANOVA followed by Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05.

GA inhibits the activity of genes and transcription factors linked to accumulation of fat and adipogenesis

To further understand the molecular process behind GA's anti-adipogenic impact, the expression levels of major transcription factors involved in adipocyte development, as well as the adipogenic genes engaged in lipid uptake and metabolism, were examined in this present study. As represented in **Figure 3**, there was a remarkable rise of AMPK and PPAR-α genes by 1.02-1.70-fold and 1.17-1.46-fold in mature adipocytes treated with GA with several doses (0.25µM, 0.5µM, 0.75µM, and 1µM) compared with the control **(Figure 3d, 3f)**. However, the expression of leptin and adiponectin genes improved considerably by 1.63-fold in mature adipocytes treated with 0.5µM of GA **(Figure 3b, 3c)**. This fatty acid also increased CPT1 expression by 1-fold, 1.26-fold, 1.18-fold and 1.01-fold on the doses of 0.25µM, 0.5µM, 0.75µM, and 1 µM of GA **(Figure 3g)**. Similarly, LPL, glucose transporter type 4 (GLUT-4), insulin all were highly expressed in GA treated groups more efficacy noted in 0.5 µM (1.83-fold, 1.54-fold, 1.35-fold) and 0.75µM group (1.66-fold, 1.48-fold,1.38-fold respectively) compared to untreated control cell **(Figure 3l, 3j, 3i)**. Research on adipogenic cell lines has demonstrated the induction of two important adipogenic transcription factors during adipocyte differentiation: PPAR-γ and SREBP-1c, whereas the GA treatment (0.25µM, 0.5µM, 0.75µM, and 1 µM) reduced the mRNA expression of those genes by 1.17-fold- 1.46-fold and 1-fold-1.07-fold in a dose-dependent manner **(Figure 3e, 3k)**. GA treatment also resulted to the inhibition of pro-inflammatory markers gene expression like IL-6 whose expression were gradually down regulated (1.20-fold, 1.37-fold, 1.49-fold, and 1.92-fold respectively) in GA treated group compared to C **(Figure 3o)**. Additionally, GA can up regulate the expression of anti-inflammatory markers such as IL-10, IL-1ra by 1.03-1.2-fold and 1.12-1.47-fold compared to C group **(Figure 3m, 3n)**.

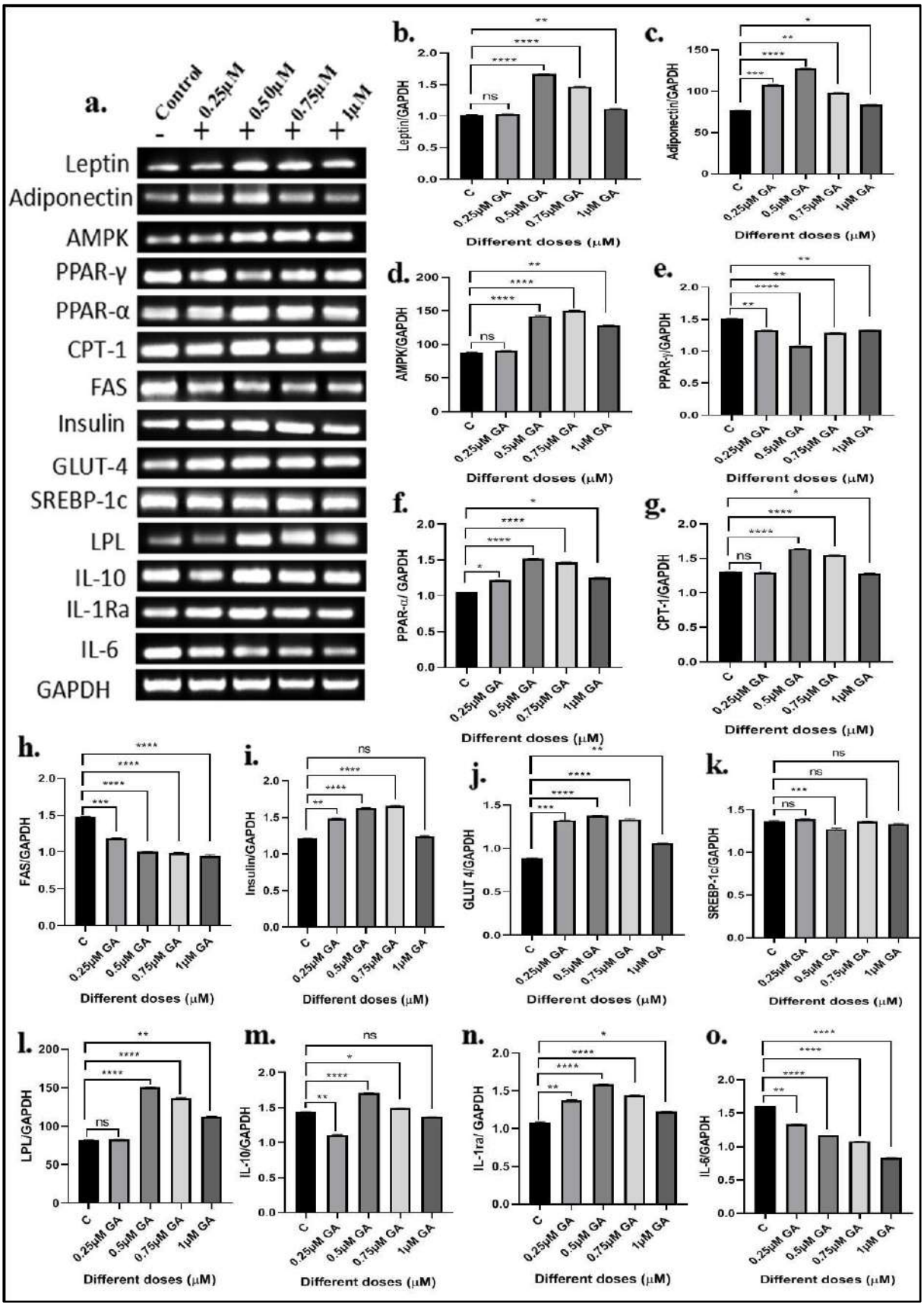


Figure 3. Semi quantitative PCR analysis of obesity related cytokines such as (b) leptin, (c) adiponectin, (d) AMPK, (e) PPAR-ɣ, (f) PPAR-α, (g) CPT-1, (h) FAS, (i) insulin, (j) GLUT-4,

(k) SREBP-1c, (l) LPL, (m) IL-10, (n) IL-1Ra, (o) IL-6 by treating with different doses (0.25,0.5, 0.75 and 1 µM) of GA on 3T3-L1 cell line (1×10^5/ml). The expression profiles of each gene are shown on the y-axis, while the effect of three doses in comparison to the control is represented on the x-axis. Values are represented as the mean ± SEM (n = 3). Statistical analysis assessed via one way ANOVA followed by Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05.

Cytokines Quantification by ELISA assay

The impacts of IL-1ra, IL-10 and TNF-α were evaluated in order to further explore how dietary fatty acids affect adipocytes' production of cytokines. Here, cells were cultured with several doses (0.25µM, 0.5µM, 0.75µM, and 1 µM) of GA. In our study, expression of pro-inflammatory markers was gradually increased by 50.61% and 42.57%. Thus, TNF-α expression was decreased after treatment of the preadipocyte cells with GA by 17.42% compared to control group. Among the all doses, treatment with 0.5µM and 0.75µM of GA showed more effectiveness **(Figure 4 a, b, c)**.

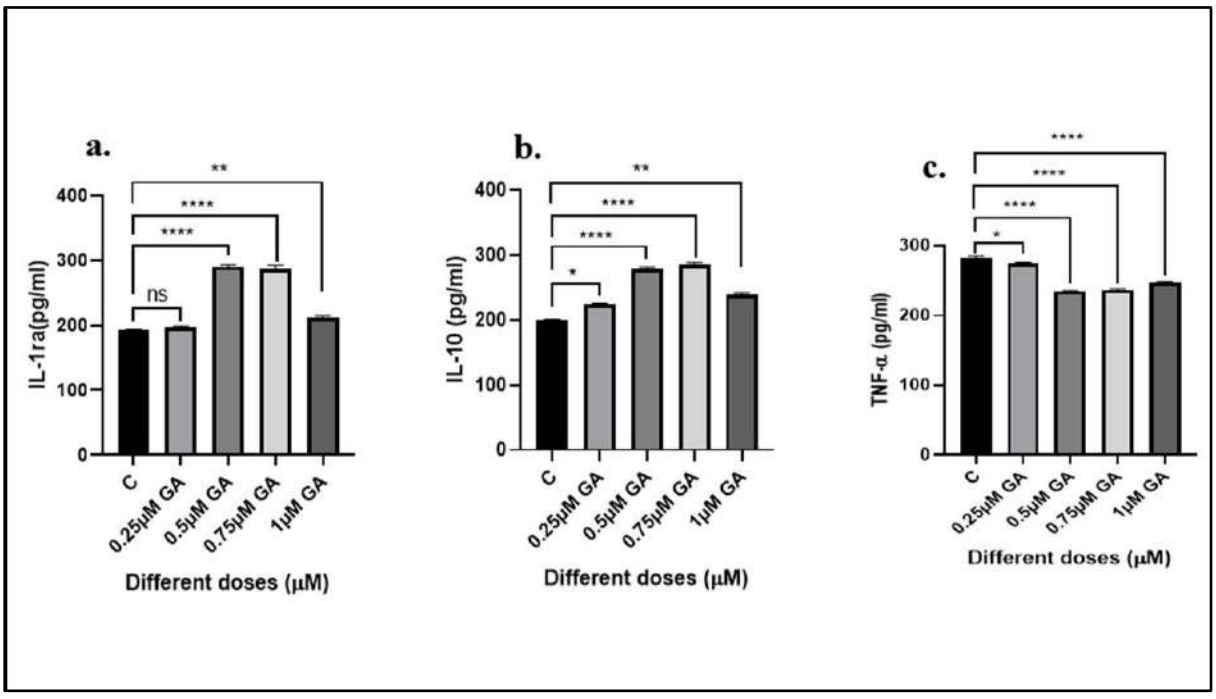


Figure 4. Quantitative evaluation of (a) IL-1ra (b) IL-10 & (c) TNF-α by treating GA with different doses (0.25, 0.5, 0.75 and 1 µM) on 3T3-L1 cell line. Values are represented as the mean ± SEM (n = 3). Statistical analysis assessed via one way ANOVA followed by Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05.

GA reduces adipogenesis in differentiated 3T3-L1 Cells

To investigate the mechanism of GA's repressive effects on fat formation, adipocyte differentiation factors were identified using the immunoblotting approach. Figure 5 shows high expression of adipocyte differentiation factors such as PPAR-ɣ, CEBP-α, and SREBP-1c in the control adipocytes. GA showed considerable dose-dependent reduction, suppressing the expression of three transcription factors by approximately 1.07-1.90-fold at 0.5µM and 0.75µM **(Figure 5e, 5f, 5c)**. GA's effects on AMPK, PPAR-α, and adiponectin, all involved in β-oxidation, were also studied. As expected, GA treatment up-regulated the expression of the genes mentioned above by around 1.05-1.48-fold **(Figure 5b, 5d, 5g)**.

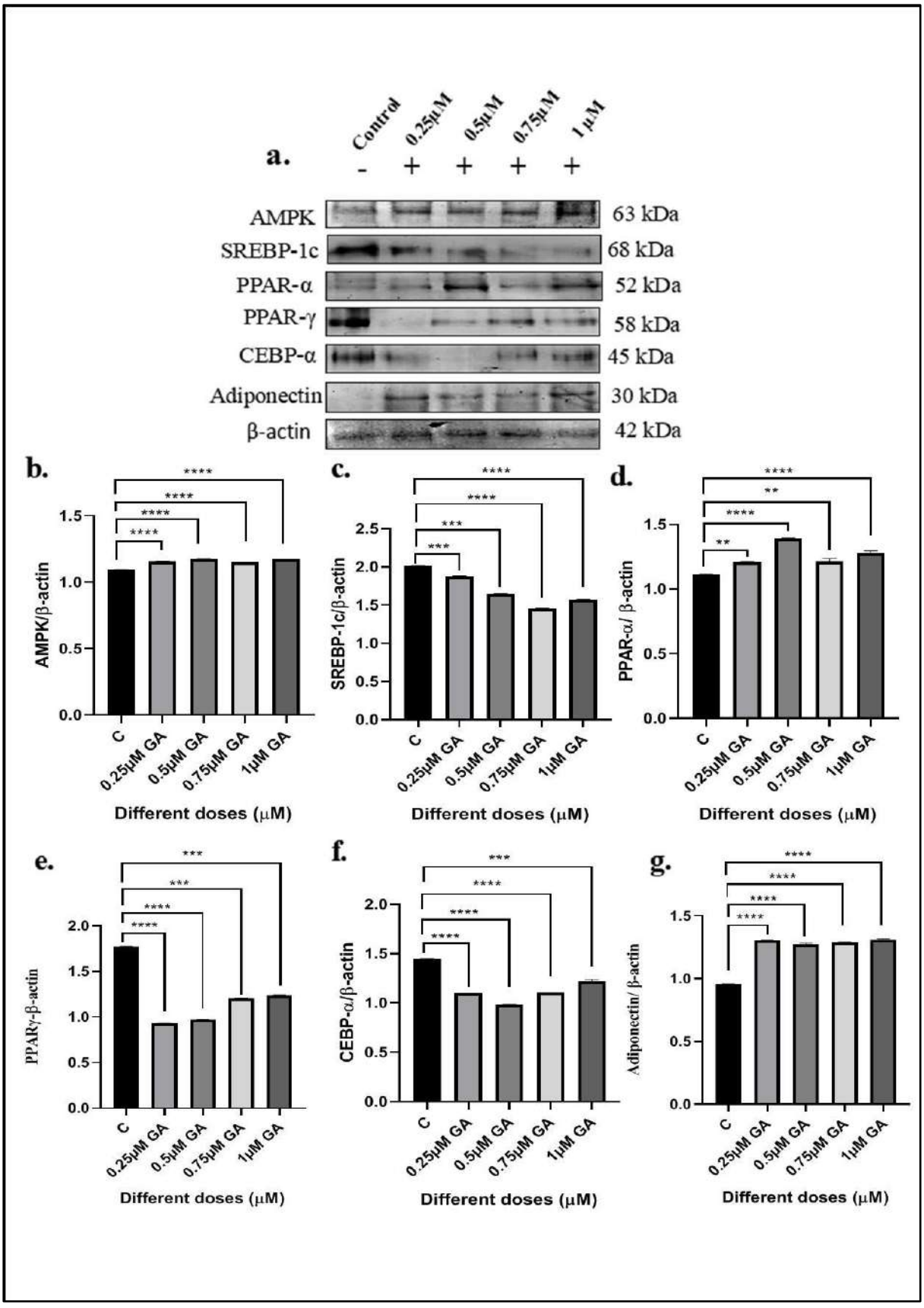


Figure 5. Effects of GA on adipogenic transcription factors and AMPK phosphorylation. (a) Relative protein expression, (b) AMPK, (c) SREBP-1c, (d)PPAR-α, (e) PPAR-γ, (f) CEBP-α

and (g) adiponectin. Confluent 3T3-L1 cells were differentiated in the presence or absence of different concentrations of GA (0.25, 0.5, 0.75 and 1 μM). After differentiation, 3T3-L1 adipocytes were lysed and measured using SDS-PAGE and Immunoblotting. β-actin protein level served as an internal reference. Values are represented as the mean ± SEM (n = 3). Statistical analysis assessed via one way ANOVA followed by Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05.

Discussion

One of the major epidemics of the twenty-first century is obesity. Several studies have shown that the degree of fat accumulation and adipocyte proliferation are linked with the development and onset of obesity. Thus, one strategy for managing obesity is to limit formation of adipocytes **(Lee et al., 2015)**. In order to address obesity, recent research have shown a strong interest in finding dietary components that can increase energy expenditure **(Dayarathne et al., 2025)**. Particularly those originating from marine sources, this family of PUFAs & MUFAs has drawn a lot of interest in the past 30 years. Cardiovascular disease, obesity, and diabetes are among the non-communicable metabolic illnesses that n-3 fatty acids may help prevent **(Todorčević & Hodson 2015)**. The molecular mechanism behind of adipogenesis is often studied using the mouse preadipocyte cell line 3T3-L1 as an in vitro model system **(Jeong et al., 2013)**. This was the very first study that we were aware of that demonstrates how GA affects lipid buildup in 3T3-L1 adipocytes. In this work, we showed that GA administration during 3T3-L1 adipocyte development increases energy expenditure through lipolysis and adipocyte browning and decreases buildup of lipids by boosting lipid metabolism. Previous study explored that, in a HFD, high MUFA increased oxidation of fatty acids and energy utilization **(Lim et al., 2024)**. In spite of that, we further found a notable decrease in TG accumulation by different doses (0.25μM, 0.5μM, 0.75μM, and 1 μM) of GA **(Figure 2B)**, while compared to the control, these dosages would not alter cell viability **(Figure 1b)**.

The efficacy of n-3 fatty acid in modulating cell differentiation is proved by Ferguson **et al. (2019).** In order to investigate how GA affects adipocyte differentiation, 3T3-L1 preadipocytes were cultivated in differentiation medium for seven days. This caused the cells to differentiate into mature adipocytes and led to a notable build-up of intracellular lipid droplets **(Lee et al., 2015)**. ORO staining was applied to colour these lipid droplets. As shown in **Figure 2A**, treatment with 0.5μM, 0.75μM of GA significantly lowered intracellular fat droplets accumulation in a dose-dependent manner. Previous studies stated that administration with DHA throughout differentiation prevented fat deposition, as seen by dose-dependent declines in droplet size and total body fat percentage. A non-cytotoxic dye called DAPI was used to

count the number of living cells. The fluorescent stain DAPI is a fat-soluble substance that aids in lipid accumulation visualization **(Chen et al., 2017)**. The nucleus of the preadipocyte cells were used in the current work to stain the cells in an unspecific manner with DAPI. In case of DAPI staining, we discovered that 0.5µM and 0.75µM dose of GA was much more potent than the other doses **(Figure 2C)**.

Many genes are involved in coordinating adipogenesis and increasing the formation of lipids **(Figure 3)**. Some study suggests that n-9 fatty acids may improve leptin sensitivity, which increases the body's sensitivity to the leptin signal and may lead to a decrease in hunger and calorie consumption **(Gonçalves-de-Albuquerque et al., 2016)**. The present research suggests that 0.5 µM of GA may enhance leptin levels by controlling fat accumulation and promoting a healthy metabolic profile **(Figure 3b)**. AT secretes the anti-inflammatory and insulin-sensitizing protein adiponectin, which can be influenced by dietary fatty acids. Obesity and related issues are inversely connected with adiponectin levels. Insulin resistance results from impaired adiponectin and insulin signalling, which is brought on by a reduction in the expression of both adiponectin and insulin receptors **(Lim et al., 2024)**. Our finding indicates that treatment with GA (0.25µM, 0.5µM, 0.75µM, and 1 µM) upregulated adiponectin **(Figure 3c)** and insulin **(Figure 3i)** level in 3T3-L1 adipocytes. The researchers discovered that GLUT-4 is primarily prevalent in insulin-sensitive tissue, such as muscle and AT, and that it increases glucose absorption in specific tissues when triggered by PUFA **(Elmendorf et al., 2004)**. As per **Figure 3j** GA can increase insulin sensitivity via activation of GLUT-4 mRNA expression at the dose of 0.5µM and 0.75µM respectively. In order to control lipid metabolism, AMPK regulates a number of processes, including the activation of BATs, the induction of mitochondrial biogenesis, and the activation of catabolic ATP-generating pathways. Furthermore, AMPK controls metabolism of fat by modifying SREBP-1c and lowering TG synthesis in adipocytes, and it suppresses adipogenesis by suppressing the levels of adipogenic genes **(Bijland et al., 2013)**. Therefore, our investigation was determining that different doses of GA can promote adipocyte browning effect was regulated by AMPK stimulation **(Figure 3d)**. During the initial phases of adipogenic commitment, important transcription factors like SREBP-1c and CEBP-β are up-regulated. These alterations raise PPAR-γ, SREBP-1c, and CEBP-α transcription **(Lefterova et al., 2010)**. We found that the mRNA expression of transcriptional regulators PPAR-γ was more inhibited by 0.5µM GA treatment in the initial phases of adipocyte formation. Nevertheless, various GA dosages (0.25µM, 0.5µM, 0.75µM, and 1 µM) also reduced the mRNA levels of adipocyte-specific genes that are controlled by PPAR-γ, including FAS and SREBP-1c **(Figure 3e, 3h, 3k)**. FAS is the primary enzyme

involved in fatty acid production and is highly expressed in AT. PPAR-α expression levels in human hepatoma cell lines are often low, therefore PPAR-α activation can prevent and reduce hepatic fat formation **(Harano et al., 2006)**. In our study, GA administration at various doses progressed the mRNA expression level of PPAR-α more at 0.5 µM and 0.75 µM dose compared with control group **(Figure 3f)**. By promoting the absorption of fatty acids from circulating lipoproteins into AT, LPL plays a crucial part in controlling lipid accumulation **(Dayarathne et al., 2025)**. Our results show that 0.5 µM and 0.75µM of GA efficiently decreases lipid accumulation and TG content in adipocytes by rising mRNA level of LPL **(Figure 3l)**. By moving long-chain fatty acids across the mitochondrial membrane, CPT-1 catalyses the rate-limiting phase of beta oxidation, making it a target for novel treatment approaches for type 2 diabetes and obesity **(Warfel et al., 2017)**. However, in our study expression of CPT-1 was increased at 0.5 µM dose of GA among the all doses **(Figure 3g).** Inflammatory cytokines and insulin sensitivity are frequently linked to adipogenic genes **(Li et al., 2017)**. Obesity and inflammatory illnesses associated with obesity are linked with elevated aberrant cytokine production and regulation of inflammatory signalling pathways. In obese individuals, adipocytes, preadipocytes, and immune cells secrete pro-inflammatory cytokines such as TNF-α and IL-6, which are released in greater amounts in adipose tissue **(Timper et al., 2017)**. While up-regulating the release of IL-1Ra, IL-10 is an anti-inflammatory cytokine that reduces the inflammatory processes brought on by TNF-α **(Figure 4a, b, c)**. Previous study stated that BMI and fasting glucose levels all have a negative correlation with IL-10 **(Yang et al., 2021)**. In our study, the inflamatory marker, IL-6 was found decreased, **(Figure 3o)** whereas the mRNA expression of IL-10 and IL-1ra were progressively upregulated in GA treated group compared with untreated control group **(Figure 3m,3n)**. These results raise the possibility that GA inhibits cytokine production via a related mechanism. Obesity develops from adipocyte hyperplasia, when preadipocytes are turned into fully grown adipocytes through the adipogenesis process, which is primarily controlled by transcription factors PPAR-ɣ and CEBP-α **(Farmer 2005)**. GA significantly increased the protein expression of AMPK, PPAR-α, and adiponectin, as further demonstrated by Western blot analysis **(Figure 5b, d, g)** as well as the inhibition of adipocyte differentiation factors such as SREBP-1c, PPAR-ɣ and CEBP-α **(Figure 5c, e, f)** in 3T3-L1 cells. In summary, GA suppresses adipocyte development and intracellular lipid buildup in 3T3-L1 cells. Additionally, our findings showed that throughout the early phases of differentiation, GA limits lipid accumulation by controlling transcriptional regulators and their downstream lipogenic targets through the stimulation of the AMPK

pathway. GA is a potential essential fatty acid that can help minimise obesity and metabolic diseases without any toxic effect.

Possible Molecular Mechanism of *P. paradiseus* oil characterized gondoic acid *in vitro* model

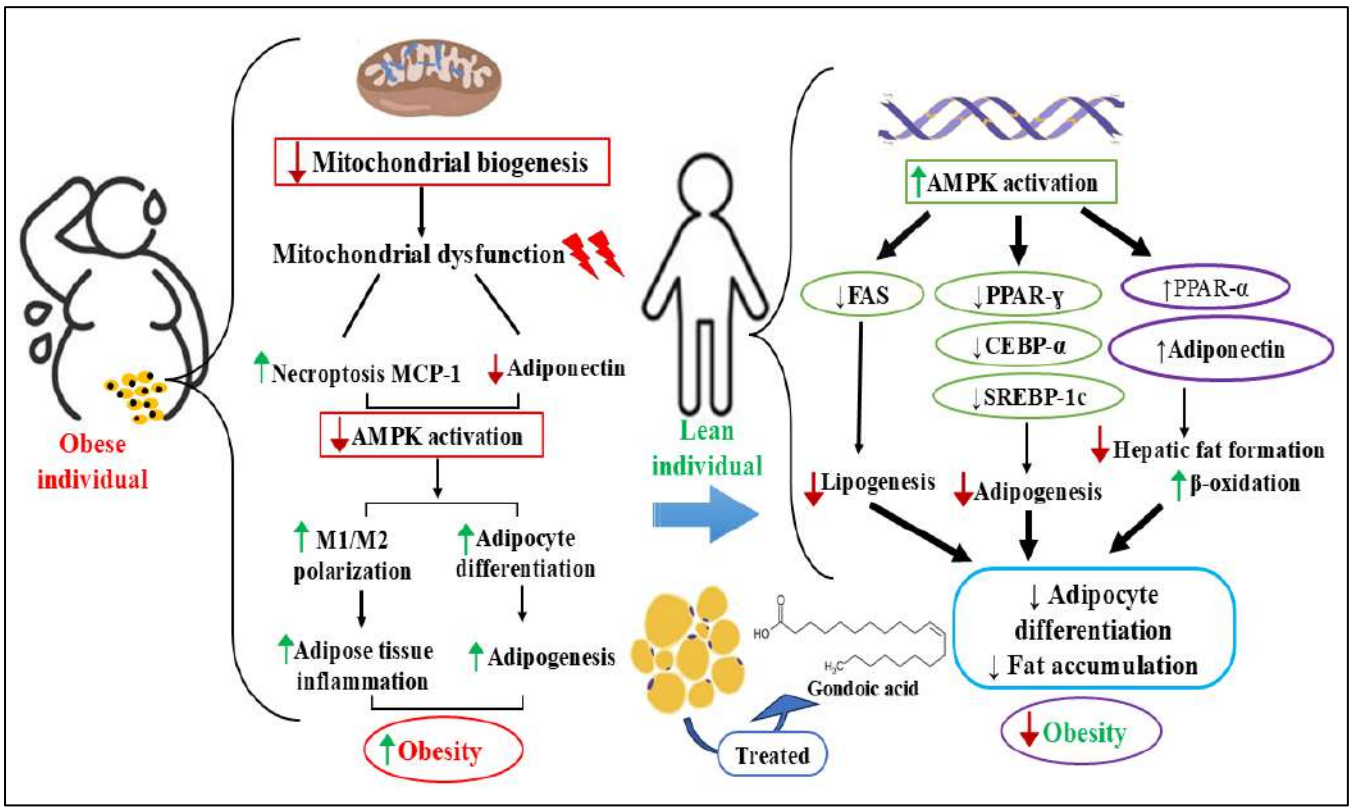


Figure 6. GA suppresses adipocyte differentiation and lipogenesis-related protein expression in 3T-3L1 cell line.

Experimental Design II: Gondoic acid ameliorates HFD-induces obesity in BALB/c mice through AMPK-mediated stimulation of thermogenesis and β-oxidation.

Materials and methods

Chemicals

To complete each experiment in this investigation, the following substances and reagents were purchased. Using treatment compound named Gondoic acid or cis-11- Eicosenoic acid (E3635-100MG), purchased from Merck, Darmstadt, Germany; RNA-Xpress Reagent, Hi-cDNA synthesis kit, 2X PCR mixture, DAB obtained from Himedia, HiGenoMB, Maharashtra, India. Graded ethanol and paraffin purchased from Histosec, Merck, Darmstadt, Germany; DAPI (4',6-Diamidino-2-Phenylindole) (Cat no. 62248) from Invitrogen; fluorescent mounting medium purchased from Dako, Hamburg, Germany. All of the PCR primers were acquired from Bioserve in India. The primary antibodies: AMPK mouse McAb (Cat No.: 66536-1-Ig), CEBP-α Mouse McAb (Cat No.: 66649-I-Ig/), SREBP-1c Mouse McAb (Cat No.: 66875-1-Ig), β- actin Mouse McAb (Cat No.:66009-1-Ig) were purchased from Proteintech (ChromoTek GmbH & Proteintech Germany) and Ob (H-5) mouse monoclonal IgG (Sc-393043), PPAR-γ mouse monoclonal IgG (Sc-7273), FAS mouse monoclonal IgG (Sc-74540) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA-USA). The secondary antibody: Goat Anti-mouse IgG-HRP (cat# 11-301) was purchased from Abgenex (Odisha, India).

Animal Care

This study was approved by the Institutional Ethics Committee, Midnapore City College (2256/PO/RE/S/23/CCSE, MCC/IAEC-SP/10/23-003) and the management of every experimental protocol agreed with the rules governing the use and care of laboratory animals. The animal data has been submitted in accordance with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) criteria. Six-week-old male BALB/c mice from Saha Enterprise, IEC No: BAEPS7949M (Habra, North 24 Pargana, West Bengal- 743271) were housed and caged individually in a room at 23 ±1°C with a 12/12-h light–dark cycle. The animals were given free access to water and a normal chow meal for one week following arrival.

Administration of diets and fatty acid

The mice were fed two different types of diets. Composition of normal chow diet was (wt/wt): 10% fat, 60% carbohydrate, 20.5% protein, 2.9% fibre, 6.6% minerals with water ad libitum. Composition of HFD was (wt/wt): 41.5% fat from lard, 35% carbohydrate, 15% protein, 5.0%

fibre, 3.5% mineral with water ad libitum. Composition of treatment compound (100 mg GA/1 ml olive oil): Gondoic acid or cis-11- Eicosenoic acid (E3635-100MG), purchased from Merck was dissolved per 1 ml of olive oil and used as treatment solution. Olive oil was co-administered to the vehicle control group (VC) and HFD-induced mice groups (Treatment-I, Treatment-II, Treatment-III) supplemented with GA by oral gavage during experiment.

Experimental protocol

Total experiment was continued for 16th weeks. Except for the control and vehicle control groups, mice were fed a HFD for 10 weeks before being administered the test oils. Six groups (n=6 of each group) were randomly divided from these acclimatized mice: (1) Normal chow feeding, control group (C); (2) Normal chow feeding and co-administered with olive oil (4 mg/kg body weight/day), vehicle control group (VC) (3) HFD feeding, obese control group (OC); (4) HFD-fed mice groups supplemented with 1mg/kg body weight/ day GA (T-I group); (5) HFD-fed mice groups supplemented with 2mg GA/kg body weight/ day (T-II group); (6) HFD-fed mice groups supplemented with 4 mg/kg body weight/ day of GA (T-III group) by oral gavage for last the 6th weeks of total experiment.

Collection of blood and tissue samples

After 16 weeks of the experiment, mice were anesthetized with ether as well as euthanized via dislocation of the cervical spine. Blood samples were taken by rupturing the orbital plexus and centrifugation at 3000 rpm for 15 minutes at 4°C, with serum kept at -20°C for biochemical evaluation. The liver, subcutaneous, and visceral adipose tissues were excised, weighed, and promptly preserved in liquid nitrogen at -20°C for further testing. Adipose fat depots were extracted, weighed, and processed according to the protocol outlined below for protein and gene expression profiling.

Assessment of daily food intake, somatic body weight index, BMI (gm/cm^2) in different experimental group

The food intake was recorded per day and bodyweight was recorded every week for sixteen weeks. Body length was measured from nasal to anal distance using a Central calliper scale (Model no. 6420) to the closest 0.1 mm. Daily food intake was measured by using food weight machine (ASIN, Model no. KS33). The standard approach was used to determine BMI, and the findings were expressed as g/cm^2 **(Dasgupta et al., 2020)**. BMI was calculated using

the standard method; by following equation given below and the results were indicated as g/cm^2.

BMI (g/cm^2) =Body weight(g)/Body length(cm^2)

Biochemical analysis

The serum levels of TC, LDL-C, VLDL-C, HDL-C, TG, serum protein level and total serum glucose level were determined using the colorimetric techniques (Enzopak, India). We also estimated the level of some toxicity marker such as SGOT, SGPT, uric acid, urea through semiauto analyser following Enzopak kit method **(Hwang et al., 2022).**

Histological analysis by HE staining

After 24 hours of oral administration of the experimental diets, liver tissues and ATs were collected from sacrificed mice instantly frozen at -80°C. Following standard procedure, tissues that were frozen were cryo-sectioned, embedded in paraffin wax, and fixed in a 10% formalin solution before being cut. HE was used to stain tissue sections 6µm thick for histological examination. Histological images were captured using the compound microscope (Olympus, Model No. CX21i) **(Esteves et al., 2020)**.

Morphological analysis by DAPI staining

Following experimental animal sacrifice, liver tissues were preserved, paraffin embedded, and dissected into 6µm slices. On a rotary microtome (Mod. RM2255; Leica-Microsystems, Wetzlar, Germany), fixed specimens were cut into serial 6 μm slices after being dried in graded ethanol and embedded in paraffin (Histosec, Merck, Darmstadt, Germany). Graded ethanol (2 × 30 sec 100%, 2 × 30 sec 90%, 2 × 30 sec 70%, and 2 × 30 sec 50%) was added to paraffin-embedded sections, which were subsequently immersed in xylene. Then five minutes of incubation with 5µg/mL DAPI (Invitrogen) in PBS followed. The slices were then covered with fluorescent mounting material (Dako, Hamburg, Germany) and rinsed three times with PBS. A fluorescent microscope was used to examine the sections (Olympus, Model No. BX43F) **(Koellensperger et al., 2013)**.

Gene expression study using semi qPCR

AT samples were used to extract whole RNA using the HiPurA Total RNA Miniprep purification kit methodology (Hi-Media Hi-GenoMB, Maharashtra, India). Reverse transcriptase was used to create cDNA, and the NanoDrop-2000 (Eppendorf)

spectrophotometer was used to measure the amount of isolated RNA. The mixture for the reverse transcription reaction will be made using the Hi-cDNA synthesis kit (Himedia, HiGenoMB, Maharashtra, India). GAPDH mRNA expression was employed as the house-keeping primer set (sense: GGTGAAGGTCGGAGTCAACG, antisense: GTGAAGACGCCAGTGGACTC). PCR will function as follows: DNA is first denaturated at 95˚C for 1 minute, then denaturated for 30 seconds, annealed at 65˚C for 45 seconds, and then extended at 72˚C for 2 minutes and finally extended for 10 minutes. Total 42 heat cycles were achieved by repeating the second and third steps. Finally, the PCR tube containing the material was kept at 4˚C. Another primer will go through the same procedure.

Table 1 Listed primer sequences used in semi q PCR analysis.

Primers	**Primary Sequences**	
	Forward Sequence	**Reverse Sequence**
AMPK	5'-AGGAAGAATCCTGTGACAAGCAC-3'	5'-CCGATCTCTGTGGAGTAGCAGT-3'
Adiponectin	5'-GTCAGTGGATCTGACGACACCAA-3'	5'-ATGCCTGCCATCCAACCTG-3'
Leptin	5'-CAAGCAGTGCCTATCCAGA-3'	5'-AAGCCCAGGAATGAAGTCCA-3'
SREBP-1c	5'-ACGGAGCCATGGATTGCACA-3'	5'-AAGGGTGCAGGTGTCACCTT-3'
GLUT 4	5'-GAGCCTGAATGCTAATGGAG-3'	5'-GAGAGAGAGCGTCCAATGTC-3'
FAS	5'-TGCTCCCAGCTGCAGGC-3'	5'-GCCCGGTAGCTCTGGGTGTA-3'
LPL	5'-CCACAGCAGCAAGACCTTC-3'	5'-AGGGCGGCCACAAGTTTG-3'
CPT-1	5'-GTGACTGGTGGGAGGAATAC-3'	5'-GAGCATCTCCATGGCGTAG-3'
Insulin	5'-CCCCAGAAAAACCTCTTCAGG-3'	5'-GTCACATTCCCAACATCGCC-3'
PPAR-α	5'-CCTGAACATCGAGTGTCGAATAT-3'	5'-GTTCTTCTTCTGAATCTTGCAGCT-3'
PPAR-γ	5'-AGGCCGAGAAGGAGAAGCTGTTG-3'	5'-TGGCCACCTCTTTGCTCTGCTC-3'

TNF-α	5’-TTCTGTCTACTGAACTTCGGGGTGATCGGTCC-3’	5’-GTATGAGATAGCAAATCGGCTGACGGTGTGG-3’
IL-10	5’-TCTCCGAGATGCCTTCAGCAGA-3’	5’-TCAGACAAGGCTTGGCAACCCA-3’
IL-1Ra	5’-GCAGCACAGGCTGGTGAATGAC-3’	5’-TGCCCCCGTGGATGCCCAAG-3’

Protein analysis by Immunoblotting assay

Using RIPA buffer, adipose tissue samples were lysed, and the Lowry method was used to measure the protein content. Protein samples weighing 50 µg were boiled for 5 minutes in loading buffer before being electrophoresed using SDS-PAGE and deposited on a nitrocellulose membrane (Bio-Rad, Model No. 165.8033FC). Then 4% BSA was next applied to block the membrane, and the primary antibody was incubated at 4°C for the whole night. The primary antibody used in this study included β-actin, Ob (H-5), PPAR-γ, FAS, SREBP-1c, CEBP-α and AMPK at a 1:1000 ratio. The membrane was then incubated for one hour at 4°C with HRP labelled anti-mouse secondary antibody 1:7500 (Abgenex) after being cleaned three times with phosphate buffer saline with tween-20 (PBST). DAB was used to identify particular protein bands. Densitometric examination utilising Image J densitometry software was used to quantify the bands **(Kar et al., 2025)**.

Statistical Analysis

The data (triplicate values) were examined by GraphPad Prism software (San Diego, CA, USA) and expressed as mean± SEM. One-way ANOVA and Tukey's multiple-comparison tests were used to compare treatment groups to the control group. Significance levels were set at $p<0.0001$, $p<0.001$, $p<0.01$, and $p< 0.05$ **(da Cunha de Sá et al., 2020)**.

Results

GA suppresses diet-induced weight gain and fat formation

To determine that GA has anti-obesity benefits, we employed HFD-fed BALB/c mice as an obese model; mice were provided with the HFD over 16 weeks, except in the C and VC groups. The successfully simulated mice were then given a suitable n-9 fatty acid at various doses (T-I, T-II, and T-III groups treated with 1mg, 2mg, and 4mg GA/kg body weight/day) for 6 sequent weeks. At the start of the experiment, mice had a mean weight of 20.2 ± 0.45 g, with no

significant difference between groups. After 16 weeks of HFD, mice in the OC group had an average body weight of 49.12 ± 0.82 g, which was significantly greater than mice in the C and VC groups ($p< 0.0001$). The mice's appearances were illustrated in **Figure 1a**, which most likely looks into how various GA dosages affect body weight and fat storage. Curiously, the overall body weight increased by the OC and T-I groups was almost equal. As shown in **(Figure 1c)**, during administration with GA, the mean body weight gain of the T-II (38± 0.32 g) and T-III (35± 0.21 g) groups were significantly reduced than those in the OC group ($p< 0.0001$). As shown in **(Figure 1b)**, the daily caloric intake of each group was the same; however, the OC group ingested a little more calorie than the other GA-treated groups. In contrast to obese mice given a HFD, animals gavage with GA usually shows a considerably reduced BMI (**Figure 1d)**. So, the BMI (g/cm^2) of mice group was as follows for C: 0.52±0.01, VC: 0.55±0.04, OC: 0.74±0.06, T-I: 0.61±0.03, T-II: 0.55±0.02; T-III: 0.52±0.02 (**Figure 1e, f, g)** presents data regarding the weight of peripheral organs in among the experimental groups. OC group tend to have larger WAT depots (2.39 ± 0.34 g) and heavier livers (1.92 ± 0.16 g) than lean mice, while the weight of the kidney (0.65 ± 0.02 g) may not change much or may even slightly increase depending on the study and degree of obesity. Furthermore, mice treated with GA at various doses (1mg, 2mg, and 4mg/kg body weight/day) tend to have lower WAT (1.80±0.04 g, 1.36±0.07 g, 0.75±0.12 g) and liver (1.76±0.05 g, 1.67±0.03 g, 1.38±0.06 g) weights than the OC group that does not received GA. Particularly, the T-III group showed more efficacy; this is because GA can help control lipid metabolism and possibly lessen the buildup of fat in these organs.

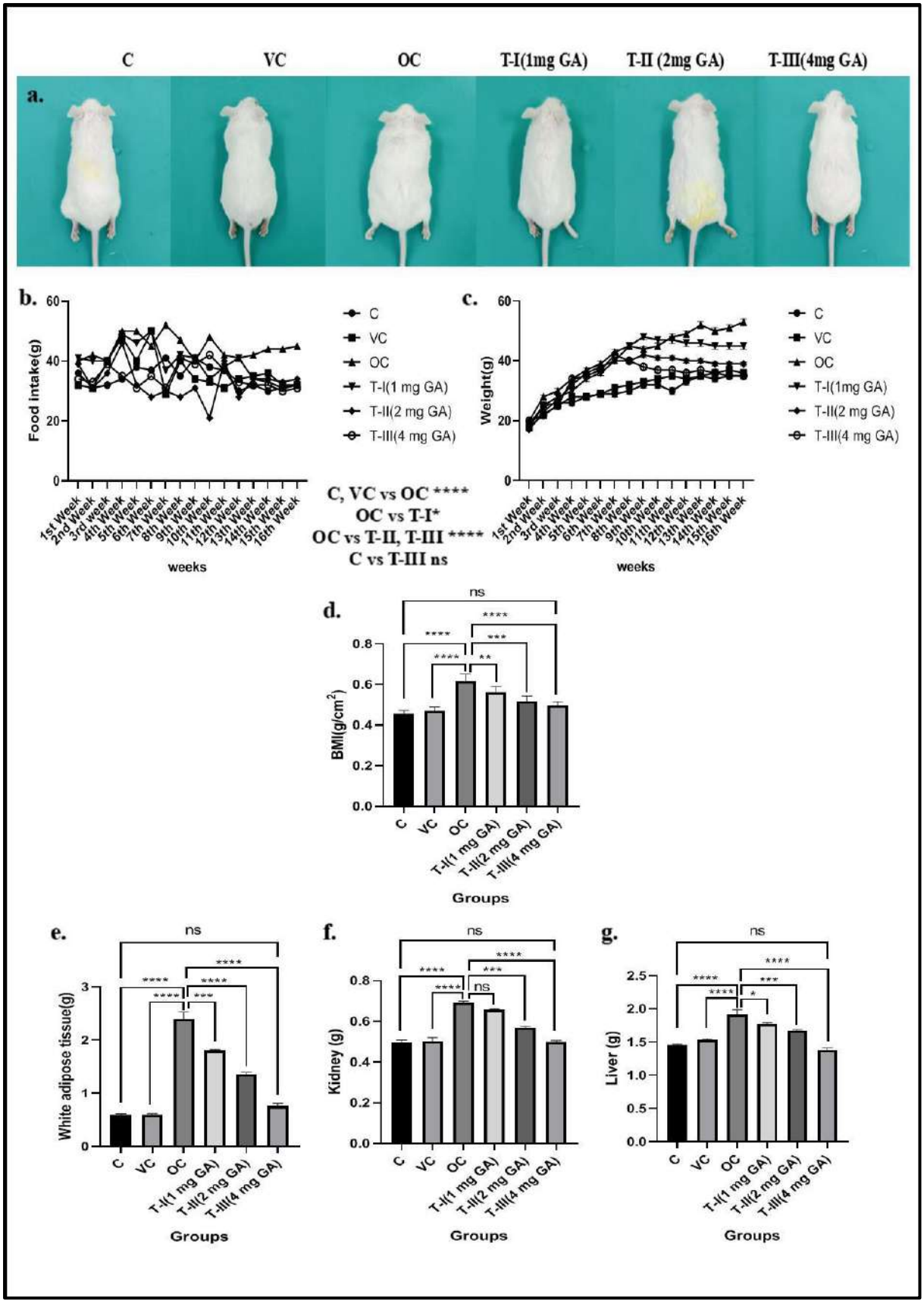

C
VC
OC
T-I(1mg GA)
T-II (2mg GA)
T-III(4mg GA)
a.
b.
Food intake(g)
weeks
C
VC
OC
T-I(1 mg GA)
T-II(2 mg GA)
T-III(4 mg GA)
C, VC vs OC ****
OC vs T-I*
OC vs T-II, T-III ****
C vs T-III ns
c.
Weight(g)
weeks
d.
BMI(g/cm²)
Groups
e.
White adipose tissue(g)
Groups
f.
Kidney (g)
Groups
g.
Liver (g)
Groups
ns

Figure 1. Impacts of GA on body and peripheral organ weight in mice consuming a high-fat diet. (a) Advancement of fat build up; (b) measurements of weekly food consumption; (c) body weight measurements taken over the study duration; (d) BMI (g/cm^2); (e) weight of WAT; (f) weight of liver; (g) weight of kidney in different experimental groups. Values are means ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA accompanied by Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05.

Efficacy of GA administration on the lipid metabolism in obese mice

Impaired glucose and lipid absorption are frequently linked to obesity induced by an HFD. The red blood cells rapidly utilise carbohydrates as their primary energy source once they are mostly absorbed in the gastrointestinal tract as glucose. But in fat cells, surplus glucose is broken down into glycerol-3-phosphate and fatty acids, which then aggregate to form TGs **(Zhou et al., 2023)**. As illustrated in **Figure 2a**, the total blood glucose readings of mice with HFD-induced obesity were 35.62% higher than those of the animals in the C and VC groups, and they were across the normal blood glucose range. Compared with that the mice treated with GA decreased serum glucose level by 16.40% in T-II and 41.57% in T-III group. Hormone-sensitive lipase (HSL) was the initially discovered lipolytic enzyme found to break down cholesterol and triglycerides both of which are essential for lipolysis. In this investigation the serum triglyceride and cholesterol level were higher in OC group by 32.69% and 23.83% compared with normal fed C group. However, this study explored that GA can maintain serum HSL homeostasis by diminished the serum triglyceride (12.68% -26.31%) and cholesterol (8.17% -20.07%) level in treated groups (**Figure 2 b, c)**. Circulating non-esterified fatty acids are highly sensitive to metabolic abnormalities in obesity. The HFD caused a notable increase in LDL and VLDL levels in the OC group by 32.69%-40.26%, but these levels were dramatically reduced in the GA treated group (T-I &T-II) by 26.32-34.08% (**Figure 2e, f)**. Furthermore, to the lower quantity of HDL, the functionality of HDL particles may be altered in OC group compare to the other experimental groups. In our study HDL level was elevated gradually by 37.25%- 51.26% in the GA treated groups (**Figure 2d)**. Subsequently, we investigated the total serum protein and some toxicity markers level in the AT and livers of mice in all diet group. The levels of uric acid, urea, SGOT and SGPT for the OC group were comparatively higher (almost 47.34-56.58%) than the C and PC group. However, serum protein level (**Figure 2g)** was lower (63.56%) in OC group. Mice groups fed HFD combined 1mg, 2mg, 4mg GA/kg body weight/day had a lower serum urea (55.61%; **Figure 2h**, uric acid

(46.37%; **Figure 2i**), SGOT (62.35%; **Figure 2j)**, and SGPT (34.14%; **Figure 2k)** levels compared to the OC group. In summary, GA might assist with obesity-related problems involving lipid and glucose metabolism.

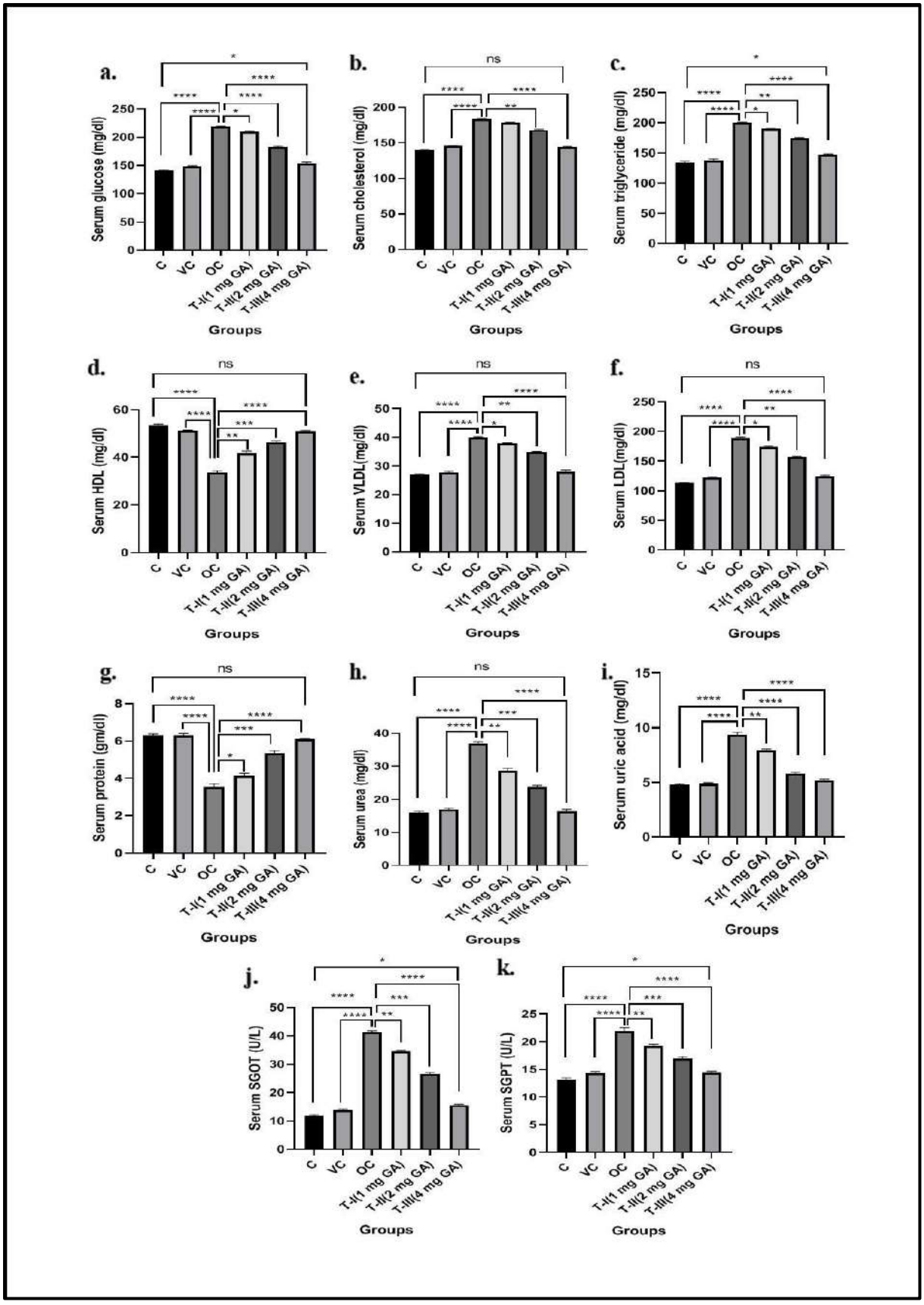


Figure 2. Efficacy of GA administration on lipid metabolism in obese mice. After the development of obesity, obese mice were segregated in to six groups, normal chow feeding,

control group (C); normal chow feeding+ olive oil, vehicle control group (VC), HFD feeding, obese control group (OC); HFD+ 1mg GA/day (T-I group); HFD+ 2mg GA/ day (T-II group); HFD+ 4 mg GA/ day (T-III group). Blood samples from every mouse were taken on the final day of the experiment. Blood samples were used to separate serum, and its concentrations were determined of (a) glucose; (b) cholesterol; (c) triglyceride; (d) HDL; (e) VLDL; (f) LDL; (g) protein; (h) urea; (i) uric acid; (j) SGOT and k) SGPT level in all the experimental group (n=6). Values represent the mean ±SEM. Statistical analysis was performed using one-way ANOVA accompanied by Tukey's multiple comparison test. ****$p<0.0001$, ***$p<0.001$, **$p<0.01$, *$p< 0.05$.

Buildup of lipids in liver tissue

Each treatment group's mice liver tissues were examined for the accumulation of lipids by HE staining (**Figure 3B**) and DAPI staining (**Figure 3C**). A major characteristic of steatosis, or fatty liver disease, that frequently arises from an accumulation of fat droplets, is cytoplasmic enlargement, which is the expansion of the liver's cells (hepatocytes). HE staining of the OC group's liver usually showed a considerable increase in big, transparent vacuoles inside hepatocytes, caused by an imbalance between the delivery, synthesis, and export of fat **(Koellensperger et al., 2013; Kar et al., 2025)**. In contrast, mice treated with GA (T-I group:1mg, T-II group: 2mg and T-III group: 4mg GA/kg body weight/ day) exhibited less liver damage and inflammation, which was frequently accompanied by a more ordered hepatic architecture. In the framework of an obesity investigation, we used DAPI staining to identify liver morphology and nuclear patterns of architecture. Due to the hyperplasia and hypertrophy of the tissue, we can observe that nuclei may appear large and that the liver tissue may have increased cellular density in the OC group. On the other hand, the liver tissue's normal cellular density appears in the C, VC, and GA treated groups. This suggests that GA with selective dose (T-III group: 4mg GA/kg body weight/ day) has a protective impact on the liver tissue (**Figure 3**).

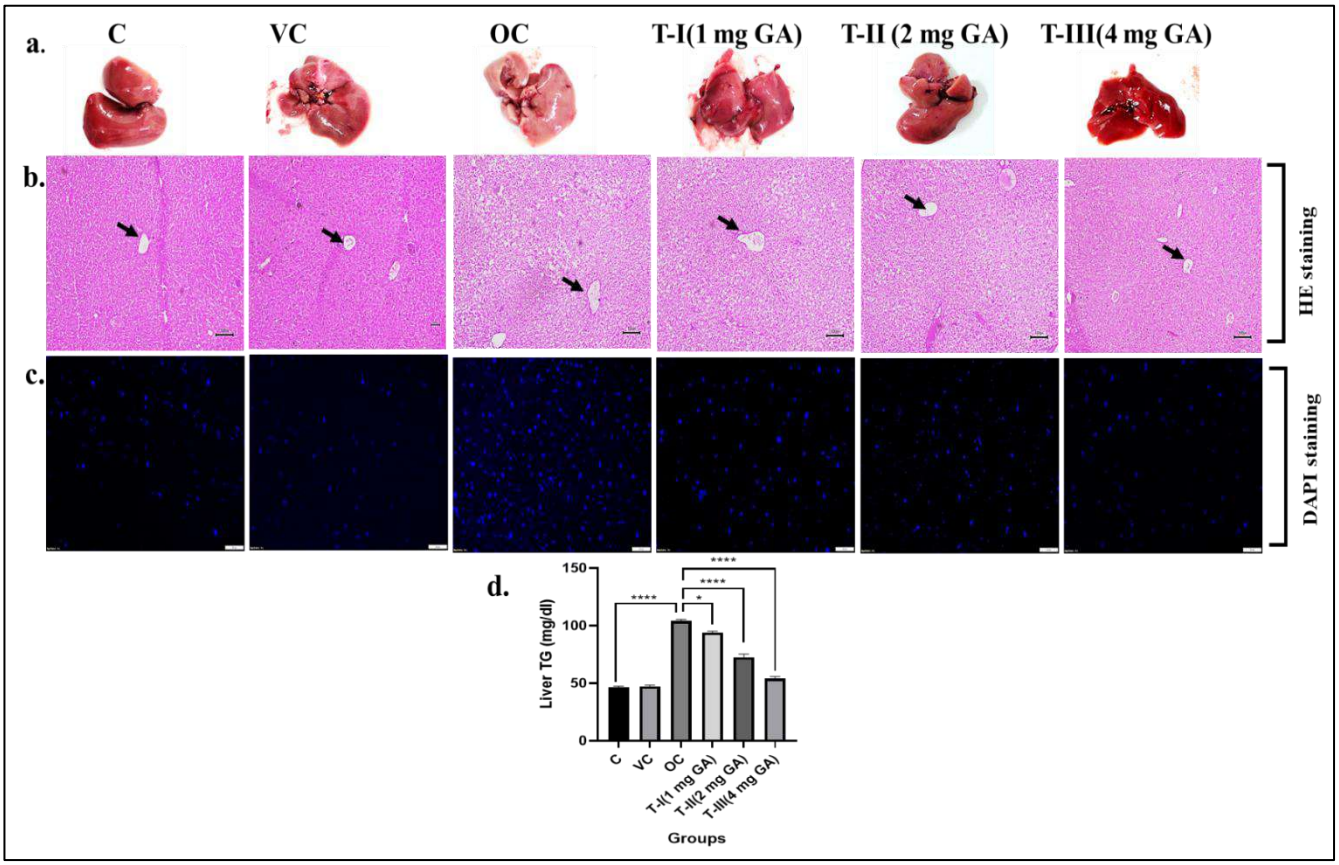


Figure 3. Histological analysis of liver tissues. Groups are separated as normal chow feeding, control group (C); normal chow feeding+ olive oil, vehicle control group (VC), HFD feeding, obese control group (OC); HFD+ 1mg GA/day (T-I group); HFD+ 2mg GA/ day (T-II group); HFD+ 4mg GA/ day (T-III group). (a) Represent the image of liver, tissue sections were stained with (b) HE & (c) Assessment of liver TG. The results for all groups (n=6) are presented as mean ± SEM. Statistical analysis was performed using one-way ANOVA accompanied by Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05. In the staining part arrows (→) represent the way of cytoplasm enlargement in several experimental groups. The scale bar correspondence to 100 µm.

GA inhibited AT's adipocyte hypertrophy

Increasing fat storage causes fat cells to enlarge. Pathological examination using HE (**Figure 4A, B)** revealed that the OC group's adipocytes in the vWAT and sWAT) were not ordered cleanly because of an abundance of lipid droplets in the cytoplasm, which were typically big and had big inner diameter fat vacuoles. However, the inner diameter of the vacuoles was smaller and the adipocytes in the C and VC groups were grouped neatly and tightly (**Fig. 4C**).

The GA treated groups showed a steady decrease in adiposity in the vWAT and sWAT as compared to the OC group, which more closely resembled findings in the C group.

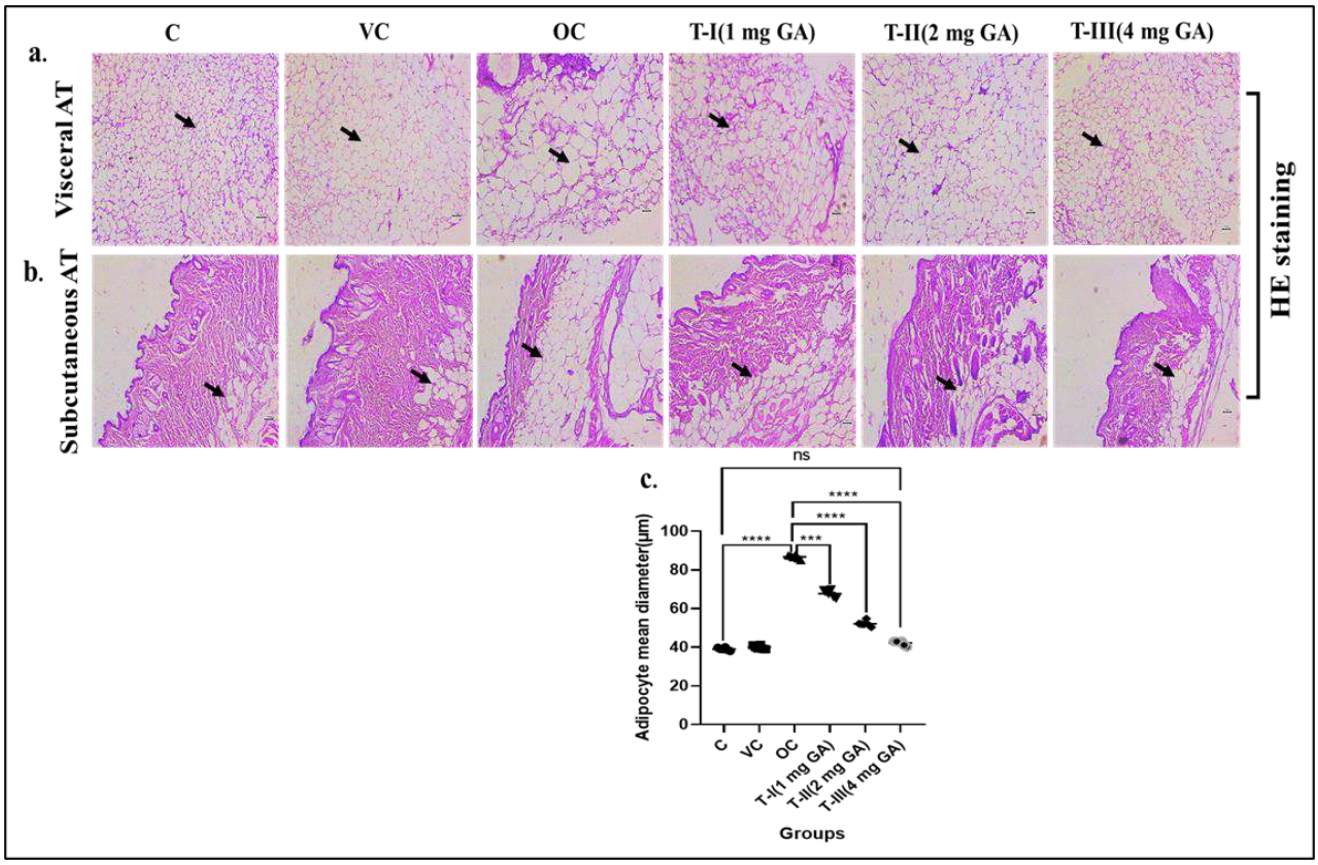


Figure 4. Effect of GA supplementation on adipose tissue in obese mice. Groups are segregated as normal chow feeding, control group (C); normal chow feeding+ olive oil, vehicle control group (VC), HFD feeding, obese control group (OC); HFD+ 1mg GA/day (T-I group); HFD+ 2mg GA/ day (T-II group); HFD+ 4 mg GA/ day (T-III group). Mass of sWAT and vWAT sections were stained with hematoxylin and eosin (HE) staining (a, b). (c) Mean adipocyte size diameter. The results for all groups (n=6) are presented as mean ± SEM. Statistical analysis was performed using one-way ANOVA accompanied by Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05. In the staining part arrows (→) represent the fat cell size (visceral AT) and fat accumulation layer (subcutaneous AT) in several experimental groups. The scale bar correspondence to 100 µm.

Gene expression of adipogenic markers

An essential regulatory organ, WAT releases a variety of chemicals known as adipokines that regulate a number of biological functions, such as vascular metabolism (adiponectin), and

body weight (leptin), regulating energy metabolism (AMPK), glucose metabolism (GLUT 4), insulin sensitivity (insulin), fatty acid oxidation (CPT-1, LPL, PPAR-α), adipocyte differentiation (PPAR-γ, SREBP-1c) and increased secretion of inflammatory cytokines (TNF-α, FAS), side by side reduced production of anti-inflammatory cytokines, including IL-10, IL-1ra among others. Leptin, adiponectin and AMPK mRNA in AT was incredibly increased by 1.71-fold,1.10- fold and 1.87- fold in the mice (T-III group) treated with 4mg/kg body weight/day of GA (**Figure 5 b, c, d)** along with HFD. In this study, HFD induced obese group was increased SREBP-1c, FAS and PPAR-γ expression by 2.23-fold, 3.36-fold and 1.75-fold individually, whereas GA treated group decreased SREBP-1C, FAS and PPAR-γ expression by 2.29-fold, 3.31-fold and 1.68-fold respectively (**Figure 5 e, g, l)**. LPL & CPT-1 expression was gradually increased in GA treated groups by 1.37-2.25-fold, and 1.06-1.28- fold individually (**Figure 5 h, i)**, on the other hand GLUT 4 and insulin expression have elevated 1.66-fold and 1.81-fold in T-III group contrasted with HFD induced OC group (**Figure 5 f, j)**. Furthermore, following intervention with GA, the T-III group's mRNA expression of related anti-inflammatory markers, such as IL-10 and IL-1ra, increased 1.76 and 1.40 times, respectively (**Figure 5 n, o)**. Although GA treatment dramatically decreased the expression up to 1.12-1.44 times in treated groups (T-I, T-II, and T-III), the pro-inflammatory marker TNF-α level was found to be higher in the OC group than in the C group (**Figure 5 m)**.

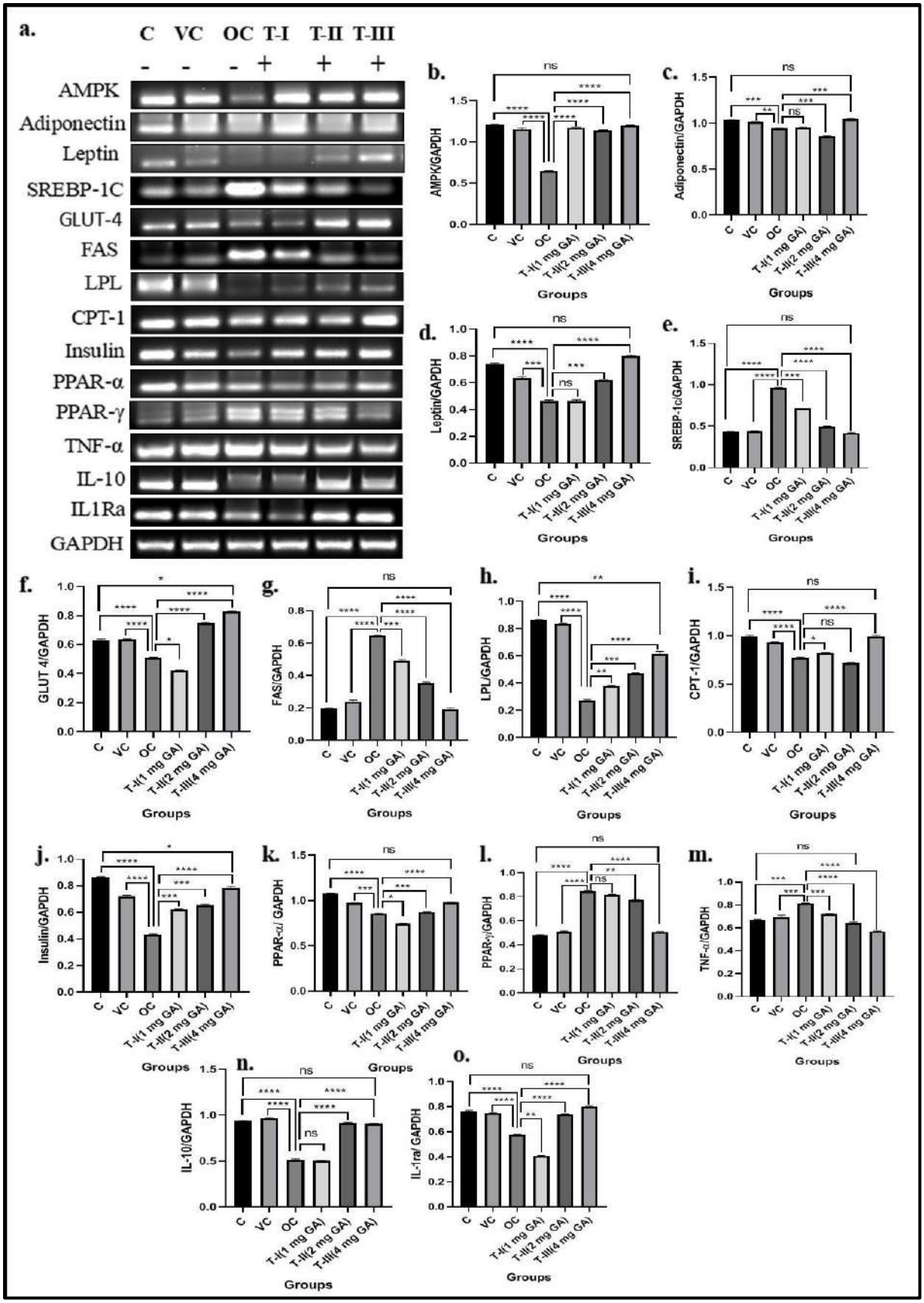

a.
C VC OC T-I T-II T-III
- - - + + +
AMPK
Adiponectin
Leptin
SREBP-1C
GLUT-4
FAS
LPL
CPT-1
Insulin
PPAR-α
PPAR-γ
TNF-α
IL-10
IL1Ra
GAPDH
b.
AMPK/GAPDH
c.
Adiponectin/GAPDH
d.
Leptin/GAPDH
e.
SREBP-1c/GAPDH
f.
GLUT 4/GAPDH
g.
FAS/GAPDH
h.
LPL/GAPDH
i.
CPT-1/GAPDH
j.
Insulin/GAPDH
k.
PPAR-α/ GAPDH
l.
PPAR-γ/GAPDH
m.
TNF-α/GAPDH
n.
IL-10/GAPDH
o.
IL-1ra/ GAPDH
C
VC
OC
T-I(1 mg GA)
T-II(2 mg GA)
T-III(4 mg GA)
Groups
ns

Figure 5. Impact of GA supplementation on obesity and inflammatory-related adipocytokines using semi-q-PCR (a) Relative gene expression level of (b) AMPK; (c) Adiponectin; (d) Leptin; (e) SREBP-1c; (f) GLUT 4; (g) FAS; (h) LPL; (i) CPT-1; (j)insulin; (k) PPAR-α; (l) PPAR-γ; (m) TNF-α; (n) IL-10 and (o) IL-1ra in AT of male BALB/c obese mice in different experimental groups (n=6) were adjusted to control gene expression level (GAPDH; b-o). The results for all groups (n=6) are presented as mean ± SEM. Statistical analysis was performed using one-way ANOVA accompanied by Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05.

Protein analysis in AT

The metabolism of dietary fats and cholesterol is strongly associated with obesity **(Zhou et al., 2023)**. The control of the utilisation of fat is significantly influenced by AMPK. In Fig. 6, GA substitution significantly activated AMPK phosphorylation and increased PPAR-γ protein expressions in HFD induced obese mice by 1.17-fold, whereas AMPK protein expression levels were upregulated by 1.11-fold and PPAR-γ protein levels were downregulated by 1.15-fold after GA administration (**Figure 6 a, e)**. Similarly, administration with GA markedly elevated the level of expression of Ob(H-5) by 1.12-fold gradually (**Figure 6 f)**. However, in the OC group, CEBP-α, SREBP-1c and FAS protein expression increased, and natural fatty acid production in fat tissues was enhanced. The expression of CEBP-α, SREBP-1c and FAS GA-treated mice was markedly downregulated by 1.11- fold, 1.15-fold, and 1.19-fold (**Figure 6 b, c, d)**. These findings demonstrate that GA treatment can limit de novo fatty acid synthesis and markedly enhance mitochondrial lipid transport and glucose utilisation in fat cells.

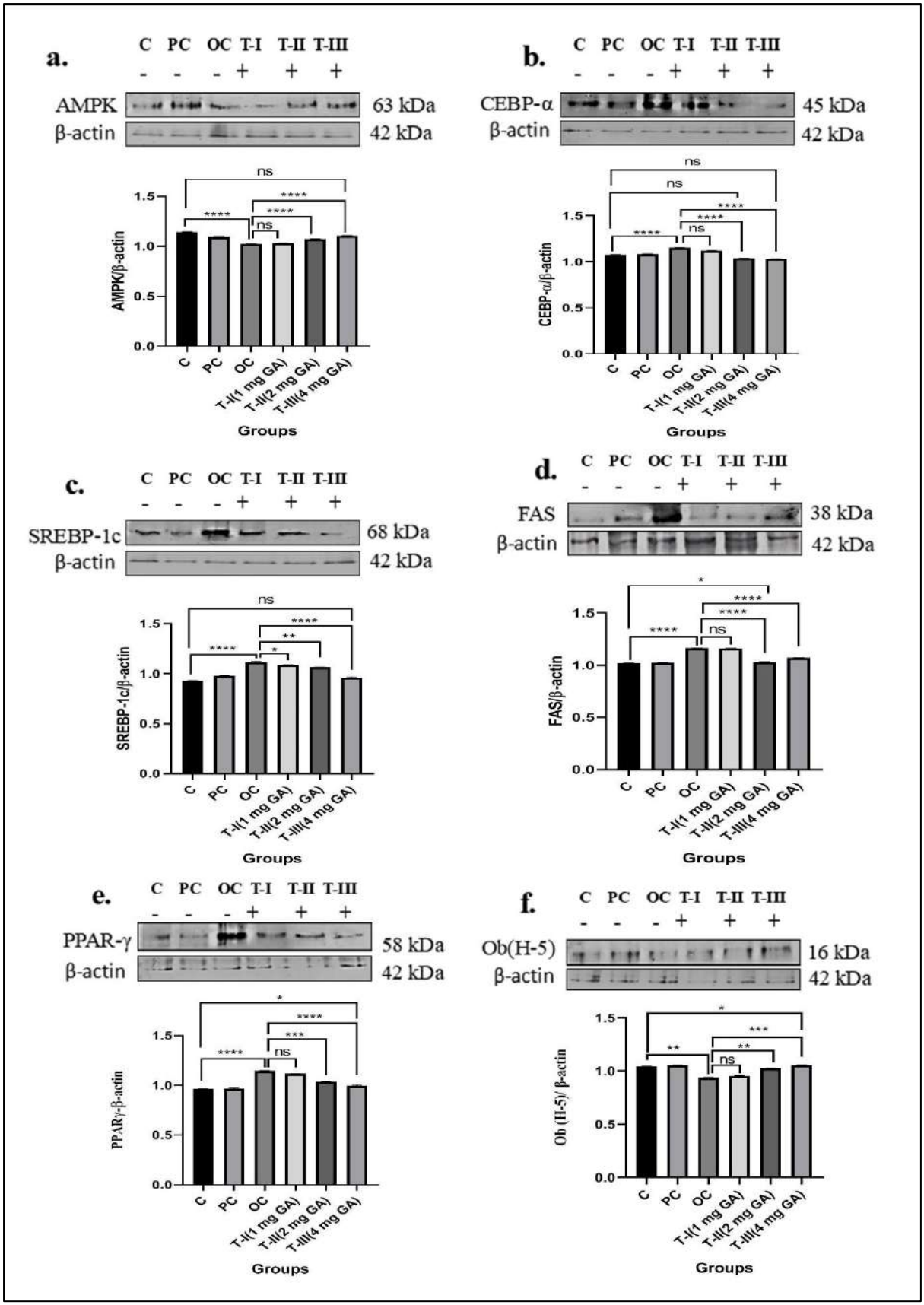

a.
C PC OC T-I T-II T-III
- - - + + +
AMPK
β-actin
63 kDa
42 kDa
AMPK/β-actin
Groups
b.
CEBP-α
β-actin
45 kDa
42 kDa
CEBP-α/β-actin
c.
SREBP-1c
β-actin
68 kDa
42 kDa
SREBP-1c/β-actin
d.
FAS
β-actin
38 kDa
42 kDa
FAS/β-actin
e.
PPAR-γ
β-actin
58 kDa
42 kDa
PPARγ-β-actin
f.
Ob(H-5)
β-actin
16 kDa
42 kDa
Ob (H-5)/ β-actin
C
PC
OC
T-I(1 mg GA)
T-II(2 mg GA)
T-III(4 mg GA)

Figure 6. Efficacy of GA intervention on protein in the WAT of nutritionally obese mice. Groups are segregated as normal chow feeding, control group (C); normal chow feeding+ olive oil, vehicle control group (VC), HFD feeding, obese control group (OC); HFD+ 1mg GA/day (T-I group); HFD+ 2mg GA/ day (T-II group); HFD+ 4mg GA/ day (T-III group). β-actin was used in order to normalise the results. (a) AMPK; (b) CEBP-α; (c) SREBP-1c; (d) FAS; (e) PPAR-γ; (f)Ob (H-5). The results for all groups (n=6) are presented as mean ± SEM. Statistical analysis was performed using one-way ANOVA accompanied by Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05

Discussion

Numerous *in vivo* studies have established the credibility of n-9 GA's anti-obesity action. However, it is well established that co-administration of n-3 PUFAs produced an anti-obesity impact by decreasing the formation of fat in animal trials employing obese animals fed HFD **(Ruzickova et al., 2004; Pérez-Matute et al., 2007)**. Furthermore, our earlier research discovered that *P. paradiseus* FO was an essential mediator of lipometabolic dysregulation in the liver and AT in HFD-fed mouse groups **(Kar et al., 2025)**. In this study, we fed the mice a HFD to create a model of physiologically obese animals. In this study, we treated mice with commercial GA (T-I group:1mg, T-II group: 2mg and T-III group: 4mg GA/kg body weight/ day) to investigate its anti-obesity function, and following dosing, the mice's daily biological state was favourable. The mice that received GA treatment had considerably decrease body weight than the animals who did not get GA treatment. Mice in the GA group consumed slightly less food during dose therapy than mice in the HFD group, however the variation was not statistically significant. Effective variation was observed in the peripheral organs (WAT, liver, kidney) among the different experimental treatment groups. By preventing fat cell growth and expansion, GA decreased body fat deposition and weight maintenance throughout the progression of obesity in BALB/c mice **(Figure 1).** SFAs are typically regarded as a risk factor for dyslipidaemia, characterised by abnormal lipid levels (e.g., cholesterol and/or fat) in the blood, frequently leading to hyperlipidaemia, it is linked to a higher chance of developing several cardiovascular conditions **(Go et al., 2016)**. Obesity causes chronic inflammation of hypertrophied adipocytes in AT, which disrupts insulin signalling and causes insulin resistance **(Yang et al., 2011)**. In our study, GA markedly lower plasma glucose **(Figure 2a)** and higher insulin levels, although the parameters did not differ in OC and T-I group. Following results explored that GA treatment can corrected the hyper insulinemic status

in male BALB/c mice **(Figure 5j)**. Consuming a high-fat meal caused weight progress, primarily in the form of retroperitoneal fat, whereas FO and its composition reversed these changes and also decreased circulating cholesterol and epididymal fat **(Esteves 2020)**. Here, we investigated the effects of GA on build-up of lipids in the livers of BALB/c mice fed a HFD evaluated with HFD+ GA treated experimental groups. Our findings demonstrated that the GA-administered group (mostly the T-III group) had considerably lower liver TC, TG, LDL, and VLDL levels, suggesting that GA could reduce the incidence of lipotoxicity. The efficacy of hyperglycemia and elevated oxidative stress may be the cause of excess urea and uric acid, which are indicators of impaired kidney function **(Figure 2 b, c, e, f)**. After the administration of GA, there was significant decrease in serum urea and uric acid level (T-III group) indicates protective effect of GA on kidney (**Figure 2h, i**). The current study reveals elevated levels of SGOT and SGPT, indicating histoarchitecture abnormalities that may have resulted from oxidative stress and tissue ischaemia related to this enzyme's leakage into the blood pool, while in our study the GA-treated groups exhibit decreased levels of SGOT and SGPT **(Figure 2j, k).** Present experiment that utilized BALB/c mice receiving HFD, elevated buildup of hepatic lipids in OC group was seen after 8 weeks of HFD intake, whereas no liver alteration noted in C and PC group. Since GA leads to corrected hepatic injury mostly in HFD+ GA treated group (T-III: 4mg/kg body weight/day). The majority of cases of hepatitis are brought on by a viral infection; however, hepatitis can also be brought on by a persistent restriction of bile flow brought on by lipid accumulation. Through the activation of many signalling pathways in hepatic parenchymal cells, studies have shown that MUFAs can aid in preventing oxidative stress, endoplasmic reticulum stress, glucose resistance, inflammatory processes, and mitochondrial breakdown **(Jhonson & Bradford 2014)**. In our present study, the histological examination revealed that GA had an inhibitory effect on the buildup of lipids through the liver **(Figure 3)**. Using HE staining, we conducted pathological section investigation on both sWAT and vWAT. The size of the lipid droplets in the T-I, T-II, and T-III groups was considerably decreased by GA treatment **(Figure 4)**. In the current study the expression of AMPK, adiponectin, leptin, SREBP-1c, GLUT-4, FAS, LPL, CPT-1, Insulin, PPAR-α, PPAR-γ, IL-6, TNF-α, IL-10 and IL-1ra, in AT were directly regulated by HFD. Additionally, we described that treatment with different doses of GA (specially T-III group treated with 4mg/kg body weight/day) was able to recover the expression of these genes **(Figure 5)**. According to certain research, n-9 fatty acids may increase leptin sensitivity, which makes the body more sensitive to the leptin signal and may result in a more reduced caloric intake and appetite **(Gonçalves-de-Albuquerque et al., 2016)**. In the current study, mice given a HFD and treated (T-III) with

GA may have higher leptin levels because it may help control fat storage and result in a more balanced metabolic state, which increases the production of leptin due to an increase in AT mass **(Figure 5 d)**.

One of the main antidiabetics and antiatherogenic adipokines is adiponectin, a hormone relea sed by adipocytes. Obesity, type 2 diabetes and insulin resistance are all associated in lower levels of plasma adiponectin. Adiponectin's insulin-sensitizing action appears to be mediated by AMPK and PPAR-α activation, which inhibits gluconeogenesis and stimulates fatty acid oxidation **(Yamauchi et al., 2007)**. Following the above statement our study showed a reduction of adiponectin level in OC group which corrected after the treatment of GA (T-III: 4 mg/kg body weight/day) **(Figure 5c)**. Side by side several studies explored that AMPK activation increases lipid oxidation through PPAR-α **(Gonçalves-de-Albuquerque et al., 2016)**. Here, GA was shown to upregulated the expression of AMPK level gradually in treated groups **(Figure 5b)**. Although positive energy balance is the primary cause of fat deposition, GA was known to have a significant effect on lipid synthesis. Numerous studies stated that, HFD increases the expression of lipogenic enzymes including FAS and ACC via promoting SREBP1-c activation through the induction of hyperinsulinemia **(Miyazaki et al., 2004; Ferre & Foufelle 2007)** Considering intrahepatic lipid generation rises when it surpasses hepatic lipid production, the mice in experiment most likely ingested a HFD that stimulated lipid formation in the liver, which in turn led to elevated TG synthesis and storage in AT **(Ter et al., 2017)**. On the other hand, GA has been demonstrated to promote lipolysis by raising hepatic PPAR-α and decrease FAS by reducing SREBP-1c expression, hence blocking TG production and fat accumulation in treated mouse groups **(Figure 5 e, g, k)**. Fatty acids and eicosanoids induce Peroxisome proliferator activated receptors (PPARs) are members of a steroid-thyroid hormone receptor superfamily that regulates gene expression in our bodies. Numerous hypothesised biological processes suggested that MUFA could be an edible PPAR-γ ligand and that its interaction with PPAR-γ could have a therapeutic effect in metabolic disorders **(Kumar & Sharma 2022)**. However, WATs from GA-treated animal groups showed a markedly lower mRNA level of PPAR-γ, a master transcription element that maintains terminal adipocyte differentiation **(Figure 5l)**.

GA treatment, suggested that the mechanism of weight reduction may be the modulation of gl yceride and cholesterol metabolism by the AMPK mechanism. In skeletal muscle, the heart, and adipocytes, GLUT 4 is the main insulin-regulated glucose transporter. The

pathophysiology of insulin resistance and type 2 diabetes begin with the downregulation of GLUT 4 in the adipocytes of obese or T2D rats and humans **(Moraes-Vieira et al., 2016)**. Moreover, GA have been alleviating obesity-induced insulin resistance and hepatic steatosis in treated groups through inducing GLUT 4 pathway **(Figure 5f)**. Correspondently, we observed at GA treated group (T-III group) **(Figure 5i)** markedly elevated mRNA levels of the gene encoding CPT-1, a rate-limiting enzyme involved in the β-oxidation pathway that transfers fatty acyl coenzymes to the inner mitochondrial membrane **(Yang et al., 2011).** Since LPL is the primary enzyme responsible for chylomicron and VLDL-TG lipolysis in AT capillary endothelial cells, it is believed to be a key regulator for adipocyte fatty acid intake from triglyceride-rich lipoprotein **(Martínez-Fernández et al., 2015)**. In the HFD+GA-fed mouse groups, the current research observed reduced adipose LPL and fat weight simultaneously with enhanced LPL and lowered plasma triacylglycerol, indicating a transition from fat accumulation to oxidation (**Figure 5 h**). Furthermore, we assessed the expression of the pro-inflammatory marker TNF-α in vWAT because chronic inflammation is linked to obesity and may aggravate insulin resistance **(Xu et al., 2023)**. The level of inflammation raised after HFD feeding, whereas GA alleviated inflammation in the treated groups (T-I, T-II, T-III) gradually **(Figure 5 m)**. On the other hand, anti-inflammatory markers such as IL-10, IL-1ra genes are responsible for reduce inflammation by supressing the macrophage activity. Here, the expressions of IL-10 and IL-1ra were raised in GA treated groups rather than OC group **(Figure 5n, o)**.

AMPK is a serine/threonine protein kinase that is extensively distributed and plays a crucial role in WAT's energy metabolism and catabolism **(Xu et al., 2023; Li et al., 2023)**. In addition, SREBP-1c and CEBP-α are crucial transcription factors in adipogenesis that can be regulated by AMPK **(Herzig & Shaw 2018)**. GA reduces endogenous cholesterol production by downregulating the expression of the proteins SREBP-1c and CEBP-α **(Figure 6 c, b)**. In addition to being abundantly produced by the liver and adipocytes, PPAR-γ also improves glucose tolerance by lowering adipose TG lipolysis **(Hsu et al., 2014).** We found that GA could activate AMPK and supress FAS and PPAR-γ expression, leading to inhibition of hepatic lipogenesis in mice model **(Figure 6 a, d, e)**. Taken together, these results demonstrated the potential antiobesity effect of GA for the first time. In summary, all of these experiments showed that in order to improve insulin resistance and control glucose homeostasis, GA triggered the AMPK pathway to combat obesity.

Conclusions

LC n-9 fatty acids have a wide range of pharmacological properties that make them a promising choice for treating a variety of diseases. With an emphasis on their effects on AT and adipocyte metabolism and function, we provide an overview of the current understanding of the advantageous effects of n-9, GA on obesity and related illnesses in this paper. Combined, our interventional trial employing HFD-induced obesity in mice revealed that treatment with GA generated large alterations in the metabolic process of obese mice and demonstrated considerable protective properties through improve fatty acid oxidation and subsequent fatty acid breakdown. The considerable decreased in body weight and diminished fat buildup in the liver in response to GA could be attributed to its high biological activity. Therefore, in addition to lifestyle adjustment, GA may be a useful adjunct to managing obesity.

Chapter VII: Objective (iv)

Objective (iv): To explore the role of isolated FA in alleviating obesity related metabolic disorder *in vivo* model.

The whole study was performed on BALB/c male mice to find out the exact underlying mechanism behind anti-obesity action of screened newly characterized FA from marine *P. paradiseus* oil.

Introduction:

Obesity is a worldwide health problem with a high incidence in all age categories that has a significant societal and economic effect, as it influences individual well-being and quality of lifestyle **(Ni et al., 2020)**. Nevertheless, obesity is a multifaceted chronic illness marked by excess fat (visceral and subcutaneous) buildup that can be detrimental for individuals' health for the development of metabolic abnormalities, which includes insulin resistance (IR) and type 2 diabetes (T2D). It also acts as a major trigger for a number of life-threatening diseases, such as non-alcoholic fatty liver disease, cardiovascular diseases, and several types of cancer. Still, it frequently occurs in conjunction with the formation of triglycerides (ectopic fat) in peripheral organs such as pancreas, muscle, and liver **(Engin 2017).** The metabolic disorder, that is determined as three of the following five characteristics are present: abdominal fat, raised TG, decreased HDL-C levels, elevated blood pressure, and high fasting blood glucose **(Aguilar et al., 2015).** In addition, as patients with obesity show decreases in inflammatory cytokine levels after losing weight, obesity can be considered a main sign of persistent low-grade inflammation. It is commonly known that AT controls metabolism by producing glycerol, esterified fatty acids, and hormones (such leptin and adiponectin) that are known to be higher in obesity. Additionally, a group of inflammatory cytokines and chemokines, including IL-6, TNF-α, IL-1β, and monocyte chemoattractant protein-1 (MCP-1), which are all commonly associated with IR, are secreted more when inflammatory cells infiltrate the liver and AT **(Monteiro et al., 2010).** The liver releases C-reactive protein (CRP), a crucial indicator of inflammation linked to a number of metabolic disorders, including type 2 diabetes and cardiovascular conditions, in reaction to increased cytokine levels. CRP also aggravates disease progression by stimulating the nuclear transcription factor kappa B (NF-κB) signalling pathway, which is straightly connected in the expression of pro-inflammatory cytokines **(Tang et al., 2017).** According to new research, n-3 fatty acids may boost mice's energy utilization by triggering UCP1-mediated no shivering thermogenesis in brown and beige adipocytes. More precisely, consumption of diets supplemented with essential fatty acids or containing FO as a fat source is linked to enhanced brown and beige adipocyte thermogenic response and elevated expression of thermogenic markers UCP1, PR domain containing 16 (PRDM16), and peroxisome proliferator activated receptor (PPAR) coactivatorα (PGC1α) **(Pahlavani et al., 2017; Ghandour et al., 2018).**

Although the primary therapy for obesity and its associated disorders is weight reduction through lifestyle changes, compliance is challenging. Adjunctive therapies for obesity

management involve medicines **(Patel 2015)**, surgery **(Arterburn & Courcoulas 2014)**, and nutritional supplements. Consequently, there is an immediate clinical requirement to gain greater insight into the biological and molecular mechanisms that relate obesity to metabolic decline in order to discover new targets for potential therapeutic approaches. Essential fatty acids and other natural bioactives may have few adverse effects, but they are safer than other methods of treating obesity. Additionally, studies found that in overweight people with a metabolic disorder, reducing carbohydrate intake raises HDL levels, lowers blood pressure and TG levels, and offers a number of potential advantages for the management of type II diabetes and obesity **(Ceja-Galicia et al., 2025).** One of the main macronutrients in an individual's diet is lipids. The type and proportion of dietary fatty acids consumed influences health and whole-body physiology **(Ratnayake et al., 2009).** Research has shown that SFA are increase inflammation as well as harmful to health, whereas MUFA and PUFA offer health benefits. It should come as no surprise that dietary supplements containing fatty fish and FO, which has a large amount of long-chain PUFAs and MUFAs, have demonstrated positive impacts on obesity and related metabolic abnormalities **(Kar et al., 2025; Albracht-Schulte et al.,2018)**. Since PUFAs like EPA and DHA are precursors to various resolving, biosynthesized lipid mediators that predominantly function in immune system cells, their various functions in the resolution of an inflammatory process have also been studied. Additionally, it has been shown that EPA and DHA may stimulate GPR120, a G-protein coupled receptor, with varying DHA concentrations, resulting in a greater response and promoting anti-inflammatory signalling that decreases the stimulation of the TNF-α mechanism in macrophages **(da Cunha de Sá et al., 2021; Ramalho et al., 2020).** The effects of MUFAs on obesity associated metabolic disorders are insufficiently recorded, although increasing evidence links MUFAs to anti-inflammatory states **(Ravaut et al., 2020)**. As we all know that, n-9 fatty acids are monounsaturated, which means they contain only one double bond. It is nine carbons away from the omega terminal of the fatty acid structure. Among other n-9 fatty acids oleic acid is the most common in the diet. However, n-9 fatty acids are not completely “essential” in the sense that they can be produced by the body. Eating foods rich in n-9 fatty acids is better than other types of fats and has many beneficial health effects **(Lopes et al.,2016; Basuny 2025)**. In comparison to mice fed an SFA-rich diet, studies conducted on mice grown on a MUFA-rich diet for 15 weeks reveal lower levels of inflammatory markers (IL-6, MCP-1, IL-1, and TNF-α) and greater concentrations of anti-inflammatory markers (IL-4 and IL-10) **(Tamer et al., 2020).** Thus, understanding n-9 fatty acids provides important insights into how everyday dietary choices influence not only personal health but also broader nutritional sustainability. GA (Gondoic acid or cis-11-

Eicosenoic acid) is a n-9 MUFA that is commonly found in plant oils and nuts. It is also found in grapeseed oil (**Basuny 2025)** and for the currently researchers found it in marine *P. paradiseus* FO **(Kar et al., 2025).** Our previous study established the effective dose (4mg/kg body weight /day) of GA to supress adiposity **(Kar et al., 2026a)**, however; our present study hypothesizes the nutritional significance of GA with varying duration has different impacts on dietary relevance and metabolic parameters altered by an HFD -induced obesity.

Materials and methods

Chemicals

The following chemicals and reagents were acquired for completing the experiments in this study. Using treatment compound named Gondoic acid or cis-11- Eicosenoic acid (E3635-100MG), purchased from Merck, Darmstadt, Germany; Hi-cDNA synthesis kit, 2X PCR mixture, DAB, RNA-express reagent obtained from Himedia, HiGenoMB, Maharashtra, India. DAPI (Cat no. 62248) from Invitrogen; fluorescent mounting medium purchased from Dako, Hamburg, Germany. Ketamine-xylazine (61763-23-3), graded ethanol and paraffin purchased from Histosec, Merck, Darmstadt, Germany; fluorescent mounting medium purchased from Dako, Hamburg, Germany; OneTouch glucometer from Johnson & Johnson, Milpitas, CA, USA. Mouse ELISA kit for TNF-α was obtained from Wuhan Fine Biotech (Wuhan, China) and IL-6, insulin was obtained from ALPCO, Salem, NH, USA. All the primers used for RT-PCR purchased from Bioserve, India. SYBR Green master mix (Cat#600882) purchased from Agilent, USA; nitrocellulose membrane from Bio-Rad, Model No. 165.8033FC. The primary antibodies: AMPK mouse McAb (Cat No.: 66536-1-Ig), SREBP-1c Mouse McAb (Cat No.: 66875-1-Ig), CEBP-α Mouse McAb (Cat No.: 66649-I-Ig/), β- actin Mouse McAb (Cat No.:66009-1-Ig) were purchased from Proteintech (ChromoTek GmbH & Proteintech Germany) and PPAR-α (H-2) (Sc-398394), PPAR-γ mouse monoclonal IgG (Sc-7273), Ob (H-5) mouse monoclonal IgG (Sc-393043), FAS mouse monoclonal IgG (Sc-74,540), PGC1α (D-5) (Sc-518025), GLUT-4 (IF8) (Sc- 53566), GLUT-3 (G-5) (Sc- 74399), GLUT-1 (A-4) (Sc-377228) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA-USA). The secondary antibody: Goat Anti-mouse IgG-HRP (cat# 11-301) was purchased from Abgenex (Odisha, India).

Animals, experimental design and diet

All experiments reported herein were approved by the Institutional Ethics Committee, Midnapore City College (2256/PO/RE/S/23/CCSE) and all experimental protocol management

was in accordance with the guidelines for the care and use of laboratory animals. Total forty two numbers of five-week-old male BALB/c mice (Saha Enterprise, Habra, North 24 Pargana, West Bengal- 743271) weighing 18 to 20 g at the beginning of the study were housed individually in plastic cages in animal quarters maintained at 23 ± 1 °C with 12-hour/12-hour dark light schedule. Total experiment was continued for 18^{th} weeks. After seven days of acclimatization, the mice were allocated into seven groups: (1) Normal chow feeding (NC) (10% fat, 60% carbohydrate, 20.5% protein, 2.9% fibre, 6.6% minerals with water ad libitum), control group (C); (2) NC- fed and co-administered with GA (4 mg/kg body weight/day), positive control group (PC); (3) NC-fed and co-administered with olive oil (4 mg/kg body weight/day), vehicle control group (VC) (4) HFD feeding (41.5% fat from lard, 35% carbohydrate, 15% protein, 5.0% fibre, 3.5% mineral with water ad libitum), obese control group (OC); (5) HFD-fed mice groups supplemented with 4 mg/kg body weight/ day GA for last 4 weeks (T-I group); (6) HFD-fed mice groups supplemented with 4mg GA/kg body weight/ day for last 5 weeks (T-II group); (7) HFD-fed mice groups supplemented with 4 mg/kg body weight/ day of GA (T-III group) by oral gavage for last 6 weeks of total experiment. Composition of treatment compound (100 mg GA/1 ml olive oil): Gondoic acid or cis-11-Eicosenoic acid (E3635-100MG), purchased from Merck was dissolved per 1 ml of olive oil and used as treatment solution.

The food intake was recorded per day and bodyweight, body length was monitored weekly. A calliper (Central; Model no. 6420) was used for measuring body length from nasal to anal distance, to the nearest 0.1 mm. Daily food intake was measured by using food weight machine (ASIN, Model no. KS33). BMI was assessed using the standard procedure, and the results were represented as gm/cm^2 **(Kar et al., 2025)**. The conventional method for calculating BMI was used; by following equation given below and the results were indicated as g/cm^2.

BMI (g/cm^2) =Body weight(g)/Body length(cm^2)

Collection of blood and tissue samples

After 18^{th} weeks of the experiment, mice were euthanized by ketamine-xylazine. Blood samples were collected by puncturing the orbital plexus and centrifuged at 3000 rpm for 15 min at 4°C and serum was stored at –80°C for biochemical analysis. The liver, subcutaneous, visceral, brown adipose tissues (BATs), liver and kidney were removed, weighed, and immediately submerged in liquid nitrogen, and being stored at −80°C for additional examination. Adipose fat depots and liver tissues were harvested, weighed, and processed as

described below for protein expression and gene expression analysis. The Lee's index was calculated using the formula: [body weight (g)] ^ (1/3) × 1000/ [body length (cm)]. AT, liver and kidney indices were calculated to assess relative organ mass. These indices were defined as the ratio of tissue weight (g) to total body weight (g) at sacrifice and were calculated using the following formula: Tissue index = [Tissue weight (g)/Body weight (g)] × 100% **(Zhang et al., 2025)**.

Biochemical measurements

The kit assay methods (Enzopak, India) were followed to analyse the serum levels of TC, HDL-C, LDL-C, VLDL-C and TG level through semi-auto analyser following Enzopak kit method **(Panchali et al., 2024).**

Measurement of metabolic parameters

In the last week of the treatment protocol, the glucose tolerance test (GTT) and the insulin tolerance test (ITT) were performed. For the GTT, mice were fasted for 12 h and then received intraperitoneal injection of glucose (1 g/kg body weight). For the ITT, mice were fasted for 4 h and intraperitoneally injected with insulin (1 U/kg body weight). Blood glucose concentrations were measured at 0, 30, 60, 90, and 120 min after glucose or insulin injection using a OneTouch glucometer (Johnson & Johnson, Milpitas, CA, USA). The homeostasis model assessment of insulin resistance (HOMA-IR) was determined by the formula: [fasting blood glucose (mmol/L) × fasting insulin (μU/mL)]/22.5. To evaluate glucose tolerance, blood glucose profiles were generated, and the area under the curve (AUC) was determined. The final concentration of adipokines such as IL-6, TNF-α and plasma insulin levels were measured by the ELISA kit (ALPCO, Salem, NH, USA) **(da Cunha de et al., 2020)**.

Histological and Immunofluorescence analysis

Frozen tissues (liver, BAT, sWAT & vWAT) were cryo-sectioned, fixed in a 4% formalin solution followed by dehydration, embedding in paraffin wax, and sliced at a thickness of 3-6 μm. Subsequently, HE staining was conducted, and the sections were analysed using a compound microscope (Olympus, Model No. CX21i). For immunofluorescence staining of liver tissue, sections embedded in paraffin were deparaffinized, and endogenous peroxidase activity was suppressed. After incubation with a blocking serum, the sections were then incubated overnight at 4◦C with primary antibodies against PPAR-α (1:1000) and PPAR-ɣ (1:1000). Following PBS washing, incubate the sample with the secondary antibody (HRP,

1:7500, Abgenex) at 37 ◦C in the absence of light for a duration of 50-60 min **(Zhang et al., 2025)**. Upon subsequent washing, the nuclei were counterstained with DAPI (G1012, Servicebio) at room temperature for 10 min. Then, they were examined under a fluorescent microscope (Olympus, Model No. BX43F).

RNA isolation and real-time PCR

Total RNA was extracted from liver tissue with TRIzol reagent (Takara, Dalian, China) according to the manufacturer's protocol. Reverse transcription reactions were performed using the a Hi-cDNA synthesis kit (Himedia, HiGenoMB, Maharashtra, India). Quantitative real-time PCR (qPCR) was carried out using SYBR Green master mix (Cat#600882) in accordance with the manufacturer's instructions. The PCR products were verified by an ABI 7500 sequencer (Applied Biosystems, Foster City, CA, USA) (Wei et al., 2020). Thermal cycling conditions were 30 s at 95 °C and 40 cycles of 10 s at 95 °C, 30 s at 58 °C, and 20 s at 72 °C. RT-qPCR efficiencies were obtained through a dilution series of cDNA and were nearly equal for all genes. Data were analysed through the relative mRNA expression ratio, using the equation $2^{-\Delta\Delta ct}$, in which $2\Delta CT$ refers to the difference between the number of cycles (CT) of the target genes and the endogenous control. The sequences of the primers are shown in **Table 1.** List of primers used in this experiment for RT- PCR.

Genes	**Primary Sequences**	
	Forward Primer	**Reverse Primer**
GAPDH	5’-GGTGAAGGTCGGAGTCAACG-3’	5’-GTGAAGACGCCAGTGGACTC-3’
AMPK	5’-AGGAAGAATCCTGTGACAAGCAC-3’	5’-CCGATCTCTGTGGAGTAGCAGT-3’
Leptin	5’-CAAGCAGTGCCTATCCAGA-3’	5’-AAGCCCAGGAATGAAGTCCA-3’
Adiponectin	5’-GTCAGTGGATCTGACGACACCAA-3’	5’-ATGCCTGCCATCCAACCTG-3’
SREBP-1c	5’-ACGGAGCCATGGATTGCACA-3’	5’-AAGGGTGCAGGTGTCACCTT-3’

PPAR-γ	5’-AGGCCGAGAAGGAGAAGCTGTTG-3’	5’-TGGCCACCTCTTTGCTCTGCTC-3’
PPAR-α	5’-CCTGAACATCGAGTGTCGAATAT-3’	5’-GTTCTTCTTCTGAATCTTGCAGCT-3’
CEBP-α	5’-TTACAACAGGCCAGGTTTCC-3’	5’-GGCTGGCGACATACAGTACA-3’
FAS	5’-TGCTCCCAGCTGCAGGC-3’	5’-GCCCGGTAGCTCTGGGTGTA-3’
GLUT-4	5’-GAGCCTGAATGCTAATGGAG-3’	5’-GAGAGAGAGCGTCCAATGTC-3’
GLUT-3	5’-ACTCAATGCTTATTGTCAACC-3’	5’-AGATCTCTCCAATGTACATAGG-3’
GLUT-1	5’-GCCATGGAGCCCAGCAGCAAG-3’	5’-CACTTGGGAATCAGCCCCCAG-3’
MCP-1	5’-ACCTCGGAACACCTCCTGGATG-3’	5’-ATCTGGTGGCTCTCCGAGTAGG-3’
CRP	5’-GCATGTTGA AGA AACGAGGGC-3’	5’-TCTGGCTGTCTCTCTGTCTCT-3’
IL-6	5’-TCAACTTCTCCAGCGTGATG-3’	5’-TCTTTCCCTCTTTTCCTCCTG-3’
TNF-α	5’-TTCTGTCTACTGAACTTCGGGGTGATCGGTCC-3’	5’-GTATGAGATAGCAAATCGGCTGACGGTGTGG-3’

Protein analysis by Immunoblotting assay

Using RIPA buffer, liver samples were lysed, and the Lowry method was used to measure the protein content. Protein samples weighing 50 µg were boiled for 5 minutes in loading buffer before being electrophoresed using SDS-PAGE and deposited on a nitrocellulose membrane (Bio-Rad, Model No. 165.8033FC). Then 4% BSA was next applied to block the membrane, and the primary antibody was incubated at 4°C for the whole night. The primary antibody used in this study included, PGC-1α, AMPK, Ob (H-5), adiponectin, SREBP-1c, PPAR-γ, PPAR-α,

CEBP-α, FAS, GLUT-4, GLUT-3, GLUT-1 and β-actin at 1:1000 ratio. The membrane was then incubated for one hour at 4 °C with HRP labelled anti-mouse secondary antibody 1:7500 (Abgenex) after being cleaned three times with phosphate buffer saline with tween-20 (PBST). DAB was used to identify particular protein bands. Densitometric examination utilising Image J densitometry software was used to quantify the bands **(Das et al., 2025b).**

Statistical Analysis

The data (triplicate values) were examined by GraphPad Prism software (San Diego, CA, USA) and expressed as mean± SEM. One-way ANOVA and Tukey's multiple-comparison tests were used to compare treatment groups to the control group. Significance levels were set at $P<0.0001$, $P<0.001$, $P<0.01$, and $P < 0.05$ **(da Cunha de et al., 2021)**.

Results

GA decreases body weight and modulates metabolic state in HFD-induced obese mice

In order to look into the potential metabolic impact of GA on obesity occurred by a HFD, we measured the body weight of mice fed, NC diet, NC+ 4mg GA, NC+ olive oil, HFD and HFD+ 4 mg GA treated experimental groups over a period of eighteen weeks. An excessive intake of calories promotes body weight gain and the buildup of abdominal fat **(Figure 1a)**. Daily food intake of all groups remained similar, however a minor variation noted between NC diet and HFD groups **(Figure 1b)**; as well as no noticeable variations observed in water consumption during experiment. The initial body weight of BALB/c male mice showed no significant difference among 7 experimental groups (C: 18± 1.24 g; PC: 18 ± 1.12 g; VC: 18± 1.1G g; OC: 17± 1.45 g; T-I: 18.5± 1.20 g; T-II: 17±1.01g; T-III: 18.6± 1.27g). Compared to the corresponding control diet group (C: 35±1.21 g; PC: 35±1.08 g; VC: 36±1.02 g), the HFD-fed OC groups gained more body weight (OC: 52±1.37 g) at the conclusion of the study protocol. Interestingly, the OC and T-I (46 ±1.21 g) groups gained nearly the same amount of total body weight. Nevertheless, the increase in body weight was lessened by selective dose of GA (4 mg/kg body weight/day) supplementation compared to the weight of the OC group, whereas the significant reduction noticed in the duration of five weeks (T-II: 40± 1.03 g) and six weeks (T-III: 37±1.05 g) GA treated group **(Figure 1c)**. In **Figure 1d** presents data regarding BMI (g/cm^2) of different experimental groups. Retroperitoneal fat was highly expressed in the OC group, and almost similar between control and GA treated groups, while epididymal fat was mostly reduced in the T-III group (duration of 42 days treatment). So, the BMI (g/cm^2) of mice group was as follows for C: 0.52±0.01, PC: 0.53±0.03, VC: 0.55±0.02, OC: 0.79±0.05, T-I:

0.63±0.03, T-II: 0.58±0.02; T-III: 0.54±0.02. Notably, GA supplement considerably decreased the weights of WAT, liver tissues and increased the weight of BAT **(Figure 1e)**. No remarkable changes noted about the weight of kidney and kidney index in all the experimental groups. The BAT and liver indexes revealed a significant decline in the HFD group, while the WAT index indicated a significant rise **(Figure 1f)**. Additionally, Lee's index was drastically diminished in NC diet fed C, PC, VC group and HFD+ GA supplemented groups (T-I, T-II, T-III) contrasted to the OC group **(Figure 1g)**.

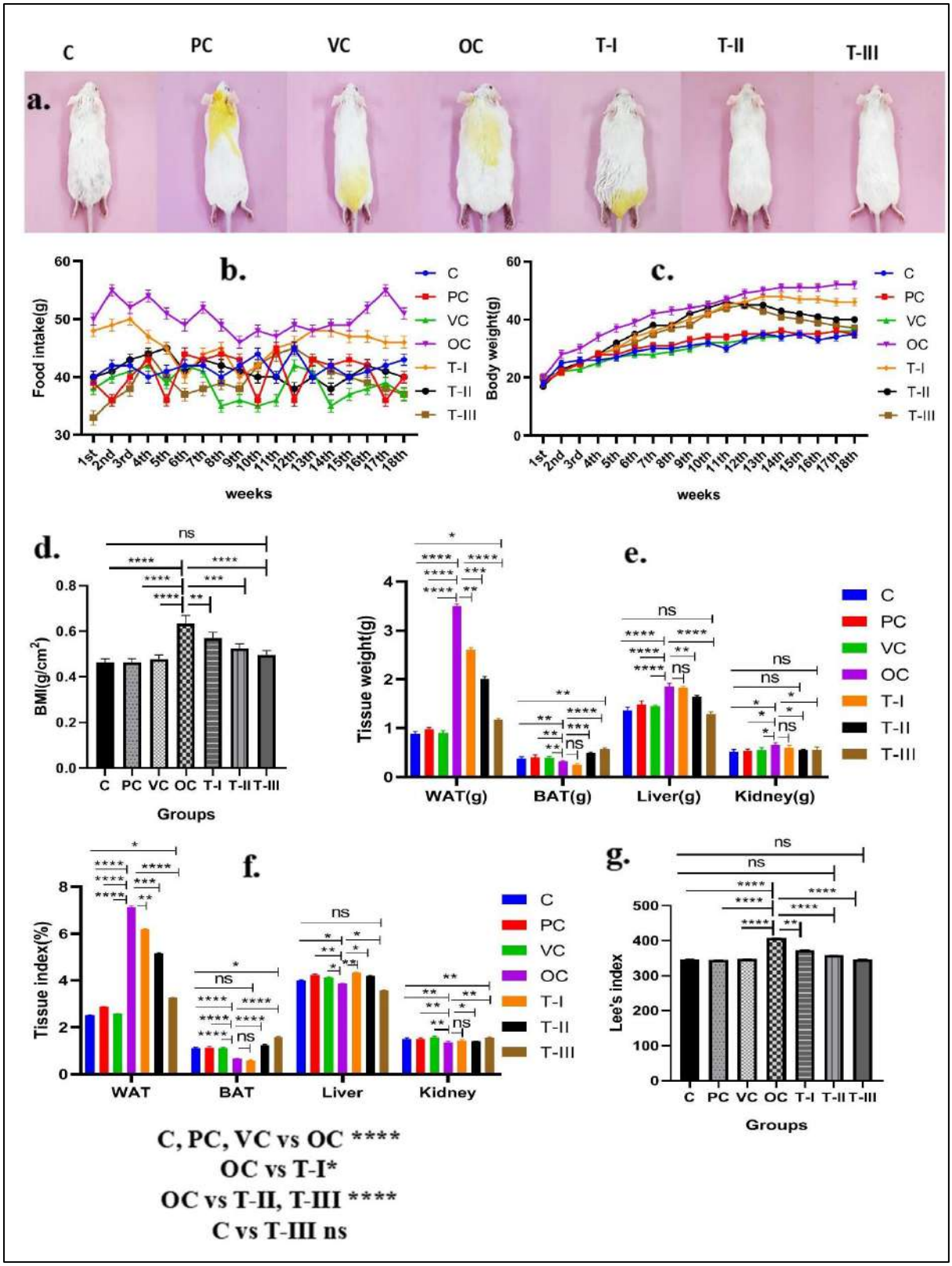


Figure 1. GA decreases fat mass and body weight *in vivo*. (a) Representative morphological images of experimental group; (b) measures of dietary intake each week; (c) assessments of body weight; (d) BMI (gm/cm^2); (e) tissue weight; (f) tissue index; (g) lee's index of different

experimental groups (n=6). Data are represented as mean ±SEM. Tukey's multiple comparison test is used following one-way ANOVA to assess statistical analysis. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05.

Impact of GA on Serum Glucose, Lipid Profile, and Insulin Resistance in HFD-Induced Obese Mice

Since blood TG is the primary component of AT, elevated serum TG levels may be a sign of metabolic abnormalities. Similarly, serum TC concentration is a prior indicator in the evaluation of obesity because higher serum TC availability increases fatty acid accumulation in the liver and AT **(de Moura e Dias et al., 2021)**. However, the effect of GA supplementation on lipid and glucose metabolism, as well as insulin resistance, was determined **(Figure 2)**. In **Figure 2i**, serum glucose concentration was markedly higher in the HFD- fed OC group (287.86 ± 2.4 mg/dl) compared to the NC diet fed C (83.92± 1.8 mg/dl), PC (83.37 ± 2.9 mg/dl) and VC (85.64 ± 2.2 mg/dl) group, while supplementation of GA at 4 mg/kg body weight with 4 weeks (T-I: 175.39 ± 3.2 mg/dl), 5 weeks (T-II: 113.4 ± 1.9 mg/dl), 6 weeks (T-III: 99.65± 2.1 mg/dl) duration considerably lowered the high blood sugar levels brought on with the HFD. The OC group showed the greatest concentrations of TC (175.73± 2.8 mg/dl, **Figure 2a**), TG (215.62± 1.7 mg/dl, **Figure 2b**), VLDL (60.78± 2.1 mg/dl, **Figure 2d)** and LDL (160.24± 2.5 mg/dl, **Figure 2e**) level. As expected, HFD-fed mice groups received 4 mg/ kg body weight GA for six weeks (T-III) were displayed a significant reduction in lipid profiles including TC (15-18.12 %), TG (28-40 %), VLDL (42.67 %), and LDL (16.4-38.76%) compared to the OC group. In contrast, HDL (44.76%) levels were markedly elevated in GA supplemented T-III group compare to OC group **(Figure 2c)**.

The HFD group had significantly higher circulating fasting blood glucose and insulin values, conversely selective dose (4mg/kg body weight/day) of GA supplementation with varying duration (T-I: 4weeks, T-II: 5 weeks, T-III: 6weeks) reduced insulin resistance gradually by 42-56.43% and 44.87-54.32% **(Figure 2f, 2g)**. As we all know HOMA-IR an indicator of insulin resistance, revealed that GA therapy significantly enhanced insulin sensitisation by 46.73%-67.33% **(Figure 2h)**. GTT revealed that 4 mg/kg body weight GA supplementation with the duration of 6 weeks (T-III) altered the high serum glucose levels following the 15th minute, serum glucose concentrations gradually decreased, similarly to the C group. The AUC induced by the HFD, indicating significantly higher glucose level. Although, the GA supplemented group showed an 52.2-63.47 % decreased sensitivity to glucose in comparison to the OC group

(Figure 2g). Additionally, adipokine levels were measured, where GA supplemented groups (T-I: 4weeks, T-II: 5 weeks, T-III: 6weeks) significantly decreasing insulin, TNF-α and IL-6 levels **(Figure 2 k, l, m)**. These results imply that GA can reverse HFD-induced dyslipidemia and glucose metabolism problems by controlling adipokine production and improving sensitivity to insulin as well as overall metabolic state.

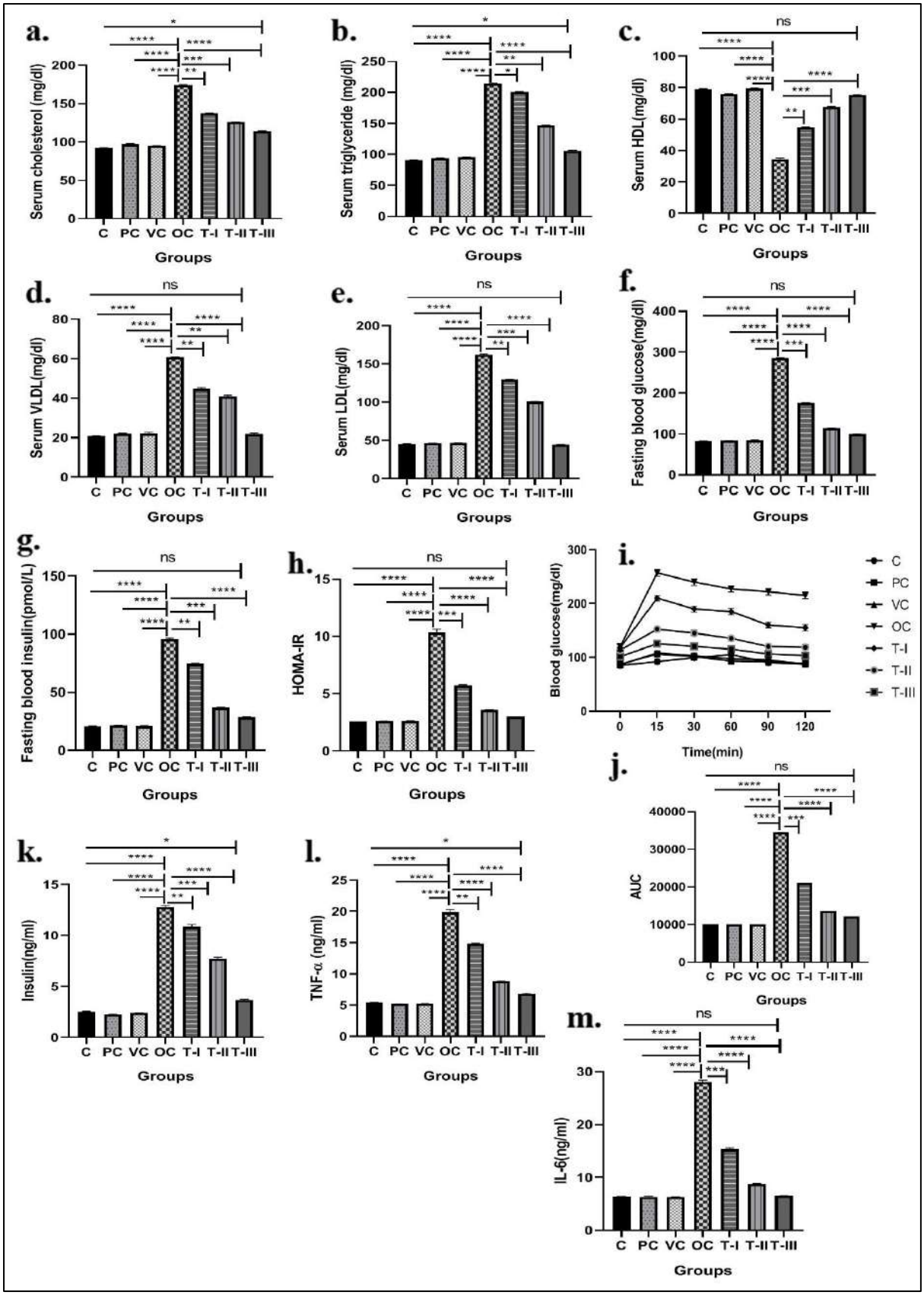


Figure 2. GA reduces insulin resistance and enhances glucose and fat metabolism. Lipid profile such as (a) TC; (b) TG; (c) HDL; (d) VLDL; (e) LDL; (f) fasting blood glucose; (g) fasting

blood insulin; (h) Homeostatic model assessment of insulin resistance (HOMA-IR) values (i, j) Glucose sensitivity tests were performed in mice, determined the area under the curve (AUC); serum (k) Insulin; (l) TNF-α; (m) IL-6 levels were estimated using ELISA kits in different experimental groups (n=6). Data are represented as mean ±SEM. Statistical analysis assessed via one way ANOVA followed by Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05.

GA reduces lipid buildup in liver and adipocyte size in subcutaneous fat

The HFD-fed OC group's liver morphology revealed a pale colour, but the HFD+ GA (4 mg/kg body weight) supplemented groups (T-I, T-II, T-III) saw a gradual restoration to normal liver colour **(Figure 3a)**. To evaluate the pathological abnormalities of the liver, HE staining was applied to liver samples. Among the GA treated groups, T-III group demonstrated a notable reduction in liver steatosis contrasted to the HFD group **(Figure 3b)**, and lipid accumulation (TG and TC) were consistent with the histological results **(Figure 3c, d).** An upward trend in adipocyte cell size could be the cause of the rise in WAT weight **(Figure 3h).** To identify the preventive adiposity mechanism in GA treated mice, the sWATs and vWATs structure were examined using measuring the fat accumulation layer as well as adipocyte cell size from WAT. The outcomes demonstrated that adipocytes size and accumulation layer dropped in the epididymal tissue of GA treated T-III (6 weeks duration) group than in OC group by HE staining **(Figure 3f, g)**. On the other hand, BAT distribution was significantly high in GA treated T-III group compare to the HFD-fed OC group **(Figure 3e)**.

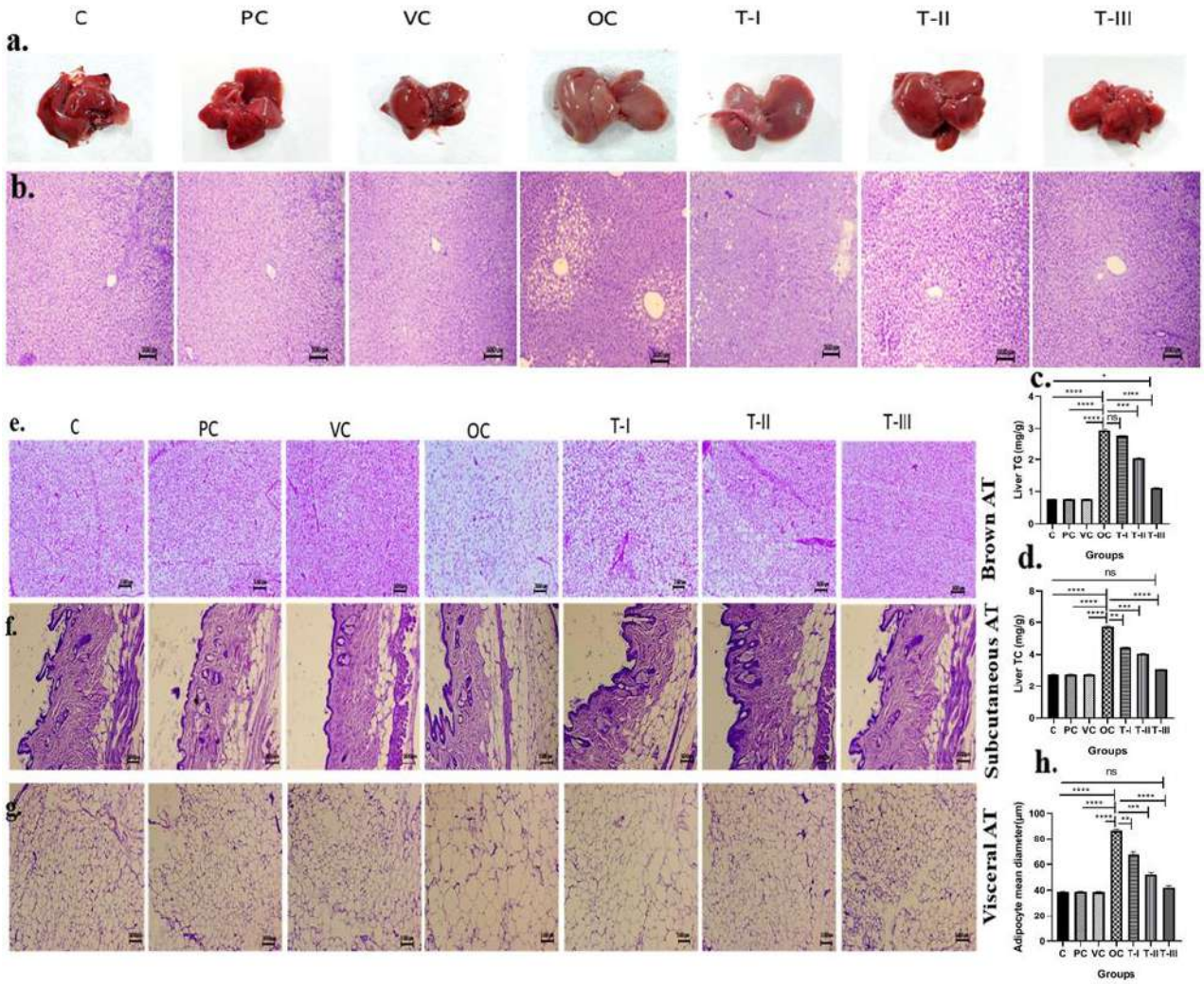


Figure 3. GA improve hepatic health and reduces adipocyte size in subcutaneous fat. (a) Represent the morphological images of liver; (b) HE staining of liver tissue; (c) Estimation of liver TG; (d) Cholesterol; (e) fat accumulation in experimental groups; stained by HE in (f) sWAT; (g) vWAT; and (h) adipocyte diameter. Results are represented as mean ± SEM for each group (n=6). The scale bar is corresponding to 100 µm.

PPAR-ɣ and PPAR-α antibody tagging in liver tissue in HFD-induced obese mice

In liver tissue sections from HFD-induced obese mice in different treatment groups, the immunofluorescence pictures in Figure 4A display the expression and localisation of PPAR-ɣ (green fluorescence) and cell nuclei (DAPI, blue fluorescence). Significantly higher amounts of green fluorescence are seen in the OC group suggesting that PPAR-ɣ is highly expressed in that liver tissue. In contrast to the HFD-induced OC group, the C, PC, VC, and T-III groups exhibit minimum to low levels of green fluorescence, as well as the combined images also reveal altered localisation or decreased PPAR-ɣ expression in those groups.

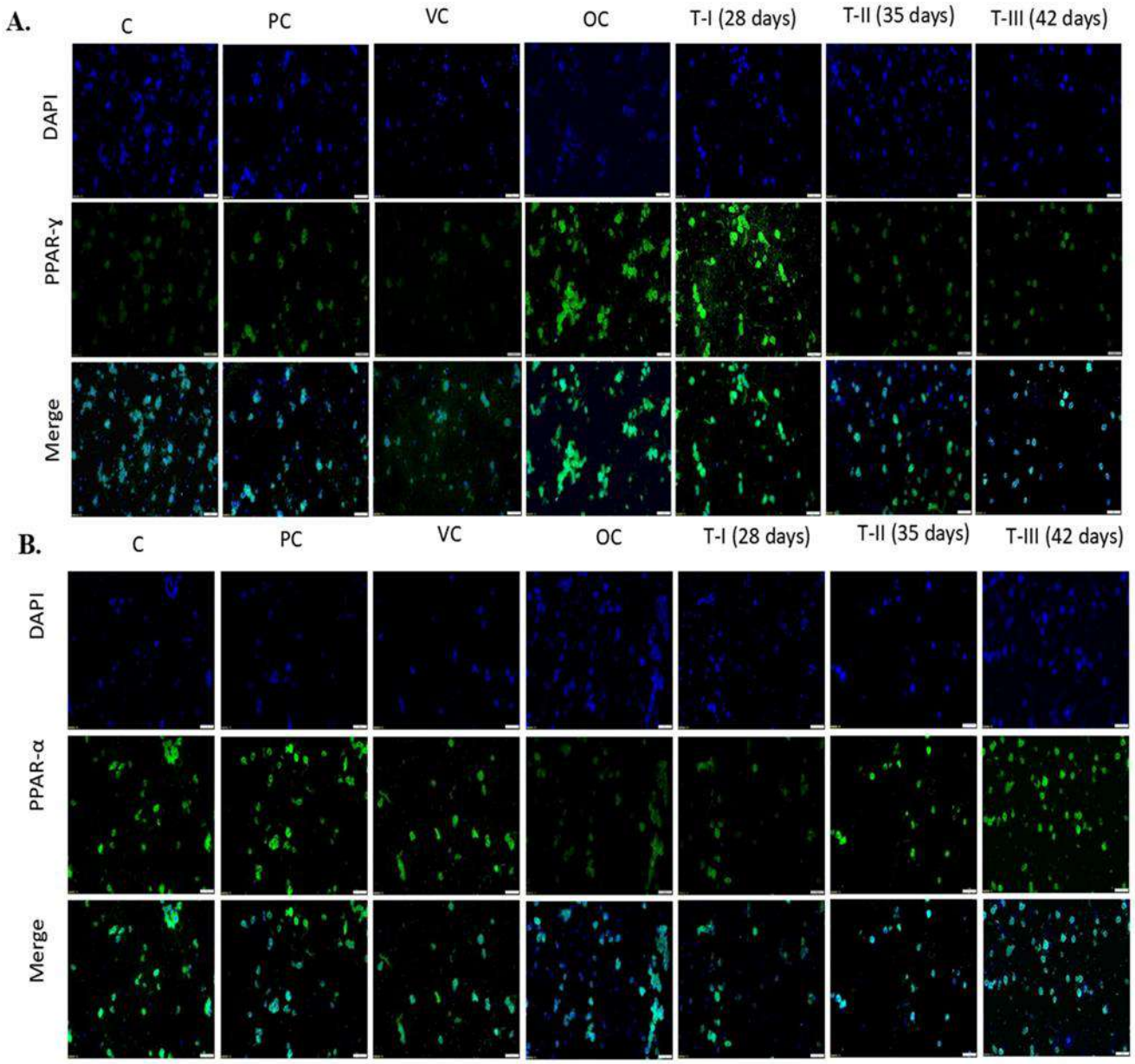


Figure 4. Representative immunofluorescence images showing the expression and localization of (A) PPAR-ɣ (green fluorescence) and cell nuclei (DAPI, blue fluorescence) in liver tissue sections. The bottom row displays merged images of DAPI and PPAR-ɣ staining, (B) PPAR-α (green fluorescence) and cell nuclei (DAPI, blue fluorescence) in liver tissue sections. The bottom row displays merged images of DAPI and PPAR-α staining.

Additionally, immunofluorescence labelling was used in Figure 4B to assess the expression of PPAR-α (green fluorescence) in the liver tissues of several experimental groups. The findings showed that DAPI nuclear staining (blue fluorescence) was consistently present in all samples from the control and treatment groups, indicating the existence of live cells under all circumstances. However, the OC group shows significantly lower levels of green fluorescence,

indicating a decrease in PPAR-α expression in the liver tissue of obese mice. The merged images confirm the localization of PPAR-α within or close to the cell nuclei in all groups, with the most prominent overlap visible in the T-II and T-III treatment groups as similar as control fed groups.

GA's mechanistic impacts on lipid metabolism and adipogenesis through gene expression in HFD-induced obesity

As represented in **Figure 5**, to investigate the anti-obesity impact of GA (4mg/kg bd wt) at the molecular level, we analysed the expression levels of AMPK, leptin, adiponectin, SREBP-1c, PPAR-γ, PPAR-α, CEBP-α, FAS, GLUT-4, GLUT-3, GLUT-1, MCP-1, CRP, IL-6 and TNF-α in liver tissue. The activities of metabolic-regulating genes AMPK, leptin, adiponectin was significantly upregulated by 1.79-2.58 -fold in the GA intervention group (T-III), as shown in **Figure 5a-c**. PPAR-α mRNA in liver was greatly elevated by 1.49-1.77- fold in the mice (T-III group) fed with a 4 mg of GA with the duration of 35-42 days **(Figure 5f)**, on the other hand FAS expression have decreased 1.33-2.96-fold f in GA treated group compared with HFD induced OC group **(Figure 5h)**. Transcription factor genes that control lipid metabolism and adipocyte development include SREBP-1c (1.02-2.25-fold), PPAR-ɣ (1.01-1.19-fold), and CEBP-α (2.03-2.94-fold) levels were downregulated gradually in GA supplemented groups (T-I, II, III) **(Figure 5d, e, g)**. Following 4 mg GA treatment (T-III), upregulated the expression of class I glucose transporter genes such as GLUT-4, GLUT-3, GLUT-1 by 1.81-fold, 1.58-fold, 1.67- fold illustrated in **Figure 5i, j, k**. In this investigation, HFD induced obese group was elevated MCP-1 by 2.56-fold, where GA received group diminished the expression by 1.60-2.38-fold respectively **(Figure 5l)**. Furthermore, the mRNA expression of related proinflammatory markers including CRP, IL-6 and TNF-α were downregulated by 1.37-1.64 -fold, 1.11-1.25-fold, and 1.65-2.70-fold in T-III group after supplemented with 4 mg GA **(Figure 5m, n, o)**.

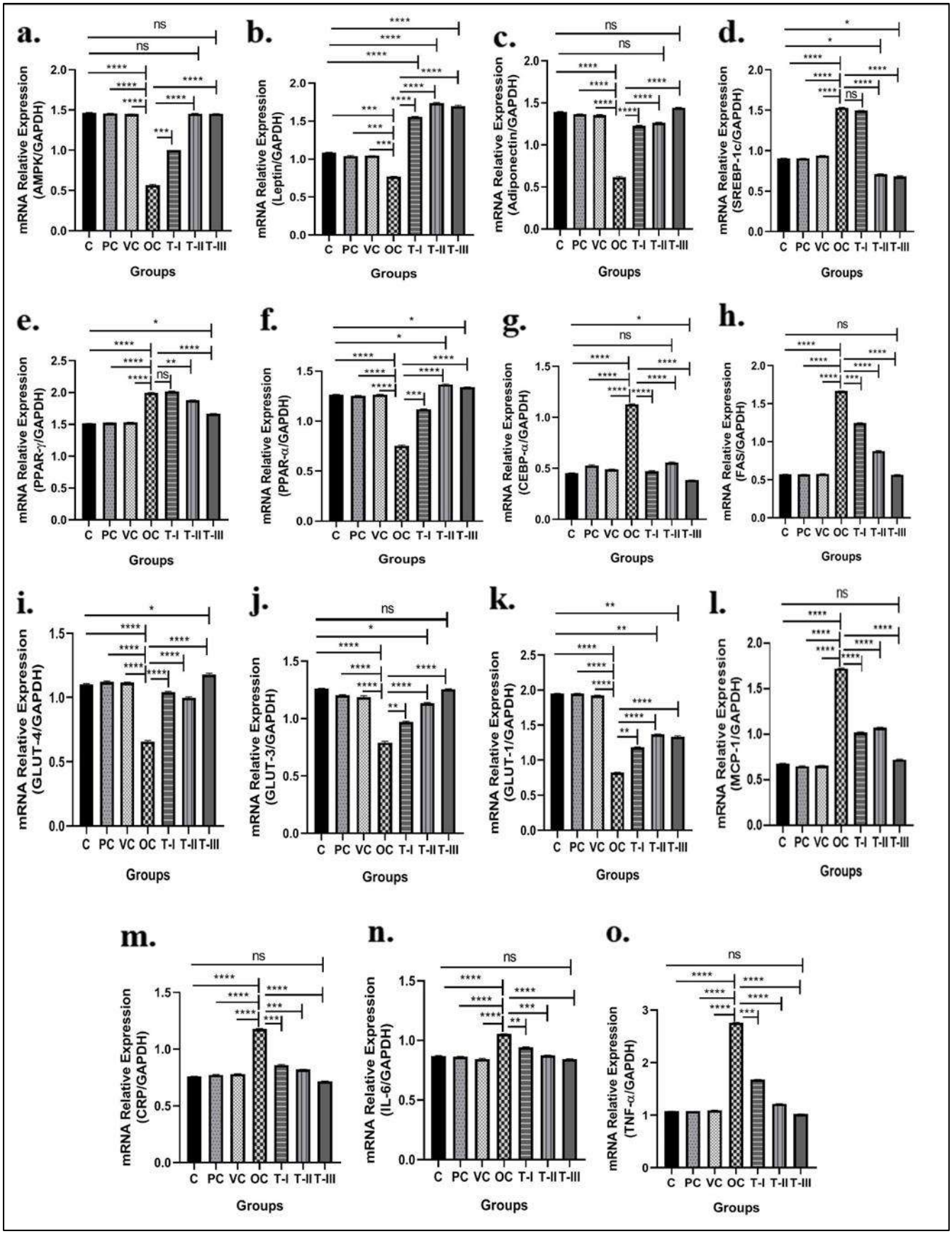

a.
mRNA Relative Expression (AMPK/GAPDH)
b.
mRNA Relative Expression (Leptin/GAPDH)
c.
mRNA Relative Expression (Adiponectin/GAPDH)
d.
mRNA Relative Expression (SREBP-1c/GAPDH)
e.
mRNA Relative Expression (PPAR-γ/GAPDH)
f.
mRNA Relative Expression (PPAR-α/GAPDH)
g.
mRNA Relative Expression (CEBP-α/GAPDH)
h.
mRNA Relative Expression (FAS/GAPDH)
i.
mRNA Relative Expression (GLUT-4/GAPDH)
j.
mRNA Relative Expression (GLUT-3/GAPDH)
k.
mRNA Relative Expression (GLUT-1/GAPDH)
l.
mRNA Relative Expression (MCP-1/GAPDH)
m.
mRNA Relative Expression (CRP/GAPDH)
n.
mRNA Relative Expression (IL-6/GAPDH)
o.
mRNA Relative Expression (TNF-α/GAPDH)
C PC VC OC T-I T-II T-III
Groups

Figure 5. Effects of GA intervention on obesogenic markers (a) AMPK; (b) leptin; (c) Adiponectin; (d) SREBP-1c; (e) PPAR-γ; (f) PPAR-α; (g) CEBP-α; (h) FAS; (i) GLUT-4; (j) GLUT-3; (k) GLUT-1; (l) MCP-1; (m) CRP; (n) IL-6; (o) TNF-α in different experimental groups (n=6). Data are represented as mean ±SEM. Statistical analysis assessed via one way ANOVA followed by Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05.

GA enhanced the expression of proteins responsible for mitochondrial biogenesis, functioning, and fatty acid oxidation in the liver of HFD-induced obese mice

We also analysed the protein content involved in the regulation of mitochondrial metabolism such as PGC1α, AMPK, Ob(H-5), adiponectin, SREBP-1c, PPAR-ɣ, PPAR-α, CEBP-α, FAS, GLUT-4, GLUT-3, and GLUT-1 **(Figure 6)**. The HFD reduced PGC1α expression, a known co-activator of PPAR-α, which improve mitochondrial biogenesis. GA supplementation (4mg/kg bd wt) eliminated the modifications in the expression of PGC1-α and PPAR-α by 1.20-fold and 1.18-fold in treatment group(T-III) **(Figure 6b, h)**. The expression levels of AMPK, Ob(H-5) and adiponectin were increased gradually by1.05-1.36-fold, 1-1.13-fold and 1.22-1.45-fold in all the treated groups **(Figure 6c, d, e)**. HFD-up regulated protein expressions of SREBP-1c, PPAR-ɣ, CEBP-α, and FAS in OC group; however, the expression were significantly down regulated in T-III (1.19-fold, 1.11-fold and 1.13-fold) group, which could be also improved by administration of GA **(Figure 6 f, g, i, j).** Our findings confirmed that GA markedly increased the levels of GLUT-4, GLUT-3 and GLUT-1 protein expression, by 1-1.17-fold demonstrating that it can induce glucose metabolism in the treatment groups. The maximum inhibitory effects on adipogenesis were noted in T-III group, that supplemented with 4mg GA for 42 days of duration.

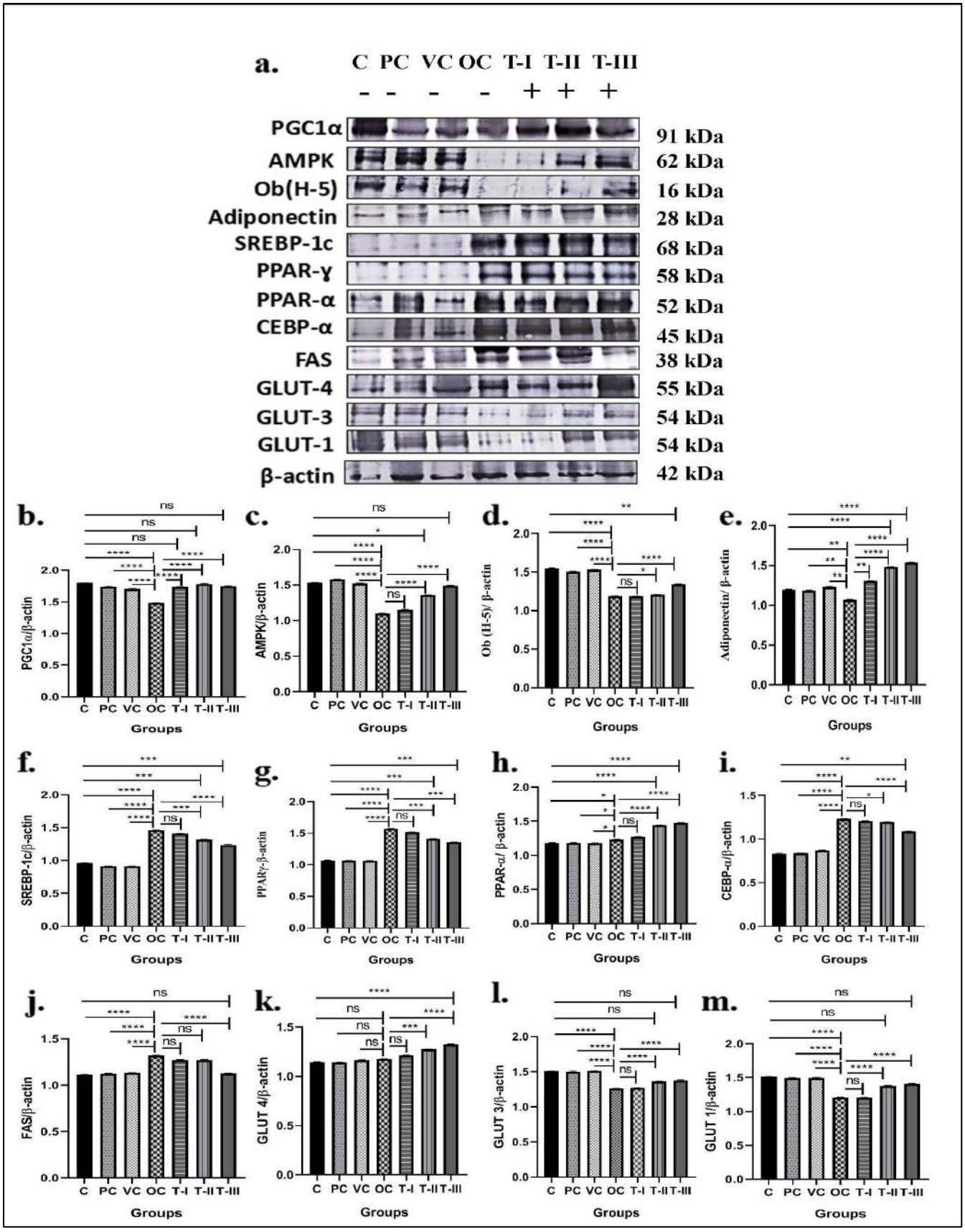


Figure 6. GA enhances mitochondrial biogenesis *in vivo* model. (a) Relative protein expression, (b) PGC1-α; (c) AMPK; (d) Ob (H-5); (e) adiponectin; (f) SREBP-1c; (g) PPAR-

γ; (h) PPAR-α; (i) CEBP-α; (j) FAS; (k) GLUT-4; (l) GLUT-3; (m) GLUT-1 in different experimental groups (n=6). Data are represented as mean ±SEM. Statistical analysis assessed via one way ANOVA followed by Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05.

Discussion:

This investigation provided novel perspectives into the mechanisms underlying GA's antiobesity impact. This research assessed the anti-obesity effects of a novel unexplored MUFA (GA) characterized from marine *P. paradiseus* FO **(Kar et al., 2025)**, in an obese mouse model generated by a HFD, with an emphasis on molecular pathways and metabolic indicators. Although Kar et al. (**2026)** have previously reported the anti-obesity impacts of GA, nothing is known about the molecular processes behind its metabolic advantages. GA treatment in a selective dose (4 mg/kg bd wt) with duration (42 days) may offer protection against obesity and related metabolic problems; these benefits were achieved by increasing fatty acid oxidation in the WAT of mice with HFD-induced obesity and 3T3-L1 adipocytes, as well as mitochondrial biogenesis in the liver. Research reveals that obesity causes a high possibility for develop several metabolic abnormalities such as T2D, hypertension, cardiovascular diseases, cancer, and non-alcoholic fatty liver disease **(Das et al., 2025a)**.

Our investigations revealed that HFD + GA received mice demonstrated a decrease in weight gain, BMI, and peripheral organ weight (WAT, liver and kidney), particularly in the T-II and T-III groups compared with that of HFD-fed mice. This outcome was in line with earlier studies showing canagliflozin's anti-obesity activities **(Wei et al., 2020)**. Moreover, GA treatment increased the weight of BAT, which is considered to perform a key role in energy expenditure and thermogenesis **(Carpentier et al., 2018)**. The liver index was also significantly reduced in GA-treated groups, indicating a potential hepatoprotective effect of GA. Lee's index, a marker of obesity, was significantly reduced in GA-treated groups, further supporting the improvement of metabolic health in obese mice by the anti-obesity effect of GA **(Figure 1)**. Obesity is also defined as abnormal AT buildup, dyslipidemia, decreased glucose tolerance, and high fasting insulin levels **(Kopelman 2000)**. Hypertriglyceridemia is caused by the chylomicron deposition of VLDL or both lipoproteins in the bloodstream, which leads to a drop in HDL levels **(Jorgensen et al., 2014)**. Therefore, high serum concentrations of TG and TC are the essential parameters for assessing obesity may indicate the presence of metabolic changes in AT and liver **(de Moura E et al., 2021).** Several studies reported that higher values of

glycaemic markers such as blood glucose **(Brahma Naidu et al., 2016),** insulin concentrations, **(Heo & Chong 2018)** and HOMA-IR index **(Rocha-Rodrigues et al., 2018)** were detected in groups that received an obesogenic diet. Our findings demonstrated that the GA treatment significantly ameliorate serum lipid profiles, fasting insulin levels, insulin sensitivity (as measured by HOMA-IR) and glucose tolerance in HFD-fed mice **(Figure 2)**. These findings highlight the GA's therapeutic value in addressing not only gaining weight but also other metabolic problems correlated with obesity. The present study demonstrates that GA, a lesser-known MUFA, mitigates lipid buildup in the liver and reduces adipocyte size in WAT (subcutaneous & visceral fat) of HFD-induced obese mice. The observed improvements in liver morphology, with a return to normal colour in GA-supplemented groups gradually (T-I, T-II, T-III), reduced hepatic TG and hepatic TC levels suggest a reduction in hepatic steatosis, a common feature of obesity-related metabolic dysfunction **(Figure 3)**. Our findings highlight the GA's therapeutic potential in treating obesity-related metabolic problems. These findings align with studies showing that MUFAs, such as oleic acid, can improve liver health by reducing ATs expansion, lipid accumulation and inflammation **(Piccinin et al., 2019; Carpentier 2006)**. According to present research by Dragano et al. **(2024)**, LC- fatty acids activate free fatty acid receptors 1 (FFAR1), which raises energy expenditure and encourages WAT browning, both of which aid in weight loss. In the current study, GA-induced FFAR1 activation results in increased thermogenesis in BAT and decreased inflammation in the T-III group **(Figure 3)**. Body fat deposition is determined by the combination of genes and metabolic processes. The consumption of a HFD resulting the impair development and distribution of obesity regulatory hormones, such as leptin, adiponectin **(Miranda et al., 2018)**. Leptin resistance can also result from a rise in serum leptin concentrations brought on by the buildup of abdominal fat **(de Moura e Dias et al., 2021)**. Furthermore, modest levels of circulating adiponectin are thought to be a separate risk indicator for endothelial cell damage **(Tortosa-Caparrós et al., 2017)**. Treatment with GA (4 mg/kg body weight) significantly modulated the expression of crucial metabolic regulators such as leptin, adiponectin in liver tissue **(Figure 5b, c)**. The energy-sensing enzyme *AMPK* regulates many physiological processes, such as the metabolism of fats and carbohydrates, maintaining energy balance. Insulin sensitivity is enhanced when AMPK activity is stimulated, however insulin resistance is linked to persistent reductions in AMPK activity in obesity **(Ruderman et al., 2013)**. However, ectopic fat buildup arises as a result of downregulation of *AMPK* **(Yuan et al., 2018)** and upregulation of SREBP-1c **(Pan et al., 2017)**, it stimulates lipogenesis and increases the production of fatty acids by the liver. However, beta-oxidation is down regulated, which raises the hepatic lipid stores

(Huang et al., 2015). Hence, the reduction in this parameter is positive for the treatment of obesity, with the expression of lipogenic genes (SREBP-1c, CEBP-α, PPAR-ɣ and FAS) conditioned **(de Moura E et al., 2021)**. At the molecular level, 4 mg dose of GA downregulated above mentioned key adipogenic and lipogenic genes (SREBP-1c, CEBP-α, PPAR-ɣ and FAS) **(Figure 5d, g, e, h)** and upregulated the fatty acid oxidation gene PPAR-α **(Figure 5f)**, particularly at the duration of 42 days. GA upregulated the expression of AMPK suggesting enhanced energy metabolism and improved insulin sensitivity. Additionally, GA enhanced the expression of glucose transporters GLUT-4, GLUT-3, and GLUT-1, suggesting improved glucose uptake and utilization **(Figure 5 i, j, k)**. In comparison to mice fed an SFA-rich diet, studies conducted on mice grown on a MUFA-rich diet for 15 weeks reveal greater circulation of anti-inflammatory markers and reduced levels of inflammatory markers **(Tamer et al., 2020)**. Obesity is linked with long term low-grade inflammation where our findings supported by the downregulation of proinflammatory markers MCP-1, CRP, IL-6, and TNF-α, highlighting GA's potential in mitigating obesity-associated inflammation **(Figure 5l, m, n, o)**. The browning of WAT engaged the differentiation of beige adipocytes, mitochondrial synthesis, non-shivering thermogenesis, and fatty acid oxidation elevated by several transcription factors, such as PGC-1α, AMPK, PPAR-α and downregulated by PPAR-γ **(Zhang et al., 2025; Martins et al., 2018)**. GA can promote mitochondrial biogenesis, enhance mitochondrial oxidative capacity, and boost beta oxidation by activating several proteins via the modulation of transcriptional factor activities (PGC-1α, AMPK, adiponectin, PPAR-α) and decrease the adipogenic proteins (SREBP-1c, PPAR-γ, CEBP-α and FAS) which responsible for induced adipogenesis. Side by side GA can improve the expression of insulin signalling protein such as GLUT-4, GLUT-3, GLUT-1 to maintaining glucose uptake **(Figure 6)**. In summary, GA (4mg/kg body weight/ day) administration substantially reversed the HFD-induced obesity, insulin resistance, and mitochondrial dysfunction by enhancing glucose metabolism, insulin signalling, and mitochondrial function in liver and AT.

Conclusion

In this present investigation, GA-treated BALB/c mice fed a HFD showed decreased body weight, boosted dyslipidemia, lessened hepatic steatosis, and increased sensitivity to glucose in liver and AT. Concentrating on the impact of n-9, GA on adipogenic and inflammatory-related adipokine levels, they could prove beneficial in early nutritional programming, performing as a preventive potential candidate against diseases characterized by obesity, low-degree chronic inflammation, diabetes and metabolic syndrome by targeting multiple

mechanism enhancing fat oxidation, reducing lipogenesis, improving glucose metabolism, and curbing inflammation. Given its selective modulation of metabolic genes, further exploration of GA's therapeutic potential in human models, is warranted.

Underlying mechanism of GA to reduce obesity

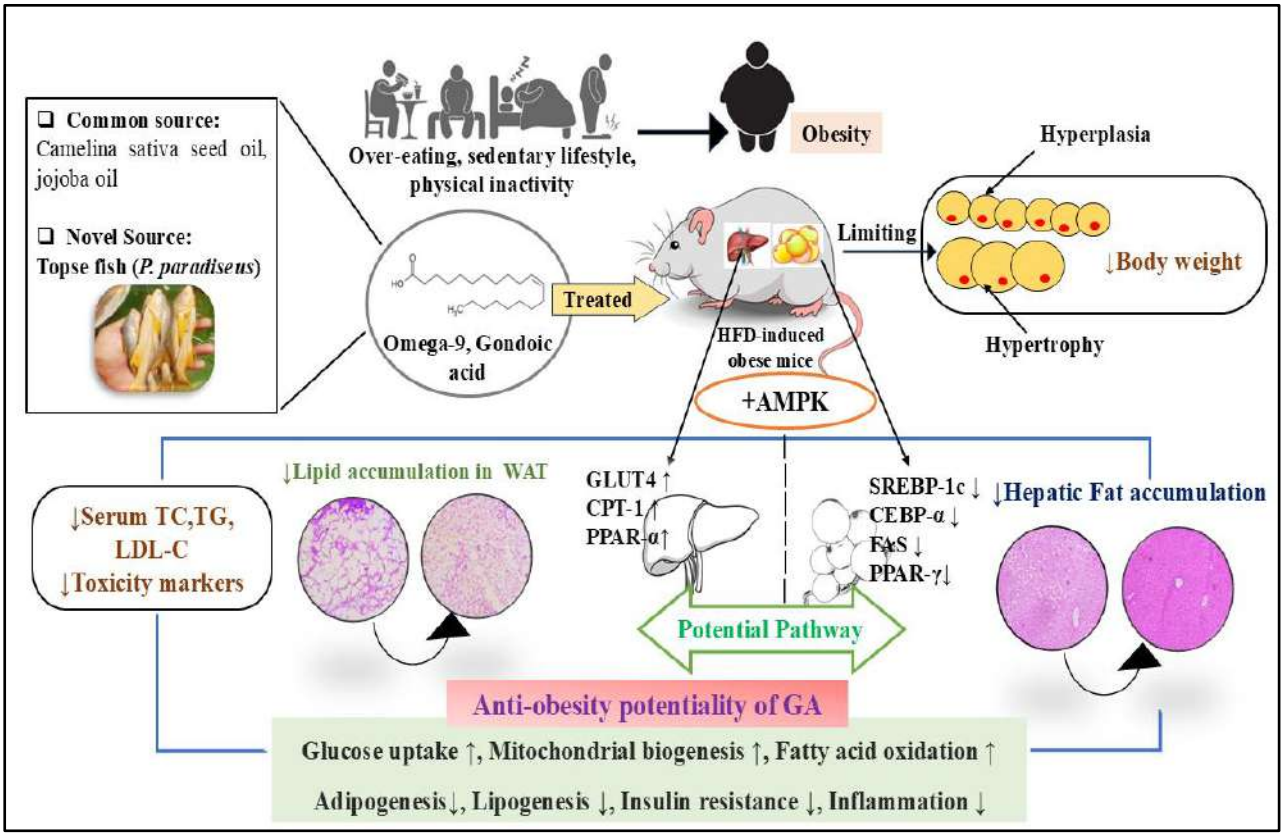


Figure 7. Effects of n-9 GA on obesity and associated disorders during *in vivo* studies. Numerous molecular and inflammatory processes that affect metabolism are influenced by obesity and its related conditions, including insulin resistance, dyslipidaemia, metabolic inflammation, dysbiosis, and non-alcoholic hepatic steatosis. GA increase β-oxidation in the liver and adipose tissue, thus limiting hypertrophy and hyperplasia via activating AMPK in these tissues. GA also decrease the production and release of lipogenic genes and proinflammatory adipokines. AMPK is one of the possible mechanisms that is shown here that could help with a better metabolic profile.

Chapter VIII: Summary & Conclusion

Summary & Conclusion

Objective (i): Extraction, isolation, and characterization of fatty acids (FAs) from *P. paradiseus* FO.

The current research investigation significantly isolated and characterised FO from *Polynemus paradiseus* using the FAME method, providing detailed information about its fatty acid composition and physicochemical properties. The extraction produced 2.75 ± 1.03 ml of crude FO from 100 g of raw fish. According to GC-MS and GC-FID study, *P. paradiseus* oil includes nine SFAs (23.81%), eleven PUFAs (51.15%), and nine MUFAs (25.04%), all of which are well-known for their cardioprotective, anti-inflammatory, anti-diabetic, anti-obesity, and neuroprotective properties. The oil's physicochemical quality measures remained within the acceptable ranges established by Codex Alimentarius standards. The acid value (4.01 ± 0.12 mg KOH/g), peroxide value (3.95 ± 0.15 mEq/kg), p-anisidine value (0.46 ± 0.19), and totox value (8.36 ± 0.27) exhibit good oxidative stability and freshness. Side by side the saponification value (145.98 ± 2.23 mg KOH/g) and iodine value (131.33 ± 3.89 mg) indicate the existence of long-chain fatty acids and high unsaturation levels of *P. paradiseus* FO. To summarise, *P. paradiseus* FO has high nutritional quality and stability, making it a promising natural source of important PUFAs and MUFAs for human consumption. To investigate its commercial and therapeutic applications, more research on purification, bioactivity assessment, and product development is recommended.

Objective (ii): To search out the anti-obesity potentiality of *P. paradiseus* FO *in vivo* model

Our second goal was to illustrate that *P. paradiseus* FO, which is high in n-3 PUFAs, n-6 PUFAs, and MUFAs, has significant anti-obesity and hypolipidemic advantages in male BALB/c mice fed HFD. In comparison to the OC group, oral FO administration at 200 mg and 400 mg/kg body weight/day successfully decreased body weight, BMI, and WAT weight gradually. Alongside this, there was an improvement in the serum lipid profile, with lower levels of TC, TG, LDL-C, VLDL-C, and glucose and higher levels of HDL-C, suggesting a reduction in the dyslipidemia and insulin resistance brought on by a HFD. *P. paradiseus* FO supplementation decreased hepatic steatosis, adipocyte hypertrophy in subcutaneous and visceral WAT, based on histological examination. Mechanistically, *P. paradiseus* FO altered the function of crucial proteins and genes related to the metabolism of glucose and fats. In the liver and AT, it upregulated β-oxidation regulators CPT-1, LPL, PPAR-α, and PGC1α while downregulating lipogenic proteins SREBP-1c, FAS, and PPAR-γ. In addition, *P. paradiseus*

FO reduced TNF-α, IL-6, and iNOS expression and increased anti-inflammatory IL-10 and IL-1Ra to inhibit obesity-associated inflammation and recover adipokine balance by raising leptin and adiponectin. The duration-dependent experiment also showed that *P. paradiseus* FO at a dose of 400 mg/kg body weight/day for 21–28 days was the most successful in lowering adiposity, enhancing hepatic and plasma lipid parameters, and recovering the liver and kidney function markers SGOT, SGPT, urea, and uric acid to normal. All of these results point to *P. paradiseus* FO as a powerful functional lipid that improves insulin sensitivity, suppresses lipogenesis, increases fatty acid oxidation, and lowers inflammation to reduce obesity and metabolic syndrome. For the management of obesity and correlated metabolic syndromes, *P. paradiseus* oil exhibits encouraging candidate as a dietary supplement or nutraceutical.

Objective (iii): To establish the anti- obesity potentiality of isolated FA both *in vitro* and *in vivo* model.

The present research displays GA, an n-9 MUFA abundant in *P. paradiseus* FO, as an effective anti-adipogenic and anti-obesity drug using complementing *in vitro* and *in vivo* methods. GA treatment dramatically reduced triglyceride content and lipid accumulation in 3T3-L1 preadipocytes without creating cytotoxicity. By increasing AMPK activation and downregulating the transcription factors PPAR-γ, CEBP-α, and SREBP-1c as well as their lipogenic target FAS, GA suppressed adipogenesis. It increased energy expenditure and fatty acid oxidation by upregulating PPAR-α, LPL, CPT-1, and GLUT-4. It additionally enhanced insulin sensitivity by boosting adiponectin and insulin mRNA levels. Furthermore, GA reduced inflammation by increasing the expression of anti-inflammatory IL-10 and IL-1Ra while decreasing TNF-α and IL-6. Oral GA supplementation at varying doses (1,2,4 mg/kg body weight/day) successfully decreased body weight growth, BMI, and vWAT and sWAT mass in HFD-induced obese BALB/c mice. GA boosted central metabolic profiles by decreasing serum glucose, TC, TG, LDL, VLDL, urea, uric acid, SGOT, and SGPT while enhancing HDL and insulin levels. Histological examination indicated the reduction of liver steatosis and adipose tissue enlargement. Together, these results show for the very first time that GA drastically decreases obesity and hyperlipidemia by promoting adipocyte browning and lipolysis, suppressing adipocyte differentiation (SREBP-1c, FAS, PPAR-γ, CEBP-α), boosting insulin responsiveness, elevating fatty acid oxidation (AMPK, PPAR-α, LPL, CPT-1), and reducing inflammation through AMPK-mediated pathways. The most significant benefits were seen at the ideal dosage of 4 mg/kg body weight/day. For the avoidance and management of obesity

and associated metabolic diseases, GA is a potentially useful dietary bioactive molecule derived from marine sources.

Objective (iv): To explore the role of isolated FA in alleviating obesity related metabolic disorder *in vivo* model.

The present objective shows that taking GA orally at a dose of 4 mg/kg body weight per day for 42 days considerably diminished body weight gain, BMI, Lee's index, and the weights of WAT and liver while raising BAT mass, which indicates increased thermogenesis and energy utilization. GA significantly enhanced metabolic state by reducing serum glucose, TC, TG, LDL-C, VLDL-C, insulin, HOMA-IR, TNF-α, and IL-6 levels while increasing HDL-C levels. This indicates that GA can alleviate HFD-induced dyslipidemia, insulin resistance, and inflammation. GA upregulated major metabolic pathways, including AMPK, PPAR-α, PGC-1α, adiponectin, leptin, and glucose transporters (GLUT-1, GLUT-3, GLUT-4), improving fatty acid oxidation, mitochondrial biogenesis, and uptake of glucose. At the same time, GA decreased pro-inflammatory markers MCP-1, CRP, IL-6, and TNF-α and downregulated lipogenic and adipogenic regulators SREBP-1c, CEBP-α, PPAR-γ, and FAS. In combination, these results demonstrate that GA is a novel bioactive MUFA that reduces inflammation, improves insulin sensitivity, promotes WAT browning and mitochondrial function, and inhibits adipogenesis and lipogenesis through AMPK-dependent mechanisms. GA is therefore a viable dietary supplement for the treatment and prevention of non-alcoholic fatty liver disease, obesity, and insulin resistance.

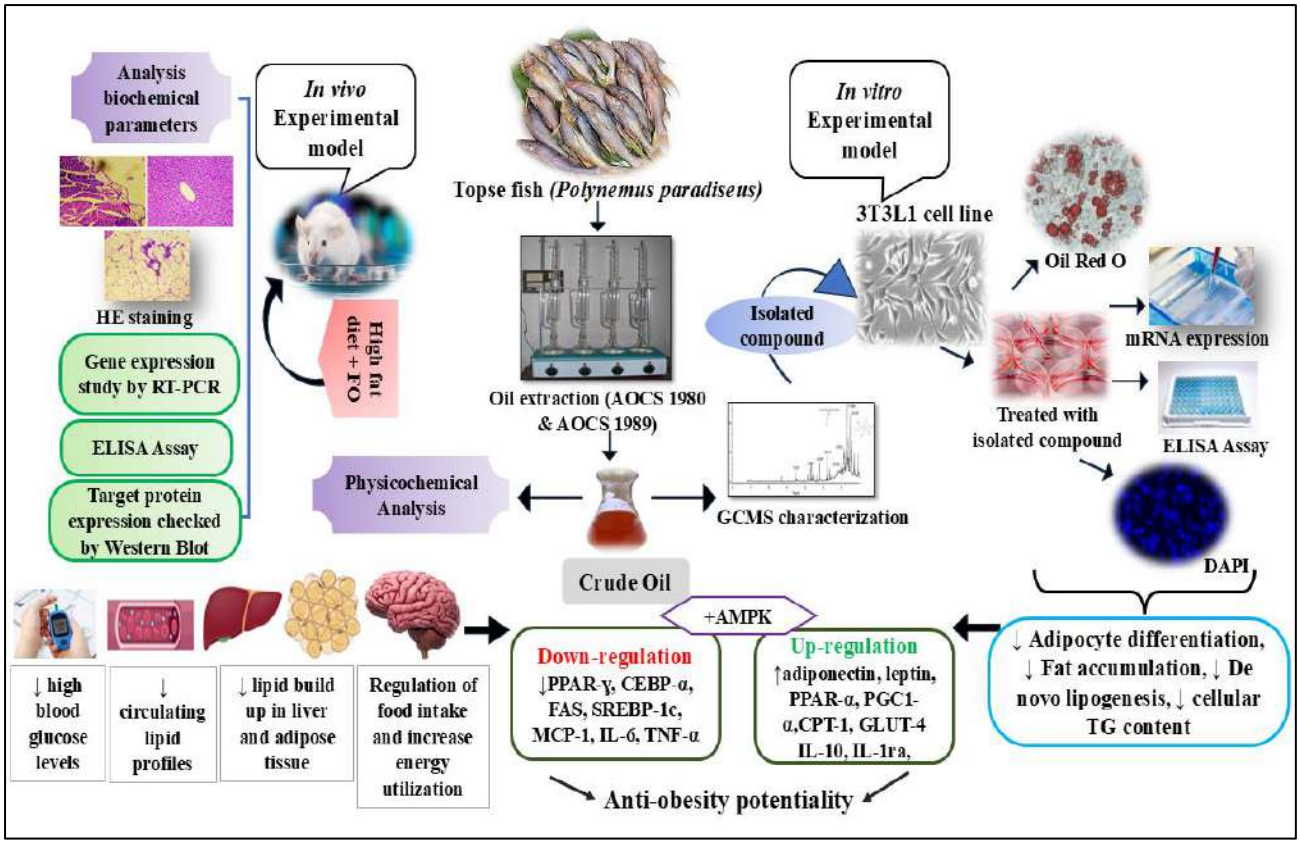


Figure 1. Exploration of antiobesity efficacy of marine *P. paradiseus* oil and its outcomes via underlying biological mechanism *in vitro* and *in vivo* study.

Chapter IX: Future Direction

Future Direction

- Considering the adverse effects of marketed drugs such as lorcaserin, orlistat, topiramate, liraglutide, there is an urgent need of alternative way with minimal side effects to combat obesity. The current evaluation makes it clear that *P. paradiseus* FO containing PUFAs and MUFAs, are the valuable and successful area of study with a promising future that help enhance our overall health.

- Following our current research we already established the anti-obesity potentiality of *P. paradiseus* FO as well as FO characterized gondoic acid *in vivo* and *in vitro* model. Given its selective modulation of metabolic genes, further exploration of GA's therapeutic potential in human models, is warranted. Next, we will develop a nutraceutical formulation with the collaboration of any pharmaceutical company to minimize the risk of obesity against the commercial drugs.

- By producing fish oil-based nutraceuticals, it also opens doors for regional economic growth as well as mitigate the global burden of obesity and metabolic abnormalities. Raising awareness of the health advantages of *P. paradiseus* fish can help local fisheries as well as local people, induce dietary modifications for better health outcomes, and increase the consumption of the fish in their daily diet.

Chapter X: Bibliography

Bibliography

Aguilar, M., Bhuket, T., Torres, S., Liu, B., & Wong, R. J. (2015). Prevalence of the metabolic syndrome in the United States, 2003-2012. *JAMA*, *313*(19), 1973–1974. https://doi.org/10.1001/jama.2015.4260

Ahirwar, R., & Mondal, P. R. (2019). Prevalence of obesity in India: A systematic review. *Diabetes & metabolic syndrome*, *13*(1), 318–321. https://doi.org/10.1016/j.dsx.2018.08.032

Aitta, E., Marsol-Vall, A., Damerau, A., & Yang, B. (2021). Enzyme-Assisted Extraction of Fish Oil from Whole Fish and by-Products of Baltic Herring (*Clupea harengus membras*). *Foods (Basel, Switzerland)*, *10*(8), 1811. https://doi.org/10.3390/foods10081811

Aiyar, A., Rahman, A., & Pingali, P. (2021). India's rural transformation and rising obesity burden. *World Development*, *138*, 105258. https://doi.org/10.1016/j.worlddev.2020.105258

Albracht-Schulte, K., Kalupahana, N. S., Ramalingam, L., Wang, S., Rahman, S. M., Robert-McComb, J., & Moustaid-Moussa, N. (2018). Omega-3 fatty acids in obesity and metabolic syndrome: a mechanistic update. *The Journal of nutritional biochemistry*, *58*, 1–16. https://doi.org/10.1016/j.jnutbio.2018.02.012

Antuna-Puente, B., Feve, B., Fellahi, S., & Bastard, J. P. (2008). Adipokines: the missing link between insulin resistance and obesity. *Diabetes & metabolism*, *34*(1), 2–11. https://doi.org/10.1016/j.diabet.2007.09.004

AOAC. (1980). In W. Horwitz (Ed.), Official methods of analysis (13th ed., p. 1038). Arlington, VA: The Association of Official Analytical Chemists.

AOCS. (1989). In D. Firestone (Ed.), Official methods and recommended practices of the American Oil Chemists Society: Method Cd 3a-63. Champaign, IL: AOCS Press.

Arai, T., Kim, H. J., Chiba, H., & Matsumoto, A. (2009). Anti-obesity effect of fish oil and fish oil-fenofibrate combination in female KK mice. *Journal of atherosclerosis and thrombosis*, *16*(5), 674–683. https://doi.org/10.5551/jat.1313

Arçari, D. P., Bartchewsky, W., dos Santos, T. W., Oliveira, K. A., Funck, A., Pedrazzoli, J., de Souza, M. F., Saad, M. J., Bastos, D. H., Gambero, A., Carvalho, P.deO., & Ribeiro, M. L. (2009). Antiobesity effects of yerba maté extract (Ilex paraguariensis) in high-fat diet-induced

obese mice. *Obesity (Silver Spring, Md.)*, *17*(12), 2127–2133. https://doi.org/10.1038/oby.2009.158

Arterburn, D. E., & Courcoulas, A. P. (2014). Bariatric surgery for obesity and metabolic conditions in adults. *BMJ (Clinical research ed.)*, *349*, g3961. https://doi.org/10.1136/bmj.g3961

Balwan, W. K., & Saba, N. (2021). Study of role of fish oil in human health. *Global Academic Journal of Medical Sciences*, *3*(1), 14-18. DOI: 10.36348/gajms.2021.v03i01.002

Banerjee, M., Gupta, S., Sharma, P., Shekhawat, J., & Gauba, K. (2020). Obesity and COVID-19: A Fatal Alliance. *Indian journal of clinical biochemistry : IJCB*, *35*(4), 410–417. https://doi.org/10.1007/s12291-020-00909-2

Basuny, A. M. (2025). Healthy omega-9 fats in diets: Nutritional importance, food sources, and sustainable health benefits. *Transnational Journal of Agriculture, Environment and Sustainability*, *1*(1). https://journals.novapexpublishers.com/sustainable-agriculture/article/view/2

Bates, S. H., & Myers, M. G., Jr (2003). The role of leptin receptor signaling in feeding and neuroendocrine function. *Trends in endocrinology and metabolism: TEM*, *14*(10), 447–452. https://doi.org/10.1016/j.tem.2003.10.003

Bijland, S., Mancini, S. J., & Salt, I. P. (2013). Role of AMP-activated protein kinase in adipose tissue metabolism and inflammation. *Clinical science (London, England: 1979)*, *124*(8), 491–507. https://doi.org/10.1042/CS20120536

Blüher, M., Fasshauer, M., Tönjes, A., Kratzsch, J., Schön, M. R., & Paschke, R. (2005). Association of interleukin-6, C-reactive protein, interleukin-10 and adiponectin plasma concentrations with measures of obesity, insulin sensitivity and glucose metabolism. *Experimental and clinical endocrinology & diabetes: official journal, German Society of Endocrinology [and] German Diabetes Association*, *113*(9), 534–537. https://doi.org/10.1055/s-2005-872851

Blüher, M., Klöting, N., Wueest, S., Schoenle, E. J., Schön, M. R., Dietrich, A., Fasshauer, M., Stumvoll, M., & Konrad, D. (2014). Fas and FasL expression in human adipose tissue is related to obesity, insulin resistance, and type 2 diabetes. *The Journal of clinical endocrinology and metabolism*, *99*(1), E36–E44. https://doi.org/10.1210/jc.2013-2488

Bonnefont, J. P., Djouadi, F., Prip-Buus, C., Gobin, S., Munnich, A., & Bastin, J. (2004). Carnitine palmitoyltransferases 1 and 2: biochemical, molecular and medical aspects. *Molecular aspects of medicine*, *25*(5-6), 495–520. https://doi.org/10.1016/j.mam.2004.06.004

Brahma Naidu, P., Uddandrao, V. V., Ravindar Naik, R., Suresh, P., Meriga, B., Begum, M. S., Pandiyan, R., & Saravanan, G. (2016). Ameliorative potential of gingerol: Promising modulation of inflammatory factors and lipid marker enzymes expressions in HFD induced obesity in rats. *Molecular and cellular endocrinology*, *419*, 139–147. https://doi.org/10.1016/j.mce.2015.10.007

Carpentier, A. C., Blondin, D. P., Virtanen, K. A., Richard, D., Haman, F., & Turcotte, É. E. (2018). Brown Adipose Tissue Energy Metabolism in Humans. *Frontiers in endocrinology*, *9*, 447. https://doi.org/10.3389/fendo.2018.00447

Carpentier, Y. A., Portois, L., & Malaisse, W. J. (2006). n-3 fatty acids and the metabolic syndrome. *The American journal of clinical nutrition*, *83*(6 Suppl), 1499S–1504S. https://doi.org/10.1093/ajcn/83.6.1499S

Ceja-Galicia, Z. A., Cespedes-Acuña, C. L. A., & El-Hafidi, M. (2025). Protection Strategies Against Palmitic Acid-Induced Lipotoxicity in Metabolic Syndrome and Related Diseases. *International journal of molecular sciences*, *26*(2), 788. https://doi.org/10.3390/ijms26020788

Chaklader, M. R., Siddik, M. A. B., Ashfaqun Nahar, A. N., Hanif, M. A., Alam, M. J., & Sultan Mahmud, S. M. (2016). Morphometric parameters and allometric growth in paradise threadfin Polynemus paradiseus (Linnaeus, 1758) from a coastal river of Bangladesh. DOI: 10.4172/2155-9546.1000417.

Chakraborty, A., Hegde, S., Praharaj, S. K., Prabhu, K., Patole, C., Shetty, A. K., Mayya, S. S., Acharya, R. V., Hande, H. M., Prabhu, M. M., & Upadhya, D. (2021). Age Related Prevalence of Mild Cognitive Impairment in Type 2 Diabetes Mellitus Patients in the Indian Population and Association of Serum Lipids With Cognitive Dysfunction. *Frontiers in endocrinology*, *12*, 798652. https://doi.org/10.3389/fendo.2021.798652

Chang, H. R., Josefs, T., Scerbo, D., Gumaste, N., Hu, Y., Huggins, L. A., ... & Goldberg, I. J. (2019). Role of LpL (lipoprotein lipase) in macrophage polarization in vitro and in

vivo. *Arteriosclerosis, thrombosis, and vascular biology*, *39*(10), 1967-1985. doi: 312389 10.1161/ATVBAHA.119.

Chen, B. H., Song, Y., Ding, E. L., Roberts, C. K., Manson, J. E., Rifai, N., Buring, J. E., Gaziano, J. M., & Liu, S. (2009). Circulating levels of resistin and risk of type 2 diabetes in men and women: results from two prospective cohorts. *Diabetes care*, *32*(2), 329–334. https://doi.org/10.2337/dc08-1625

Chen, H., Zhang, Z. Q., Zhao, B., Ning, W. L., Wang, X. Y., & Zhou, F. (2017). Clinical significance of ALDH1 combined with DAPI expression in patients with esophageal carcinoma. *Oncology letters*, *14*(4), 4878–4882. https://doi.org/10.3892/ol.2017.6743

Chiu, C. Y., Chang, T. C., Liu, S. H., & Chiang, M. T. (2017). The regulatory effects of fish oil and chitosan on hepatic lipogenic signals in high-fat diet-induced obese rats. *Journal of food and drug analysis*, *25*(4), 919–930. https://doi.org/10.1016/j.jfda.2016.11.015

Chiu, C. Y., Wang, L. P., Liu, S. H., & Chiang, M. T. (2018). Fish Oil Supplementation Alleviates the Altered Lipid Homeostasis in Blood, Liver, and Adipose Tissues in High-Fat Diet-Fed Rats. *Journal of agricultural and food chemistry*, *66*(16), 4118–4128. https://doi.org/10.1021/acs.jafc.8b00529

Choi, H., Eo, H., Park, K., Jin, M., Park, E. J., Kim, S. H., Park, J. E., & Kim, S. (2007). A water-soluble extract from Cucurbita moschata shows anti-obesity effects by controlling lipid metabolism in a high fat diet-induced obesity mouse model. *Biochemical and biophysical research communications*, *359*(3), 419–425. https://doi.org/10.1016/j.bbrc.2007.05.107

Choi, Y. J., Jung, J. I., Lim, S., Kim, C. S., Park, D. W., Lee, S. H., Hur, N., Bae, J., Lee, J. K., & Kim, E. J. (2025). Steamed Ginger Extract (GGE03) Attenuates Obesity and Improves Metabolic Parameters in Association with AMPK Activation and Lipid Metabolism Regulation in High-Fat Diet-Induced Obese Mice. *International journal of molecular sciences*, *26*(18), 8950. https://doi.org/10.3390/ijms26188950

Christiansen, E., Watterson, K. R., Stocker, C. J., Sokol, E., Jenkins, L., Simon, K., Grundmann, M., Petersen, R. K., Wargent, E. T., Hudson, B. D., Kostenis, E., Ejsing, C. S., Cawthorne, M. A., Milligan, G., & Ulven, T. (2015). Activity of dietary fatty acids on FFA1 and FFA4 and characterisation of pinolenic acid as a dual FFA1/FFA4 agonist with potential effect against metabolic diseases. *The British journal of nutrition*, *113*(11), 1677–1688. https://doi.org/10.1017/S000711451500118X

Cohen, P., Miyazaki, M., Socci, N. D., Hagge-Greenberg, A., Liedtke, W., Soukas, A. A., Sharma, R., Hudgins, L. C., Ntambi, J. M., & Friedman, J. M. (2002). Role for stearoyl-CoA desaturase-1 in leptin-mediated weight loss. *Science (New York, N.Y.)*, *297*(5579), 240–243. https://doi.org/10.1126/science.1071527

Colletti, A., Cravotto, G., Citi, V., Martelli, A., Testai, L., & Cicero, A. F. G. (2021). Advances in Technologies for Highly Active Omega-3 Fatty Acids from Krill Oil: Clinical Applications. *Marine drugs*, *19*(6), 306. https://doi.org/10.3390/md19060306

Cui, C., Li, Y., Gao, H., Zhang, H., Han, J., Zhang, D., Li, Y., Zhou, J., Lu, C., & Su, X. (2017). Modulation of the gut microbiota by the mixture of fish oil and krill oil in high-fat diet-induced obesity mice. *PloS one*, *12*(10), e0186216. https://doi.org/10.1371/journal.pone.0186216

D'Angelo, S., Motti, M. L., & Meccariello, R. (2020). ω-3 and ω-6 polyunsaturated fatty acids, obesity and cancer. *Nutrients*, *12*(9), 2751. https://doi.org/10.3390/nu1209275.

da Cunha de Sá, R. D. C., Cruz, M. M., de Farias, T. M., da Silva, V. S., de Jesus Simão, J., Telles, M. M., & Alonso-Vale, M. I. C. (2020). Fish oil reverses metabolic syndrome, adipocyte dysfunction, and altered adipokines secretion triggered by high-fat diet-induced obesity. *Physiological reports*, *8*(4), e14380. https://doi.org/10.14814/phy2.14380

da Cunha de Sá, R. D. C., Simão, J. J., Silva, V. S. D., Farias, T. M., Cruz, M. M., Antraco, V. J., Armelin-Correa, L., & Alonso-Vale, M. I. (2021). Fish Oil Enriched in EPA, but Not in DHA, Reverses the Metabolic Syndrome and Adipocyte Dysfunction Induced by a High-Fat Diet. *Nutrients*, *13*(3), 754. https://doi.org/10.3390/nu13030754

da Cunha de Sá, R. D., Cruz, M. M., de Farias, T. M., da Silva, V. S., de Jesus Simão, J., Telles, M. M., & Alonso-Vale, M. I. C. (2020). Fish oil reverses metabolic syndrome, adipocyte dysfunction, and altered adipokines secretion triggered by high-fat diet-induced obesity. *Physiological Reports*, *8*(4), e14380. https://doi.org/10.14814/phy2.14380.

Das, P., Dutta, A., Panchali, T., Khatun, A., Kar, R., Das, T. K., ... & Pradhan, S. (2024). Advances in therapeutic applications of fish oil: A review. *Measurement: Food*, *13*, 100142. https://doi.org/10.1016/j.meafoo.2024.100142

Das, P., Kar, R., Panchali, T., Dutta, A., Phoujdar, M., Ghosh, K., & Pradhan, S. (2025). Mechanistic insights on effective ratio of Linoelaidic and Docosapentaenoic acid by modulating adipogenic and inflammatory biomarkers in 3T3-L1 preadipocytes. *Advances in Biomarker Sciences and Technology*. https://doi.org/10.1016/j.abst.2025.11.005

Das, P., Kar, R., Panchali, T., Dutta, A., Phoujdar, M., Ghosh, K., & Pradhan, S. (2025a). Mechanistic insights on effective ratio of Linoelaidic and Docosapentaenoic acid by modulating adipogenic and inflammatory biomarkers in 3T3-L1 preadipocytes. *Advances in Biomarker Sciences and Technology*. https://doi.org/10.1016/j.abst.2025.11.005

Das, P., Kar, R., Panchali, T., Dutta, A., Phoujdar, M., Ghosh, K., & Pradhan, S. (2025b). Antiobesity Efficacy of Marine Tapra Fish (Opisthopterus tardoore) Oil in High-Fat Diet-Induced Obese Mice by Activating Lipolysis and Suppressing Adipose Inflammation. *Molecular nutrition & food research*, e70341. Advance online publication. https://doi.org/10.1002/mnfr.70341

Dasgupta, A., Bandyopadhyay, G. K., Ray, I., Bandyopadhyay, K., Chowdhury, N., De, R. K., & Mahata, S. K. (2020). Catestatin improves insulin sensitivity by attenuating endoplasmic reticulum stress: In vivo and in silico validation. *Computational and structural biotechnology journal*, *18*, 464-481. https://doi.org/10.1016/j.csbj.2020.02.005.

Dasilva, G., Pazos, M., García-Egido, E., Gallardo, J. M., Ramos-Romero, S., Torres, J. L., Romeu, M., Nogués, M. R., & Medina, I. (2017). A lipidomic study on the regulation of inflammation and oxidative stress targeted by marine ω-3 PUFA and polyphenols in high-fat high-sucrose diets. *The Journal of nutritional biochemistry*, *43*, 53–67. https://doi.org/10.1016/j.jnutbio.2017.02.007

Dayarathne, L. A., Ko, S. C., Yim, M. J., Lee, J. M., Kim, J. Y., Oh, G. W., ... & Je, J. Y. (2025). Neptunea cumingii Extract Inhibits Adipogenesis and Stimulates Browning of Adipocytes in 3T3-L1 Preadipocytes. *Journal of Food Biochemistry*, *2025*(1), 3632548.

de Moura E Dias, M., Dos Reis, S. A., da Conceição, L. L., Sediyama, C. M. N. O., Pereira, S. S., de Oliveira, L. L., Gouveia Peluzio, M. D. C., Martinez, J. A., & Milagro, F. I. (2021). Diet-induced obesity in animal models: points to consider and influence on metabolic markers. *Diabetology & metabolic syndrome*, *13*(1), 32. https://doi.org/10.1186/s13098-021-00647-2

Delgado, G. E., Krämer, B. K., Lorkowski, S., März, W., von Schacky, C., & Kleber, M. E. (2017). Individual omega-9 monounsaturated fatty acids and mortality-The Ludwigshafen Risk and Cardiovascular Health Study. *Journal of clinical lipidology*, *11*(1), 126–135.e5. https://doi.org/10.1016/j.jacl.2016.10.015

Dongho Dongmo, F. F., Fogang Mba, A. R., Manz Koule, J. C., Njike Ngamga, F. H., Simo Noutsa, B., Ngo Hagbe, D., ... & Gouado, I. (2023). Physicochemical characteristics and nutritional and biological properties of fish oils in Cameroon: an overview. *Journal of Food Quality*, *2023*(1), 7847288. https://doi.org/10.1155/2023/7847288

Dragano, N. R. V., Milbank, E., Haddad-Tóvolli, R., Garrido-Gil, P., Nóvoa, E., Fondevilla, M. F., Capelli, V., Zanesco, A. M., Solon, C., Morari, J., Pires, L., Estevez-Salguero, Á., Beiroa, D., González-García, I., Barca-Mayo, O., Diéguez, C., Nogueiras, R., Labandeira-García, J. L., Rexen Ulven, E., Ulven, T., … López, M. (2024). Hypothalamic free fatty acid receptor-1 regulates whole-body energy balance. *Molecular metabolism*, *79*, 101840. https://doi.org/10.1016/j.molmet.2023.101840

Dranca, F., Ursachi, F., & Oroian, M. (2020). Bee Bread: Physicochemical Characterization and Phenolic Content Extraction Optimization. *Foods (Basel, Switzerland)*, *9*(10), 1358. https://doi.org/10.3390/foods9101358

Dutta, A., Panchali, T., Khatun, A., Jarapala, S. R., Das, K., Ghosh, K., Chakrabarti, S., & Pradhan, S. (2023). Anti-cancer potentiality of linoelaidic acid isolated from marine Tapra fish oil (Ophisthopterus tardoore) via ROS generation and caspase activation on MCF-7 cell line. *Scientific reports*, *13*(1), 14125. https://doi.org/10.1038/s41598-023-34885-3

Edwards, P. A., Tabor, D., Kast, H. R., & Venkateswaran, A. (2000). Regulation of gene expression by SREBP and SCAP. *Biochimica et biophysica acta*, *1529*(1-3), 103–113. https://doi.org/10.1016/s1388-1981(00)00140-2

Elmendorf J. S. (2004). Fluidity of insulin action. *Molecular biotechnology*, *27*(2), 127–138. https://doi.org/10.1385/MB:27:2:127

Engin A. (2017). The Pathogenesis of Obesity-Associated Adipose Tissue Inflammation. *Advances in experimental medicine and biology*, *960*, 221–245. https://doi.org/10.1007/978-3-319-48382-5_9

Esteves, G. P., Manca, C. S., Veida-Silva, H. P., Ovidio, P. P., Holland, H., Matsuo, F. S., ... & Jordao, A. A. (2020). A fish oil-rich diet leads to lower adiposity and serum triglycerides but increases liver lipid peroxidation in fructose-fed rats. *Egyptian Liver Journal*, *10*(1), 35. https://doi.org/10.1186/s43066-020-00045-9
Esteves, G. P., Manca, C. S., Veida-Silva, H. P., Ovidio, P. P., Holland, H., Matsuo, F. S., ... & Jordao, A. A. (2020). A fish oil-rich diet leads to lower adiposity and serum triglycerides but

increases liver lipid peroxidation in fructose-fed rats. *Egyptian Liver Journal*, *10*(1), 35. https://doi.org/10.1186/s43066-020-00045-9.

Fan, G., Li, Y., Liu, Y., Suo, X., Jia, Y., & Yang, X. (2022). Gondoic acid alleviates LPS-induced Kupffer cells inflammation by inhibiting ROS production and PKCθ/ERK/STAT3 signaling pathway. *International Immunopharmacology*, *111*, 109171. https://doi.org/10.1016/j.intimp.2022.109171

Fan, R., Kim, J., Kim, Y. C., & Chung, S. (2025). Effects of Fish Oil with Heat Treatment on Obesity, Inflammation, and Gut Microbiota in Ovariectomized Mice. *Nutrients*, *17*(3), 549. https://doi.org/10.3390/nu17030549Ferreira, S. R. G., Macotela, Y., Velloso, L. A., & Mori, M. A. (2024). Determinants of obesity in Latin America. *Nature metabolism*, *6*(3), 409–432. https://doi.org/10.1038/s42255-024-00977-1

Farag, M. A., & Gad, M. Z. (2022). Omega-9 fatty acids: Potential roles in inflammation and cancer management. *Journal of Genetic Engineering and Biotechnology*, *20*(1), 48. https://doi.org/10.1186/s43141-022-00329-0

Farmer S. R. (2005). Regulation of PPARgamma activity during adipogenesis. *International journal of obesity (2005)*, *29 Suppl 1*, S13–S16. https://doi.org/10.1038/sj.ijo.0802907

Feillet-Coudray, C., Aoun, M., Fouret, G., Bonafos, B., Ramos, J., Casas, F., Cristol, J. P., & Coudray, C. (2013). Effects of long-term administration of saturated and n-3 fatty acid-rich diets on lipid utilisation and oxidative stress in rat liver and muscle tissues. *The British journal of nutrition*, *110*(10), 1789–1802. https://doi.org/10.1017/S0007114513001311

Feng, H., Zheng, L., Feng, Z., Zhao, Y., & Zhang, N. (2013). The role of leptin in obesity and the potential for leptin replacement therapy. *Endocrine*, *44*(1), 33–39. https://doi.org/10.1007/s12020-012-9865-y

Ferguson, J. F., Roberts-Lee, K., Borcea, C., Smith, H. M., Midgette, Y., & Shah, R. (2019). Omega-3 polyunsaturated fatty acids attenuate inflammatory activation and alter differentiation in human adipocytes. *The Journal of nutritional biochemistry*, *64*, 45–49. https://doi.org/10.1016/j.jnutbio.2018.09.027

Ferre, P., & Foufelle, F. (2007). SREBP-1c transcription factor and lipid homeostasis: clinical perspective. *Hormone research*, *68*(2), 72-82. https://doi.org/10.1159/000100426.

Ferreira, S. R. G., Macotela, Y., Velloso, L. A., & Mori, M. A. (2024). Determinants of obesity in Latin America. *Nature metabolism*, *6*(3), 409–432. https://doi.org/10.1038/s42255-024-00977-1

Galcheva, S. V., Iotova, V. M., Yotov, Y. T., Bernasconi, S., & Street, M. E. (2011). Circulating proinflammatory peptides related to abdominal adiposity and cardiometabolic risk factors in healthy prepubertal children. *European journal of endocrinology*, *164*(4), 553–558. https://doi.org/10.1530/EJE-10-1124

Gao, H., Geng, T., Huang, T., & Zhao, Q. (2017). Fish oil supplementation and insulin sensitivity: a systematic review and meta-analysis. *Lipids in health and disease*, *16*(1), 131. https://doi.org/10.1186/s12944-017-0528-0

GBD 2021 Adult BMI Collaborators (2025). Global, regional, and national prevalence of adult overweight and obesity, 1990-2021, with forecasts to 2050: a forecasting study for the Global Burden of Disease Study 2021. *Lancet (London, England)*, *405*(10481), 813–838. https://doi.org/10.1016/S0140-6736(25)00355-1

Ghandour, R. A., Colson, C., Giroud, M., Maurer, S., Rekima, S., Ailhaud, G., Klingenspor, M., Amri, E. Z., & Pisani, D. F. (2018). Impact of dietary ω3 polyunsaturated fatty acid supplementation on brown and brite adipocyte function. *Journal of lipid research*, *59*(3), 452–461. https://doi.org/10.1194/jlr.M081091

Go, R. E., Hwang, K. A., Park, G. T., Lee, H. M., Lee, G. A., Kim, C. W., ... & Choi, K. C. (2016). Effects of microalgal polyunsaturated fatty acid oil on body weight and lipid accumulation in the liver of C57BL/6 mice fed a high fat diet. *Journal of Biomedical Research*, *30*(3), 234. https://doi.org/10.7555/JBR.30.2016K0004.

Goel, S., Chaudhary, M., Khedkar, R., & Khandelwal, S. (2018). The Effect of Long Chain N-3 PUFA on Obese Individuals-Desk Review. *Nutri Food Sci Int J*, *7*(3), 555714. DOI: 10.19080/NFSIJ.2018.07.555714.

Gonçalves, R. M., Petenuci, M. E., Maistrovicz, F. C., Galuch, M. B., Montanher, P. F., Pizzo, J. S., Gualda, I. P., & Visentainer, J. V. (2021). Lipid profile and fatty acid composition of marine fish species from Northeast coast of Brazil. *Journal of food science and technology*, *58*(3), 1177–1189. https://doi.org/10.1007/s13197-020-04631-y

Gonçalves-de-Albuquerque, C. F., Medeiros-de-Moraes, I. M., Oliveira, F. M., Burth, P., Bozza, P. T., Castro Faria, M. V., Silva, A. R., & Castro-Faria-Neto, H. C. (2016). Omega-9

Oleic Acid Induces Fatty Acid Oxidation and Decreases Organ Dysfunction and Mortality in Experimental Sepsis. *PloS one*, *11*(4), e0153607. https://doi.org/10.1371/journal.pone.0153607

Gopalan, C., Sastri, B.V., Balasubramanian, S.C. (2004). Nutritive value of Indian foods. Hyderabad: National Institute of Nutrition Publications, ICMR.

Guggisberg, D., Burton-Pimentel, K. J., Walther, B., Badertscher, R., Blaser, C., Portmann, R., Schmid, A., Radtke, T., Saner, H., Fournier, N., Bütikofer, U., & Vergères, G. (2022). Molecular effects of the consumption of margarine and butter varying in trans fat composition: a parallel human intervention study. *Lipids in health and disease*, *21*(1), 74. https://doi.org/10.1186/s12944-022-01675-1

Habib, S. S., Al-Khlaiwi, T., Al-Khliwi, H., Habib, S. M., Habib, S. A., Habib, S. H., & Khan, A. (2024). Adiponectin and TNFα in relation to glucometabolic control in patients with type 2 diabetes mellitus. *Journal of family medicine and primary care*, *13*(7), 2741–2745. https://doi.org/10.4103/jfmpc.jfmpc_1896_23

Halade, G. V., Rahman, M. M., Bhattacharya, A., Barnes, J. L., Chandrasekar, B., & Fernandes, G. (2010). Docosahexaenoic acid-enriched fish oil attenuates kidney disease and prolongs median and maximal life span of autoimmune lupus-prone mice. *Journal of immunology (Baltimore, Md.: 1950)*, *184*(9), 5280–5286. https://doi.org/10.4049/jimmunol.0903282

Harano, Y., Yasui, K., Toyama, T., Nakajima, T., Mitsuyoshi, H., Mimani, M., Hirasawa, T., Itoh, Y., & Okanoue, T. (2006). Fenofibrate, a peroxisome proliferator-activated receptor alpha agonist, reduces hepatic steatosis and lipid peroxidation in fatty liver Shionogi mice with hereditary fatty liver. *Liver international : official journal of the International Association for the Study of the Liver*, *26*(5), 613–620. https://doi.org/10.1111/j.1478-3231.2006.01265.x

Haugaard, S. B., Madsbad, S., Høy, C. E., & Vaag, A. (2006). Dietary intervention increases n-3 long-chain polyunsaturated fatty acids in skeletal muscle membrane phospholipids of obese subjects. Implications for insulin sensitivity. *Clinical endocrinology*, *64*(2), 169-178. https://doi.org/10.1111/j.1365-2265.2006.02444.x

Heir, R., & Stellwagen, D. (2020). TNF-Mediated Homeostatic Synaptic Plasticity: From *in vitro* to *in vivo* Models. *Frontiers in cellular neuroscience*, *14*, 565841. https://doi.org/10.3389/fncel.2020.565841

Hensler, M., Bardova, K., Jilkova, Z. M., Wahli, W., Meztger, D., Chambon, P., Kopecky, J., & Flachs, P. (2011). The inhibition of fat cell proliferation by n- 3 fatty acids in dietary obese mice. Lipids in Health and Disease, 10, 128. https:// doi. org/ 10. 1186/ 1476- 511X- 10- 128

Heo, M. G., & Choung, S. Y., (2018). Anti-obesity effects of Spirulina maxima in high fat diet induced obese rats via the activation of AMPK pathway and SIRT1. *Food & function*, *9*(9), 4906–4915. https://doi.org/10.1039/c8fo00986d

Herzig, S., & Shaw, R. J. (2018). AMPK: guardian of metabolism and mitochondrial homeostasis. *Nature reviews Molecular cell biology*, *19*(2), 121-135. https://doi.org/10.1038/nrm.2017.95.

Hollie, N. I., & Hui, D. Y. (2011). Group 1B phospholipase A_2 deficiency protects against diet-induced hyperlipidemia in mice. *Journal of lipid research*, *52*(11), 2005–2011. https://doi.org/10.1194/jlr.M019463

Hsu, W. H., Chen, T. H., Lee, B. H., Hsu, Y. W., & Pan, T. M. (2014). Monascin and ankaflavin act as natural AMPK activators with PPARα agonist activity to down-regulate nonalcoholic steatohepatitis in high-fat diet-fed C57BL/6 mice. *Food and Chemical Toxicology*, *64*, 94-103. https://doi.org/10.1016/j.fct.2013.11.015.

Hu, S., Wang, L., Yang, D., Li, L., Togo, J., Wu, Y., Liu, Q., Li, B., Li, M., Wang, G., Zhang, X., Niu, C., Li, J., Xu, Y., Couper, E., Whittington-Davies, A., Mazidi, M., Luo, L., Wang, S., Douglas, A., … Speakman, J. R. (2018). Dietary Fat, but Not Protein or Carbohydrate, Regulates Energy Intake and Causes Adiposity in Mice. *Cell metabolism*, *28*(3), 415–431.e4. https://doi.org/10.1016/j.cmet.2018.06.010

Huang, C. W., Chien, Y. S., Chen, Y. J., Ajuwon, K. M., Mersmann, H. M., & Ding, S. T. (2016). Role of n-3 Polyunsaturated Fatty Acids in Ameliorating the Obesity-Induced Metabolic Syndrome in Animal Models and Humans. *International journal of molecular sciences*, *17*(10), 1689. https://doi.org/10.3390/ijms17101689

Huang, K., Liang, X. C., Zhong, Y. L., He, W. Y., & Wang, Z. (2015). 5-Caffeoylquinic acid decreases diet-induced obesity in rats by modulating PPARα and LXRα transcription. *Journal of the science of food and agriculture*, *95*(9), 1903–1910. https://doi.org/10.1002/jsfa.6896

Hwang, S. M., Kim, Y. U., Kim, J. K., Chun, Y. S., Kwon, Y. S., Ku, S. K., & Song, C. H. (2022). Preventive and Therapeutic Effects of Krill Oil on Obesity and Obesity-Induced

Metabolic Syndromes in High-Fat Diet-Fed Mice. *Marine drugs*, *20*(8), 483. https://doi.org/10.3390/md20080483

Izuddin, W. I., Loh, T. C., Nayan, N., Akit, H., Noor, A. M., & Foo, H. L. (2023). Blood lipid profiles, fatty acid deposition and expression of hepatic lipid and lipoprotein metabolism genes in laying hens fed palm oils, palm kernel oil, and soybean oil. *Frontiers in veterinary science*, *10*, 1192841. https://doi.org/10.3389/fvets.2023.1192841

Jeong, M. Y., Kim, H. L., Park, J., An, H. J., Kim, S. H., Kim, S. J., So, H. S., Park, R., Um, J. Y., & Hong, S. H. (2013). Rubi Fructus (Rubus coreanus) Inhibits Differentiation to Adipocytes in 3T3-L1 Cells. *Evidence-based complementary and alternative medicine : eCAM*, *2013*, 475386. https://doi.org/10.1155/2013/475386

Johnson, M. A. C. B., & Bradford, C. (2014). Omega-3, omega-6 and omega-9 fatty acids: implications for cardiovascular and other diseases. *J Glycomics Lipidomics*, *4*(123), 2153-0637. doi:10.4172/2153-0637.1000123.

Johnston, L. W., Harris, S. B., Retnakaran, R., Zinman, B., Giacca, A., Liu, Z., Bazinet, R. P., & Hanley, A. J. (2016). Longitudinal Associations of Phospholipid and Cholesteryl Ester Fatty Acids With Disorders Underlying Diabetes. *The Journal of clinical endocrinology and metabolism*, *101*(6), 2536–2544. https://doi.org/10.1210/jc.2015-4267

Jørgensen, A. B., Frikke-Schmidt, R., Nordestgaard, B. G., & Tybjærg-Hansen, A. (2014). Loss-of-function mutations in APOC3 and risk of ischemic vascular disease. *The New England journal of medicine*, *371*(1), 32–41. https://doi.org/10.1056/NEJMoa1308027

Kar, R., Das, P., Panchali, T., Dutta, A., Phoujdar, M., & Pradhan, S. (2026). Mechanistic Insights Into the Anti-Obesity Effects of Gondoic Acid: Regulation of AMPK Signalling and Obesity Related Gene Expression in Obese Mice. *Applied biochemistry and biotechnology*, 10.1007/s12010-025-05488-7. Advance online publication. https://doi.org/10.1007/s12010-025-05488-7

Kar, R., Das, P., Panchali, T., Dutta, A., Phoujdar, M., Ghosh, K., & Pradhan, S. (2025). Anti-obese potentiality of marine Topse (*Polynemus paradiseus*) fish oil by inhibiting the expression of SREBP-1c & promoting β-oxidation of fat through upregulating PPAR-α. *Adipocyte*, *14*(1), 2524640. https://doi.org/10.1080/21623945.2025.2524640

Karri, S., Sharma, S., Hatware, K., & Patil, K. (2019). Natural anti-obesity agents and their therapeutic role in management of obesity: A future trend perspective. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie*, *110*, 224–238. https://doi.org/10.1016/j.biopha.2018.11.076

Kasbi Chadli, F., Andre, A., Prieur, X., Loirand, G., Meynier, A., Krempf, M., Nguyen, P., & Ouguerram, K. (2012). n-3 PUFA prevent metabolic disturbances associated with obesity and improve endothelial function in golden Syrian hamsters fed with a high-fat diet. *The British journal of nutrition*, *107*(9), 1305–1315. https://doi.org/10.1017/S0007114511004387

Kaur, N., Chugh, V., & Gupta, A. K. (2014). Essential fatty acids as functional components of foods- a review. *Journal of food science and technology*, *51*(10), 2289–2303. https://doi.org/10.1007/s13197-012-0677-0

Kern, P. A., Ranganathan, S., Li, C., Wood, L., & Ranganathan, G. (2001). Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. *American journal of physiology. Endocrinology and metabolism*, *280*(5), E745–E751. https://doi.org/10.1152/ajpendo.2001.280.5.E745

Kim, S. K., & Kong, C. S. (2010). Anti-adipogenic effect of dioxinodehydroeckol via AMPK activation in 3T3-L1 adipocytes. *Chemico-biological interactions*, *186*(1), 24–29. https://doi.org/10.1016/j.cbi.2010.04.003

Kinkel, A. D., Fernyhough, M. E., Helterline, D. L., Vierck, J. L., Oberg, K. S., Vance, T. J., Hausman, G. J., Hill, R. A., & Dodson, M. V. (2004). Oil red-O stains non-adipogenic cells: a precautionary note. *Cytotechnology*, *46*(1), 49–56. https://doi.org/10.1007/s10616-004-3903-4

Koellensperger, E., Niesen, W., Kolbenschlag, J., Gramley, F., Germann, G., & Leimer, U. (2013). Human adipose tissue derived stem cells promote liver regeneration in a rat model of toxic injury. *Stem cells international*, *2013*(1), 534263. https://doi.org/10.1155/2013/534263.

Kojadinovic, M. I., Arsic, A. C., Debeljak-Martacic, J. D., Konic-Ristic, A. I., Kardum, N. D., Popovic, T. B., & Glibetic, M. D. (2017). Consumption of pomegranate juice decreases blood lipid peroxidation and levels of arachidonic acid in women with metabolic syndrome. *Journal of the science of food and agriculture*, *97*(6), 1798–1804. https://doi.org/10.1002/jsfa.7977

Komal, F., Khan, M. K., Imran, M., Ahmad, M. H., Anwar, H., Ashfaq, U. A., Ahmad, N., Masroor, A., Ahmad, R. S., Nadeem, M., & Nisa, M. U. (2020). Impact of different omega-3

fatty acid sources on lipid, hormonal, blood glucose, weight gain and histopathological damages profile in PCOS rat model. *Journal of translational medicine*, *18*(1), 349. https://doi.org/10.1186/s12967-020-02519-1

Kopelman P. G. (2000). Obesity as a medical problem. *Nature*, *404*(6778), 635–643. https://doi.org/10.1038/35007508

Kumar, J. S., & Sharma, B. (2022). A review on neuropharmacological role of erucic acid: An omega-9 fatty acid from edible oils. *Nutritional Neuroscience*, *25*(5), 1041-1055. https://doi.org/10.1080/1028415X.2020.1831262.

Kwok, K. H. M., Rydén, M., Andersson, D. P., Beauchef, G., Guere, C., Vie, K., Bergman, O., Lundbäck, V., Arner, P., & Dahlman, I. (2020). Prospective analyses of white adipose tissue gene expression in relation to long-term body weight changes. *International journal of obesity (2005)*, *44*(2), 377–387. https://doi.org/10.1038/s41366-019-0385-1

Lan, Y. L., Lou, J. C., Lyu, W., & Zhang, B. (2019). Update on the synergistic effect of HSL and insulin in the treatment of metabolic disorders. *Therapeutic advances in endocrinology and metabolism*, *10*, 2042018819877300. https://doi.org/10.1177/2042018819877300

Lee, H. W., Rhee, D. K., Kim, B. O., & Pyo, S. (2018). Inhibitory effect of sinigrin on adipocyte differentiation in 3T3-L1 cells: Involvement of AMPK and MAPK pathways. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie*, *102*, 670–680. https://doi.org/10.1016/j.biopha.2018.03.124

Lee, J. H., Kim, T., Lee, J. J., Lee, K. J., Kim, H. K., Yun, B., Jeon, J., Kim, S. K., & Ma, J. Y. (2015). The Herbal Medicine KBH-1 Inhibits Fat Accumulation in 3T3-L1 Adipocytes and Reduces High Fat Diet-Induced Obesity through Regulation of the AMPK Pathway. *PloS one*, *10*(12), e0142041. https://doi.org/10.1371/journal.pone.0142041

Lee, M. J., Wu, Y., & Fried, S. K. (2013). Adipose tissue heterogeneity: implication of depot differences in adipose tissue for obesity complications. *Molecular aspects of medicine*, *34*(1), 1–11. https://doi.org/10.1016/j.mam.2012.10.001

Lefterova, M. I., Steger, D. J., Zhuo, D., Qatanani, M., Mullican, S. E., Tuteja, G., Manduchi, E., Grant, G. R., & Lazar, M. A. (2010). Cell-specific determinants of peroxisome proliferator-activated receptor gamma function in adipocytes and macrophages. *Molecular and cellular biology*, *30*(9), 2078–2089. https://doi.org/10.1128/MCB.01651-09

Li, J., Wu, K., Zhong, Y., Kuang, J., Huang, N., Guo, X., ... & Sun, R. (2023). Si–Ni-SAN ameliorates obesity through AKT/AMPK/HSL pathway-mediated lipolysis: network pharmacology and experimental validation. *Journal of Ethnopharmacology*, *302*, 115892. https://doi.org/10.1016/j.jep.2022.115892.

Li, L., Spranger, L., Stobäus, N., Beer, F., Decker, A. M., Wernicke, C., Brachs, S., Brachs, M., Spranger, J., & Mai, K. (2021). Fetuin-B, a potential link of liver-adipose tissue cross talk during diet-induced weight loss-weight maintenance. *Nutrition & diabetes*, *11*(1), 31. https://doi.org/10.1038/s41387-021-00174-z

Li, Y., Rong, Y., Bao, L., Nie, B., Ren, G., Zheng, C., Amin, R., Arnold, R. D., Jeganathan, R. B., & Huggins, K. W. (2017). Suppression of adipocyte differentiation and lipid accumulation by stearidonic acid (SDA) in 3T3-L1 cells. *Lipids in health and disease*, *16*(1), 181. https://doi.org/10.1186/s12944-017-0574-7

Lim, S. S., Vos, T., Flaxman, A. D., Danaei, G., Shibuya, K., Adair-Rohani, H., Amann, M., Anderson, H. R., Andrews, K. G., Aryee, M., Atkinson, C., Bacchus, L. J., Bahalim, A. N., Balakrishnan, K., Balmes, J., Barker-Collo, S., Baxter, A., Bell, M. L., Blore, J. D., Blyth, F., … Memish, Z. A. (2012). A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet (London, England)*, *380*(9859), 2224–2260. https://doi.org/10.1016/S0140-6736(12)61766-8

Lim, S. Y., & Chien, Y. W. (2024). Effects of Polyunsaturated Fatty Acid/Saturated Fatty Acid Ratio and Different Amounts of Monounsaturated Fatty Acids on Adipogenesis in 3T3-L1 Cells. *Biomedicines*, *12*(9), 1980. https://doi.org/10.3390/biomedicines12091980

Liu, L., Jin, R., Hao, J., Zeng, J., Yin, D., Yi, Y., Zhu, M., Mandal, A., Hua, Y., Ng, C. K., Egilmez, N. K., Sauter, E. R., & Li, B. (2020). Consumption of the Fish Oil High-Fat Diet Uncouples Obesity and Mammary Tumor Growth through Induction of Reactive Oxygen Species in Protumor Macrophages. *Cancer research*, *80*(12), 2564–2574. https://doi.org/10.1158/0008-5472.CAN-19-3184

Lopes, L. L., Peluzio, M. D. C. G., & Hermsdorff, H. H. M. (2016). Monounsaturated fatty acid intake and lipid metabolism. *Jornal Vascular Brasileiro*, *15*, 52-60. https://doi.org/10.1590/1677-5449.008515

Maeda, H., Hosokawa, M., Sashima, T., & Miyashita, K. (2007). Dietary combination of fucoxanthin and fish oil attenuates the weight gain of white adipose tissue and decreases blood

glucose in obese/diabetic KK-Ay mice. *Journal of agricultural and food chemistry*, *55*(19), 7701–7706. https://doi.org/10.1021/jf071569n

Mahapatra, B. K., Sarkar, U. K., & Lakra, W. S. (2014). A Review on status, potentials, threats and challenges of the fish biodiversity of West Bengal. *J. Biodivers. Biopros. Dev*, *2*(140), 2376-0214. doi:10.4172/2376-0214.1000140

Majumder, T. H., Chowdhury, A. J., Sarwer, M. G., & Hasan, M. M. (2016). Proximate composition of estuarine fish paradise threadfin, Polynemus paradiseus (Linnaeus, 1758). *American journal of food nutrition and health*, *6*(4), 117-125. doi:10.5251/ajfn.2016.6.4.123.131

Marchi, J., Berg, M., Dencker, A., Olander, E. K., & Begley, C. (2015). Risks associated with obesity in pregnancy, for the mother and baby: a systematic review of reviews. *Obesity reviews : an official journal of the International Association for the Study of Obesity*, *16*(8), 621–638. https://doi.org/10.1111/obr.12288

Martin, P. G., Guillou, H., Lasserre, F., Déjean, S., Lan, A., Pascussi, J. M., Sancristobal, M., Legrand, P., Besse, P., & Pineau, T. (2007). Novel aspects of PPARalpha-mediated regulation of lipid and xenobiotic metabolism revealed through a nutrigenomic study. *Hepatology (Baltimore, Md.)*, *45*(3), 767–777. https://doi.org/10.1002/hep.21510

Martínez-Fernández, L., Laiglesia, L. M., Huerta, A. E., Martínez, J. A., & Moreno-Aliaga, M. J. (2015). Omega-3 fatty acids and adipose tissue function in obesity and metabolic syndrome. *Prostaglandins & other lipid mediators*, *121*, 24-41. https://doi.org/10.1016/j.prostaglandins.2015.07.003.

Martins, A. R., Crisma, A. R., Masi, L. N., Amaral, C. L., Marzuca-Nassr, G. N., Bomfim, L. H. M., Teodoro, B. G., Queiroz, A. L., Serdan, T. D. A., Torres, R. P., Mancini-Filho, J., Rodrigues, A. C., Alba-Loureiro, T. C., Pithon-Curi, T. C., Gorjao, R., Silveira, L. R., Curi, R., Newsholme, P., & Hirabara, S. M. (2018). Attenuation of obesity and insulin resistance by fish oil supplementation is associated with improved skeletal muscle mitochondrial function in mice fed a high-fat diet. *The Journal of nutritional biochemistry*, *55*, 76–88. https://doi.org/10.1016/j.jnutbio.2017.11.012

Maury, E., Ehala-Aleksejev, K., Guiot, Y., Detry, R., Vandenhooft, A., & Brichard, S. M. (2007). Adipokines oversecreted by omental adipose tissue in human obesity. *American journal of physiology. Endocrinology and metabolism*, *293*(3), E656–E665. https://doi.org/10.1152/ajpendo.00127.2007

McLaughlin, T., Liu, L. F., Lamendola, C., Shen, L., Morton, J., Rivas, H., Winer, D., Tolentino, L., Choi, O., Zhang, H., Hui Yen Chng, M., & Engleman, E. (2014). T-cell profile in adipose tissue is associated with insulin resistance and systemic inflammation in humans. *Arteriosclerosis, thrombosis, and vascular biology*, *34*(12), 2637–2643. https://doi.org/10.1161/ATVBAHA.114.304636

Minokoshi, Y., Kim, Y. B., Peroni, O. D., Fryer, L. G., Müller, C., Carling, D., & Kahn, B. B. (2002). Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. *Nature*, *415*(6869), 339–343. https://doi.org/10.1038/415339a

Miotto, P. M., Horbatuk, M., Proudfoot, R., Matravadia, S., Bakovic, M., Chabowski, A., & Holloway, G. P. (2017). α-Linolenic acid supplementation and exercise training reveal independent and additive responses on hepatic lipid accumulation in obese rats. *American journal of physiology. Endocrinology and metabolism*, *312*(6), E461–E470. https://doi.org/10.1152/ajpendo.00438.2016

Miralles-Pérez, B., Nogués, M. R., Sánchez-Martos, V., Taltavull, N., Méndez, L., Medina, I., Ramos-Romero, S., Torres, J. L., & Romeu, M. (2021). The Effects of the Combination of Buckwheat D-Fagomine and Fish Omega-3 Fatty Acids on Oxidative Stress and Related Risk Factors in Pre-Obese Rats. *Foods (Basel, Switzerland)*, *10*(2), 332. https://doi.org/10.3390/foods10020332

Miranda, J., Eseberri, I., Lasa, A., & Portillo, M. P. (2018). Lipid metabolism in adipose tissue and liver from diet-induced obese rats: a comparison between Wistar and Sprague-Dawley strains. *Journal of physiology and biochemistry*, *74*(4), 655–666. https://doi.org/10.1007/s13105-018-0654-9

Miyazaki, M., Dobrzyn, A., Man, W. C., Chu, K., Sampath, H., Kim, H. J., & Ntambi, J. M. (2004). Stearoyl-CoA desaturase 1 gene expression is necessary for fructose-mediated induction of lipogenic gene expression by sterol regulatory element-binding protein-1c-dependent and-independent mechanisms. *Journal of Biological Chemistry*, *279*(24), 25164-25171. https://doi.org/10.1074/jbc.M402781200.

Monteiro, R., & Azevedo, I. (2010). Chronic inflammation in obesity and the metabolic syndrome. *Mediators of inflammation*, *2010*, 289645. https://doi.org/10.1155/2010/289645

Moraes-Vieira, P. M., Saghatelian, A., & Kahn, B. B. (2016). GLUT4 expression in adipocytes regulates de novo lipogenesis and levels of a novel class of lipids with antidiabetic and anti-inflammatory effects. *Diabetes*, *65*(7), 1808-1815. https://doi.org/10.2337/db16-0221.

Moseti, D., Regassa, A., & Kim, W. K. (2016). Molecular Regulation of Adipogenesis and Potential Anti-Adipogenic Bioactive Molecules. *International journal of molecular sciences*, *17*(1), 124. https://doi.org/10.3390/ijms17010124

Muppala, S., Konduru, S. K. P., Merchant, N., Ramsoondar, J., Rampersad, C. K., Rajitha, B., Mukund, V., Kancherla, J., Hammond, A., Barik, T. K., Mannarapu, M., Alam, A., Basha, R., Bramhachari, P. V., Verma, D., Sushma, P. S., Pattnaik, S., & Nagaraju, G. P. (2017). Adiponectin: Its role in obesity-associated colon and prostate cancers. *Critical reviews in oncology/hematology*, *116*, 125–133. https://doi.org/10.1016/j.critrevonc.2017.06.003

Murphy, M., Robertson, W., & Oyebode, O. (2017). Obesity in International Migrant Populations. *Current obesity reports*, *6*(3), 314–323. https://doi.org/10.1007/s13679-017-0274-7

Nadia, L. O. M. H., Windarsih, A., Riswanto, F. D. O., Putri, A. R., Rohman, A., & Ambardini, S. (2023). Fatty acid composition, biological activity and authentication of marine fish oil. *Food Research*, *7*(6), 187-196. https://doi.org/10.26656/fr.2017.7(6).472

Ng, M., Fleming, T., Robinson, M., Thomson, B., Graetz, N., Margono, C., Mullany, E. C., Biryukov, S., Abbafati, C., Abera, S. F., Abraham, J. P., Abu-Rmeileh, N. M., Achoki, T., AlBuhairan, F. S., Alemu, Z. A., Alfonso, R., Ali, M. K., Ali, R., Guzman, N. A., Ammar, W., … Gakidou, E. (2014). Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet (London, England)*, *384*(9945), 766–781. https://doi.org/10.1016/S0140-6736(14)60460-8

Ni, Y., Ni, L., Zhuge, F., Xu, L., Fu, Z., & Ota, T. (2020). Adipose Tissue Macrophage Phenotypes and Characteristics: The Key to Insulin Resistance in Obesity and Metabolic Disorders. *Obesity (Silver Spring, Md.)*, *28*(2), 225–234. https://doi.org/10.1002/oby.22674

Noutsa, B. S., Tchabong, S. R., Djitieu, A. D. D., Dongmo, F. F. D., Ngamga, F. H. N., Zokou, R., Tamgue, O., Ngane, R. A. N., & Tchoumbougnang, F. (2022). Chemical Characterization and Antibacterial Properties of *Fontitrygon margarita* (Günther, 1870) Liver Oil. *Journal of lipids*, *2022*, 9369387. https://doi.org/10.1155/2022/9369387

Oliart Ros, R. M., Rodríguez, I. S., Sánchez Otero, M. G., Gil, O. M. L., Escobar-Sagastume, I., & Alexander-Aguilera, A. (2018). Comparative Effect between Sardine Oil and Fish Oil Rich in Omega-3 Fatty Acids on Hypertension and the Membrane Composition of Adipocytes in SHR Rats. *Journal of nutritional science and vitaminology*, *64*(3), 179–184. https://doi.org/10.3177/jnsv.64.179

Ondo-Azi, A. S., Kumulungui, B. S., Mewono, L., Koumba, A. M., & Missang, C. E. (2013). Proximate composition and microbiological study of five marine fish species consumed in Gabon. *African Journal of Food Science*, *7*(8), 227-231. doi: 10.5897/AJFS12.177

Ouchi, N., Kihara, S., Funahashi, T., Nakamura, T., Nishida, M., Kumada, M., Okamoto, Y., Ohashi, K., Nagaretani, H., Kishida, K., Nishizawa, H., Maeda, N., Kobayashi, H., Hiraoka, H., & Matsuzawa, Y. (2003). Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue. *Circulation*, *107*(5), 671–674. https://doi.org/10.1161/01.cir.0000055188.83694.b3

Pahlavani, M., Razafimanjato, F., Ramalingam, L., Kalupahana, N. S., Moussa, H., Scoggin, S., & Moustaid-Moussa, N. (2017). Eicosapentaenoic acid regulates brown adipose tissue metabolism in high-fat-fed mice and in clonal brown adipocytes. *The Journal of nutritional biochemistry*, *39*, 101–109. https://doi.org/10.1016/j.jnutbio.2016.08.012

Pal, J., Shukla, B. N., Maurya, A. K., Verma, H. O., Pandey, G., & Amitha, A. (2018). A review on role of fish in human nutrition with special emphasis to essential fatty acid. *International journal of fisheries and aquatic studies*, *6*(2), 427-430. E-ISSN: 2347-5129 P-ISSN: 2394-0506

Pan, M. H., Yang, G., Li, S., Li, M. Y., Tsai, M. L., Wu, J. C., Badmaev, V., Ho, C. T., & Lai, C. S. (2017). Combination of citrus polymethoxyflavones, green tea polyphenols, and Lychee extracts suppresses obesity and hepatic steatosis in high-fat diet induced obese mice. *Molecular nutrition & food research*, *61*(11), 10.1002/mnfr.201601104. https://doi.org/10.1002/mnfr.201601104

Panchali, T., Dutta, A., Das, P., Khatun, A., Kar, R., Mondal, S., Mondal, K. C., Chakrabarti, S., Ghosh, K., & Pradhan, S. (2024). Amelioration of obesity induction by a high-fat diet and related inflammation by Phasa fish (Setipinna phasa) oil in BALB/c mice. *Journal of applied biomedicine*, *22*(1), 49–58. https://doi.org/10.32725/jab.2024.003

Patel D. (2015). Pharmacotherapy for the management of obesity. *Metabolism: clinical and experimental*, *64*(11), 1376–1385. https://doi.org/10.1016/j.metabol.2015.08.001

Pedersen, M. H., Mølgaard, C., Hellgren, L. I., Matthiessen, J., Holst, J. J., & Lauritzen, L. (2011). The effect of dietary fish oil in addition to lifestyle counselling on lipid oxidation and body composition in slightly overweight teenage boys. *Journal of nutrition and metabolism*, *2011*(1), 348368. doi:10.1155/2011/348368.

Pena Muniz, M. A., Ferreira Dos Santos, M. N., da Costa, C. E., Morais, L., Lamarão, M. L., Ribeiro-Costa, R. M., & Silva-Júnior, J. O. (2015). Physicochemical characterization, fatty acid composition, and thermal analysis of Bertholletia excelsa HBK oil. *Pharmacognosy magazine*, *11*(41), 147–151. https://doi.org/10.4103/0973-1296.149730

Peng, W., Zhang, L., Wen, F., Tang, X., Zeng, L., Chen, J., & Wang, Y. (2023). Trends and disparities in non- communicable diseases in the Western Pacific region. The Lancet Regional Health–Western Pacific, The Lancet Regional Health–Western Pacific, 43(100938), 1–17. https://doi.org/10.1016/j.lanwpc.2023.100938

Pérez-Echarri, N., Pérez-Matute, P., Marcos-Gómez, B., Martínez, J. A., & Moreno-Aliaga, M. J. (2008). Effects of eicosapentaenoic acid ethyl ester on visfatin and apelin in lean and overweight (cafeteria diet-fed) rats. *British Journal of Nutrition*, *101*(7), 1059-1067. https://doi.org/10.1017/S0007114508048307.

Pérez-Matute, P., Pérez-Echarri, N., Martínez, J. A., Marti, A., & Moreno-Aliaga, M. J. (2007). Eicosapentaenoic acid actions on adiposity and insulin resistance in control and high-fat-fed rats: role of apoptosis, adiponectin and tumour necrosis factor-α. *British Journal of Nutrition*, *97*(2), 389-398. https://doi.org/10.1017/S0007114507207627.

Piccinin, E., Cariello, M., De Santis, S., Ducheix, S., Sabbà, C., Ntambi, J. M., & Moschetta, A. (2019). Role of Oleic Acid in the Gut-Liver Axis: From Diet to the Regulation of Its Synthesis via Stearoyl-CoA Desaturase 1 (SCD1). *Nutrients*, *11*(10), 2283. https://doi.org/10.3390/nu11102283

Pradhan, A. D., Manson, J. E., Rifai, N., Buring, J. E., & Ridker, P. M. (2001). C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA*, *286*(3), 327–334. https://doi.org/10.1001/jama.286.3.327

Pradhan, S., Panchali, T., Paul, B., Khatun, A., Rao Jarapala, S., Mondal, K. C., Ghosh, K., & Chakrabarti, S. (2020). Anti-obesity potentiality of Tapra fish (Opisthopterus tardoore) oil. *Journal of food biochemistry*, *44*(11), e13448. https://doi.org/10.1111/jfbc.13448

Rahman, N., Hashem, S., Akther, S., & Jothi, J. S. (2023). Impact of various extraction methods on fatty acid profile, physicochemical properties, and nutritional quality index of Pangus fish oil. *Food science & nutrition*, *11*(8), 4688–4699. https://doi.org/10.1002/fsn3.3431

Ramalho, T., Pahlavani, M., Kalupahana, N., Wijayatunga, N., Ramalingam, L., Jancar, S., & Moustaid-Moussa, N. (2020). Eicosapentaenoic Acid Regulates Inflammatory Pathways through Modulation of Transcripts and miRNA in Adipose Tissue of Obese Mice. *Biomolecules*, *10*(9), 1292. https://doi.org/10.3390/biom10091292

Ramanjaneya, M., Chen, J., Brown, J. E., Tripathi, G., Hallschmid, M., Patel, S., Kern, W., Hillhouse, E. W., Lehnert, H., Tan, B. K., & Randeva, H. S. (2010). Identification of nesfatin-1 in human and murine adipose tissue: a novel depot-specific adipokine with increased levels in obesity. *Endocrinology*, *151*(7), 3169–3180. https://doi.org/10.1210/en.2009-1358

Ratnayake, W. M., & Galli, C. (2009). Fat and fatty acid terminology, methods of analysis and fat digestion and metabolism: a background review paper. *Annals of nutrition & metabolism*, *55*(1-3), 8–43. https://doi.org/10.1159/000228994

Ravaut, G., Légiot, A., Bergeron, K. F., & Mounier, C. (2020). Monounsaturated Fatty Acids in Obesity-Related Inflammation. *International journal of molecular sciences*, *22*(1), 330. https://doi.org/10.3390/ijms22010330

Ray, M. K., Sylvester, M. D., Helton, A., Pittman, B. R., Wagstaff, L. E., McRae, T. R., 3rd, Turan, B., Fontaine, K. R., Amthor, F. R., & Boggiano, M. M. (2019). The effect of expectation on transcranial direct current stimulation (tDCS) to suppress food craving and eating in individuals with overweight and obesity. *Appetite*, *136*, 1–7. https://doi.org/10.1016/j.appet.2018.12.044

Raynor, H. A., & Champagne, C. M. (2016). Position of the Academy of Nutrition and Dietetics: Interventions for the Treatment of Overweight and Obesity in Adults. *Journal of the Academy of Nutrition and Dietetics*, *116*(1), 129–147. https://doi.org/10.1016/j.jand.2015.10.031

Rocha-Rodrigues, S., Gonçalves, I. O., Beleza, J., Ascensão, A., & Magalhães, J. (2018). Physical exercise mitigates high-fat diet-induced adiposopathy and related endocrine alterations in an animal model of obesity. *Journal of physiology and biochemistry*, *74*(2), 235–246. https://doi.org/10.1007/s13105-018-0609-1

Rocke D. M. (2004). Design and analysis of experiments with high throughput biological assay data. *Seminars in cell & developmental biology*, *15*(6), 703–713. https://doi.org/10.1016/j.semcdb.2004.09.007

Rossignoli, C. P., Dechandt, C. R. P., Souza, A. O., Sampaio, I. H., Vicentini, T. M., Teodoro, B. G., Neto, M. P. C., Ferrari, G. D., Couto-Lima, C. A., & Alberici, L. C. (2018). Effects of intermittent dietary supplementation with conjugated linoleic acid and fish oil (EPA/DHA) on body metabolism and mitochondrial energetics in mice. *The Journal of nutritional biochemistry*, *60*, 16–23. https://doi.org/10.1016/j.jnutbio.2018.07.001

Ruderman, N. B., Carling, D., Prentki, M., & Cacicedo, J. M. (2013). AMPK, insulin resistance, and the metabolic syndrome. *The Journal of clinical investigation*, *123*(7), 2764–2772. https://doi.org/10.1172/JCI67227

Ruiz-Ojeda, F. J., Rupérez, A. I., Gomez-Llorente, C., Gil, A., & Aguilera, C. M. (2016). Cell Models and Their Application for Studying Adipogenic Differentiation in Relation to Obesity: A Review. *International journal of molecular sciences*, *17*(7), 1040. https://doi.org/10.3390/ijms17071040

Ruxton, C. H., Reed, S. C., Simpson, M. J., & Millington, K. J. (2004). The health benefits of omega-3 polyunsaturated fatty acids: a review of the evidence. *Journal of human nutrition and dietetics : the official journal of the British Dietetic Association*, *17*(5), 449–459. https://doi.org/10.1111/j.1365-277X.2004.00552.x

Ruzickova, J., Rossmeisl, M., Prazak, T., Flachs, P., Sponarova, J., Vecka, M., ... & Kopecky, J. (2004). Omega-3 PUFA of marine origin limit diet-induced obesity in mice by reducing cellularity of adipose tissue. *Lipids*, *39*(12), 1177-1185. https://doi.org/10.1007/s11745-004-1345-9.

Samane, S., Christon, R., Dombrowski, L., Turcotte, S., Charrouf, Z., Lavigne, C., Levy, E., Bachelard, H., Amarouch, H., Marette, A., & Haddad, P. S. (2009). Fish oil and argan oil intake differently modulate insulin resistance and glucose intolerance in a rat model of dietary-induced obesity. *Metabolism: clinical and experimental*, *58*(7), 909–919. https://doi.org/10.1016/j.metabol.2009.02.013

Sato, T., Ida, T., Nakamura, Y., Shiimura, Y., Kangawa, K., & Kojima, M. (2014). Physiological roles of ghrelin on obesity. *Obesity research & clinical practice*, *8*(5), e405–e413. https://doi.org/10.1016/j.orcp.2013.10.002

Schwartz, M. W., Seeley, R. J., Zeltser, L. M., Drewnowski, A., Ravussin, E., Redman, L. M., & Leibel, R. L. (2017). Obesity Pathogenesis: An Endocrine Society Scientific Statement. *Endocrine reviews*, *38*(4), 267–296. https://doi.org/10.1210/er.2017-00111

Serra, D., Mera, P., Malandrino, M. I., Mir, J. F., & Herrero, L. (2013). Mitochondrial fatty acid oxidation in obesity. *Antioxidants & redox signaling*, *19*(3), 269–284. https://doi.org/10.1089/ars.2012.4875

Sewter, C., Berger, D., Considine, R. V., Medina, G., Rochford, J., Ciaraldi, T., Henry, R., Dohm, L., Flier, J. S., O'Rahilly, S., & Vidal-Puig, A. J. (2002). Human obesity and type 2 diabetes are associated with alterations in SREBP1 isoform expression that are reproduced ex vivo by tumor necrosis factor-alpha. *Diabetes*, *51*(4), 1035–1041. https://doi.org/10.2337/diabetes.51.4.1035

Stephens J. M. (2012). The fat controller: adipocyte development. *PLoS biology*, *10*(11), e1001436. https://doi.org/10.1371/journal.pbio.1001436

Sun, D., Krishnan, A., Zaman, K., Lawrence, R., Bhattacharya, A., & Fernandes, G. (2003). Dietary n-3 fatty acids decrease osteoclastogenesis and loss of bone mass in ovariectomized mice. *Journal of bone and mineral research: the official journal of the American Society for Bone and Mineral Research*, *18*(7), 1206–1216. https://doi.org/10.1359/jbmr.2003.18.7.1206

Tamer, F., Ulug, E., Akyol, A., & Nergiz-Unal, R. (2020). The potential efficacy of dietary fatty acids and fructose induced inflammation and oxidative stress on the insulin signaling and fat accumulation in mice. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association*, *135*, 110914. https://doi.org/10.1016/j.fct.2019.110914

Tang, Q. Q., Otto, T. C., & Lane, M. D. (2003). CCAAT/enhancer-binding protein beta is required for mitotic clonal expansion during adipogenesis. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(3), 850–855. https://doi.org/10.1073/pnas.0337434100

Tang, Y., Fung, E., Xu, A., & Lan, H. Y. (2017). C-reactive protein and ageing. *Clinical and experimental pharmacology & physiology*, *44 Suppl 1*, 9–14. https://doi.org/10.1111/1440-1681.12758

Ter Horst, K. W., & Serlie, M. J. (2017). Fructose consumption, lipogenesis, and non-alcoholic fatty liver disease. *Nutrients*, *9*(9), 981. https://doi.org/10.3390/nu9090981.

Timper, K., Denson, J. L., Steculorum, S. M., Heilinger, C., Engström-Ruud, L., Wunderlich, C. M., Rose-John, S., Wunderlich, F. T., & Brüning, J. C. (2017). IL-6 Improves Energy and Glucose Homeostasis in Obesity via Enhanced Central IL-6 trans-Signaling. *Cell reports*, *19*(2), 267–280. https://doi.org/10.1016/j.celrep.2017.03.043

Todorčević, M., & Hodson, L. (2015). The Effect of Marine Derived n-3 Fatty Acids on Adipose Tissue Metabolism and Function. *Journal of clinical medicine*, *5*(1), 3. https://doi.org/10.3390/jcm5010003

Tortosa-Caparrós, E., Navas-Carrillo, D., Marín, F., & Orenes-Piñero, E. (2017). Anti-inflammatory effects of omega 3 and omega 6 polyunsaturated fatty acids in cardiovascular disease and metabolic syndrome. *Critical reviews in food science and nutrition*, *57*(16), 3421–3429. https://doi.org/10.1080/10408398.2015.1126549

Vaidya, H., & Cheema, S. K. (2014). Sea cucumber and blue mussel: new sources of phospholipid enriched omega-3 fatty acids with a potential role in 3T3-L1 adipocyte metabolism. *Food & function*, *5*(12), 3287–3295. https://doi.org/10.1039/c4fo00330f

Valenzuela, C. A., Baker, E. J., Miles, E. A., & Calder, P. C. (2019). Eighteen-carbon trans fatty acids and inflammation in the context of atherosclerosis. *Progress in lipid research*, *76*, 101009. https://doi.org/10.1016/j.plipres.2019.101009

van Deuren, T., Blaak, E. E., & Canfora, E. E. (2022). Butyrate to combat obesity and obesity-associated metabolic disorders: Current status and future implications for therapeutic use. *Obesity reviews : an official journal of the International Association for the Study of Obesity*, *23*(10), e13498. https://doi.org/10.1111/obr.13498

Varda, N. M., Medved, M., & Ojsteršek, L. (2020). The associations between some biological markers, obesity, and cardiovascular risk in Slovenian children and adolescents. *BMC pediatrics*, *20*(1), 81. https://doi.org/10.1186/s12887-020-1978-5

Venkatrao, M., Nagarathna, R., Majumdar, V., Patil, S. S., Rathi, S., & Nagendra, H. (2020). Prevalence of Obesity in India and Its Neurological Implications: A Multifactor Analysis of a Nationwide Cross-Sectional Study. *Annals of neurosciences*, *27*(3-4), 153–161. https://doi.org/10.1177/0972753120987465

Waehler R. (2023). Fatty acids: facts vs. fiction. *International journal for vitamin and nutrition research. Internationale Zeitschrift fur Vitamin- und Ernahrungsforschung. Journal international de vitaminologie et de nutrition*, *93*(3), 268–288. https://doi.org/10.1024/0300-9831/a000713

Wakutsu, M., Tsunoda, N., Mochi, Y., Numajiri, M., Shiba, S., Muraki, E., & Kasono, K. (2012). Improvement in the high-fat diet-induced dyslipidemia and adiponectin levels by fish oil feeding combined with food restriction in obese KKAy mice. *Bioscience, biotechnology, and biochemistry*, *76*(5), 1011-1014. https://doi.org/10.1271/bbb.110743.

Wang, F., Hu, M., Zhu, H., Yang, C., Xia, H., Yang, X., Yang, L., & Sun, G. (2021). MyD88 determines the protective effects of fish oil and perilla oil against metabolic disorders and inflammation in adipose tissue from mice fed a high-fat diet. *Nutrition & diabetes*, *11*(1), 23. https://doi.org/10.1038/s41387-021-00159-y

Warfel, J. D., Vandanmagsar, B., Dubuisson, O. S., Hodgeson, S. M., Elks, C. M., Ravussin, E., & Mynatt, R. L. (2017). Examination of carnitine palmitoyltransferase 1 abundance in white adipose tissue: implications in obesity research. *American journal of physiology. Regulatory, integrative and comparative physiology*, *312*(5), R816–R820. https://doi.org/10.1152/ajpregu.00520.2016

Wei, D., Liao, L., Wang, H., Zhang, W., Wang, T., & Xu, Z. (2020). Canagliflozin ameliorates obesity by improving mitochondrial function and fatty acid oxidation via PPARα in vivo and in vitro. *Life sciences*, *247*, 117414. https://doi.org/10.1016/j.lfs.2020.117414

Wei, W., Hu, M., Huang, J., Yu, S., Li, X., Li, Y., & Mao, L., (2021). Anti-obesity effects of DHA and EPA in high fat-induced insulin resistant mice. *Food & function*, *12*(4), 1614–1625. https://doi.org/10.1039/d0fo02448a

World Health Organization. (2016). *The double burden of malnutrition: policy brief* (No. WHO/NMH/NHD/17.3). World Health Organization. https://www.who.int/publications/i/item/WHO-NMH-NHD-17.3

World Obesity Federation. World Obesity Atlas 2026, Childhood Obesity 2nd edition. London: World Obesity Federation, 2026. https://data.worldobesity.org/publications/?cat=24

Xi, Y., Wu, M., Li, H., Dong, S., Luo, E., Gu, M., Shen, X., Jiang, Y., Liu, Y., & Liu, H. (2015). Baicalin Attenuates High Fat Diet-Induced Obesity and Liver Dysfunction: Dose-Response and

Potential Role of CaMKKβ/AMPK/ACC Pathway. *Cellular physiology and biochemistry: international journal of experimental cellular physiology, biochemistry, and pharmacology*, *35*(6), 2349–2359. https://doi.org/10.1159/000374037

Xu, M., Xue, H., Kong, L., Lin, L., & Zheng, G. (2023). Smilax china L. polyphenols improves insulin resistance and obesity in high-fat diet-induced mice through IRS/AKT-AMPK and NF-κB signaling pathways. *Plant Foods for Human Nutrition*, *78*(2), 299-306. https://doi.org/10.1007/s11130-023-01052-y.

Yamauchi, T., Nio, Y., Maki, T., Kobayashi, M., Takazawa, T., Iwabu, M., ... & Kadowaki, T. (2007). Targeted disruption of AdipoR1 and AdipoR2 causes abrogation of adiponectin binding and metabolic actions. *Nature medicine*, *13*(3), 332-339. https://doi.org/10.1038/nm1557.

Yang, P. K., Chou, C. H., Huang, C. C., Wen, W. F., Chen, H. F., Shun, C. T., Ho, H. N., & Chen, M. J. (2021). Obesity alters ovarian folliculogenesis through disrupted angiogenesis from increased IL-10 production. *Molecular metabolism*, *49*, 101189. https://doi.org/10.1016/j.molmet.2021.101189

Yang, T., Feng, F., Zhan, K., Ma, X., Jiang, M., Datsomor, O., Zhu, X., Huo, Y., & Zhao, G. (2022). Effect of the Tea Tree Oil on Growth Performance, Meat Quality, Serum Biochemical Indices, and Antioxidant Capacity in Finishing Pigs. *Frontiers in veterinary science*, *9*, 916625. https://doi.org/10.3389/fvets.2022.916625

Yang, Z. H., Miyahara, H., Mori, T., Doisaki, N., & Hatanaka, A. (2011). Beneficial effects of dietary fish-oil-derived monounsaturated fatty acids on metabolic syndrome risk factors and insulin resistance in mice. *Journal of agricultural and food chemistry*, *59*(13), 7482–7489. https://doi.org/10.1021/jf201496h

Yao-Borengasser, A., Rassouli, N., Varma, V., Bodles, A. M., Rasouli, N., Unal, R., Phanavanh, B., Ranganathan, G., McGehee, R. E., Jr, & Kern, P. A. (2008). Stearoyl-coenzyme A desaturase 1 gene expression increases after pioglitazone treatment and is associated with peroxisomal proliferator-activated receptor-gamma responsiveness. *The Journal of clinical endocrinology and metabolism*, *93*(11), 4431–4439. https://doi.org/10.1210/jc.2008-0782

Yuan, E., Duan, X., Xiang, L., Ren, J., Lai, X., Li, Q., Sun, L., & Sun, S. (2018). Aged Oolong Tea Reduces High-Fat Diet-Induced Fat Accumulation and Dyslipidemia by Regulating the AMPK/ACC Signaling Pathway. *Nutrients*, *10*(2), 187. https://doi.org/10.3390/nu10020187

Zhang, H., Xiong, P., Zheng, T., Hu, Y., Guo, P., Shen, T., & Zhou, X. (2025). Combination of Berberine and Evodiamine Alleviates Obesity by Promoting Browning in 3T3-L1 Cells and High-Fat Diet-Induced Mice. *International journal of molecular sciences*, *26*(9), 4170. https://doi.org/10.3390/ijms26094170

Zhang, J., Yi, C., Han, J., Ming, T., Zhou, J., Lu, C., Li, Y., & Su, X. (2020). Novel high-docosahexaenoic-acid tuna oil supplementation modulates gut microbiota and alleviates obesity in high-fat diet mice. *Food science & nutrition*, *8*(12), 6513–6527. https://doi.org/10.1002/fsn3.1941

Zhou, M., Huang, J., Zhou, J., Zhi, C., Bai, Y., Che, Q., Cao, H., Guo, J., & Su, Z. (2023). Anti-Obesity Effect and Mechanism of Chitooligosaccharides Were Revealed Based on Lipidomics in Diet-Induced Obese Mice. *Molecules (Basel, Switzerland)*, *28*(14), 5595. https://doi.org/10.3390/molecules28145595

Chapter XI: Publications

SCI indexed Publication in international journal:

As 1st author

1. **Kar, R.,** Das, P., Panchali, T., Dutta, A., Kumar Das, T., Ghosh, K., & Pradhan, S. (2026b). Isolation, characterisation and antimicrobial activities of marine topse (*Polynemus paradiseus*) fish oil: an overview. *Natural product research*, 1–7. Advance online publication. https://doi.org/10.1080/14786419.2026.2625445 **IF: 2.0**
2. **Kar, R.,** Das, P., Panchali, T., Dutta, A., Phoujdar, M., & Pradhan, S. (2026a). Mechanistic Insights Into the Anti-Obesity Effects of Gondoic Acid: Regulation of AMPK Signalling and Obesity Related Gene Expression in Obese Mice. *Applied Biochemistry and Biotechnology*, 1-21. https://doi.org/10.1007/s12010-025-05488-7 **IF: 3.4.**
3. **Kar, R.,** Das, P., Panchali, T., Dutta, A., Phoujdar, M., Ghosh, K., & Pradhan, S. (2025). Anti-obese potentiality of marine Topse (*Polynemus paradiseus*) fish oil by inhibiting the expression of SREBP-1c & promoting β-oxidation of fat through upregulating PPAR-α. *Adipocyte*, *14*(1), 2524640. https://doi.org/10.1080/21623945.2025.2524640 **IF: 3.4.**
4. **Kar, R.,** Panchali, T., Das, P., Dutta, A., Phoujdar, M., & Pradhan, S. (2024). Overview of the therapeutic efficacy of marine fish oil in managing obesity and associated metabolic disorders. *Physiological reports*, *12*(19), e70019. https://doi.org/10.14814/phy2.70019 **IF: 1.9**

As co-author

1. Panchali, T., **Kar, R**., Das, P., Dutta, A., Phoujdar, M., Ghosh, K., & Pradhan, S. (2026). Effect of Icosa-11, 14, 17-Trienoic Acid from Setipinna phasa Oil on Lipogenesis and Adipose Inflammation on Mice with High Fat Diet Induced Obesity. *Metabolites*. *16*(6), 384; **https://doi.org/10.3390/metabo16060384 IF: 3.7**
2. Dutta, A., **Kar, R**., Das, P., Panchali, T., Phoujdar, M., Raut, S., ... & Pradhan, S. (2026). Linoelaidic acid exhibits antitumor activity towards MDA-MB-231 cancer cell-induced tumor mice model through modulation of glutathione level and activation of Caspase family. *Systems Ethnopharmacology and Sustainable Bioresources*, 100013. https://doi.org/10.1016/j.sesb.2026.100013

3. Das, P., **Kar, R**., Panchali, T., Dutta, A., Phoujdar, M., Ghosh, K., & Pradhan, S. (2025). Antiobesity Efficacy of Marine Tapra Fish (Opisthopterus tardoore) Oil in High-Fat Diet-Induced Obese Mice by Activating Lipolysis and Suppressing Adipose Inflammation. *Molecular nutrition & food research*, e70341. https://doi.org/10.1002/mnfr.70341 **IF: 4.2**
4. Das, P., **Kar, R**., Panchali, T., Dutta, A., Phoujdar, M., Ghosh, K., & Pradhan, S. (2025). Mechanistic insights on effective ratio of Linoelaidic and Docosapentaenoic acid by modulating adipogenic and inflammatory biomarkers in 3T3-L1 preadipocytes. *Advances in Biomarker Sciences and Technology*. https://doi.org/10.1016/j.abst.2025.11.005
5. Mangal, S., Jana, S., Dutta, A., **Kar, R**., Are, V., Pradhan, S., Biswas, S., Indu, R., & Ray, M. (2025). Antiproliferative effect of Filopaludina bengalensis fluid inducing apoptosis-necroptosis synergy with immunogenic remodelling in triple-negative breast cancer. *Scientific reports*, *15*(1), 41864. https://doi.org/10.1038/s41598-025-25810-x **IF: 3.9**
6. Panchali, T., Dutta, A., Das, P., Khatun, A., **Kar, R.,** Mondal, S., Mondal, K. C., Chakrabarti, S., Ghosh, K., & Pradhan, S. (2024). Amelioration of obesity induction by a high-fat diet and related inflammation by Phasa fish (Setipinna phasa) oil in BALB/c mice. *Journal of applied biomedicine*, *22*(1), 49–58. https://doi.org/10.32725/jab.2024.003 **IF: 2.0**
7. Das, P., Dutta, A., Panchali, T., Khatun, A., **Kar, R**., Das, T. K., ... & Pradhan, S. (2024). Advances in therapeutic applications of fish oil: A review. *Measurement: Food*, *13*, 100142. https://doi.org/10.1016/j.meafoo.2024.100142 **IF: 3.6**

Check for updates

Received: 3 June 2024 | Revised: 5 August 2024 | Accepted: 14 August 2024

DOI: 10.14814/phy2.70019

The Physiological Society | american physiological society | PHYSIOLOGICAL REPORTS

REVIEW

Overview of the therapeutic efficacy of marine fish oil in managing obesity and associated metabolic disorders

Riya Kar[1,2] | **Titli Panchali**[1,2] | **Pipika Das**[1,2] | **Ananya Dutta**[1,2] | **Manisha Phoujdar**[1,2] | **Shrabani Pradhan**[2]

[1]Biodiversity and Environmental Studies Research Center, Midnapore City College, affiliated to Vidyasagar University, Midnapore, West Bengal, India

[2]Central Research Laboratory, Department of Paramedical and Allied Health Sciences, Midnapore City College, Midnapore, West Bengal, India

Correspondence
Shrabani Pradhan, Central Research Laboratory, Department of Paramedical and Allied Health Sciences, Midnapore City College, Midnapore, Paschim Medinipur 721129, West Bengal, India.
Email: shrabanipradhan@mcconline.org.in

Abstract

In the present scenario, obesity is a challenging health problem and its prevalence along with comorbidities are on the rise around the world. Ingestion of fish becomes trendy in daily meals. Recent research has shown that marine fish oil (FO) (found in tuna, sardines, and mackerel) may offer an alternative method for reducing obesity and problems associated with it. Marine FO rich in long-chain omega-3 polyunsaturated fatty acids (LC n-3 PUFA) and long-chain omega-6 polyunsaturated fatty acids (LC n-6 PUFA) plays an important role in reducing abnormalities associated with the metabolic syndrome and has a variety of disease-fighting properties, including cardioprotective activity, anti-atherosclerotic, anti-obesity, anti-cancer, anti-inflammatory activity. Studies in rodents and humans have indicated that LC n-3 PUFA potentially elicit a number of effects which might be useful for reducing obesity, including suppression of appetite, improvements in circulation, enhanced fat oxidation, energy expenditure, and reduced fat deposition. This review discusses the interplay between inflammation and obesity, and their subsequent regulation via the beneficial role of marine FO, suggesting an alternative dietary strategy to ameliorate obesity and obesity-associated chronic diseases.

KEYWORDS

fat oxidation, marine FO, metabolic disorders, obesity, omega-3 polyunsaturated fatty acids

1 | INTRODUCTION

Recently obesity is becoming the pandemic of the 21st century and its prevalence rate growing very rapidly. Obesity is considered as a heavier problem of our society due to its associated complications (Ray et al., 2019). In spite of above statement as per 2021, Ministry of Statistic and Programme Implementation stated that total population of India is around 1.39 billion more than 135 million individuals are affected by obesity, however the prevalence rate of obesity is 40.3% (Venkatrao et al., 2020). Based on the 2023 World Obesity Atlas, obesity prevalence is expected to increase globally over the next 10 years (Peng et al., 2023). According to Ferreira et al. (2024), it should come as no surprise that obesity has gained prominence on the global public health

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
© 2024 The Author(s). *Physiological Reports* published by Wiley Periodicals LLC on behalf of The Physiological Society and the American Physiological Society.

agenda and has drawn funding from a variety of societal sectors in an effort to slow down this grave health issue. The metabolism of fat and glucose is greatly influenced by adipose tissue (AT), which is a primary location of energy storage. Adipokines, such as leptin, factor-alpha, interleukin-6, and monocyte chemoattractant protein-1, are known to be expressed and secreted by AT (Antuna-Puente et al., 2008). Due to the disruption of adipokine secretion from white adipose tissue (WAT), an excessive amount of WAT contributes to obesity and obesity-related disorders like diabetes mellitus, hypertension, dyslipidemia, and cardiovascular disease in the current nutritionally abundant environment (Walker et al., 2007). Clinical practitioners occupy a crucial role in the front line of obesity and its related comorbidities management, however currently recommended therapies with evidence-based support are lifestyle intervention, pharmacotherapy, and bariatric surgery (Lorente-Cebrián et al., 2013). Only modest benefits have been seen from therapeutic approaches focused on dietary and lifestyle modifications. Pharmacotherapy typically boosts catabolism or improves satiety and suppresses hunger (Gjermeni et al., 2021). In modern times, several drugs, such as orlistat, lorcaserin, liraglutide, phentermine-topiramate, are available in the market for the treatment of obesity and overweight patient (Cui et al., 2017) but these drugs are not free from side effects (Pradhan et al., 2020). Numerous studies stated that marine FO could be a fruitful substitute to reduce obesity and obesity-related metabolic disorders (de Sá et al., 2016). Fish is regarded as a budget-friendly source of a wide range of beneficial elements, mostly high in fat and protein, both of which are important components of a human diet. Fish protein is particularly vital for muscle tissue growth and repair, as well as for immune system enhancement (Pal et al., 2018). The oil obtained from fish contain triglycerides, phospholipids, fatty acids, wax esters, sterols, other minor compounds like glyceryl esters, glycolipids, hydrocarbons like squalene, sulfolipids. While phospholipids and sterols are structural elements of the cell membrane, other lipids function as energy reserves. Marine FO contains a more diverse range of fatty acids, with a particular emphasis on polyunsaturated and monounsaturated fatty acids (PUFA and MUFA) (Das et al., 2024). Dietary sources of long-chain polyunsaturated fatty acids (LC n-3 PUFAs), such as eicosa pentaenoic acid (EPA) and docosahexaenoic acid (DHA), from marine FO have been shown to have hypotriglyceridemic, anti-inflammatory, and cardioprotective effects (Carpentier et al., 2006). Thus, it is particularly interesting to see how marine FO (LC n-3 PUFAs) affects body weight and body composition. We provide an update on the impact of marine FO (LC n-3 PUFAs) on obesity and its related disorders in this review, emphasizing the possible mechanisms for LC n-3 PUFAs in improving body composition, lowering body weight, and mitigating the negative metabolic effects of obesity.

2 | BASIC PATHOMECHANISM OF OBESITY

The basic pathogenesis of obesity involves controlling cellular processes, physical activity, and other factors to either upregulate appetite or downregulate calorie utilization. This dysregulation causes an abundance of adipocytes to form, which boosts the release of cytokines and causes the development of vascular complications. The excess blood level of fatty acids and triglycerol causes accumulation of adipocytes which leads to an increase in oxidative stress, hypertriglyceridemia, lipotoxicity, diabetes, and various metabolic syndromes. Thus, lowering oxidative stress, which is a common etiological factor in many pathological conditions, may be advantageous in minimizing the risky effects of obesity and other complications (Karri et al., 2019). The adipocytes are responsible to stimulating the release of adipocytokines, such as leptin, adiponectin and vastatin, where adiponectin may responsible for cytotoxic autophagy in breast-, colon-, prostate-, and female-specific carcinogenesis (Muppala et al., 2017). These hormones regulate hunger, satiety, and body fat, and their dysregulation can lead to obesity (Karri et al., 2019) (Figure 1).

3 | HORMONAL REGULATION IN OBESITY THROUGH ENERGY BALANCE

Compared to lean individuals, obese people exhibit a different hormonal profile, which may affect energy balance and contribute to maintaining a higher weight. Hormonal and metabolic alterations take place in response to diet-induced weight loss that upsets this energy balance equilibrium (Sumithran et al., 2011).

3.1 | Energy imbalance in obesity

Energy intake, expenditure, and storage are the three main elements of an energy balance. The only time that a person's body weight can change is when their daily energy intake is greater than their daily energy expenditure (Bray & Bouchard, 2014). After increasing body weight when the storage capacity of white AT became

2051817x, 2024, 19, Downloaded from https://physoc.onlinelibrary.wiley.com/doi/10.14814/phy2.70019, Wiley Online Library on [02/10/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

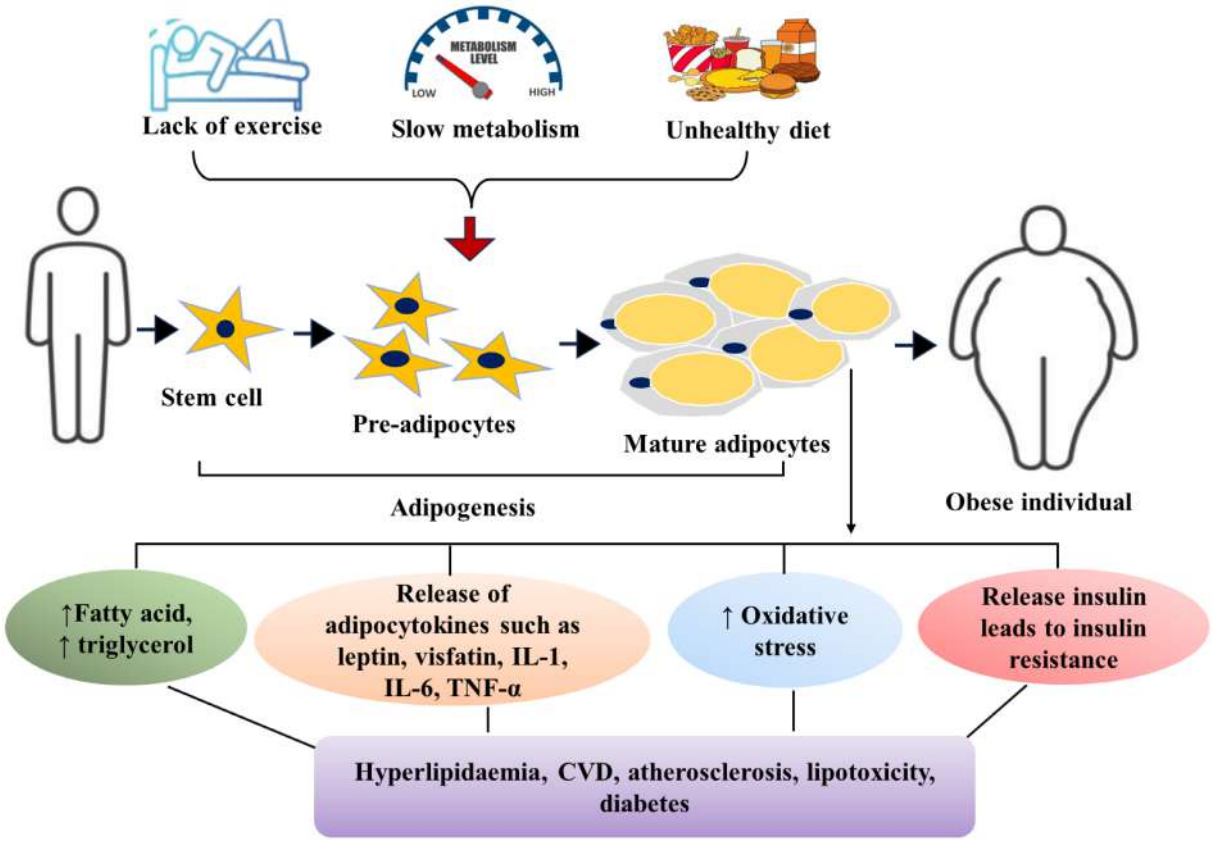


FIGURE 1 Overview of alterations in AT with obesity-associated adipogenesis that responsible for release of some adipocytokines, free fatty acid, triglycerol, and raised the risk of several metabolic disorders in an obese individual.

saturated, lipid accumulation started in triglycerides from our peripheral organs (liver, muscle, pancreas etc.) and the excess fat enter into the non-oxidative pathway. Throughout this process, toxic reactive lipid species, such as diacylglycerols, ceramides, are produced and ultimately causes tissue damage as well as lipotoxicity (Cao, 2014) and side by side upregulate the expression of pro-inflammatory adipokines, downregulate the expression of anti-inflammatory adipokines that ultimately leads to develop obesity and obesity-associated disorders (Figure 2).

3.2 | Long-acting hormonal regulation in energy balance

It has been demonstrated that a number of hormones can influence satiety in both humans and animals by directly affecting the central nervous system (CNS) and the peripheral nervous system. According to Gjermeni et al. (2021), leptin and insulin are the primary players in controlling energy balance as a result of long-term energy reserves. Leptin's physiological function is to convey nutritional status, especially low energy. Thus, a decrease in leptin levels is linked to fasting and losing weight (Busebee et al., 2023). Leptin binds to receptors in the brainstem, hypothalamus, and arcuate nucleus which express pro-opiomelanocortin (POMC) to affect hunger and calorie consumption. POMC undergoes posttranslational processing to produce melanocortin peptides (alpha, beta, and gamma MSH), which act as agonists for melanocortin 3 and 4 receptors. By a variety of melanocortin-dependent and -independent mechanisms, leptin signaling affects energy balance. Energy intake and expenditure are regulated by these hypothalamic pathways in conjunction with other brain regions. Severe obesity is caused by homozygous loss-of-function mutations in POMC that affect signaling through the leptin receptor or reduce leptin production, secretion, or biologic activity. According to studies, targeted genetic disruption of MC4R in mice results in increased food intake, increased lean mass, and linear development, characteristics that completely coincide with those reported in humans with loss-of-function mutations in MC4R (van der Klaauw & Farooqi, 2015). Hormones linked to feeding circulate during the process of food digestion and absorption, which can impact feelings of fullness and the need to stop eating. After eating, and especially after consuming glucose, insulin is secreted. There seems to be a hunger response associated with

2051817x, 2024, 19, Downloaded from https://physoc.onlinelibrary.wiley.com/doi/10.14814/phy2.70019, Wiley Online Library on [02/10/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

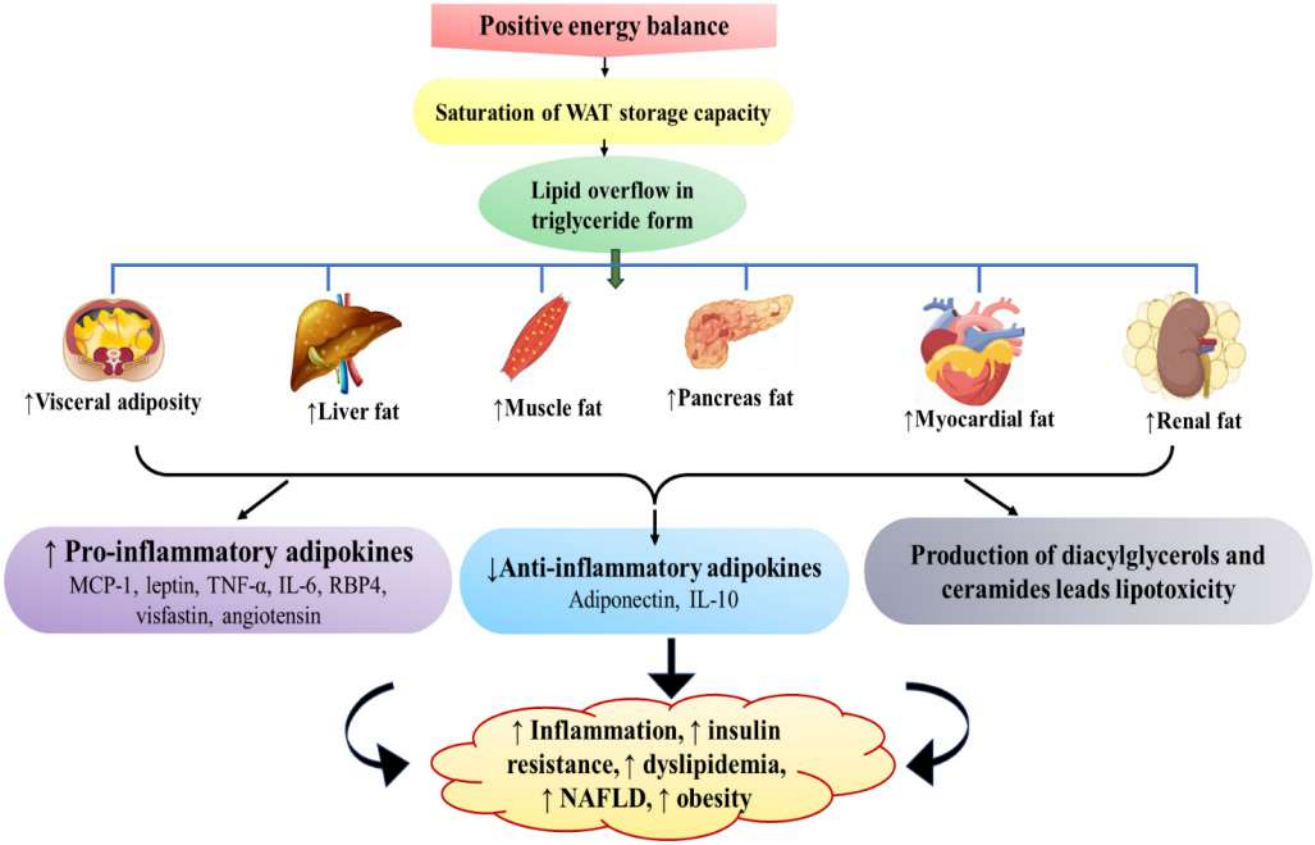


FIGURE 2 Contribution of WAT to obesity and its associated metabolic complications via lipotoxicity, upregulation, and downregulation of some adipokines. Due to alteration of positive energy balance, excessive lipid accumulated in the peripheral organs that leads tissue damage.

this postprandial raise in insulin. Throughout the brain, insulin receptors are present, and insulin can pass the blood–brain barrier (Honda et al., 2007).

3.3 | Short-acting hormonal regulation in positive energy balance: Orexigenic

Dietary consumption, utilization of energy, and metabolism of glucose all are regulated by POMC, agouti-related peptide, and neuropeptide Y (AgRP/NPY) neurons located in the arcuate nucleus. Insulin activates AgRP/NPY and POMC neurons, which carry insulin receptors (Belfort-DeAguiar & Seo, 2018). These are basically antagonistic pathways that converge at targets such as cells in the paraventricular nucleus of the hypothalamus that express melanocortin MC4R. The POMC neurons appear to trigger MC4R-expressing cells in the paraventricular nucleus, which induce satiety. Conversely, the orexigenic hormone ghrelin is mostly released by the stomach (Date et al., 2000). According to Higgins et al. (2007), ghrelin levels rise before a meal and then fall to their baseline levels thereafter. Furthermore, ghrelin induces the release of growth hormone. It causes changes in many gastrointestinal functions and increases food intake by stimulating the vagus nerve.

3.4 | Short-acting hormonal regulation in negative energy balance: Anorexigenic

GLP-1, also known as glucagon-like peptide, is released by L-cell enteroendocrine cells in the colon and small intestine in reaction to intraluminal nutrients, specifically fat and glucose. By promoting satiety and reducing calorie intake, GLP-1 activates myenteric neurons and vagal afferent nerves, which lower the rate of gastric emptying (GE) and have anorexigenic effects. Similar effects are seen by other enteroendocrine-produced peptides, such as cholecystokinin (CCK), which similarly slows GE, stimulates vagal afferents, and encourages meal termination (Makaronidis & Batterham, 2019). Insulin and amylin are released together by pancreatic beta cells. According to Li et al. (2015), it is additionally produced in the lateral hypothalamus, where it works in concert with leptin to decrease calorie intake. Aside from its effect on leptin activity, amylin also raises energy expenditure and affects hedonic elements of eating, which

2051817x, 2024, 19, Downloaded from https://physoc.onlinelibrary.wiley.com/doi/10.14814/phy2.70019, Wiley Online Library on [02/10/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

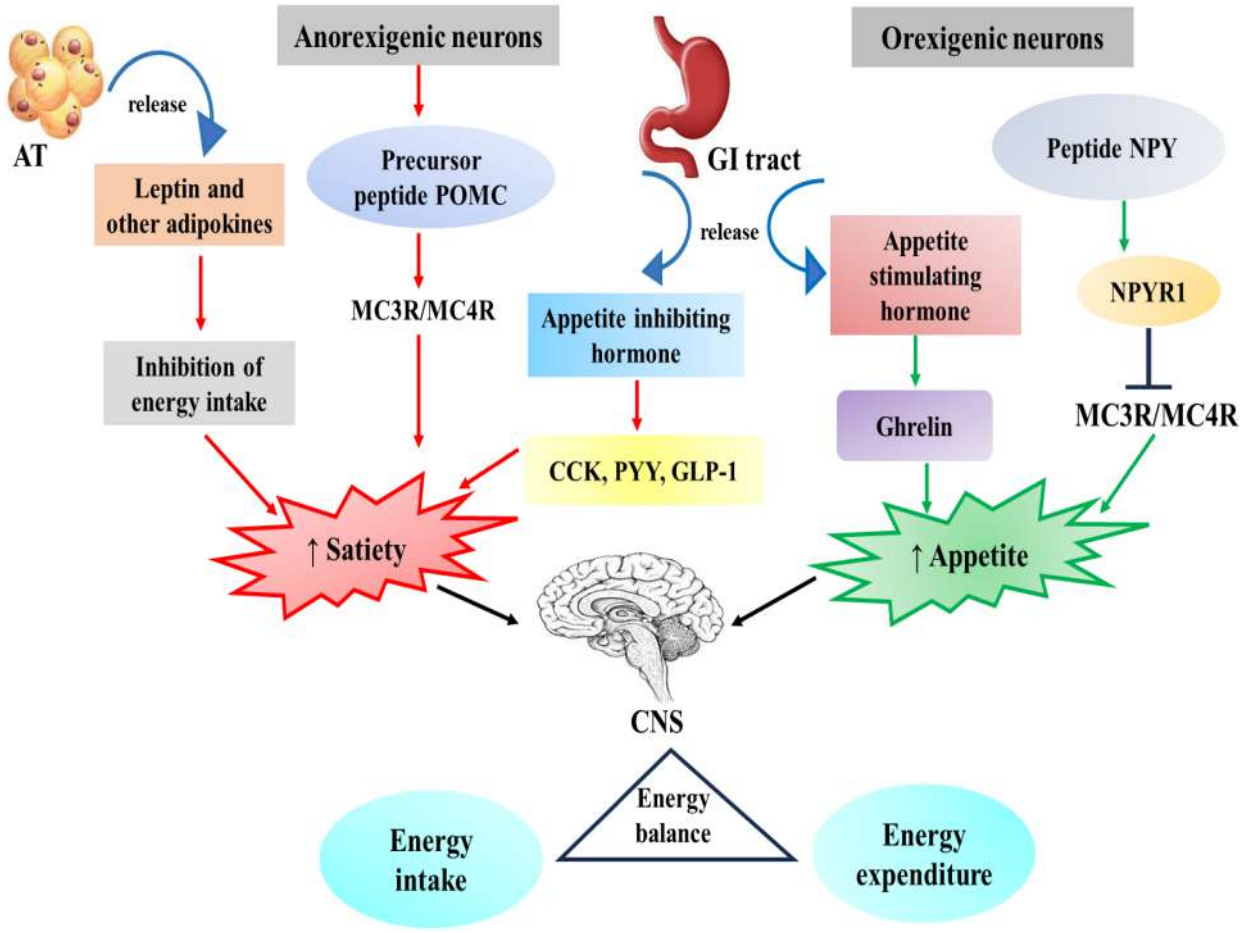


FIGURE 3 A summary of the ways that peripheral hormones regulate hunger and satiety in relation to orexigenic and anorexigenic stimuli. PYY, GLP-1, and CCK are examples of endogenous gut hormones that the peripheral hormones use to control hunger and cause anorexia. In humans, ghrelin has an orexigenic action and increases appetite. Pointed arrows indicate activation; Blocked arrows indicate inhibition. AT, adipose tissue; CCK, cholecystokinin; GLP-1, glucagon like peptide-1; NPY, neuropeptide Y; POMC, propiomelanocortin; PYY, peptide YY; NPY, neuropeptide Y; CNS, central nervous system.

could result in choosing a particular meal type (Whiting et al., 2017) (Figure 3).

4 | ROLE OF AT IN OBESITY

Adipocytes, preadipocytes, endothelial cells, and immune cells are among the various cell types found in AT, which is the primary and significant location for the storage of surplus energy in the form of triglycerides. It has been determined that there are three main varieties of AT: beige, brown adipose tissue (BAT), and WAT. WAT is known as the main energy reservoir of our body and responsible for secretes a huge number of hormones and cytokines that regulate metabolism and insulin resistance. Moreover, BAT, specialized in energy expenditure through no shivering thermogenesis via the mitochondrial uncoupling protein1 (UCP-1) (Mathieu et al., 2010) (Table 1).

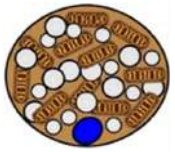
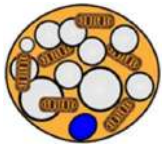
Basically, AT dysfunction is accumulation of ectopic fat including visceral obesity or central obesity (a surplus of fat accumulated in the abdomen, especially from excess visceral fat, is designated as central obesity), which is further characterized by changes in the cellular composition, increased lipid storage and impaired insulin sensitivity in adipocytes, and secretion of a proinflammatory, atherogenic, and diabetogenic adipokine pattern (Blüher et al., 2005). Obesity is the propagation form of WAT with the help of lipolysis process WAT increases release of FFAs that leads to raise of serum fatty acid levels. This over-abundance of lipids has been considered a major reason for obesity-associated insulin resistance and hepatosteatosis (Samuel et al., 2010) (Figure 4).

5 | ROLE OF ADIPOCYTOKINES IN OBESITY AND OBESITY-INDUCED METABOLIC DISORDERS

Leptin play important role to regulate hepatic lipogenesis by suppressing the expression of key enzymes in the

2051817x, 2024, 19, Downloaded from https://physoc.onlinelibrary.wiley.com/doi/10.14814/phy2.70019, Wiley Online Library on [02/10/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 1 Characteristics of white, brown, and beige adipocytes.

Adipocytes	Lipid droplets	Mitochondrial density	Deposition	UCP1 expression	Secreted factors	Function	Changes occur during obesity
 White adipose tissue	Large unilocular	Low	It is extensively spread throughout the body's viscera and subcutaneous tissue	Negative	Leptin, adiponectin, resistin	Lipogenesis, lipolysis, glucose uptake, adipokine secretion	Hyperplasia, hypertrophy, immune cell, infiltration, secretion of vasoconstrictors
 Brown adipose tissue	Small multilocular	High	It is mainly distributed in the interscapular region	Positive	FGF-21, IL-6	Thermogenesis, lipid clearance, glucose uptake, batokine secretion	Potentially resistant to obesity induced inflammation
 Beige	Small multilocular	High	In adults, it's beneath the skin close to the collarbone and spine	Positive	FGF-21, IL-6, SLIT-2	Thermogenesis, catabolism	Loss of UCP1 expression

2051817x, 2024, 19, Downloaded from https://physoc.onlinelibrary.wiley.com/doi/10.14814/phy2.70019, Wiley Online Library on [02/10/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

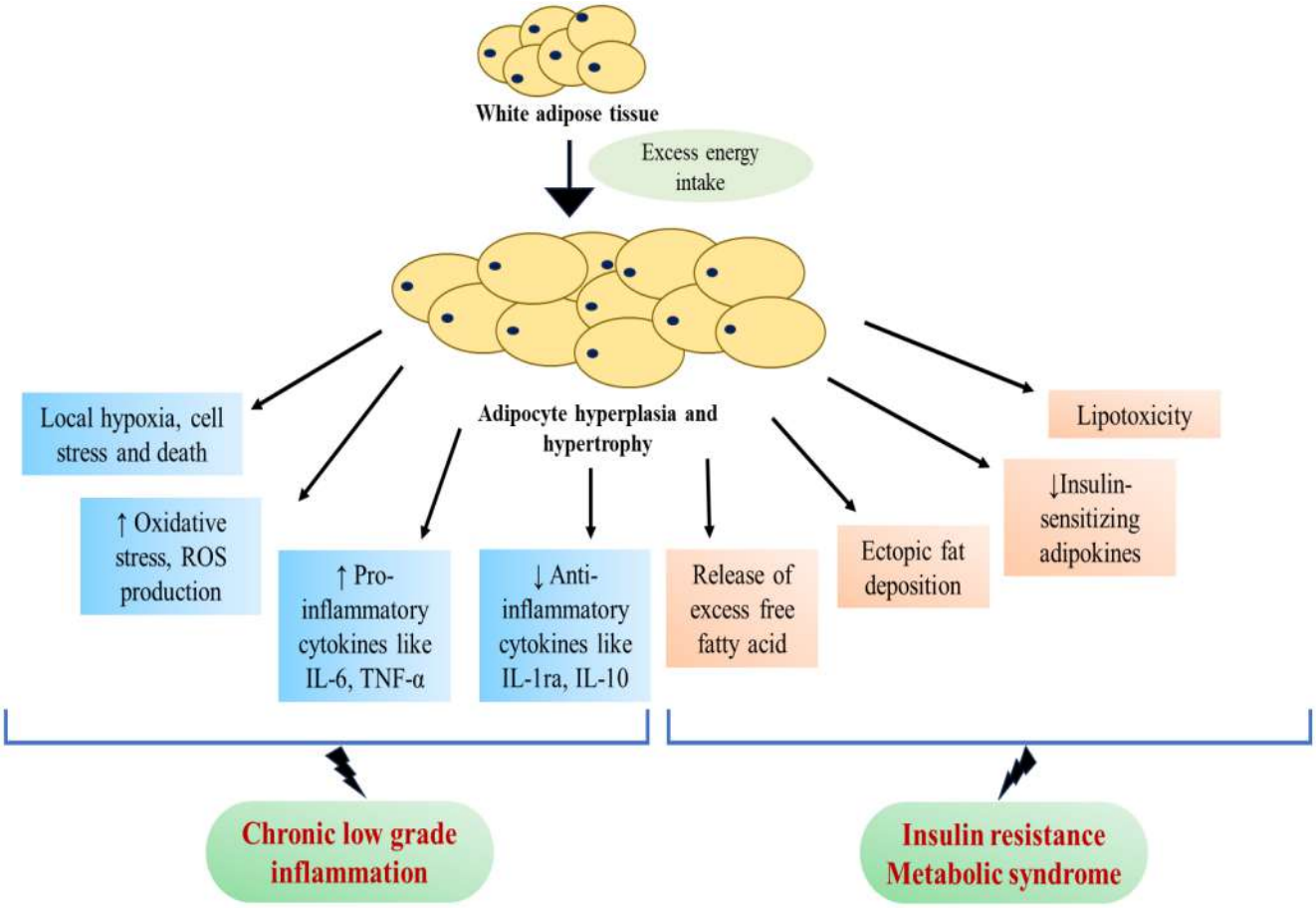


FIGURE 4 Adipocytokines and metabolic inflammation in adipose tissue. The interplay between immune cells and adipocytes that leads to AT malfunction produces adipocytokines, which are produced from AT. Obese patients frequently have changed levels and actions of adipocytokines, which can lead to diseases generated by obesity.

fatty acid synthesis pathway (Cohen et al., 2002). AMP-activated protein kinase (AMPK) is a crucial cellular energy sensor that is activated by leptin, which enhances muscle fatty acid oxidation (Minokoshi et al., 2002). In obesity, metabolic stress is diminished by protective role of adiponectin through its anti-inflammatory effects. Tumor necrosis factor-alpha (TNF-α) production in obese mice is inhibits by adiponectin. Low levels of plasma adiponectin are associated with C-reactive protein in humans (Ouchi et al., 2003). Adiponectin levels are negatively correlated with the adiposity and fasting glucose. The main role of TNF-α is promotes insulin resistance of obesity and non-insulin dependent diabetes mellitus (NIDDM). Specifically, in muscle and fat tissues insulin sensitivity improves through neutralization of TNF-α by enhancing the activity of the insulin receptor tyrosine kinase (Heir & Stellwagen, 2020). Studies stated that increasing circulating level of resistin increases the risk of type 2 diabetes, inflammatory markers, atherosclerosis (Chen et al., 2009). IL-6 is one of the major pro-inflammatory cytokines as well as an anti-inflammatory myokine, in obese patients IL-6 serum level was found to be increased that resulting the development of chronic inflammatory conditions and serum lipid concentrations abnormalities (Galcheva et al., 2011). According to Blüher et al. (2005), IL-10 is inversely linked with BMI and fasting blood sugar levels. Low levels of IL-10 are associated with both the metabolic syndrome and T2D. Fatty acid synthase (FAS) is a central enzyme in lipogenesis which identified as a candidate gene for determining body fat. Sterol regulatory element binding protein-1c (SREBP-1c) is a member of a family of transcription factors that was initially engaged in the control of genes by the cellular availability of cholesterol (Sewter et al., 2002). Main function of lipoprotein lipase (LPL) is hydrolysis of triglyceride in chylomicron and VLDL and clearance of plasma triglyceride rich particles (Gonzales & Orlando, 2007) those are used for metabolic energy or re-esterified into triglyceride which stored in AT. Moreover, peroxisome proliferator-activated receptors alpha (PPAR-α) reduces endogenous fatty acid synthesis by blocking enzymes like acetyl-CoA carboxylase and FASN. Ghrelin has various physiological functions, such as the stimulation of growth hormone release and of appetite, and fat accumulation. Ghrelin is the

2051817x, 2024, 19, Downloaded from https://physoc.onlinelibrary.wiley.com/doi/10.14814/phy2.70019, Wiley Online Library on [02/10/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

only peripheral hormone to transmit satiety signal (Sato et al., 2014).

6 | BASIC COMPOSITION OF MARINE FO AND SYNTHESIS OF PRESENT ESSENTIAL FATTY ACIDS (N-3 FATTY ACIDS AND N-6 FATTY ACIDS)

LC n-6 PUFAs and LC n-3 PUFAs are the two major PUFA families that are important to human health. Within most diets, the PUFAs with the largest concentrations are α-linolenic acid (ALA, 18:3 n-3) and linoleic acid (LA, 18:2 n-6) (Djuricic & Calder, 2021). LA and ALA are essential fatty acids that cannot be synthesized by the human body and must be taken from outside (Al-Khalaifah & Al-Nasser, 2019). The LC n–3 PUFAs EPA, 20:5 n-3, and DHA, 22:6 n-3 can be synthesized very slowly from ALA in the human body with very little amounts (Kocatepe & Turan, 2018). For this reason, we need to consume foods rich in these oils in order to ensure that enough EPA and DHA are synthesized from our body. Additionally, vitamins A and D, which are essential for children's growth and development, are typically present in marine FO (Kaur et al., 2014). Marine FO provides micronutrients comprising selenium, iodine, potassium also. The European Food Safety Authority (EFSA) states that the ratio of LC n-3 to LC n-6 fatty acids ranges from 1.3 to 21.2. The balance of n-6/n-3 LC-PUFAs is an important determinant in homeostasis maintenance, normal development, and mental health throughout the life cycle. Therefore, eating more fish may significantly improve the body's ability to absorb LC n-3 fatty acids and maximize the use of EPA and DHA, which will benefit numerous physiological processes (Das et al., 2024). In order to keep the human body healthy, it is also crucial to take into account the ratio of dietary n-6 to n-3 fatty acids. However, the ratio of n-6: n-3 in the typical western diet is 10–20:1. A high n-6/n-3 ratio and excessive n-6 PUFA consumption have been linked to an increased risk of several diseases, such as cancer, autoimmune diseases, obesity, inflammation, and cardiovascular disease (CVD). Conversely, a low n-6/n-3 ratio or an increase in high n-3 PUFA intake have a suppressive impact. The ideal ratio may vary depending on the disease, but usually a 3:1–4:1 ratio is suggested to prevent many pathological diseases brought on by the modern western diet. For instance, a 4/1 ratio is linked to a 70% decrease in overall CVD mortality. The 2.5/1 ratio may inhibit rectal cell proliferation in patients with colorectal cancer. A lower n-6/n-3 ratio was linked to a lower risk in breast cancer-affected women, and a 2–3/1 ratio was shown to be effective in reducing patients' obesity and its related inflammation (Kartikasari et al., 2024). Many studies have found low conversion rates of ALA to EPA and DPA, and little to no DHA synthesis; hence, any direct benefits of these very long-chain fatty acids depend on dietary intake (Pawlosky et al., 2001). Both dietary intake and fatty acid desaturase activity determine plasma n-3 PUFA levels (Vessby, 2003) (Figure 5).

7 | ROLE OF MARINE FO ON OBESITY AND OBESITY-ASSOCIATED METABOLIC DISORDERS

Recently marine FO has attracted special attention for its fruitful health beneficial effect. Marine FO exerts many defensive properties against several diseases such as obesity, cardiovascular diseases, rheumatoid arthritis, depression, cognitive decline, and neurological disorders, such as Alzheimer's, thought to have anti-thrombotic and anti-inflammatory properties (Hostalek et al., 2015). Daily intake of fish through diet can diminish the risk of coronary heart disease for the presence of protective components such as EPA and DHA (Iso et al., 2006). LC n-3 PUFAs are major ligands of PPARs. Their binding induced transcription of specific genes encoding inflammation in AT. Marine FO play important role to activated PPARα (Wang et al., 2021). β-Oxidation is a catabolic process in myocytes that enhanced by EPA as well as it can responsible for ameliorate intramyocellular triacylglycerol lowering associated with compression of lipogenesis (Yu et al., 2011). DHA is incorporated from dietary marine FO in the hippocampus, where it controls the expression of genes implicated in the prevention of neurological diseases and can slow the age-related cognitive decline (Denis et al., 2013). Consumption of dietary marine FO can play a significant impact in ghrelin modulation, which may affect feeding behavior and energy intake. Saidpour et al. (2012) found that a high-fat meal combined with marine FO had the strongest stimulatory effect on fasting ghrelin expression and plasma level. However, Cao et al. (2022) investigated whether marine FO containing DHA could reduce diet-induced insulin resistance in obese individuals, in part by regulating gut microbiome (e.g., lactobacillus) and promoting colonic PYY expression (via the mediation of short-chain fatty acid receptor FFA4 in colon), both of which play important roles in linking diet, gut microbiome, and host metabolism. Research suggests that consuming natural fatty acids, such as marine FO (tuna, sardine, mackerel, and tapra), may help reduce obesity (Das et al., 2009). However, in order to support the current findings and explore the long-term benefits of n-3 fatty acid supplementation in managing overweight and

2051817x, 2024, 19, Downloaded from https://physoc.onlinelibrary.wiley.com/doi/10.14814/phy2.70019, Wiley Online Library on [02/10/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

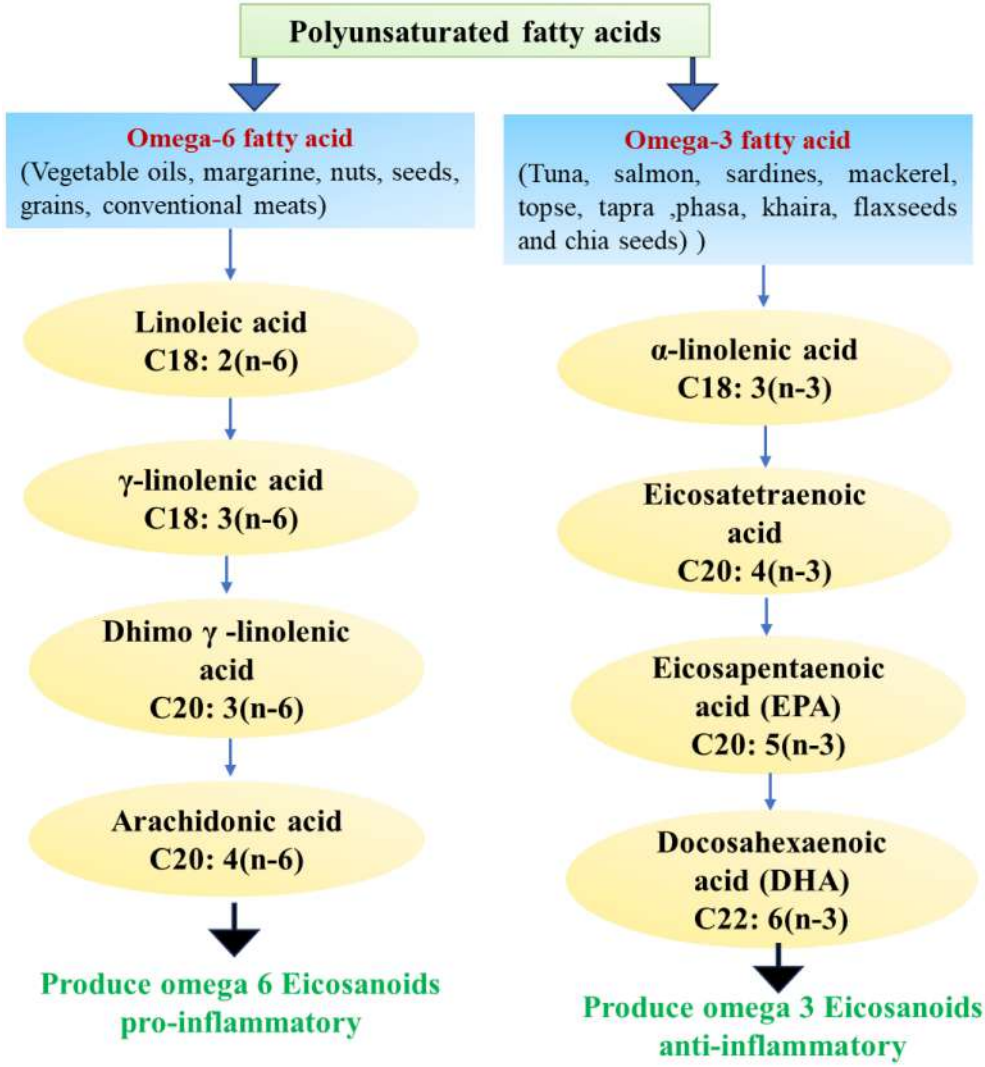


FIGURE 5 Classification of LC-PUFAs. PUFAs are subdivided into two subclasses: n-6 and n-3 PUFAs. This flowchart represents general pathway of LC n-3 and n-6 fatty acids synthesis in human body. AA and proinflammatory eicosanoids are produced by the metabolism of LA, an important LC n-6 PUFA. The necessary LC n-3 PUFA ALA is broken down into EPA and DHA. Though they are thought to be more anti-inflammatory, eicosanoids produced from the metabolism of EPA and DHA also help to regulate inflammation.

obesity, a large-scale, multi-center, placebo-controlled long-term trial should be carefully planned. The researchers found that n-3 PUFA supplementation could serve as an effective approach to lower triglyceride levels and waist circumference (Zhang et al., 2017). It has been suggested that tapra FO (a marine fish found in the Bay of Bengal) supplementation may reduce levels of body weight, BMI, serum total cholesterol, triglycerides, low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL) in obese mice model where the mice was treated with 12.5 mg/kg body weight /day amount of tapra FO for 4 weeks of total experiment. However, marine FO contains various essential fatty acids which may increase lipid oxidation, decrease lipid synthesis, and inhibit lipogenesis (Pradhan et al., 2020). WAT produces leptin, which binds to brain receptors such as anandamide and neuropeptide Y (both of which are appetite promoters), reducing hunger and inducing satiety. In this way, it communicates with the brain to regulate food and energy consumption (Al-Hussaniy et al., 2021). Panchali et al. (2024) explored that marine FO(treated with Phasa FO, 12.5 mg/kg body weight/day) containing conjugated fatty acids (specially LC n-3 PUFAs) can inhibit the protein and mRNA expression of leptin by correlated with the inhibition of fat accumulation and adipocyte proliferation due to disruption of leptin signaling (Figure 6). According to US Food and Drug Administration guidelines, including up to 3 gm of marine FO/day in the diet is generally considered safe. Increased bleeding time and drug interactions are two possible side effects that may be concerning at larger doses, but these symptoms are usually not regarded as clinically significant (Fetterman Jr & Zdanowicz, 2009).

2051817x, 2024, 19, Downloaded from https://physoc.onlinelibrary.wiley.com/doi/10.14814/phy2.70019, Wiley Online Library on [02/10/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

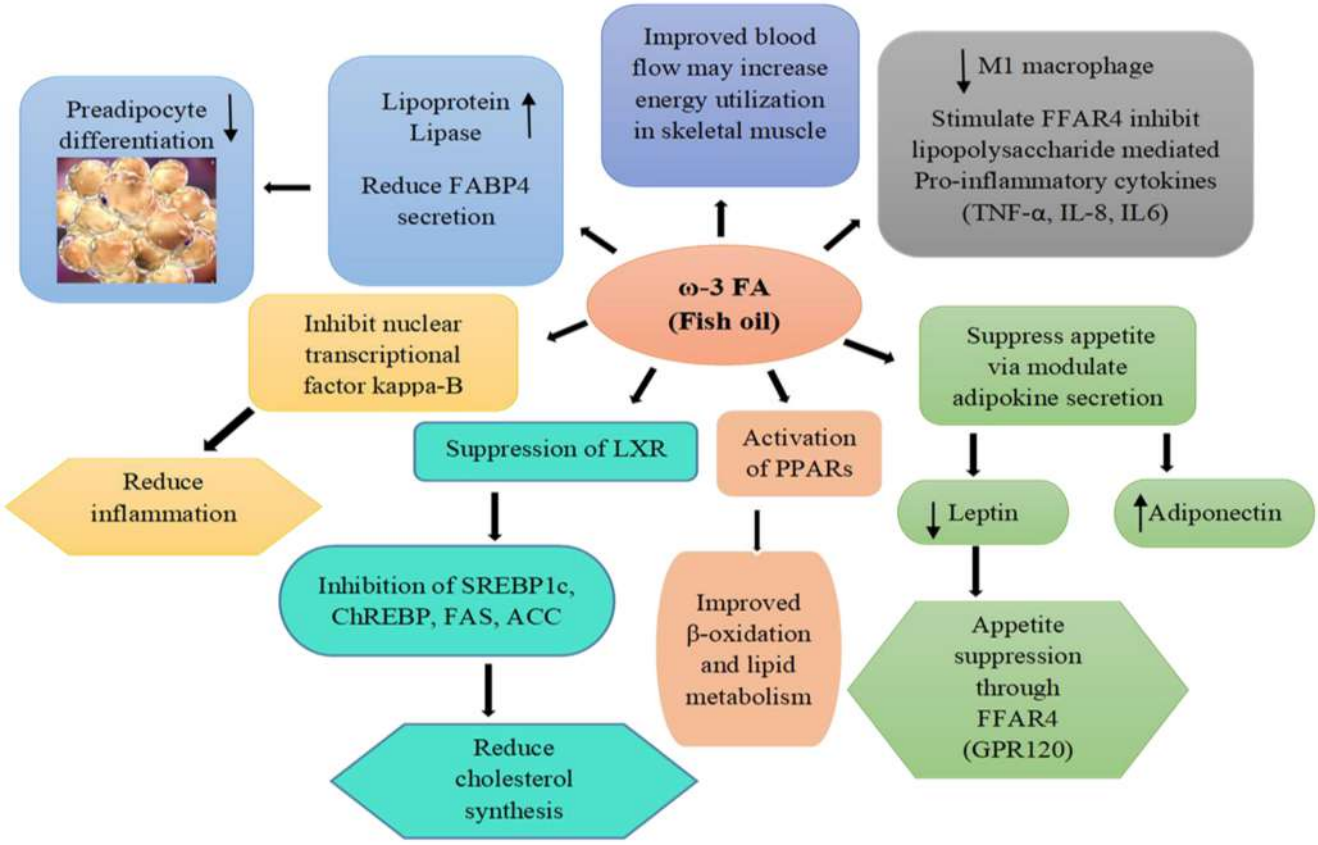


FIGURE 6 Beneficial effects of marine FO on obesity and obesity-associated metabolic disorders. Marine FO containing n-3 PUFAs can modulate adipokine secretion by suppressing the function of LXR, FABP4, SREBP-1c, FAS, ChREBP. Eventually, inflammatory cytokines are expressed resulting in chronic inflammatory conditions in adipocytes of WAT. Dietary marine FO may have preventive effects on these events, leading to health benefits.

8 | MECHANISM OF N-3 PUFAS IMPROVE ADIPOSITY AND OBESITY THROUGH LIPID METABOLISM VIA THE PPAR- γ AND AMPK PATHWAYS

There are several proposed mechanisms by which n-3 PUFA could work in reducing body weight and improving the metabolic profile. AT expansion in obesity occurs via adipocyte hypertrophy (enlargement of adipocytes) and hyperplasia (increase in adipocyte number due to adipogenesis). LC n-3 PUFAs alter expression and nuclear localization of both the transcription factor SREBP-1 and the ChREBP, which control several lipogeneic genes, including those regulating cholesterol and fatty acid synthesis. Furthermore, n-3 PUFAs suppress hepatic lipogenesis by reducing both messenger RNA (mRNA) and active protein expression of SREBP-1c, which results in reduced expression of many genes involved in lipogenesis, including FAS and acetyl-coA carboxylase. PPARs are transcription factors that form heterodimers with retinoid X receptors in the promoter regions of several genes involved in lipid and glucose metabolism. For example, LC n-3 PUFA activation of PPARα decreases lipogenesis by suppressing FAS activity (Kaur et al., 2011). It has been suggested that PPARγ plays a significant role in the ability of n-3 PUFA, specifically DHA, to stimulate M2 macrophage polarization and thereby reduce inflammation. LC n-3 PUFAs have been shown to increase mitochondrial biogenesis and fatty acid oxidation in the liver, AT and small intestine of rodents, possibly through PPARα and COX3 induction (Hensler et al., 2011). PUFA-controlled genes involved in lipid oxidation and thermogenesis include mitochondrial HMG-CoA synthase, peroxisomal acyl-CoA oxidase, hepatic CPT-1, FABP and fatty acid transporter proteins. Activation of PPARα can also increase fatty acid oxidation. Increases in fatty acid oxidation by n-3 PUFA may also be mediated by AMPK, a known regulator of cellular energy metabolism. AMPK upregulation by n-3 PUFA has been proved in both AT and cultured adipocytes. As a central energy sensor, the AMPK has now been explored to play a paramount role in fat synthesis and catabolism, especially in regulating the energy expenditure of brown/beige adipose tissue and the browning of WAT (Kasbi Chadli et al., 2012) (Figure 7).

2051817x, 2024, 19, Downloaded from https://physoc.onlinelibrary.wiley.com/doi/10.14814/phy2.70019, Wiley Online Library on [02/10/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

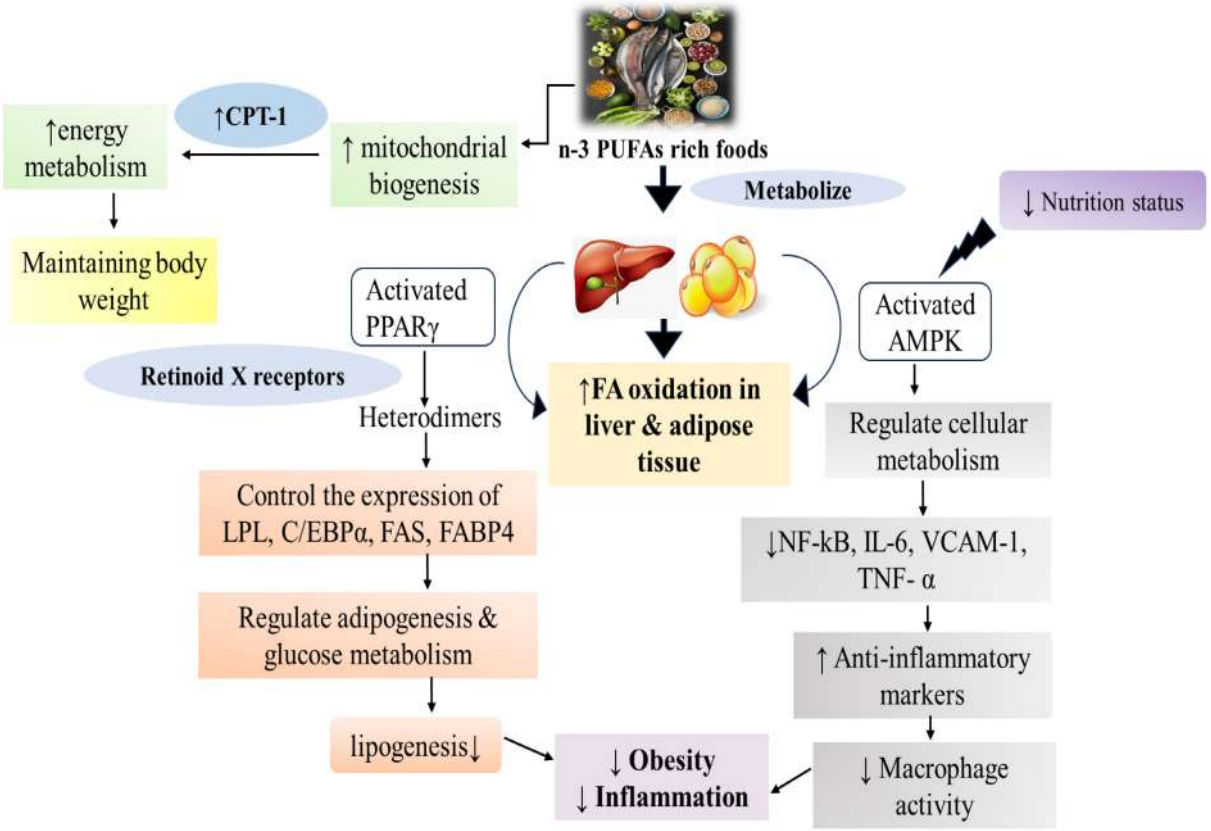


FIGURE 7 Mechanisms mediating effects of n-3 PUFA on liver and AT metabolism. LC n-3 PUFA increase fatty acid oxidation in the liver and adipose tissue, thus limiting fat storage in these tissues. LC n-3 PUFA also decrease the production and release of pro-inflammatory adipokines by two major pathways PPAR- γ and AMPK pathways. All mechanisms depicted here contribute to an improved metabolic profile.

9 | FUTURE TRENDS

According to the present assessment, marine FO is an useful productive field of research with bright future prospects. Thus, by using FO as the only source of dietary oil and varying the amount or type of oil in the diet, marine fish can be readily farmed to yield fillets with levels of oil, 22:6n-3 and 20:5n-3 as required to suit consumer demand and human nutritional and health requirements. Modem fish farming, and especially marine fish farming, has much to offer, whether nutritionally, economically, or ethically, for optimizing the exploitation of limited global marine resources of FO. On the basis of current situation, it has been predicted that during the decade to come, marine FO might be revolutionize for the management for multiple illnesses.

10 | CONCLUSION

Marine FO has received a lot of attention in medical science over the last few decades, and some experts recommend it because of its functional properties. Marine FO is a growing source of LC n-3 fatty acids, such as DHA and EPA, which can mitigate the negative effects of long-term illnesses. This review work was done to look after how marine FO fight against obesity and obesity-associated inflammation via several mechanism of LC n-3 fatty acids. We may draw the conclusion that, in order to improve society, a greater portion of the populace needs to be made aware of the quantity, significance, and health impact of marine FO.

AUTHOR CONTRIBUTIONS

Riya Kar: Writing—review, editing & preparing figures and table, Writing—original draft, visualization, resources, project administration, investigation, formal analysis. **Titli Panchali:** Writing—review & editing, formal analysis. **Pipika Das:** Writing—original draft, investigation, formal analysis. **Ananya Dutta** and **Manisha Phoujdar:** Performed formal analysis. **Shrabani Pradhan:** Conceptualized the study, supervised the review, editing and approved the final manuscript.

ACKNOWLEDGMENTS

The authors are thankful to the Director, Dr. Pradip Ghosh, Midnapore City College for provide all essential

2051817x, 2024, 19, Downloaded from https://physoc.onlinelibrary.wiley.com/doi/10.14814/phy2.70019, Wiley Online Library on [02/10/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

supports to carry out this work. The authors also acknowledge to Vidyasagar University for providing affiliation of research centre to Midnapore City College as Biodiversity and Environmental Studies Research centre. The current research study presented in this manuscript is a part of the PhD research work of the first author.

FUNDING INFORMATION

This work was supported by own fundings, Biodiversity and Environmental Studies Research Center, Midnapore City College, Bhadutala, Paschim Medinipur, 721129, West Bengal, India.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of the study are available from the corresponding author upon request.

ETHICS STATEMENT

This work does not involve trials on any human or animals.

ORCID

Shrabani Pradhan https://orcid.org/0000-0002-9796-8952

REFERENCES

Al-Hussaniy, H. A., Alburghaif, A. H., & Naji, M. A. (2021). Leptin hormone and its effectiveness in reproduction, metabolism, immunity, diabetes, hopes and ambitions. *Journal of Medicine and Life*, *14*(5), 600–605. https://doi.org/10.25122/jml-2021-0153

Al-Khalaifah, H., & Al-Nasser, A. (2019). Role of poultry research in increasing consumption of PUFA in humans. In *Nutrition in health and disease-our challenges now and forthcoming time*. IntechOpen.

Antuna-Puente, B., Feve, B., Fellahi, S., & Bastard, J. P. (2008). Adipokines: The missing link between insulin resistance and obesity. *Diabetes & Metabolism*, *34*(1), 2–11. https://doi.org/10.1016/j.diabet.2007.09.004

Belfort-DeAguiar, R., & Seo, D. (2018). Food cues and obesity: Overpowering hormones and energy balance regulation. *Current Obesity Reports*, *7*(2), 122–129. https://doi.org/10.1007/s13679-018-0303-1

Blüher, M., Fasshauer, M., Tönjes, A., Kratzsch, J., Schön, M. R., & Paschke, R. (2005). Association of interleukin-6, C-reactive protein, interleukin-10 and adiponectin plasma concentrations with measures of obesity, insulin sensitivity and glucose metabolism. *Experimental and Clinical Endocrinology & Diabetes*, *113*(9), 534–537. https://doi.org/10.1055/s-2005-872851

Bray, G., & Bouchard, C. (Eds.). (2014). *Handbook of obesity-volume 2: Clinical applications* (Vol. *2*). CRC Press.

Busebee, B., Ghusn, W., Cifuentes, L., & Acosta, A. (2023). Obesity: A review of pathophysiology and classification. *Mayo Clinic Proceedings*, *98*(12), 1842–1857. https://doi.org/10.1016/j.mayocp.2023.05.026

Cao, H. (2014). Adipocytokines in obesity and metabolic disease. *The Journal of Endocrinology*, *220*(2), T47–T59. https://doi.org/10.1530/JOE-13-0339

Cao, W., Liu, F., Li, R. W., Chin, Y., Wang, Y., Xue, C., & Tang, Q. (2022). Docosahexaenoic acid-rich fish oil prevented insulin resistance by modulating gut microbiome and promoting colonic peptide YY expression in diet-induced obesity mice. *Food Science and Human Wellness*, *11*(1), 177–188.

Carpentier, Y. A., Portois, L., & Malaisse, W. J. (2006). n-3 fatty acids and the metabolic syndrome. *The American Journal of Clinical Nutrition*, *83*(6 Suppl), 1499S–1504S. https://doi.org/10.1093/ajcn/83.6.1499S

Chen, B. H., Song, Y., Ding, E. L., Roberts, C. K., Manson, J. E., Rifai, N., Buring, J. E., Gaziano, J. M., & Liu, S. (2009). Circulating levels of resistin and risk of type 2 diabetes in men and women: Results from two prospective cohorts. *Diabetes Care*, *32*(2), 329–334. https://doi.org/10.2337/dc08-1625

Cohen, P., Miyazaki, M., Socci, N. D., Hagge-Greenberg, A., Liedtke, W., Soukas, A. A., Sharma, R., Hudgins, L. C., Ntambi, J. M., & Friedman, J. M. (2002). Role for stearoyl-CoA desaturase-1 in leptin-mediated weight loss. *Science (New York, N.Y.)*, *297*(5579), 240–243. https://doi.org/10.1126/science.1071527

Cui, C., Li, Y., Gao, H., Zhang, H., Han, J., Zhang, D., Li, Y., Zhou, J., Lu, C., & Su, X. (2017). Modulation of the gut microbiota by the mixture of fish oil and krill oil in high-fat diet-induced obesity mice. *PLoS One*, *12*(10), e0186216. https://doi.org/10.1371/journal.pone.0186216

Das, P., Dutta, A., Panchali, T., Khatun, A., Kar, R., Das, T. K., Phoujdar, M., Chakrabarti, S., Ghosh, K., & Pradhan, S. (2024). Advances in therapeutic applications of fish oil: A review. *Measurement: Food*, *100142*, 1–17.

Das, S., Paul, B. N., Sengupta, J., & Datta, A. K. (2009). Beneficial effects of fish oil to human health: A review. *Agricultural Reviews*, *30*(3), 199–205.

Date, Y., Kojima, M., Hosoda, H., Sawaguchi, A., Mondal, M. S., Suganuma, T., Matsukura, S., Kangawa, K., & Nakazato, M. (2000). Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. *Endocrinology*, *141*(11), 4255–4261. https://doi.org/10.1210/endo.141.11.7757

de Sá, R. D., Crisma, A. R., Cruz, M. M., Martins, A. R., Masi, L. N., do Amaral, C. L., Curi, R., & Alonso-Vale, M. I. (2016). Fish oil prevents changes induced by a high-fat diet on metabolism and adipokine secretion in mice subcutaneous and visceral adipocytes. *The Journal of Physiology*, *594*(21), 6301–6317. https://doi.org/10.1113/JP272541

Denis, I., Potier, B., Vancassel, S., Heberden, C., & Lavialle, M. (2013). Omega-3 fatty acids and brain resistance to ageing and stress: Body of evidence and possible mechanisms. *Ageing Research Reviews*, *12*(2), 579–594. https://doi.org/10.1016/j.arr.2013.01.007

Djuricic, I., & Calder, P. C. (2021). Beneficial outcomes of omega-6 and omega-3 polyunsaturated fatty acids on human health: An update for 2021. *Nutrients*, *13*(7), 2421. https://doi.org/10.3390/nu13072421

Ferreira, S. R. G., Macotela, Y., Velloso, L. A., & Mori, M. A. (2024). Determinants of obesity in Latin America. *Nature*

2051817x, 2024, 19, Downloaded from https://physoc.onlinelibrary.wiley.com/doi/10.14814/phy2.70019, Wiley Online Library on [02/10/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Metabolism, *6*(3), 409–432. https://doi.org/10.1038/s42255-024-00977-1

Fetterman, J. W., Jr., & Zdanowicz, M. M. (2009). Therapeutic potential of n-3 polyunsaturated fatty acids in disease. *American Journal of Health-System Pharmacy: AJHP*, *66*(13), 1169–1179. https://doi.org/10.2146/ajhp080411

Galcheva, S. V., Iotova, V. M., Yotov, Y. T., Bernasconi, S., & Street, M. E. (2011). Circulating proinflammatory peptides related to abdominal adiposity and cardiometabolic risk factors in healthy prepubertal children. *European Journal of Endocrinology*, *164*(4), 553–558. https://doi.org/10.1530/EJE-10-1124

Gjermeni, E., Kirstein, A. S., Kolbig, F., Kirchhof, M., Bundalian, L., Katzmann, J. L., Laufs, U., Blüher, M., Garten, A., & Le Duc, D. (2021). Obesity-an update on the basic pathophysiology and review of recent therapeutic advances. *Biomolecules*, *11*(10), 1426. https://doi.org/10.3390/biom11101426

Gonzales, A. M., & Orlando, R. A. (2007). Role of adipocyte-derived lipoprotein lipase in adipocyte hypertrophy. *Nutrition & Metabolism*, *4*, 22. https://doi.org/10.1186/1743-7075-4-22

Heir, R., & Stellwagen, D. (2020). TNF-mediated homeostatic synaptic plasticity: From *in vitro* to *in vivo* models. *Frontiers in Cellular Neuroscience*, *14*, 565841. https://doi.org/10.3389/fncel.2020.565841

Hensler, M., Bardova, K., Jilkova, Z. M., Wahli, W., Meztger, D., Chambon, P., Kopecky, J., & Flachs, P. (2011). The inhibition of fat cell proliferation by n-3 fatty acids in dietary obese mice. *Lipids in Health and Disease*, *10*, 128. https://doi.org/10.1186/1476-511X-10-128

Higgins, S. C., Gueorguiev, M., & Korbonits, M. (2007). Ghrelin, the peripheral hunger hormone. *Annals of medicine*, *39*(2), 116–136. https://doi.org/10.1080/07853890601149179

Honda, K., Kamisoyama, H., Saneyasu, T., Sugahara, K., & Hasegawa, S. (2007). Central administration of insulin suppresses food intake in chicks. *Neuroscience Letters*, *423*(2), 153–157. https://doi.org/10.1016/j.neulet.2007.07.004

Hostalek, U., Czarnecka, D., & Koch, E. M. (2015). Treatment of hypertensive patients with a fixed-dose combination of Bisoprolol and amlodipine: Results of a cohort study with more than 10,000 patients. *Cardiology and Therapy*, *4*(2), 179–190. https://doi.org/10.1007/s40119-015-0045-z

Iso, H., Kobayashi, M., Ishihara, J., Sasaki, S., Okada, K., Kita, Y., Kokubo, Y., Tsugane, S., & JPHC Study Group. (2006). Intake of fish and n3 fatty acids and risk of coronary heart disease among Japanese: The Japan public health center-based (JPHC) study cohort I. *Circulation*, *113*(2), 195–202. https://doi.org/10.1161/CIRCULATIONAHA.105.581355

Karri, S., Sharma, S., Hatware, K., & Patil, K. (2019). Natural anti-obesity agents and their therapeutic role in management of obesity: A future trend perspective. *Biomedicine & Pharmacotherapy*, *110*, 224–238. https://doi.org/10.1016/j.biopha.2018.11.076

Kartikasari, L., Geier, M., Hughes, R., Bastian, S., & Gibson, R. (2024). Assessment of omega-3 and omega-6 fatty acid profiles and ratio of omega-6/omega-3 of white eggs produced by laying hens fed diets enriched with omega-3 rich vegetable oil. *Open Agriculture*, *9*(1), 20220274. https://doi.org/10.1515/opag-2022-0274

Kasbi Chadli, F., Andre, A., Prieur, X., Loirand, G., Meynier, A., Krempf, M., Nguyen, P., & Ouguerram, K. (2012). n-3 PUFA prevent metabolic disturbances associated with obesity and improve endothelial function in golden Syrian hamsters fed with a high-fat diet. *The British Journal of Nutrition*, *107*(9), 1305–1315. https://doi.org/10.1017/S0007114511004387

Kaur, G., Sinclair, A. J., Cameron-Smith, D., Barr, D. P., Molero-Navajas, J. C., & Konstantopoulos, N. (2011). Docosapentaenoic acid (22:5n-3) down-regulates the expression of genes involved in fat synthesis in liver cells. *Prostaglandins, Leukotrienes, and Essential Fatty Acids*, *85*(3–4), 155–161. https://doi.org/10.1016/j.plefa.2011.06.002

Kaur, N., Chugh, V., & Gupta, A. K. (2014). Essential fatty acids as functional components of foods- a review. *Journal of Food Science and Technology*, *51*(10), 2289–2303. https://doi.org/10.1007/s13197-012-0677-0

Kocatepe, D., & Turan, H. (2018). Balık yağları, DHA, EPA ve sağlık. *Türkiye Klinikleri Public Health-Special Topics Journal*, *4*(1), 62–67.

Li, Z., Kelly, L., Gergi, I., Vieweg, P., Heiman, M., Greengard, P., & Friedman, J. M. (2015). Hypothalamic amylin acts in concert with leptin to regulate food intake. *Cell Metabolism*, *22*(6), 1059–1067.

Lorente-Cebrián, S., Costa, A. G., Navas-Carretero, S., Zabala, M., Martínez, J. A., & Moreno-Aliaga, M. J. (2013). Role of omega-3 fatty acids in obesity, metabolic syndrome, and cardiovascular diseases: A review of the evidence. *Journal of Physiology and Biochemistry*, *69*(3), 633–651. https://doi.org/10.1007/s13105-013-0265-4

Makaronidis, J. M., & Batterham, R. L. (2019). The role of gut hormones in the pathogenesis and management of obesity. *Current Opinion in Physiology*, *12*, 1–11.

Mathieu, P., Lemieux, I., & Després, J. P. (2010). Obesity, inflammation, and cardiovascular risk. *Clinical Pharmacology and Therapeutics*, *87*(4), 407–416. https://doi.org/10.1038/clpt.2009.311

Minokoshi, Y., Kim, Y. B., Peroni, O. D., Fryer, L. G., Müller, C., Carling, D., & Kahn, B. B. (2002). Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. *Nature*, *415*(6869), 339–343. https://doi.org/10.1038/415339a

Muppala, S., Konduru, S. K. P., Merchant, N., Ramsoondar, J., Rampersad, C. K., Rajitha, B., Mukund, V., Kancherla, J., Hammond, A., Barik, T. K., Mannarapu, M., Alam, A., Basha, R., Bramhachari, P. V., Verma, D., Sushma, P. S., Pattnaik, S., & Nagaraju, G. P. (2017). Adiponectin: Its role in obesity-associated colon and prostate cancers. *Critical Reviews in Oncology/Hematology*, *116*, 125–133. https://doi.org/10.1016/j.critrevonc.2017.06.003

Ouchi, N., Kihara, S., Funahashi, T., Nakamura, T., Nishida, M., Kumada, M., Okamoto, Y., Ohashi, K., Nagaretani, H., Kishida, K., Nishizawa, H., Maeda, N., Kobayashi, H., Hiraoka, H., & Matsuzawa, Y. (2003). Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue. *Circulation*, *107*(5), 671–674. https://doi.org/10.1161/01.cir.0000055188.83694.b3

Pal, J., Shukla, B. N., Maurya, A. K., Verma, H. O., Pandey, G., & Amitha, A. (2018). A review on role of fish in human nutrition with special emphasis to essential fatty acid. *International Journal of Fisheries and Aquatic Studies*, *6*(2), 427–430.

Panchali, T., Dutta, A., Das, P., Khatun, A., Kar, R., Mondal, S., Mondal, K. C., Chakrabarti, S., Ghosh, K., & Pradhan, S. (2024). Amelioration of obesity induction by a high-fat diet and related inflammation by Phasa fish (*Setipinna phasa*) oil in BALB/c mice. *Journal of Applied Biomedicine*, *22*(1), 49–58. https://doi.org/10.32725/jab.2024.003

2051817x, 2024, 19, Downloaded from https://physoc.onlinelibrary.wiley.com/doi/10.14814/phy2.70019, Wiley Online Library on [02/10/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Pawlosky, R. J., Hibbeln, J. R., Novotny, J. A., & Salem, N., Jr. (2001). Physiological compartmental analysis of alpha-linolenic acid metabolism in adult humans. *Journal of Lipid Research*, *42*(8), 1257–1265.

Peng, W., Zhang, L., Wen, F., Tang, X., Zeng, L., Chen, J., & Wang, Y. (2023). Trends and disparities in non-communicable diseases in the Western Pacific region. *The Lancet Regional Health–Western Pacific, The Lancet Regional Health–Western Pacific*, *43*(100938), 1–17. https://doi.org/10.1016/j.lanwpc.2023.100938

Pradhan, S., Panchali, T., Paul, B., Khatun, A., Rao Jarapala, S., Mondal, K. C., Ghosh, K., & Chakrabarti, S. (2020). Anti-obesity potentiality of Tapra fish (*Opisthopterus tardoore*) oil. *Journal of Food Biochemistry*, *44*(11), e13448. https://doi.org/10.1111/jfbc.13448

Ray, M. K., Sylvester, M. D., Helton, A., Pittman, B. R., Wagstaff, L. E., McRae, T. R., III, … Boggiano, M. M. (2019). The effect of expectation on transcranial direct current stimulation (tDCS) to suppress food craving and eating in individuals with overweight and obesity. *Appetite*, *136*, 1–7.

Saidpour, A., Kimiagar, M., Zahediasl, S., Ghasemi, A., Vafa, M., Abadi, A., Daneshpour, M., & Zarkesh, M. (2012). The modifying effects of fish oil on fasting ghrelin mRNA expression in weaned rats. *Gene*, *507*(1), 44–49. https://doi.org/10.1016/j.gene.2012.07.015

Samuel, V. T., Petersen, K. F., & Shulman, G. I. (2010). Lipid-induced insulin resistance: Unravelling the mechanism. *Lancet (London, England)*, *375*(9733), 2267–2277. https://doi.org/10.1016/S0140-6736(10)60408-4

Sato, T., Ida, T., Nakamura, Y., Shiimura, Y., Kangawa, K., & Kojima, M. (2014). Physiological roles of ghrelin on obesity. *Obesity Research & Clinical Practice*, *8*(5), e405–e413. https://doi.org/10.1016/j.orcp.2013.10.002

Sewter, C., Berger, D., Considine, R. V., Medina, G., Rochford, J., Ciaraldi, T., Henry, R., Dohm, L., Flier, J. S., O'Rahilly, S., & Vidal-Puig, A. J. (2002). Human obesity and type 2 diabetes are associated with alterations in SREBP1 isoform expression that are reproduced ex vivo by tumor necrosis factor-alpha. *Diabetes*, *51*(4), 1035–1041. https://doi.org/10.2337/diabetes.51.4.1035

Sumithran, P., Prendergast, L. A., Delbridge, E., Purcell, K., Shulkes, A., Kriketos, A., & Proietto, J. (2011). Long-term persistence of hormonal adaptations to weight loss. *The New England Journal of Medicine*, *365*(17), 1597–1604. https://doi.org/10.1056/NEJMoa1105816

van der Klaauw, A. A., & Farooqi, I. S. (2015). The hunger genes: Pathways to obesity. *Cell*, *161*(1), 119–132. https://doi.org/10.1016/j.cell.2015.03.008

Venkatrao, M., Nagarathna, R., Majumdar, V., Patil, S. S., Rathi, S., & Nagendra, H. (2020). Prevalence of obesity in India and its neurological implications: A multifactor analysis of a nationwide cross-sectional study. *Annals of Neurosciences*, *27*(3–4), 153–161. https://doi.org/10.1177/0972753120987465

Vessby, B. (2003). Dietary fat, fatty acid composition in plasma and the metabolic syndrome. *Current Opinion in Lipidology*, *14*(1), 15–19. https://doi.org/10.1097/00041433-200302000-00004

Walker, C. G., Zariwala, M. G., Holness, M. J., & Sugden, M. C. (2007). Diet, obesity and diabetes: A current update. *Clinical Science (London, England: 1979)*, *112*(2), 93–111. https://doi.org/10.1042/CS20060150

Wang, F., Hu, M., Zhu, H., Yang, C., Xia, H., Yang, X., Yang, L., & Sun, G. (2021). MyD88 determines the protective effects of fish oil and perilla oil against metabolic disorders and inflammation in adipose tissue from mice fed a high-fat diet. *Nutrition & Diabetes*, *11*(1), 23. https://doi.org/10.1038/s41387-021-00159-y

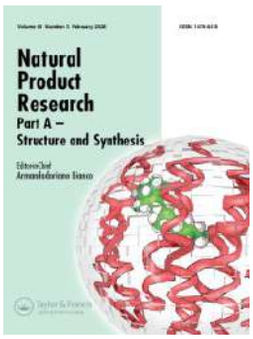
Whiting, L., McCutcheon, J. E., Boyle, C. N., Roitman, M. F., & Lutz, T. A. (2017). The area postrema (AP) and the parabrachial nucleus (PBN) are important sites for salmon calcitonin (sCT) to decrease evoked phasic dopamine release in the nucleus accumbens (NAc). *Physiology & Behavior*, *176*, 9–16. https://doi.org/10.1016/j.physbeh.2017.03.023

Yu, Y. H., Wu, S. C., Cheng, W. T., Mersmann, H. J., Shen, T. L., & Ding, S. T. (2011). The function of porcine PPARγ and dietary fish oil effect on the expression of lipid and glucose metabolism related genes. *The Journal of Nutritional Biochemistry*, *22*(2), 179–186. https://doi.org/10.1016/j.jnutbio.2010.01.006

Zhang, Y. Y., Liu, W., Zhao, T. Y., & Tian, H. M. (2017). Efficacy of omega-3 polyunsaturated fatty acids supplementation in managing overweight and obesity: A meta-analysis of randomized clinical trials. *The Journal of Nutrition, Health & Aging*, *21*(2), 187–192. https://doi.org/10.1007/s12603-016-0755-5

How to cite this article: Kar, R., Panchali, T., Das, P., Dutta, A., Phoujdar, M., & Pradhan, S. (2024). Overview of the therapeutic efficacy of marine fish oil in managing obesity and associated metabolic disorders. *Physiological Reports*, *12*, e70019. https://doi.org/10.14814/phy2.70019

2051817x, 2024, 19, Downloaded from https://physoc.onlinelibrary.wiley.com/doi/10.14814/phy2.70019, Wiley Online Library on [02/10/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License



Natural Product Research

Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: www.tandfonline.com/journals/gnpl20

Isolation, characterisation and antimicrobial activities of marine topse (*Polynemus paradiseus*) fish oil: an overview

Riya Kar , Pipika Das , Titli Panchali , Ananya Dutta , Tridip Kumar Das , Kuntal Ghosh & Shrabani Pradhan

To cite this article: Riya Kar , Pipika Das , Titli Panchali , Ananya Dutta , Tridip Kumar Das , Kuntal Ghosh & Shrabani Pradhan (05 Feb 2026): Isolation, characterisation and antimicrobial activities of marine topse (*Polynemus paradiseus*) fish oil: an overview, Natural Product Research, DOI: 10.1080/14786419.2026.2625445

To link to this article: https://doi.org/10.1080/14786419.2026.2625445

View supplementary material

Published online: 05 Feb 2026.

Submit your article to this journal

View related articles

View Crossmark data

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=gnpl20

NATURAL PRODUCT RESEARCH
https://doi.org/10.1080/14786419.2026.2625445

Taylor & Francis
Taylor & Francis Group

Check for updates

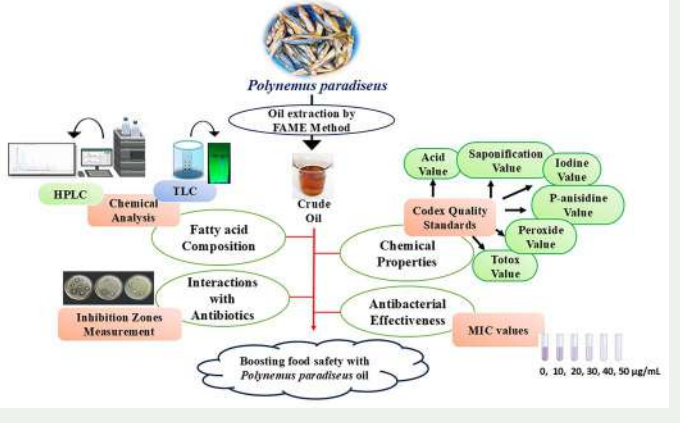
Isolation, characterisation and antimicrobial activities of marine topse (*Polynemus paradiseus*) fish oil: an overview

Riya Kar[a,b], Pipika Das[a,b], Titli Panchali[a,b], Ananya Dutta[a,b], Tridip Kumar Das[c], Kuntal Ghosh[c] and Shrabani Pradhan[b*]

[a]Biodiversity and Environmental Studies Research Center, affiliated to Vidyasagar University, Midnapore City College, Bhadutala, India; [b]Central Research Laboratory, Department of Paramedical and Allied Health Sciences, Midnapore City College, Bhadutala, India; [c]Department of Biological Sciences, Midnapore City College, Bhadutala, India

ABSTRACT

In the past few decades, there has been a growing interest in fish oils because of their significant health benefits. Marine *Polynemus paradiseus* fish locally named as topse fish are indigenous source which thrives in West Bengal's marine areas, India. Furthermore, there is a limitation of current study on *P. paradiseus* FO. The present study aimed to investigate *P. paradiseus* FO's chemical index and antibacterial effectiveness against the pathogenic microorganisms. The quality indexes of oil were conformed to Codex Alimentarius Standard. The broth microdilution method was used to assess the antibacterial properties of *P. paradiseus* FO and its interaction with antibiotics. Inhibition zones and MIC value of *P. paradiseus* FO against bacterial strains ranged from 13 to 18 mm and from 15 to 55 µg/mL, respectively. Our findings indicated *P. paradiseus* FO may be regarded as a viable antibacterial agent for enhancing food safety through the management of foodborne infections.



ARTICLE HISTORY
Received 30 April 2024
Accepted 27 January 2026

KEYWORDS
Fish oil; *Polynemus paradiseus*; HPLC analysis; antibacterial agent; polyunsaturated fatty acids

CONTACT Shrabani Pradhan shrabanipradhan@mcconline.org.in
Supplemental data for this article can be accessed online at https://doi.org/10.1080/14786419.2026.2625445.
*__Present address:__ Central Research Laboratory, Department of Paramedical and Allied Health Sciences, Midnapore City College, Bhadutala, Paschim Medinipur, 721129 West Bengal, India.
© 2026 Informa UK Limited, trading as Taylor & Francis Group

1. Introduction

Encouragement of increased fish consumption is necessary, particularly in developing nations where food security is a major issue. Additionally, more people need to know about the quality of the proteins, essential fatty acids and other substances found in fish, fish-based foods, and nutraceuticals (Dongho Dongmo et al. 2023). Several studies reported that marine FO contains plenty amount of polyunsaturated fatty acids (PUFA) (eicosapentaenoic acid/EPA, docosahexaenoic acid/DHA, arachidonic acid, etc.), mono-unsaturated fatty acids (MUFA) (gondoic acid, palmitoleic acid, oleic acid, etc.), and least amount of saturated fatty acids (SFA) (palmitic acid, stearic acid, etc.) (Kar et al. 2025). Since ancient times, fish has been a significant component of the diets of the people in our nation, India, particularly in Bengal (Majumder et al. 2016). Although local fish are highly productive and wide-ranging, not much has been researched about FOs from marine *Polynemus paradiseus*, particularly regarding their physico-chemical traits, nutritional value, and biological qualities. Systematic position of this species is as follows Phylum: Chordata, Class: Actinopterygii (Ray-finned fishes), Order: Perciformes (Perches), Family: Polynemidae (Threadfins), Genus: *Polynemus* and Species: *P. paradiseus* (Linnaeus 1758). The Topse fish (*P. paradiseus*), commonly known as the *paradise threadfin*, has an elongated, slightly compressed body and small eyes with filamentous pectoral fin rays and a sharply forked tail. The fish is typically golden with greyish fins, and it has a complete lateral line. In this species pectoral fins are separated into two parts: the upper section has the longest three unbranched rays, while the bottom part has seven free filamentous rays. This species can be found throughout the Indian subcontinent, including Bangladesh, India, Pakistan, and Sri Lanka. It has also been observed at the Bay of Bengal's Indo-Pacific Ocean confluence (Chaklader et al. 2016). An overview based on characterisation of *P. paradiseus* FO has already been undertaken previously by Kar et al. (2025). Our present study attempts to demonstrate the antibacterial properties of *P. paradiseus* FO against common pathogenic bacteria that promote food-borne disease.

2. Results and discussion

2.1. Sample collection and oil extraction by FAME method

The FAME (Fatty Acid Methyl Ester) method for fish oil extraction involves a multi-step transesterification process includes preparing the fish sample, sun drying, moisture elimination fish dust preparation, Soxhlet extraction, centrifugation, purification and final product filtration. FO is extracted using the FAME method because it is a quick, accurate, and efficient way to identify the different fatty acids in the oil and the FO extraction process was carried out using Das et al. 2025). Following the extraction procedure, 100 g of raw fish yielded approximately 2.75 ± 1.03 ml of crude fish oil (FO), corresponding to 2.53 ± 0.95 g of oil (Figure S1). Oil yields of 4.9–9.0 g/100 g for whole fish and 4.6–9.5 g/100 g for byproducts are reported by Aitta et al. (2021), with recovery percentages in relation to solvent extraction from the Baltic herring (*Clupea harengus membras*), one of the most prevalent commercially fished fish species from the Baltic Sea.

2.2. Chemical quality index of P. paradiseus FO

As part of chemical characterisation, the following values are assessed: acid value, saponification value, iodine value, peroxide value, p-anisidine value, and total oxidation value (Totox value). The results are shown in Table S1, where all of the parameters remained within the range defined by the Codex Alimentarius International Food Standards (Panchali et al. 2024; Pena-Muniz et al. 2015). The acid value 4.01 ± 0.12 mg KOH/g, an indicator of the oils quality and freshness where our result implies good stability of oil and this result was comparable to that of earlier research (Panchali et al. 2024) on *Setipinna phasa* oil. Next our experimental oil's composition and quality, especially the average molecular weight of its fatty acids, are determined by the saponification value. The *P. paradiseus* oil has a high percentage of long chain fatty acids, as indicated by the saponification number of 145.98 ± 2.23 mg KOH/g. Similarly, Pradhan et al. (2020) explored that the saponification number of *Opisthopterus tardoore* fish oil was 129.8 ± 5.09 mg KOH/g indicating that the oil contained polyunsaturated fatty acids in a large proportion. The peroxide result was 3.95 ± 0.15 mEq/kg, falling within the Codex Alimentarius's 10.0 mEq/kg standard and totox value was 8.36 ± 0.27 mg. Peroxide value and lower totox value indicates the good shelf-life and nutritional value of the experimental oil side by side a lower p-anisidine value (0.46 ± 0.19) indicates better quality and less oxidation of oil also and this outcome was similar to previous studies on *Rastrelliger kanagurta* oil (Agnesia et al. 2023). We observed that iodine value was 131.33 ± 3.89 mg, signifies that the FO was more unsaturated in nature and this result is consistent with few previous investigations (Pradhan et al. 2020; Dongho Dongmo et al. 2023; Rahman et al. 2023). The total quality control assessment results showed the *P. paradiseus* FO's advantageous physicochemical properties and bioactivity, laying the groundwork for future purification and possible uses.

2.3. HPLC analysis

Reversed-phase HPLC (RP-HPLC) is a widely used technique for determining fatty acid composition, particularly for MUFA and PUFA, purines in FOs due to its reliability, simplicity, and repeatability (Roy et al. 2013). This method yields results comparable to GC techniques (Dołowy and Pyka 2015). Previous GC-MS and GC-FID analyses of P. paradiseus FO revealed 9 SFAs (23.81%), 11 PUFAs (51.15%), and 9 MUFAs (25.04%) (Kar et al. 2025). MUFAs, especially omega-9 fatty acids, have gained attention for their biological benefits, including anti-inflammatory effects on liver Kupffer cells (Fan et al. 2022). In this present study we focused on gondoic acid (GA, cis-11-Eicosenoic acid, $C_{20}H_{38}O_2$, MW 310.51 g/mol), a 20-carbon omega-9 MUFA (6.38% in FO), as a key standard due to its very few explorations in biological field. HPLC chromatograms (Figure S2) confirmed GA presence in FO, with retention times: FO (1–25 min), GA standard (1–15 min), and FO+GA sample (1–4 min and 14–15 min), verifying essential fatty acid content in *P. paradiseus* FO. Similarly, a preliminary characterisation of triglycerides (TAGs) in crude tuna fish oil analysed by HPLC, that was reported by Indelicato et al. (2023).

2.4. Thin-layer chromatography (TLC) verification of GA presence in P. paradiseus FO

In this study we used *P. paradiseus* FO along with pure compound (GA). The separation of GA geometric isomers as per its quantity of double bonds in the trans configuration can be accomplished using TLC, a chromatography technique that balances efficiency, affordability, and simplicity (Jenkins et al. 2008). Our results showed that this FO included a sizable amount of GA, which was clearly recognised through its distinctive blue/green fluorescence under UV transilluminator where the R_f value was 0.83 (Figure S3). According to Nahar et al. (2020) quantitative and qualitative analysis of lipid classes from *Rhodococcus* and *Williamsia* species were preliminarily screened by TLC.

2.5. Anti-microbial effect of P. paradiseus FO

Antimicrobial investigations commonly use *Aeromonas hydrophila* ATCC 13442*, Vibrio parahemolyticus* ATCC 14035*, and Escherichia coli* ATCC 8739 because they cause serious risks to both people and the environment. The antimicrobial properties of the experimental oil altered depending on the bacterial strains and the method of preparing the test sample was followed by Noutsa et al. (2022). The standard antibiotic ciprofloxacin is typically effective (susceptible) against *Aeromonas hydrophila* ATCC 13442*, Vibrio parahemolyticus* ATCC 14035*, and E. coli* ATCC 8739 (Bazgosha et al. 2025). The zone of inhibition of *P. paradiseus* FO was 14.8 ± 0.09, 17.2 ± 0.12 and 15.3 ± 0.11 for *E. coli* ATCC 8739, *Aeromonas hydrophila* ATCC 13442 and *Vibrio parahemolyticus* ATCC 14035 respectively (Table S2). So, our result confirmed that *P. paradiseus* FO had minimum to moderate antibacterial activity against the selected pathogens. Similarly, Shin et al. (2007) established that essential fatty acids such as EPA and DHA have antibacterial activities against foodborne pathogenic bacteria determining the inhibition zone and MIC values.

2.6. Minimal inhibitory concentration (MIC) of FO against bacterial strain

Aeromonas hydrophila ATCC 13442 can cause severe diseases such gastroenteritis and wound infections in humans exposed to polluted water or seafood, while *E. coli* ATCC 8739 is a common indicator for tracking faecal contamination in food and water. According to studies, *Vibrio parahemolyticus* ATCC 14035 is a major global source of seafood-borne gastroenteritis, and eating seafood is frequently associated with illness (Elmahdi et al. 2016; Jeamsripong et al. 2025). In this study, MIC values were used to evaluate the qualitative and quantitative effects of FO's *in vitro* antibacterial activity against above foodborne pathogenic bacteria. According to the findings shown in Figure S4, among the FO concentrations (10, 20, 30, 40 and 50 µg/mL) 40 µg/mL concentration displayed significant antibacterial properties against following gram-negative bacteria including *E. coli* ATCC 8739, *Aeromonas hydrophila* ATCC 13442 and *Vibrio parahemolyticus* ATCC 14035. So, the dietary supplements of *P. paradiseus* FO can fight against food infections. This outcome is consistent with

some earlier studies (Al-Hajj et al. 2017; Anjum et al. 2021). Desbois and Lawlor (2013) stated that natural compounds deserve further evaluation for the treatment of infections caused by harmful pathogenic bacteria and long chain fatty acids could be applied in combination with some currently available treatments to enhance therapeutic efficacy.

3. Experimental

See Supplementary Material.

4. Conclusion

This investigation demonstrated that the Codex Alimentarius Standard validated the extraction yields and quality indexes of *P. paradiseus* FO. The largest amounts of PUFAs and MUFAs are found in *P. paradiseus* FO, which often has antimicrobial properties. Yet, this experimental FO increases Ciprofloxacin's effectiveness against some food poisoning-causing pathogenic bacteria. In order to increase the efficacy of certain medications fighting foodborne diseases, this FO could be used as a nutritional additive.

These findings demonstrate *P. paradiseus* FO's potential as a natural antibacterial agent for enhancing food safety, warranting further exploration for pharmaceutical and food applications.

Acknowledgements

The authors are thankful to the Director, Dr. Pradip Ghosh, Midnapore City College for provide all essential supports to carry out this work. The current research study presented in this manuscript is a part of the PhD research work of the first author.

Author contributions

CRediT: **Riya Kar**: Data curation, Methodology, Project administration, Visualization, Writing – original draft; **Pipika Das**: Data curation, Formal analysis, Resources; **Titli Panchali**: Data curation, Formal analysis, Resources; **Ananya Dutta**: Formal analysis, Investigation, Visualization; **Tridip Kumar Das**: Data curation, Formal analysis, Methodology; **Kuntal Ghosh**: Resources, Software, Writing – review & editing; **Shrabani Pradhan**: Investigation, Methodology, Supervision, Validation, Writing – review & editing.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by own fundings, Biodiversity and Environmental Studies Research Centre, Midnapore City College, Bhadutala, Paschim Medinipur, 721129, West Bengal, India.

References

Agnesia P, Herawati E, Puspaningrum UH, Praseptiangga D. 2023. Physicochemical properties and antioxidant activity of fish oil from Indian mackerel (*Rastrelliger kanagurta*). Curr Appl Sci Technol. 26(1):e0265241. https://doi.org/10.55003/cast.2025.265241

Aitta E, Marsol-Vall A, Damerau A, Yang B. 2021. Enzyme-assisted extraction of fish oil from whole fish and by-products of baltic herring (*Clupea harengus membras*). Foods. 10(8):1811. https://doi.org/10.3390/foods10081811

Al-Hajj NQ et al. 2017. Antibacterial activities of the essential oils of some aromatic medicinal plants to control pathogenic bacteria and extend the shelf-life of seafood. Turk J Fish Aquat Sci. 17(1):181–191. https://doi.org/10.4194/1303-2712-v17

Anjum MF, Schmitt H, Börjesson S, Berendonk TU. 2021. The potential of using *E. coli* as an indicator for the surveillance of antimicrobial resistance (AMR) in the environment. Curr Opin Microbiol. 64:152–158. https://doi.org/10.1016/j.mib.2021.09.011

Bazgosha M et al. 2025. Augmentation of antibacterial activity of ciprofloxacin by bisabololoxide A encapsulated in polymeric nanoparticles against uropathogenic *Escherichia coli*. BMC Biotechnol. 25(1):140. https://doi.org/10.1186/s12896-025-01070-5

Chaklader MR et al. 2016. Morphometric parameters and allometric growth in paradise threadfin *Polynemus paradiseus* (Linnaeus, 1758) from a coastal river of Bangladesh. J Aquac Res Dev. 7(3):1000417. https://doi.org/10.4172/2155-9546.1000417

Das P et al. 2025. Antiobesity Efficacy of Marine Tapra Fish (*Opisthopterus tardoore*) Oil in High-Fat Diet-Induced Obese Mice by Activating Lipolysis and Suppressing Adipose Inflammation. Mol Nutr Food Res. 70(1):e70341. https://doi.org/10.1002/mnfr.70341

Desbois AP, Lawlor KC. 2013. Antibacterial activity of long-chain polyunsaturated fatty acids against *Propionibacterium acnes* and *Staphylococcus aureus*. Mar Drugs. 11(11):4544–4557. https://doi.org/10.3390/md11114544

Dołowy M, Pyka A. 2015. Chromatographic methods in the separation of long-chain mono-and polyunsaturated fatty acids. J Chem. 2015(1):120830. https://doi.org/10.1155/2015/120830

Dongho Dongmo FF et al. 2023. Physicochemical characteristics and nutritional and biological properties of fish oils in Cameroon: an overview. J Food Qual. 2023:1–23. https://doi.org/10.1155/2023/7847288

Elmahdi S, DaSilva LV, Parveen S. 2016. Antibiotic resistance of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in various countries: a review. Food Microbiol. 57:128–134. https://doi.org/10.1016/j.fm.2016.02.008

Fan G et al. 2022. Gondoic acid alleviates LPS-induced Kupffer cells inflammation by inhibiting ROS production and PKCθ/ERK/STAT3 signaling pathway. Int Immunopharmacol. 111:109171. https://doi.org/10.1016/j.intimp.2022.109171

Indelicato S et al. 2023. HPLC/HRMS and GC/MS for triacylglycerols characterization of Tuna fish Oils obtained from green extraction. Foods. 12(6):1193. https://doi.org/10.3390/foods12061193

Jeamsripong S et al. 2025. Global spread and antimicrobial resistance of *Aeromonas hydrophila* in aquatic food animals: a systematic review and meta-analysis. Sci Rep. 15(1):28441. https://doi.org/10.1038/s41598-025-14498-8

Jenkins DJ et al. 2008. Fish-oil supplementation in patients with implantable cardioverter defibrillators: a meta-analysis. CMAJ. 178(2):157–164. https://doi.org/10.1503/cmaj.070261

Kar R et al. 2025. Anti-obese potentiality of marine Topse (*Polynemus paradiseus*) fish oil by inhibiting the expression of SREBP-1c & promoting β-oxidation of fat through upregulating PPAR-α. Adipocyte. 14(1):2524640. https://doi.org/10.1080/21623945.2025.2524640

Majumder TH, Chowdhury AJ, Sarwer MG, Hasan MM. 2016. Proximate composition of estuarine fish paradise threadfin, *Polynemus paradiseus* (Linnaeus, 1758). American Journal of Food and Nutrition. 6(4):123–131. https://doi.org/10.5251/ajfn.2016.6.4.123.131

Nahar A, Baker AL, Nichols DS, Bowman JP, Britz ML. 2020. Application of thin-layer chromatography-flame ionization detection (TLC-FID) to total lipid quantitation in mycolic-acid

synthesizing *Rhodococcus* and *Williamsia* species. Int J Mol Sci. 21(5):1670. https://doi.org/10.3390/ijms21051670

Noutsa BS et al. 2022. Chemical characterization and antibacterial properties of *Fontitrygon margarita* (Günther, 1870) liver oil. J Lipids. 2022(1):9369387–9369310. https://doi.org/10.1155/2022/9369387

Panchali T et al. 2024. Amelioration of obesity induction by a high-fat diet and related inflammation by Phasa fish (*Setipinna phasa*) oil in BALB/c mice. J Appl Biomed. 22(1):49–58. 1 https://doi.org/10.32725/jab.2024.003

Pena-Muniz MA et al. 2015. Physicochemical characterization, fatty acid composition, and ther mal analysis of *Bertholletia excelsa* HBK oil. Pharmacogn Mag. 11(41):147–151. https://doi.org/10.4103/0973-1296.149730

Pradhan S et al. 2020. Anti-obesity potentiality of Tapra fish (*Opisthopterus tardoore*) oil. J Food Biochem. 44(11):e13448. https://doi.org/10.1111/jfbc.13448

Rahman N, Hashem S, Akther S, Jothi JS. 2023. Impact of various extraction methods on fatty acid profile, physicochemical properties, and nutritional quality index of Pangus fish oil. Food Sci Nutr. 11(8):4688–4699. https://doi.org/10.1002/fsn3.3431

Roy L, Harrell CC, Ryan AS, Thorsteinsson T, Sancilio FD. 2013. Development and validation of a single HPLC method for analysis of purines in fish oil supplements. FNS. 04(12):1255–1259. https://doi.org/10.4236/fns.2013.412160

Shin SY, Bajpai VK, Kim HR, Kang SC. 2007. Antibacterial activity of bioconverted eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) against foodborne pathogenic bacteria. Int J Food Microbiol. 113(2):233–236. https://doi.org/10.1016/j.ijfoodmicro.2006.05.020

SUPPLEMENTARY MATERIAL

Isolation, characterization and antimicrobial activities of marine topse (*Polynemus paradiseus*) fish oil: An Overview

Riya Kar[a,b], Pipika Das[a,b], Titli Panchali[a,b], Ananya Dutta[a,b], Tridip Kumar Das[c], Kuntal Ghosh[c], Shrabani Pradhan[b]*

[a] Biodiversity and Environmental Studies Research Center, affiliated to Vidyasagar University, Midnapore City College, Bhadutala, Paschim Medinipur, 721129, West Bengal, India

[b] Central Research Laboratory, Department of Paramedical and Allied Health Sciences, Midnapore City College, Bhadutala, Paschim Medinipur, 721129, West Bengal, India

[c] Department of Biological Sciences, Midnapore City College, Bhadutala, Paschim Medinipur, 721129, West Bengal, India

Correspondence

Shrabani Pradhan, Central Research Laboratory, Department of Paramedical and Allied Health Sciences, Midnapore City College, Bhadutala, Paschim Medinipur, 721129, West Bengal, India.

Email: shrabanipradhan@mcconline.org.in

Present address

Shrabani Pradhan, Central Research Laboratory, Department of Paramedical and Allied Health Sciences, Midnapore City College, Bhadutala, Paschim Medinipur, 721129, West Bengal, India.

Abstract

In the past few decades, there has been a growing interest in fish oils because of their significant health benefits. Marine *Polynemus paradiseus* fish locally named as topse fish are indigenous source which thrives in West Bengal's marine areas, India. Furthermore, there is a limitation of current study on *P. paradiseus* FO. The present study aimed to investigate *P. paradiseus* FO's chemical index and antibacterial effectiveness against the pathogenic microorganisms. The quality indexes of oil were conformed to Codex Alimentarius Standard. The broth microdilution method was used to assess the antibacterial properties of *P. paradiseus* FO and its interaction with antibiotics. Inhibition zones and MIC value of *P. paradiseus* FO against bacterial strains ranged from 13 to 18 mm and from 15 to 55 μg/mL, respectively. Our findings indicated *P. paradiseus* FO may be regarded as a viable antibacterial agent for enhancing food safety through the management of foodborne infections.

Keywords: Fish oil, *Ploynemus paradiseus*, HPLC analysis, antibacterial agent, polyunsaturated fatty acids

List of Supplementary Material

3. Experimental

Table S1. Physicochemical Properties of *P. paradiseus* FO

Table S2. Inhibition zone measurements against some pathogenic bacterial strain.

Figure S1. Flow chart of oil extraction process using FAME method, followed by a variety of analyses and assessments.

Figure S2. The chromatograms of FO (A), standard solution (GA) (B), sample solution (FO+GA) (C).

Figure S3. TLC of fractions with mobile phase methanol: chloroform: *n*-hexane (7:2:1, *v/v*) for confirm the presence of GA.

Figure S4. MIC of crude FO against pathogenic bacterial strains (a. *E. coli* ATCC 8739, *b. Vibrio parahemolyticus* ATCC 14035 *and c. Aeromonas hydrophila* ATCC 13442). OD_{540nm} was used to calculate growth curves at different concentrations. The values are plotted as mean± SE obtained from one representative experiment performed in triplicate.

3. Experimental

3.1 Reagents & Chemicals

The following substances and reagents were obtained to complete the experiments in this investigation. Isopropanol, *n*-hexane, chloroform, DMSO, Gondoic acid or cis-11- Eicosenoic acid (E3635-100MG), TLC plates and the solvents used in estimation of chemical quality of

oil, purchased from Merck, Darmstadt, Germany. Positive control antibiotic named Ciprofloxacin and the reference bacterial strains used in this study including *Escherichia coli* ATCC 8739, *Aeromonas hydrophilia* ATCC 13442 and *Vibrio parahemolyticus* ATCC 14035 were obtained from the Laboratory of Microbiology, Midnapore City College, West Bengal, India.

3.2 Sample collection & characterization

Firstly, marine Topse fish was collected with their overall length (12–15 cm) and weight (25–30 g) from the Digha Mohana fish landing center, these are approximately 21°63' N and 87°54' E. The altitude ranges of coastal plains of Purba Medinipur from 5 to 7 meters above mean sea level. The species were collected with the help of fishermen during October-December 2023 and January-February 2024. After that fish dust will be prepare by using solvent extraction (*n*-hexane /isopropanol by 3:2 *v/v* per 10 g fish dust) followed by shaker at 140 rpm (3.286×g) for 24 hours, Soxhlet apparatus for 12 hours, centrifugation for 20 minutes at 2,500 rpm (1048.12×g). The rotary evaporator is employed as a last processing step to separate the oil from the more volatile solvent, after which fish oil was collected for further examination.

3.3 Determination of fish oil chemical quality

Quality Indexes of FO such as acid value, saponification value, peroxide value, totox value p-anisidine value and iodine values were evaluated according to standard methods of AOAC (1980) and AOCS (1989). The acid value was a measurement of the free fatty acids (FFA) in the fat or oil and indicates how many milligrams of KOH are needed to neutralise the organic acids in 1g of fat. The saponification value was the weight of KOH needed to saponify 1g of fat under particular circumstances. The average molecular weight (or chain length) of all the fatty acids that are present in the sample as triglycerides was measured by this. Iodine value was the mass of iodine used by 100g of oil and was a measure of the unsaturation of oil. The peroxide value was defined as the reactive oxygen content expressed in mEq of free iodine per kg of oil. It quantifies the amount of primary oxidation in an oil sample; the amount of secondary oxidation can be evaluated using the p anisidine test. Totox value was measured as following formula.

TOTOX=2 Peroxide value+ Anisidine value.

All the analyses were done in triplicate.

3.4 Analytical technique

Our previous study performed GC-MS and GC-FID studies for total fatty acid screening from marine *P. paradiseus* FO (Kar et al., 2025). High performance liquid chromatography (HPLC), combined with electrospray ionization mass spectrometry (ESI–MS), was used for analyses of

intact fatty acids from *P. paradiseus* crude oil. Chromatographic separation was carried out on an Agilent 1260 infinity II. For fatty acid analysis selected column was Agilent Pursuit C18 (4.6 x 150 mm, 5 µm) with a guard column (Agilent, USA). The HPLC was operated with a gradient mobile phase system consisting of water (phase A, 10%) and acetonitrile (phase B, 90%) at a flow rate of 1 mL/min. Total gradient time was 25 min. A 10 µL sample was injected into the system with the auto-sampler conditioned at 4°C and column temperature maintained at 35°C. Variable wavelength detector was 203 nm. Icos-11-enoic acid or gondoic acid (standard) was accurately weighed and dissolved in DMSO at 310.5 ug/mL and 0.5g of the crude oil added with 1 mL DMSO, then filtered with the help of 0.45 µm filters. The system control and data analyses were performed by Lab Solutions software.

3.5 Geometric isomer separation via thin-layer chromatography (TLC)

TLC was used to separate the FAMEs fraction, which includes isomers of several unsaturated fatty acids, according to the quantity of *trans* double bonds. During 30 minutes, a silver nitrate solution (10% w/v in acetonitrile) was used to saturate TLC plates (silica gel, 20 cm 20 cm, Merck, Darmstadt, Germany). Plates were eluted with methanol/chloroform/hexane (7:2:1 by *v/v*). A newly isolated compound namely Icos-11-enoic acid or gondoic acid fractions were scraped off the plate in the following formats: mono-, di-, tri-, tetra-, penta-, and hexa-*trans*. The FAMEs were then recovered by adding 5mL of a 1% NaCl in methanol: water 90:10 (*v/v*) solution, followed by two extractions with 2 mL of *n*-hexane (Fournier et al. 2007).

3.6 Evaluation of antibacterial properties

The bacteria used in this study consisted of Gram -ve bacteria, including strains of *Escherichia coli, Aeromonas hydrophilia* and *Vibrio parahemolyticus*. The reference strains (ATCC 8739, ATCC 13442 and ATCC 14035) were obtained from the Laboratory of Microbiology, Midnapore City College, West Bengal, India. Ciprofloxacin antibiotic was prepared at a concentration of 256µg/mL (Noutsa et al., 2022). The disc diffusion method (Murray et al., 1995) was used to conduct antibacterial testing using 10 ml of a suspension containing 10^5 CFU/mL of bacteria on nutrient broth (NB) agar. Separately, the discs (6 mm in diameter) were impregnated with 1.5 µL fish oil and placed on inoculated agar. The inoculation plates were incubated at 37°C for 24 hours. The diameter of inhibition zones against the test microorganisms was measured to assess antibacterial activity (Schneedorferová et al., 2015). In this experiment, each assay for FO was done in triplicate.

3.7 Minimum inhibitory concentration (MIC) of FO

The two-fold dilution approach was used to test the minimum inhibitory doses of the crude FO (Keita et al., 2022). A loopful of bacterial culture from the NB slant was inoculated in Nutrient

broth and cultured at 37◦ C for 24 hours before using the two-fold serial dilution procedure described below. The pure compound was first dissolved in 5 % dimethyl sulfoxide (DMSO). This solution was further diluted with DMSO and was added to NB to final concentration of 10 µg/ 100 µL, 20 µg/ 100 µL, 30 µg/ 100 µL, 40 µg/ 100 µL and 50 µg/ 100 µL.

3.8 Statistical Analysis

The results were expressed as mean ±standard error. The means were compared by the ANOVAI test to the 5%probability threshold using Graph Pad InStat software version2000 (Noutsa et al., 2022).

Table S1. Physicochemical Properties of *P. paradiseus* FO

Parameters	**Value (Mean ±SE)**	**Codex Alimentarius standard values**
Acid value (mg KOH/g)	4.01±0.12	4-5 mg KOH/g
Saponification value (mg KOH/g)	145.98±2.23	140-260 mg KOH/g
Peroxide value (mEq/kg) (PV)	3.95±0.15	10 mEq/kg
P- anisidine value (AV)	0.46 ± 0.19	<10
Totox value (mg)(2*PV+AV)	8.36±0.27	-
Iodine value (mg)	131.33± 3.89	120-139 mg

Table S2. Inhibition zone measurements against some pathogenic bacterial strain

Name of pathogenic bacteria	**Strains**	**Gram (+/-)**	**Zone of inhibition (Mean ±SE)**	
			P. paradiseus	**Ciprofloxacin**
a) E. coli	ATCC 8739	_	14.8±0.09 mm	27.3±0.07 mm
b) Aeromonas hydrophilia	ATCC 13442	_	17.2±0.12 mm	19.7±0.10 mm
c) Vibrio parahemolyticus	ATCC 14035	_	15.3± 0.11 mm	17.2±0.08 mm

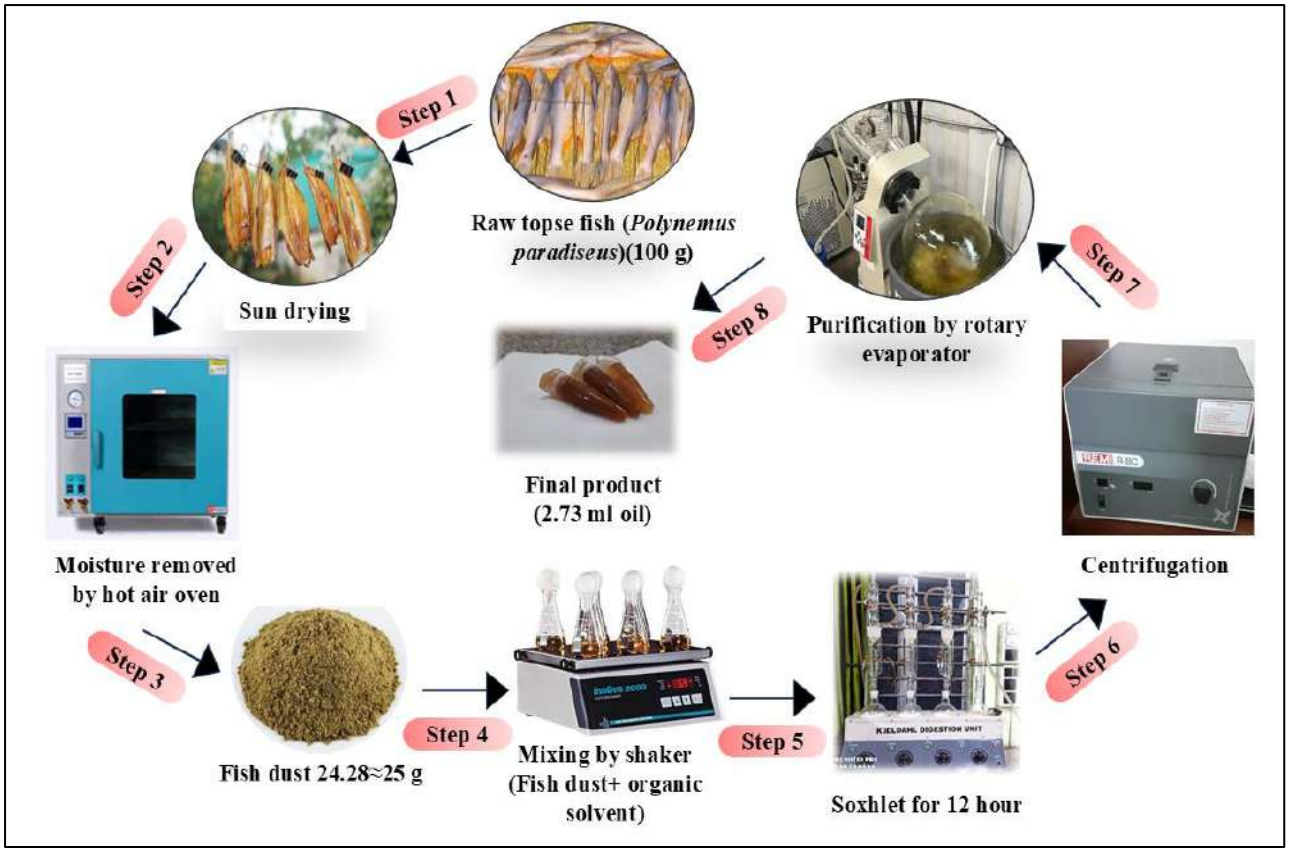


Figure S1. Flow chart of oil extraction process using FAME method, followed by a variety of analyses and assessments.

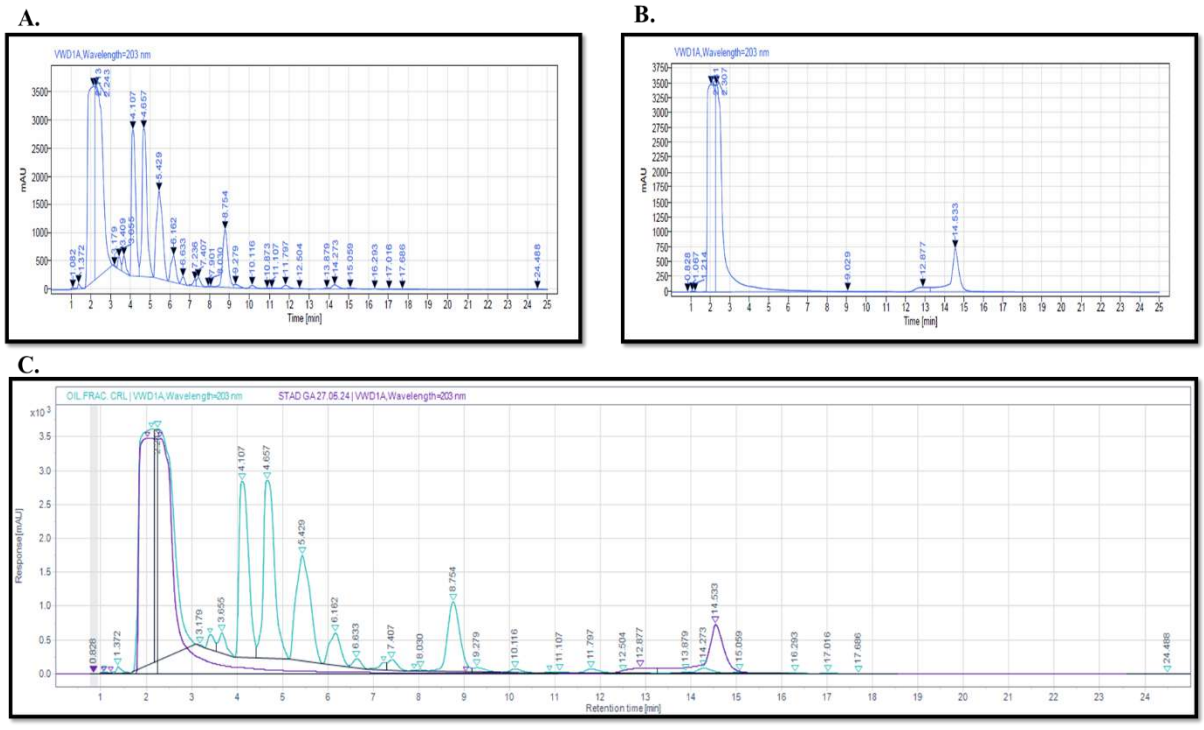


Figure S2. The chromatograms of FO (A), standard solution (GA) (B), sample solution (FO+GA) (C).

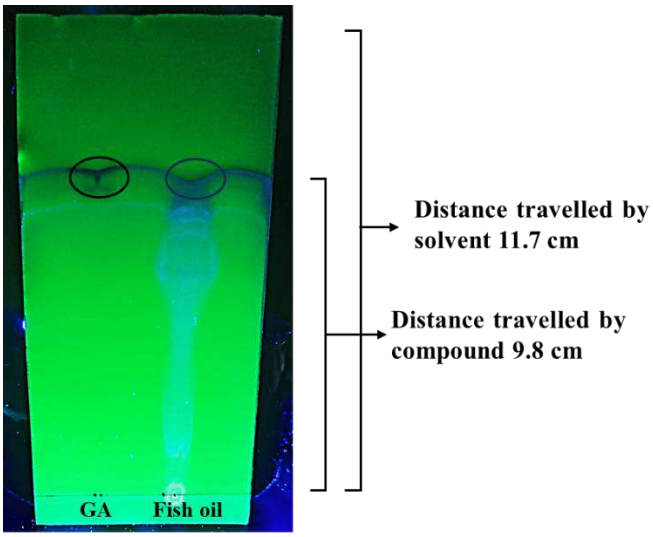


Figure S3. TLC of fractions with mobile phase methanol: chloroform: *n*-hexane (7:2:1, *v/v*) for confirm the presence of GA.

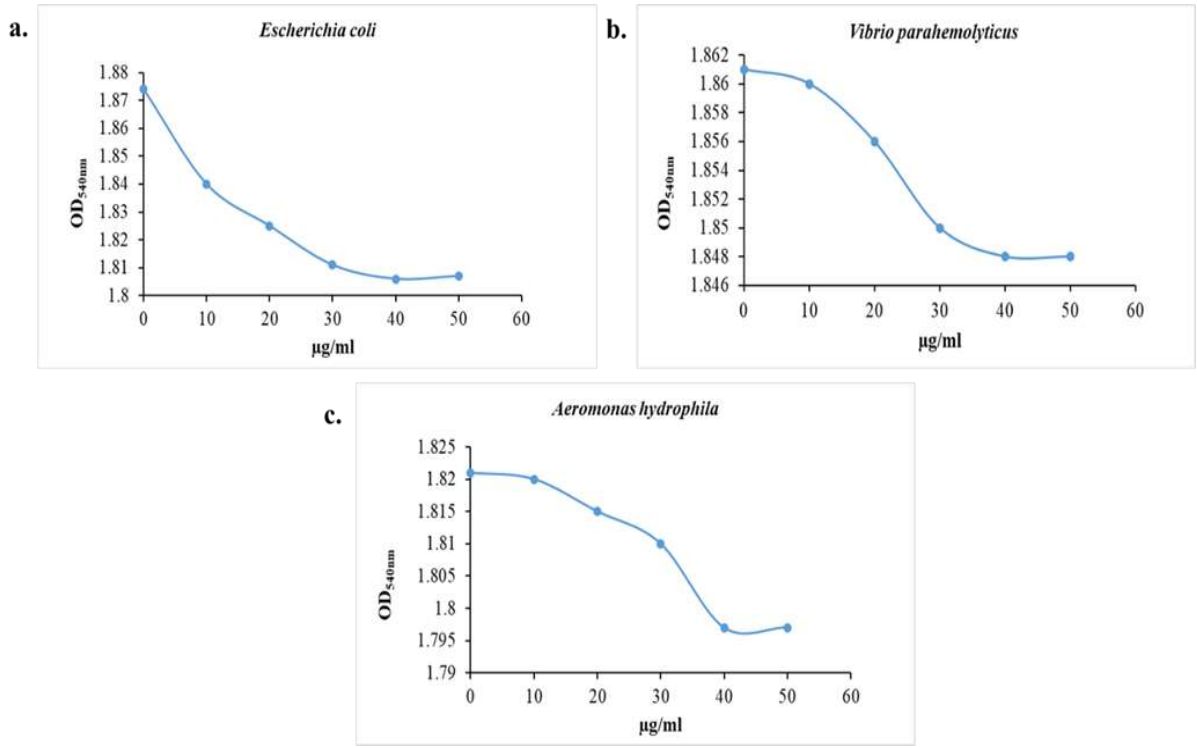


Figure S4. MIC of crude FO against pathogenic bacterial strains (a. *E. coli* ATCC 8739, *b. Vibrio parahemolyticus* ATCC 14035 *and c. Aeromonas hydrophila* ATCC 13442). OD_{540nm} was used to calculate growth curves at different concentrations. The values are plotted as mean± SE obtained from one representative experiment performed in triplicate.

References

AOAC. (1980). In W. Horwitz (Ed.), Official methods of analysis (13th ed., p. 1038). Arlington, VA: The Association of Official Analytical Chemists.

AOCS. (1989). In D. Firestone (Ed.), Official methods and recommended practices of the American Oil Chemists Society: Method Cd 3a-63. Champaign, IL: AOCS Press.

Fournier V, Destaillats F, Hug B, Golay PA, Joffre F, Juanéda P, Sémon E, Dionisi F, Lambelet P, Sébédio JL, Berdeaux O. 2007. Quantification of eicosapentaenoic and docosahexaenoic acid geometrical isomers formed during fish oil deodorization by gas–liquid chromatography. Journal of Chromatography A. 1154(1-2):353-9. https://doi.org/10.1016/j.chroma.2007.03.099

Kar R, Das P, Panchali T, Dutta A, Phoujdar M, Ghosh K, Pradhan S. 2025. Anti-obese potentiality of marine Topse (*Polynemus paradiseus*) fish oil by inhibiting the expression of SREBP-1c & promoting β-oxidation of fat through upregulating PPAR-α. Adipocyte. 14(1):2524640. DOI: 10.1080/21623945.2025.2524640

Keita K, Darkoh C, Okafor F. 2022. Secondary plant metabolites as potent drug candidates against antimicrobial-resistant pathogens. SN Appl Sci. 4(8):209. doi:10.1007/s42452-022- 05084-y.

Noutsa BS, Tchabong SR, Djitieu AD, Dongmo FF, Ngamga FH, Zokou R, Tamgue O, Ngane RA, Tchoumbougnang F. 2022. Chemical characterization and antibacterial properties of *Fontitrygon margarita* (Günther, 1870) liver oil. Journal of Lipids. 2022(1):9369387. https://doi.org/10.1155/2022/9369387

Schneedorferová I, Tomčala A, Valterová I. 2015. Effect of heat treatment on the n-3/n-6 ratio and content of polyunsaturated fatty acids in fish tissues. Food Chemistry. 176:205-11. https://doi.org/10.1016/j.foodchem.2014.12.058



Adipocyte

ISSN: 2162-3945 (Print) 2162-397X (Online) Journal homepage: www.tandfonline.com/journals/kadi20

Anti-obese potentiality of marine Topse (*Polynemus paradiseus*) fish oil by inhibiting the expression of SREBP-1c & promoting β-oxidation of fat through upregulating PPAR-α

Riya Kar , Pipika Das , Titli Panchali , Ananya Dutta , Manisha Phoujdar , Kuntal Ghosh & Shrabani Pradhan

To cite this article: Riya Kar , Pipika Das , Titli Panchali , Ananya Dutta , Manisha Phoujdar , Kuntal Ghosh & Shrabani Pradhan (2025) Anti-obese potentiality of marine Topse (*Polynemus paradiseus*) fish oil by inhibiting the expression of SREBP-1c & promoting β-oxidation of fat through upregulating PPAR-α, Adipocyte, 14:1, 2524640, DOI: 10.1080/21623945.2025.2524640

To link to this article: https://doi.org/10.1080/21623945.2025.2524640

© 2025 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.

Published online: 28 Jun 2025.

Submit your article to this journal

View related articles

View Crossmark data

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=kadi20

ADIPOCYTE
2025, VOL. 14, NO. 1, 2524640
https://doi.org/10.1080/21623945.2025.2524640

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

OPEN ACCESS Check for updates

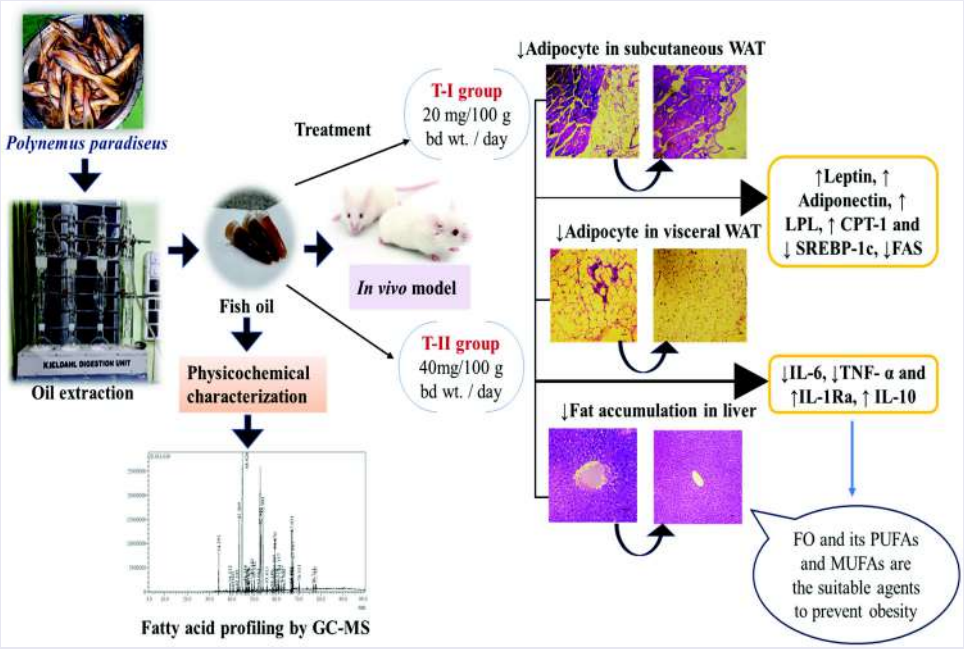
Anti-obese potentiality of marine Topse (*Polynemus paradiseus*) fish oil by inhibiting the expression of SREBP-1c & promoting β-oxidation of fat through upregulating PPAR-α

Riya Kar[a,b], Pipika Das[a,b], Titli Panchali[a,b], Ananya Dutta[a,b], Manisha Phoujdar[a,b], Kuntal Ghosh[c], and Shrabani Pradhan[b]

[a]Biodiversity and Environmental Studies Research Center Affiliated to Vidyasagar University, Midnapore City College, West Bengal, India; [b]Central Research Laboratory, Department of Paramedical and Allied Health Sciences, Midnapore City College, West Bengal, India; [c]Department of Biological Sciences, Midnapore City College, West Bengal, India

ABSTRACT

Considering the adverse effects of marketed drugs, we isolated and analysed topse fish oil (FO) in this study for the first time and examined its effect on obesity. Topse, scientifically known as *Polynemus paradiseus*, is a common fish species found in the maritime environment of the West Bengal region. To explore the role of marine P. paradiseus FO in alleviating obesity-related metabolic disorders in vivo model. Twenty-four male BALB/c mice with a standard body weight of 18.2 ± 2.1 g were taken and randomly divided into four groups: control group (C), normal chow feeding; obese control (OC), high fat diet (HFD) feeding; Treatment I (T-I) and Treatment II (T-II) group received 200 mg and 400 mg crude oil/kg body weight/day by gavage along with HFD. Here, we examined the effects of P. paradiseus oil on white adipose tissue (WAT) weight, lipid profiles, blood glucose, and adipokine expression levels in the OC group compared to the treated groups to evaluate the anti-obesity effects of FO. Compared to the HFD-induced OC group, the treated obese mice group (T-I and T-II) showed a significant reduction in body weight, Body Mass Index (BMI), and serum lipid profiles following the application of FO. The FO-treated HFD-induced obese mice group showed a moderate reduction in obesity and inflammatory-related adipocytokines compared to the OC group. Topse FO was enhanced with a large amount of essential fatty acids (FAs) and it might be administered as a dietary supplement to prevent obesity.



ARTICLE HISTORY
Received 19 November 2024
Revised 13 June 2025
Accepted 19 June 2025

KEYWORDS
Obesity; dietary FO; GC-MS analysis; high fat diet; adipocytokines

CONTACT Shrabani Pradhan shrabanipradhan@mcconline.org.in Central Research Laboratory, Department of Paramedical and Allied Health Sciences, Midnapore City College, Bhadutala, Paschim Medinipur, West Bengal 721129, India
*Present address: Shrabani Pradhan, Central Research Laboratory, Department of Paramedical and Allied Health Sciences, Midnapore City College, Bhadutala, Paschim Medinipur, 721129, West Bengal, India.

© 2025 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.
This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (http://creativecommons.org/licenses/by-nc/4.0/), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited. The terms on which this article has been published allow the posting of the Accepted Manuscript in a repository by the author(s) or with their consent.

1. Introduction

Obesity is a metabolic syndrome caused by an energy imbalance, including excessive calorie consumption and reduced energy utilization [1]. More than 2.1 billion people globally, including more than half a billion without regard to age or gender, having a body mass index (BMI) between 25.0 and 30.0, according to a survey. Obesity, which is defined as persistent inflammation of fat or decreased satiety signalling, is associated with a number of chronic illnesses, including cardiovascular conditions, type II diabetes, hypertension, and multiple cancer types [2]. The metabolism of fat and glucose is greatly influenced by the adipose tissue (AT), which is the primary location of energy storage. Furthermore, adipokines, which are biologically active mediators released by AT, are increasingly being acknowledged as a key endocrine and secretory organs [3]. Disruption of adipokine production from excess white adipose tissue (WAT) leads to obesity and obesity-related illnesses in a nutritionally abundant environment.

Fish oil (FO) has been used for centuries as a dietary supplement for improving insulin tolerance and has strong anti-inflammatory, hypolipidemic, cardiovascular protection, and body weight-reduction effects because it contains a high level of *n*-3 polyunsaturated fatty acids (*n*-3 PUFAs), eicosapentaenoic acid (EPA, 20:5 *n*-3), and docosahexaenoic acid (DHA, 22:6 *n*-3) [4]. Supplementation with marine FO may help to lower the waist–hip ratio in overweight and obese people by changing body fat composition. Some studies have reported that FO may help to reduce abdominal fat by reducing fat cell size [5]. Therefore, adding fatty fish and FO to our diet may be more beneficial for preventing and treating metabolic diseases linked to obesity, along with obesity itself [6]. Since ancient times, fish have been a significant component of the diets of the people in India, particularly in Bengal. One of the most significant sources of animal protein, fish, contributes to 16% of all animal protein ingested by people worldwide and is regarded as a superior supply of essential elements for maintaining a healthy body [7]. Thus, it was affordable for both the wealthy and poor to eat fish on a regular basis. One of the most popular marine fishes is the topse fish, which is scientifically known as *Polynemus paradiseus* and is found in the marine areas of West Bengal, India. *P. paradiseus*, a fish that is essential for commerce in a large portion of West Bengal and Bangladesh, is a member of the family Polynemidae and the order Perciformes. The local names of this fish are muni, rishi, bairagi, topse, ramsos, tapasi, riksha, and tapasi. This fish is the second-most significant fishery for the Hilsha in the West Bengal Estuary [8]. However, scientists have not paid much attention to the biological activities of *P. paradiseus* fish up until now. The Indian Council of Medical Research (ICMR) has only established proximate analysis reports for this fish [9]; there is a shortage of knowledge related to biological activity so we concentrated on that gap and wished to establish some biological activities of *P. paradiseus* FO. To determine whether marine *P. paradiseus* FO has a favourable effect on obesity and obesity-associated metabolic disorders, we focused on PUFAs and MUFAs isolated from topse FO and studied their influence on obesity and inflammatory markers linked to metabolic syndrome in diet-induced obese (BALB/C) mice. In this study, we calculated the weight of WAT, BMI (gm/cm2), and total lipid profile in BALB/C mice administered with FO. We also examined the expression of adipocytokines related to obesity and inflammation in the AT of BALB/C male mice.

2. Methodology

2.1. Reagents & chemicals

The following chemicals and reagents were acquired for completing the experiments in this study. Hexane, isopropanol, chloroform, and the solvents used to estimate the physicochemical properties were purchased from Merck, Darmstadt, Germany; RNA-Xpress Reagent, Hi-cDNA synthesis kit, 2X PCR mixture, and 3,3′,4,4′-tetraaminobiphenyl tetrahydrochloride (DAB) were obtained from Himedia, HiGenoMB, Maharashtra, India. All primers used for PCR were purchased from Bioserve (India). The primary antibodies: Ob (H-5) mouse monoclonal IgG (Sc-393,043), PPAR-γ mouse monoclonal IgG (Sc-7273), PPAR-α mouse monoclonal IgG (Sc-398,394) and Fatty acid synthase (FAS) mouse monoclonal IgG (Sc-74,540) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA-USA). The secondary antibody, Goat Anti-mouse IgG-HRP (cat# 11–301) was purchased from Abgenex (Odisha, India). Precision Plus Protein Dual Color Standards (cat# 1610374) were acquired from Bio-RAD, Hercules, California, United States.

2.2. Collection of fish samples

Samples were collected from the coastline region of Purba Medinipur in the Indian state of West Bengal. The collected specimens were then assessed for their overall length (15–16 cm) and weight (28–30 g) before being placed immediately in an icebox. The samples were then transported to the laboratory for further examination. Before being processed into fish dust, the samples were cleaned with water and dried out for 24 h at 60°C in a hot air oven.

2.3. Extraction of P. paradiseus FO

AOCS [10] methodology was employed to extract FO. Briefly, fish dust was mixed with hexane/isopropanol (3:2 by volume) and shaken at 140 rpm (3.286 × g) for 24 h before being-extraction using the Soxhlet method for 12 h. The leftover material was cooled and then centrifuged for 20 min at 2,500 rpm (1048.12 × g) to separate the supernatants, which were then filtered through Whatman No. 1 filter paper. The filtrate product was placed in a rotary evaporator to evaporate or remove volatile compounds, and the FO was retained for additional analysis.

2.4. GC–MS instrumentation and conditions of P. paradiseus FO

FO was analysed using a GC ultra-gas chromatograph (Thermo Fisher Scientific S.P.A., Milan, Italy, Model: Trace 1300 series, S/N – 717100576), and a Mass Spectrometer was used for fatty acid analysis in FO (Model: ISQ QD Single Quadrupole Mass Spectrometer). The material was passed through a stationary phase and a TG-WAXMS column containing 5% phenyl polysilphenylene siloxane (30 m × 0.25 mm ID × 0.25 m film thickness). Helium gas was flowed at a rate of 1.5 ml/min during the experiment. The column temperature was 50°C for the first 10 min. It was then elevated to 220°C (5°C/min) and maintained there for another 10 min. The MS was carried out in electron ionization mode (EI) at 70 eV with a temperature of 250°C and a detector setup of 40–600 D. The mass spectrum from GC-MS was evaluated using the National Institute of Standards and Technology (NIST) database. Molecular weights of the compounds were determined using the XCALIBUR program. The absolute quantification of FAMEs was carried out by internal standardization, using methyl ester of tricosanoic acid (23:0) (Sigma, USA). Theoretical correction factors for GC-FID (Gas chromatography – flame ionization detection) determine fatty acid concentration on samples in mg g-1 of total lipids, according to equation given below

$$FA = A_X W_{IS} CF_X / A_{IS} W_X CF_{AE} \times 100$$

being, FA the fatty acids concentration in mg per g of total lipids, A_X the peak area (fatty acids), A_{IS} the peak area of internal pattern methyl ester of tricosanoic acid (23:0), W_{IS} the mass of the internal pattern (in mg) added to the sample, W_X the sample mass (in mg), CF_X the theoretical correction factor and CF_{AE} the conversion factor required to express the results in mg of fatty acids [11].

2.5. Physicochemical characteristics

The AOAC [12] and AOCS [12] protocols were used to evaluate the acid, saponification, peroxide, and totox values.

2.6. In vivo experimental design

All experimental protocols were approved by the Institutional Annual Ethical Committee (IAEC) of, Midnapore City College (2256/PO/Re/S/23/CCSEA, MCC/IAEC-SP/10/23–003). The animal data have been reported in adherence with the Animal Research: Reporting of In vivo Experiments (ARRIVE) guidelines. Twenty-four inbreed male BALB/c mice (5–6-week-old) were randomly separated into four groups (six mice per group housed in four different cage) after a week of acclimatization to a normal chow diet (Hindustan Lever, Mumbai, India) at an average weight of 18.2 ± 2.1 g. After that, each group was then placed in a controlled environment (12 h light/dark cycle, 32 ± 2°C temperature, 50 ± 5% humidity) with free availability of food and water for 6 weeks. The four groups were as follows: a. C group: normal chow feeding (protein with 20% kcal, carbohydrate with 70% kcal, fat with 10% kcal) with water ad libitum; b. Obese control (OC) group, high fat diet (HFD) feeding (protein with 20% kcal, carbohydrate with 35% kcal, fat from lard with 45% kcal) with water ad libitum; c. Treatment-I (T-I) group, HFD feeding and received 200 mg/kg body weight/day of *P. paradiseus* FO by gavage; d. Treatment-II (T-II) group, HFD feeding and received 400 mg/kg body weight/day of *P. paradiseus* FO through oral gavage. The mice's body weight monitored every 7 days and food consumption were monitored for daily basis. After 12 weeks, the mice were anesthetized with ether and euthanized by cervical dislocation. Blood samples were collected by puncturing the orbital plexus, centrifuged at 3000 rpm for 15 min at 4°C, and serum

was stored at −20°C for biochemical analysis. The liver, subcutaneous white adipose tissue (sWAT), and visceral white adipose tissue (vWAT) were removed, weighed, and immediately submerged in liquid nitrogen, and stored at −20°C for further examination.

2.7. Assessment of somatic body weight index

Changes in body weight were recorded once per week for up to 12 weeks according to the standard protocol. A caliper (Central; Model no. 6420) was used to measure body length from the nasal to anal distance, to the nearest 0.1 mm. BMI was assessed using the standard procedure, and the results are represented as gm/cm^2.

2.8. Examination of total lipid profile and serum glucose level

The kit assay methods (Enzopak, India) were used to analyse the serum levels of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), triglycerides, and total serum glucose levels.

2.9. Histological examination

Liver tissues and ATs (visceral and subcutaneous) were fixed in 10% formalin and then were embedded in paraffin. Tissue sections of 5 µm thickness were stained with Oil Red O and- haematoxylin and eosin (HE) for histological analysis. Histological images were captured using a compound microscope (Model No. CX21i).

2.10. RNA isolation and semi qPCR for the expression of genes related to obesity and obesity associated inflammation

Complete RNA was extracted from adipose tissue samples using the HiPurA Total RNA Miniprep purification kit methodology (Hi-Media Hi-GenoMB, Maharashtra, India), the complete RNA was extracted from adipose tissue samples. The isolated RNA was quantified using a NanoDrop-2000 (Eppendorf) spectrophotometer, and cDNA was synthesized using reverse transcriptase. The reverse transcription reaction mixture was prepared using a Hi-cDNA synthesis kit (Himedia, HiGenoMB, Maharashtra, India). mRNA expression of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the housekeeping primer set (sense: GGTGAAGGTCGGAGTCAACG, antisense: GTGAAGACGCCAGTGGACTC). The genes used in this experiment are listed below:

2.11. ELISA assay

Following sacrifice of the mice, plasma was extracted from the blood and subjected to an ELISA kit (Wuhan Fine Biotech Co. Ltd, China) to measure the levels of several inflammatory cytokines in accordance with the manufacturer's instructions. Complete blots of 32-cytokine arrays were imaged using a FluorChem E System (Protein Simple, Santa Clara, CA, USA). The concentrations of leptin, IL-10, TNF-α and granulocyte macrophage colony- stimulating factor (GM-CSF) in the mouse plasma were determined using the Mouse Inflammatory 4-Plex Magnetic Bead Panel (Invitrogen, Carlsbad, CA, USA) on a Luminex 200 system (Luminex, Austin, TX, USA).

2.12. Protein analysis by immunoblotting assay

Using radioimmunoprecipitation assay (RIPA) buffer, adipose tissue samples were lysed, and the Lowry method was used to measure the protein content. Sodium dodecyl-sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to electrophorese 50 µg of protein samples after they had been boiled for 3 min in loading buffer and then moved to a nitrocellulose membrane (Bio-Rad, Model No. 165.8033FC). After blocking the membrane with 4% bovine serum albumin (BSA), the primary antibody was incubated at 4°C for the whole night.

In this investigation, β-actin, Ob (H-5), PPAR-γ, PPAR-α, and FAS were employed as the main antibodies in a 1:1000 ratio. Next, the membrane was incubated for 1 hour at 4°C with horseradish peroxidase (HRP)-labelled anti-mouse secondary antibody 1:7500 (Abgenex) after being cleaned three times with phosphate buffer saline containing tween-20 (PBST). Individual protein bands were identified via 3,3′,4,4′-tetraaminobiphenyl tetrahydrochloride (DAB).

2.13. Statistical analysis

The data were evaluated using one-way ANOVA, followed by Tukey's multiple-comparison tests to compare each treatment group to the control group. The results (triplicate values) are expressed as mean ± SEM. Statistical analysis was performed using GraphPad Prism software. Values were considered significant at $p < 0.0001$, $p < 0.001$, $p < 0.01$, and $p < 0.05$.

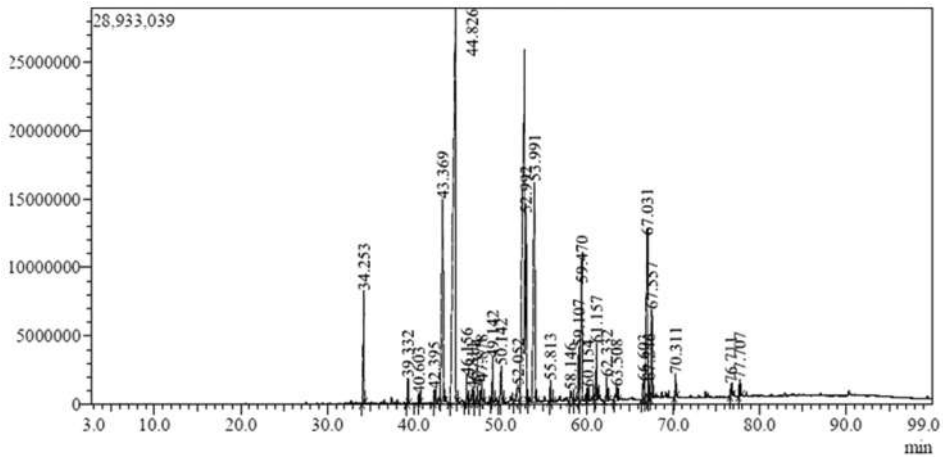


Figure 1. GC-MS chromatograms analysis of *P. paradiseus* FO. This chromatogram showing the presence of nine SFA, eleven PUFAs and nine MUFAs.

Table 1a. Total percentage of fatty acids composition from *P. paradiseus* FO measured using GC-FID.

Sl.no	FA (%)	Types of fatty acid	*P. paradiseus*
1.	Methyl myristate	SFA	2.91
2.	Tetradecanoic acid	SFA	2.32
3.	Pentadecanoic acid	SFA	1.02
4.	Methyl palmitate	SFA	11.28
5.	Phosphoric acid-3TMS	SFA	0.42
6.	Methyl margarate	SFA	2.32
7.	Nonadecanoic acid	SFA	0.38
8.	Tetracosanoic acid	SFA	1.11
9.	Methyl pentacosanoate or Pentacosanoic acid	SFA	2.05
SFA 23.81%			
10.	Methyl ganma-linolenate; Methyl linolenate or (9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid (Linolenic acid)	*n*-6 PUFA	1.2
11.	Methyl linoleate (linoleic acids); Methyl linolelaidate	*n*-6 PUFA	2.05
12.	(9E,12E)-octadeca-9,12-dienoic acid (Linoeladic acid)	*n*-6 PUFA	4.12
13.	Octadeca-6,9,12-trienoic acid	*n*-6 PUFA	0.44
14.	(5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraenoic acid	*n*-6 PUFA	0.82
n-6 PUFA 8.63%			
15.	Methyl arachidonate	*n*-3 PUFA	6.48
16.	Methyl cis-5,8,11,14,17-Eicosapentaenoate or Icosa-5,8,11,14,17-pentaenoic acid	*n*-3 PUFA	7.22
17.	Methyl cis-4,7,10,13,16,19-Docosahexaenoate	*n*-3 PUFA	8.03
18.	Methyl cis-7,10,13,16,19-docosapentaenoate	*n*-3 PUFA	6.52
19.	(7Z,10E,13E,16E,19E)-docosa-7,10,13,16,19-pentaenoic acid (Clupanodonic acid)	*n*-3 PUFA	2.38
20.	Docosa-4,7,10,13,16,19-hexaenoic acid (DHA)	*n*-3 PUFA	11.89
n-3 PUFA 42.52%			
21.	Methyl palmitoleate (Palmetoleic acid); Hexadec-9-enoic acid	MUFA	4.56
22.	Methyl cis-vaccenate	MUFA	1.84
23.	(Z)-octadec-11-enoic acid; Methyl cis-10-heptadecenoate	MUFA	2.02
24.	Heptadec-10-enoic acid	MUFA	1.56
25.	Icos-11-enoic acid or gondoic acid	MUFA	6.38
26.	Methyl cis-12-Octadecenoate	MUFA	0.28
27.	(Z)-octadec-12-enoic acid (Petroselinic acid)	MUFA	4.31
28.	Methyl cis-11-icosenoate	MUFA	0.89
29.	Methyl cis-12-Octadecenoate	MUFA	3.2
MUFA 25.04%			

3. Results

3.1. Chromatographic analysis and GC-MS characterization of P. paradiseus FO

GC-MS analysis clearly identified the presence of 9 SFA,11 PUFAs, and 9 MUFAs (Figure 1 and Table 1a) in this oil. GC-FID was used in this investigation to determine the overall proportion of FAs from marine *P. paradiseus* FO. SFAs include Methyl myristate, Tetradecanoic acid, Pentadecanoic acid, Methyl palmitate, Phosphoric acid-3TMS, Methyl margarate, Nonadecanoic acid, Tetracosanoic acid, Methyl pentacosanoate or Pentacosanoic acid. However, PUFAs include methyl ganma-linolenate, methyl linolenate, methyl linoleate, methyl linolelaidate, (9E,12E)-octadeca-9,12-dienoic acid, methyl arachidonate, methyl cis-5,8,11,14,17-eicosapentaenoate, methyl cis-4,7,10,13,16,19-docosahexaenoate, methyl cis-

Table 1b. List of PUFAs from *P. paradiseus* FO with their RT value, molecular weight and biological significance listed after GC-MS analysis.

RT Value	PUFAs	Molecular weight (g/mol)	Formula	Biological significance
42.395	Methyl ganma-linolenate; Methyl linolenate or (9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid (Linolenic acid)	292.46	C19H32O2	The prevention of hepatic fat buildup and diminished insulin signaling can be achieved independently by either alpha-linolenic acid supplementation or exercising training [13].
52.053	Methyl linoleate (linoleic acids); Methyl linolelaidate	294.5	C19H34O2	In the present investigation, meals supplemented with tea tree oil had lowered SFA (lauric acid and palmitic acid) and raised PUFA (primarily methyl linolelaidate) levels in meat [14].
52.053	(9E,12E)-octadeca-9,12-dienoic acid (Linoeladic acid)	280.45	C18H32O2	Overall, research on humans, animals, and *in vitro* suggests that Trans fatty acids (Trans vaccenic acid, elaidic acid, rumenic acid, trans 10, cis 12, conjugated linoleic acid, and linoelaidic acid) have little or minimally positive effects on metabolic parameters, inflammatory markers, and cardiovascular health [15].
59.103	Methyl arachidonate	318.5	C21H34O2	In the current study, it was examined whether consuming pomegranate juice, which is high in polyphenols, for six weeks may reduce the risk of overall metabolic syndromes. Additionally, the plasma's SFA level increased while arachidonic acid levels considerably reduced [16].
59.470	Methyl cis-5,8,11,14,17-Eicosapentaenoate or Icosa-5,8,11,14,17-pentaenoic acid	316.5	C21H32O2	Diet-induced weight loss increases the number of unsaturated FAs such as cis-5,8,11,14,17-Eicosapentaenoate (C20:5 *n*-3) and linolenate (C18:3 *n*-3) in the femoral muscle, suggesting that it lessens the symptoms of obesity and may enhance the quality of rabbit meat [17]
60.153	Methyl cis-4,7,10,13,16,19-Docosahexaenoate	342.5	C23H34O2	Not reported clearly.
63.512	Methyl cis-7,10,13,16,19-docosapentaenoate	344.53	C23H36O2	Not reported clearly.
66.603	(7Z,10E,13E,16E,19E)-docosa-7,10,13,16,19-pentaenoic acid (Clupanodonic acid)	330.50	C22H34O2	Not reported clearly.
67.031	Docosa-4,7,10,13,16,19-hexaenoic acid (DHA)	328.48	C22H32O2	Additionally, 1% DHA and 1% EPA reduce GPR120, which inhibits adipogenesis; via upregulating PPARγ, 4% DHA promotes browning of WAT and enhances IR and inflammatory infiltration [18].
67.245	Octadeca-6,9,12-trienoic acid	278.4	C18H30O2	A study reported that bee bread was characterized in terms of its physicochemical composition where the most abundant fatty acids were C18:3 (all-cis-9,12,15) octadeca-6,9,15-trienoic acid, followed by C16:1 (9Z)-hexadec-9-enoic acid, C21:0 heneicosanoic acid and C18:2 (all-cis-9,12) (9Z,12Z)-octadeca-9,12-dienoic acid, respectively [19].
67.553	(5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraenoic acid	304.47	C20H32O2	Not reported clearly.

Table 1c. List of MUFAs from *P. paradiseus* FO with their RT value, molecular weight and biological significance listed after GC-MS analysis.

RT Value	MUFAs	Molecular weight (g/mol)	Formula	Biological significance
43.370	Methyl palmitoleate (Palmetoleic acid); Hexadec-9-enoic acid	268.4	C17H32O2	Palmitoleic acid (POA) has demonstrated anti-inflammatory and lipid-lowering benefits linked to prevention of metabolic syndrome. This finding implies that POA may be activating AMPK to produce at least part of its positive benefits [20].
43.370	Methyl cis-vaccenate	296.5	C19H36O2	According to the findings of this study, Canadians who are at risk for diabetes found that higher levels of saturated and *n*-6 fatty acids and lower levels of cis-vaccenic acid consistently correlated with lower insulin sensitivity and cell function, with the majority of correlations occurring in the PL (Phospoloipid) fraction [21].
43.370	(Z)-octadec-11-enoic acid; Methyl cis-10-heptadecenoate	282.46	C18H34O2	The most prevalent TFAs in butter and margarines, which are (E)-octadec-11-enoic acid (C18:1 t11; trans vaccenic acid) and (E)-octadec-9-enoic acid (C18:1 t9; elaidic acid), were reflected in the significantly different serum levels of TFAs measured after the dietary interventions [22].
46.812	Heptadec-10-enoic acid	268.4	C17H32O2	Not reported.
47.878	Icos-11-enoic acid or gondoic acid	310.5	C20H38O2	Oleic acid (OA), gondoic acid (GA), and nervonic acid (NA) all showed inverse correlations with LDL-C, HDL-C, and eGFR in a partial correlation analysis that was controlled for age and gender but direct connections with markers of inflammation and endothelial activation as well as heart failure [23].
52.995	Methyl cis-12-Octadecenoate	296.5	C19H36O2	Not reported.
52.995	(Z)-octadec-12-enoic acid (Petroselinic acid)	282.46	C18H34O2	The medium- and long-chain NEFA receptor FFA4 (free fatty acid receptor 4, formerly known as GPR120) has been associated with insulin-sensitizing and anti-inflammatory effects. The effects of oleic acid (18: 1n-9), petroselinic acid (18: 1n-12), and cis-vaccenic acid (18: 1n-7) on FFA4 were reduced [24].
61.153	Methyl cis-11-icosenoate	324.54	C21H40O2	Not reported.
76.712	Methyl cis-12-Octadecenoate	296.5	C19H36O2	Not reported.

7,10,13,16,19-docosapentaenoate, (7Z,10E,13E,16E,19E)-docosa-7,10,13,16,19-pentaenoic acid, docosa-4,7,10,13,16,19-hexaenoic acid (DHA), octadeca-6,9,12-trienoic acid, and (5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraenoic acid. The MUFAs identified were methyl cis-vaccenate, methyl cis-10-heptadecenoate, heptadec-10-enoic acid, gondoic acid, methyl cis-12-Octadecenoate; (Z)-octadec-12-enoic acid, methyl cis-11-icosenoate, and methyl cis-12-octadecenoate. Additionally, Table 1b & 1c highlight the presence of total PUFAs and MUFAs in *P. paradiseus* FO, along with their RT values, molecular weight, and biological relevance.

3.2. Physicochemical characteristics of *P. paradiseus* FO

Acid and peroxide indices were used to evaluate the quality of the oil. The oil's acid value, which was 4.53 ± 0.23 mg KOH/g, was within the acceptable range of 5.0 mg KOH set by the National Agency of Sanitary Surveillance. The peroxide value was 3.66 ± 0.33 mEq/kg, which falls within the acceptable range of 5.0 milliequivalents of active oxygen/kg oil set by the Codex Alimentarius Committee and the World Health Organization (2017). The oil's high saponification value (135.52 ± 2.68 mg KOH/g) confirmed that it contained a lot of polyunsaturated fatty acids. The totox (8.71 ± 0.15 mg) and *p*- anisidine value (0.51 ± 0.21) were within the range established by the National Agency of Sanitary Surveillance (Table 2).

Table 2. Physicochemical properties of *P. paradiseus* FO.

Parameters	Value (Mean ±SE)
Acid value (mg KOH/g)	4.53 ± 0.23
Saponification value (mg KOH/g)	135.52 ± 2.68
Peroxide value (mEq/kg)	3.66 ± 0.33
Totox value (mg)	8.71 ± 0.15
P- anisidine value	0.51 ± 0.21

3.3. Assessment of food intake, somatic body weight, BMI and weight of WATs in HFD induced BALB-c obese mice treated with P. paradiseus FO

To assess the anti-obesity benefits of *P. paradiseus* FO, we examined alterations in body weight and serum lipid profile of HFD-induced obese mice administered varying doses of *P. paradiseus* FO for 12 weeks. The preliminary body weights of the mice in each group were almost similar (C, 16.88 ± 1.05 g; OC, 16.86 ± 0.98 g; T-I, 15.69 ± 0.85 g; T-II, 16.01 ± 1.28 g). As shown in Figure 2b, mice fed a HFD acquired greater weight than the other groups after 12 weeks. The final mean body weights in C, OC, T-I, and T-II were 32 ± 2.87 g, 45.13 ± 2.66 g, 38.14 ± 3.54 g and 34.32 ± 2.35 g after 4 weeks of the FO treatment (T-I, 200

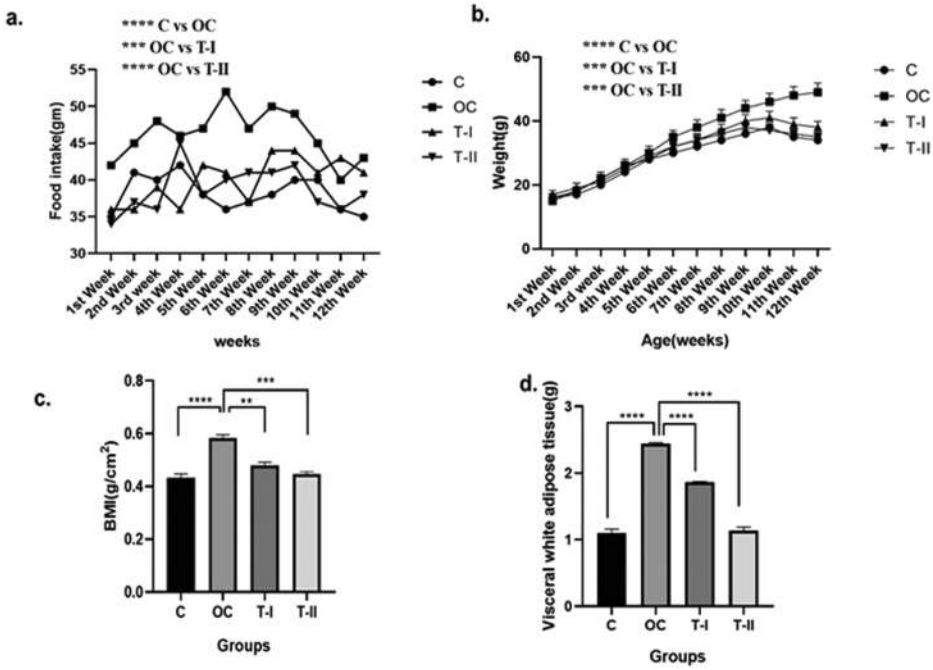


Figure 2. FO supplementation reduces body weight increase and fat formation in HFD-fed mice. (a) measurements of weekly food consumption (b) variation in the body weight (c) BMI (gm/cm^2) (d) weight of visceral WAT (gm) in different experimental group (n = 6) values are expressed as mean ±SEM. Statistical analysis evaluated via one way ANOVA followed by Tukey's multiple comparison test. ****p <0.0001, ***p <0.001, **p <0.01, *p < 0.05.

mg/kg body weight/day; T-II, 400 mg/kg body weight/day). Food intake was also monitored every week, and the variation in food intake in the different experimental groups is shown in Figure 2a Among the four groups, the OC group had a substantially higher energy intake than the other groups. BMI was computed by applying the standard procedure and was expressed as g/cm^2. On the basis of Figure 2c BMI was significantly higher in the OC group than in the C group. Conversely, although BMI was reduced after treatment with different doses of FO. After sacrificing the mice, the weight of visceral WAT was also measured, where the weight of WAT was higher in the OC group (2.41 g ± 0.10) than in the C group (0.843 g ± 0.01). In addition, HFD with FO showed significant efficacy in decreasing the weight of WAT in the treatment groups (T-I and T-II) (Figure 2d).

3.4. Assessment of lipid profile and total serum glucose level of HFD induced obese mice treated with P. paradiseus FO

Tested serum lipid profiles such as TC, TG, LDL, VLDL were 15–30% higher and HDL levels were 50% lower in OC group (Figure 3a-e). Similarly, after the administration of topse FO, TC levels (Figure 3a), and TG (Figure 3b) in the liver were decreased up to 25% in the treatment group. Furthermore, the level of serum LDL cholesterol was diminished (20–30%) due to FO supplementation, and $p < 0.05$ compared with the OC groups (Figure 3d). Additionally, a decrease (18%) in VLDL cholesterol was also observed in the both T-I and T-II groups, in contrast to OC (Figure 3e), though there was a deliberate increase in HDL cholesterol levels (55%) after *P. paradiseus* FO feeding (Figure 3c). Total serum glucose level was 51.19% higher in OC group compared to the C group (Figure 3f). These findings demonstrate that FO therapy has a more pronounced effect on blood and hepatic metabolic parameters in obese mice.

3.5. Fat droplet accumulation in liver

Figure 4(a–d) demonstrates the impacts of FO on livertissue, as determined by HE staining. Lipid metabolism is mostly regulated by the liver. The expanded cytoplasm of the HFD-fed group was packed with numerous fat droplets, although receiving FO therapy, accumulation of fat droplets was reduced in both the T-I and T-II groups. Between the two doses of FO, the T-II group showed a significant difference compared with the other groups. In the T-II group, sheets of hepatocytes were arranged in a uniform manner, and the central vein was proper in shape, similar to the control group. As anticipated, mice fed a high-fat diet

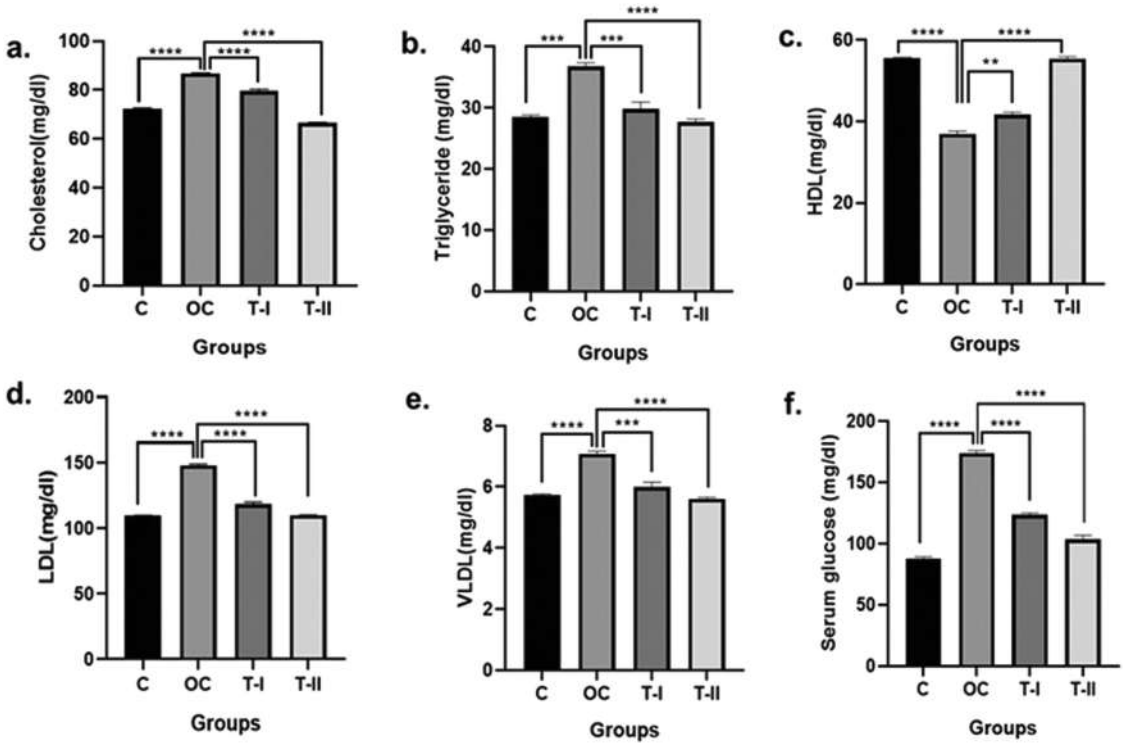


Figure 3. Assessments of total lipid profiles such as a) cholesterol, b) triglyceride, c) HDL, d) LDL, e) VLDL and f) total serum glucose level in different experimental group (n=6) values are expressed as mean ±SEM. Followed by one way ANOVA and Tukey's multiple comparison test. ****$p < 0.0001$, ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$.

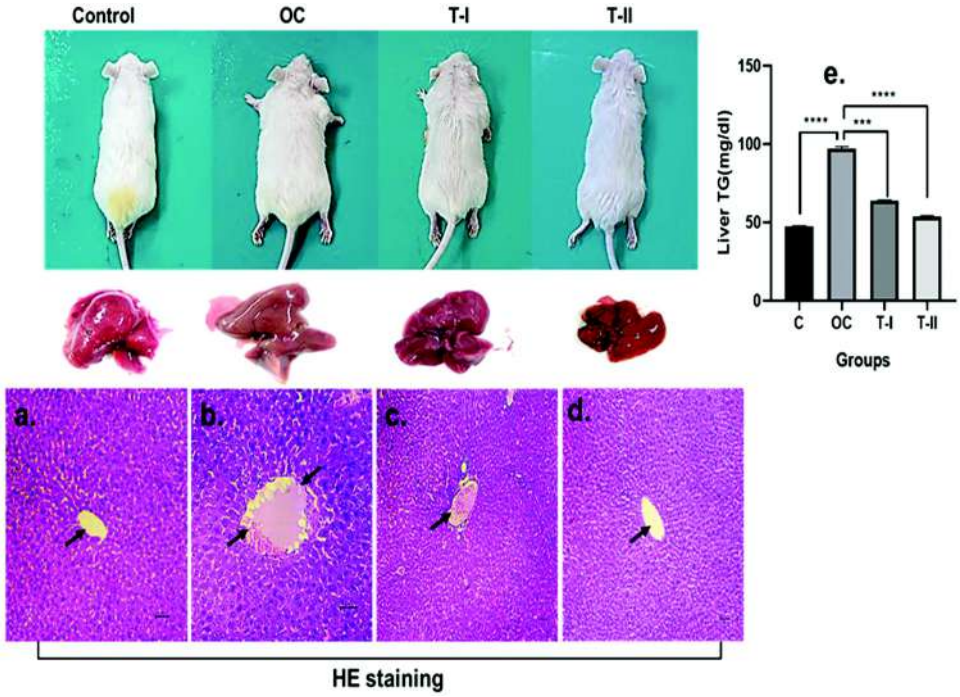


Figure 4. Effects of FO on hepatic lipid profile of HFD-fed mice groups. Groups are separated as a) control; b) obese control; c) T-I (HFD feeding and received 200 mg/kg body weight/day of *P. paradiseus* oil) and d) T-II (HFD feeding and received 400 mg/kg body weight/day of *P. paradiseus* crude oil). Tissue sections were stained with haematoxylin and eosin. (e) Estimation of liver TG in different experimental groups. Results are expressed as mean ± SEM for each group ($n = 6$). Arrows () indicate the enlargement of cytoplasm in different experimental groups.

(HFD) and supplemented with 400 mg/kg body weight/day FO showed a significant 38–45% decrease in TG as compared to the OC group (Figure 4e).

3.6. Effect of P. paradiseus oil on abdominal fat accumulation in HFD induced mice

We used HE staining for histopathology to examine the impact of *P. paradiseus* oil on the abdominal section of the skin and visceral AT (Figure 5a,b). The adipocytes in the subcutaneous and visceral AT of the OC group were not uniform in size, but they were generally large and contained fat vacuoles with large inner diameters. On the other hand, the adipocytes in the C group were arranged properly and neatly, and their vacuole inner diameter was smaller. Following FO treatment, the T-II group showed a greater reduction in abdominal fat, which was equivalent to the C group.

3.7. Impact of dietary P. paradiseus oil on mRNA expression in AT

Therefore, we quantified the degree of expression levels of some obesity- and inflammation- related adipocytokines mRNA, such as leptin, adiponectin, sterol regulatory element-binding protein-1c (SREBP-1c), fatty acid synthase (FAS), lipoprotein lipase (LPL), carnitine palmitoyl transferase 1 (CPT-1), tumour necrosis factor (TNF-α), interleukin 1 receptor antagonist (IL-1ra), interleukin 10 (IL-10), and interleukin 6 (IL-6) in the AT of the mice groups using semi- quantitative PCR (Figure 6). In this study, leptin mRNA and adiponectin mRNA levels were decreased by 1.45-fold and 1.27-fold, respectively, in the OC group compared to the control group. Leptin mRNA and adiponectin mRNA levels in AT increased significantly in mice fed 200 mg/kg body weight/day of *P. paradiseus* oil and 400 mg/kg body weight/day of *P. paradiseus* oil (Figure 6b,c). The HFD diet, combined with FO, reduced mRNA expression of the lipogenic gene SREBP-1c in AT and liver by 1.36-fold (T-I) and 1.57-fold (T-II), respectively, compared to the HFD group (Figure 6d). The HFD increased FAS expression by 1.06-fold in the OC group compared to that in the control group. In contrast, topse FO decreased FAS level by 1.13-fold and 1.48-fold, respectively, in the T-I and T-II groups compared to the obese group (Figure 6e). Inconsistently, the expression of CPT-1 and LPL decreased in the OC group by 0.54-fold and 1.07-fold, respectively. After treatment with crude oil, the mRNA expression levels of CPT-1 and LPL

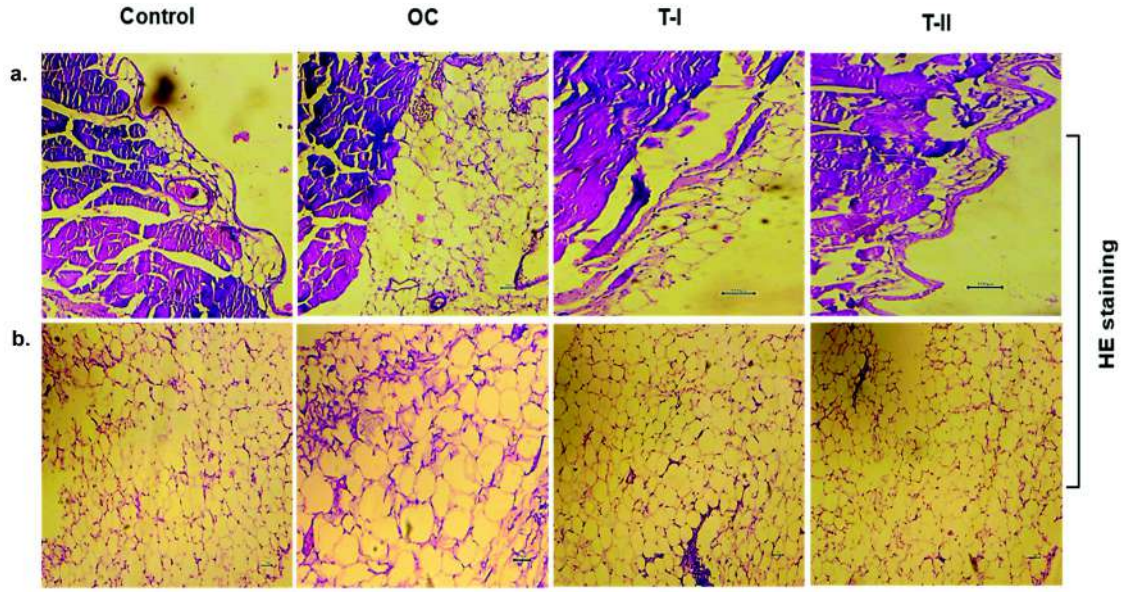


Figure 5. The impact of *P. paradiseus* oil on skin and vWAT histology. Groups are separated as control group (feed with normal diet), OC group (received HFD), T-I group (HFD feeding and received 200 mg/kg body weight/day of *P. paradiseus* oil) and T-II group (HFD feeding and received 400 mg/kg body weight/day of *P. paradiseus* crude oil). (A) sWAT and (B) vWAT sections were stained with hematoxylin and eosin (HE) staining. Results are expressed as mean ± SEM for each group (n=6). The scale bar correspondence to 10 µm. Arrows () indicate the size of vWAT and accumulation of sWAT in different experimental groups.

increased by 1.14- and 1.83-fold, respectively, in the treated groups (Figure 6f,g). Additionally, the mRNA expression of associated anti-inflammatory markers, including IL-1ra and IL-10, was increased by 1.48 -fold and 1.68 -fold in the T-II group after treatment (Figure 6h,j). The pro-inflammatory markers, TNF-α and IL-6 were upregulated in the OC group by 1.02-fold, whereas *P. paradiseus* FO treatment significantly reduced the expression of TNF-α and IL-6 by 1.05- fold and 1.11- fold (Figure 6i,k).

3.8. Expression of leptin, IL-10 and TNF-α level in HFD-induced obese mice by ELISA assay

We assessed the levels of an important metabolic regulators leptin that plays an important role in regulation of appetite, here Leptin levels were down regulated by 43.28% in OC group compared to control group where as in FO treated group leptin levels were up regulated gradually by 30–52.6% due to proper regulation of leptin (Figure 7a). IL-10 which is known to be an anti-inflammatory cytokine decreased the generation of pro-inflammatory cytokines that interrupt the proper function of insulin. As expected, IL-10 was reduced by 60.27% in the OC group compared with control. Compared to OC group, IL-10 levels were elevated by 67.65% after treatment with *P. paradiseus* oil (Figure 7b). Conversely, we assessed the TNF-α levels, which are known to be generated during the initial stages of inflammation. TNF-α was, as assumed, higher in the OC group than in the control group by 35.86%, whereas supplementation of FO reduced the inflammation by 20.39% (Figure 7c).

3.9. Protein analysis for ob (H-5), PPAR-γ, PPAR-α and FAS

To explore the effect of FO supplementation on energy expenditure, we analysed the protein expression of ob (H-5), PPAR-γ, PPAR-α, and FAS genes in adipose tissue. In contrast to the C group, in the HFD group, the ratio of ob (H-5)/β-actin and PPAR-α/β- actin was diminished in WAT by 1.29-fold and1.16-fold. FO supplementation in the HFD + FO groups (T-I, T-II) significantly reversed the HFD effects by increasing the ob (H-5)/β- actin and PPAR- α/β- actin ratio by 1.56-fold to 1.67- fold and 1.48-fold to 1.51-fold in WAT, respectively. However, the ratio of PPAR-γ/β- actin and FAS/β- actin was raised in the OC group by approximately 2-fold whereas FO administration decreased the protein expression of PPAR-γ and FAS serially in the T-I group by 1.15- fold,1.56- fold and in the T-II group by 1.29- fold,1.67- fold (Figure 8).

4. Discussion

P. paradiseus is a common fish that is mostly found in the coastal regions of West Bengal. It is a locally available indigenous food that is affordable to all

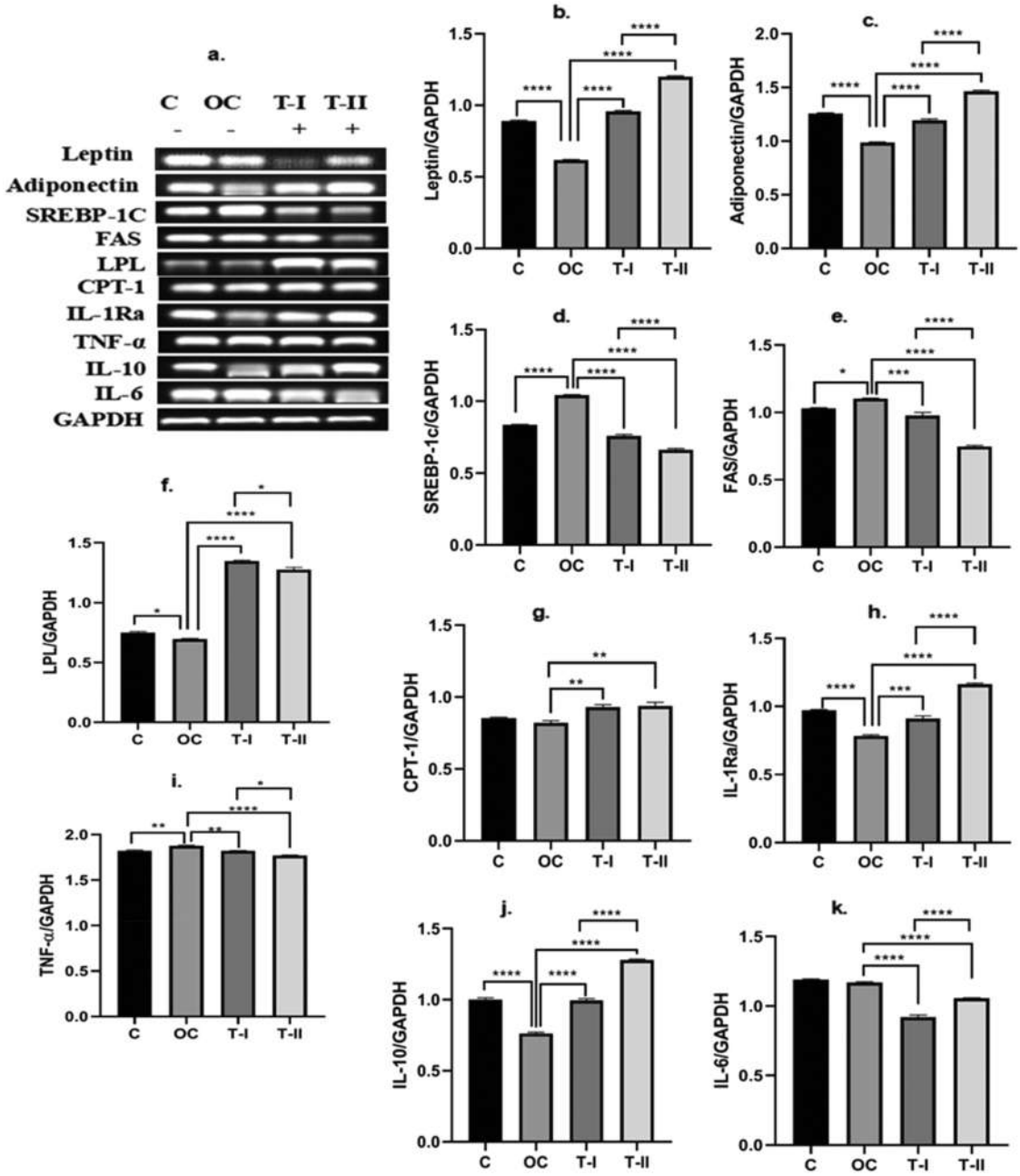


Figure 6. Expression of obesity, inflammatory and anti-inflammatory related adipocytokines by semi q-PCR. (a) Relative gene expression level of (b) leptin, (c) adiponectin, (d) SREBP-1c, (e) Fas, (f) LPL (g) CPT-1 (h) IL-1Ra, (i) TNF-α, (j) IL-10, and (k) IL-6 in adipose tissue of male albino BALB/c mice in different experimental groups (n=6) were adjusted to control gene expression level (GAPDH; b-k). Values are expressed as mean ±SEM. Statistical analysis evaluated via one way ANOVA followed by Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05.

communities. In the study, we isolated and analysed *P. paradiseus* FO and assessed its effects on adiposity. Table 2 shows the results of the physicochemical investigation of *P. paradiseus* FO. *P. paradiseus* FO exhibited minimal acid readings, indicating that triacylglycerol was not degraded, which could imply high stability. The increased saponification value of fish oil demonstrated that a significant amount of medium-chain fatty acids was present in the oil. The good nature of the oil and a favourable state of preservation were demonstrated by the reduced peroxide value. In contrast, the totox value range indicates the existence of both high primary and secondary oxidative stability [25]. Marine fish oils are generally acknowledged as

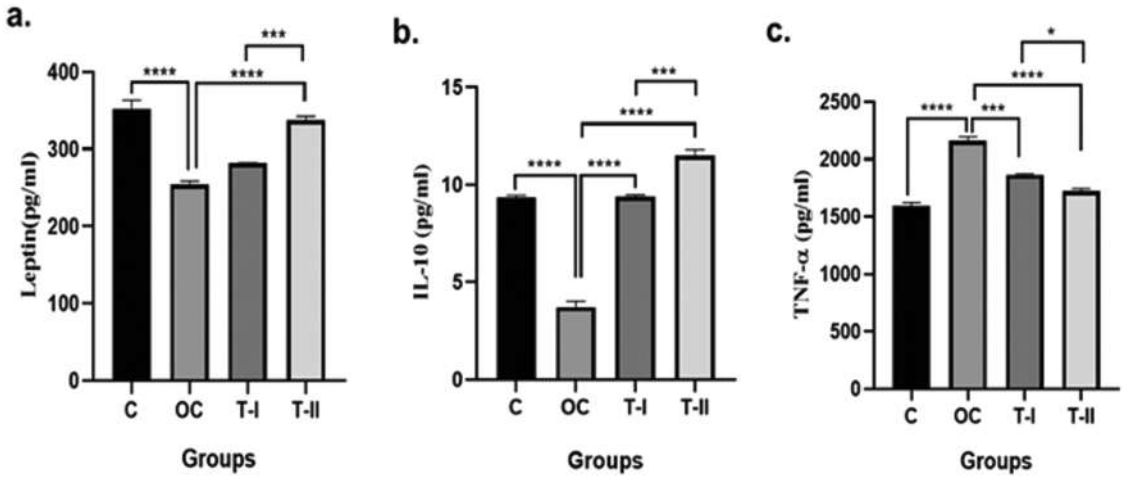


Figure 7. ELISA was used to investigate the impact of *P. paradiseus* oil on the synthesis of a) leptin b) IL-10 and c) TNF-α in different experimental groups (C, OC, T-I, and T-II). Values are expressed as mean± SEM. Statistical analysis evaluated via one way ANOVA followed by Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05.

wholesome and extremely nutrient-dense food additives. In general, the quantities of polyunsaturated fatty acids (PUFAs) in marine fish samples were very high, with alpha-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid having the most positive impacts on human health [26]. As per the statement, our present study explored the presence of diverse range of PUFAs (51.15%) and MUFAs (25.04%) in *P. paradiseus* FO (Table 1a) determined using GC-MS (Figure 1) (Table 1b,c). Some of them have been broadly discussed owing to their biological activity and health benefits in humans [27].

In the current study, we investigated the effects of *P. paradiseus* FO on lipid metabolism in a male BALB/c mouse model. Although the bioactive substances and health benefits of various marine FOs have been well documented [25], but the biological activity of *P. paradiseus* oil has not been fully investigated. In this study, we isolated *P. paradiseus* FO and assessed its potential in preventing obesity. The HFD-fed mice (OC group) experienced the greatest weight increase at the completion of the 12-week experimental period (Figure 2a), and they eventually achieved the highest BMI in comparison to the other groups (Figure 2b). After *P. paradiseus* FO feeding, mice in the HFD-induced treatment groups (T-I and T-II) showed noticeable weight loss and decreased BMI. The weight of WAT changed for body weight and was considerably lower in the mice groups that were fed two different dosages of FO compared to the OC group (Figure 2c). This could be because *P. paradiseus* FO contains various anti-obesity ingredients, particularly fatty acids that are involved in fat metabolism. To investigate the anti-obesity potential of *P. paradiseus* FO, we analysed the total serum lipid profile and total serum glucose levels of each experimental group. According to our findings, a HFD causes insulin resistance and promotes the onset of lipid metabolism. Furthermore, insulin resistance is a major factor in fat degradation in AT, which can lead to the transport of excess free fatty acids (FFAs) and increase the threshold of lipotoxicity. Following this statement in our study, total serum glucose level was increased in OC group up to 51.19% compared to control group, and it was diminished 28.57–38.09% in both the FO treated groups. Additionally, dyslipidemia is highly correlated with the levels of TC, TG, LDL-C, VLDL-C, and HDL-C [28]. Our findings demonstrated that in the FO treated group compared to the OC group, liver TC, TG, serum LDL-C, VLDL-C levels were significantly lower (Figure 3). In contrast, blood HDL-C concentrations increased dramatically following *P. paradiseus* FO administration, indicating that *P. paradiseus* FO could reduce the occurrence of lipotoxicity. Both doses of FO markedly lowered the total serum glucose levels between the treated groups compared to the OC group. This finding suggests that FO ingestion can reduce the hyper-insulinemic state of BALB/c mice [29].

The liver is the primary organ that controls lipid metabolized [30]. Figure 4(a–d) demonstrates the effect of fish oil on liver properties, as revealed by HE staining. HFD-fed mice exhibited a qualitative increase in lipid accumulation in the liver. The aberrant build-up of fat in the liver may be significantly reduced through daily intake of 200 mg and 400 mg of crude oil/kg of

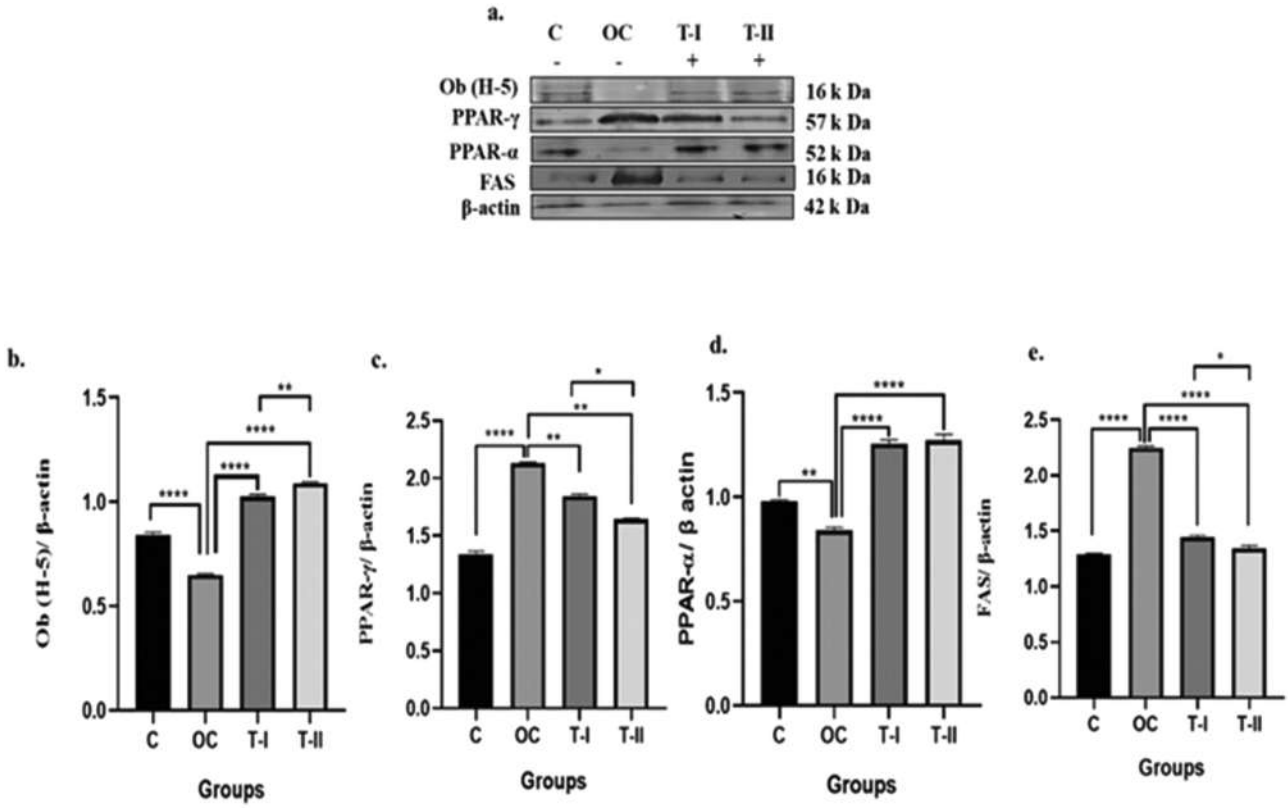


Figure 8. Protein expression analysis of ob (H-5), PPAR-α, PPAR-γ and FAS in WAT. Groups are separated as control group (feed with normal diet), OC group (received HFD), T-I group (HFD feeding and received 200 mg/kg body weight/day of *P. paradiseus* oil) and T-II group (HFD feeding and received 400 mg/kg body weight/day of *P. paradiseus* crude oil). Results were normalized with respect to β-actin. Representative images of western blot WAT (a) relative protein expression, (b) ob (H-5), (c) PPAR-γ, (d)PPAR-α and (e) FAS. Values are expressed as mean ±SEM. Statistical analysis evaluated via one-way ANOVA followed by Tukey's multiple comparison test. ****p<0.0001, ***p<0.001, **p<0.01, *p< 0.05.

body weight (Figure 4e). Adipocyte hypertrophy and hyperplasia are both considered symptoms of obesity, which is defined as an excessive build-up of lipids [31]. We used haematoxylin and eosin (HE) (Figure 5a,b) staining for histological sections analysis of sWAT and vWAT. Adipocytes in the sWAT and vWAT of the OC group contained fat vacuoles with wide inner diameters and were not homogeneous in size. The T-II group showed a substantial difference in fat deposition in the epidermis between the two treatment groups.

Semi-quantitative PCR was used to analyse the mRNA expression of genes associated with glucose and lipid metabolism to determine how FO consumption modifies metabolic syndrome risk factors. Obesity is frequently associated with lipid metabolism problems [4]. Higher levels of circulating leptin act to suppress hunger and promote energy utilization in normal homoeostasis when fat storage rise. Although a lower energy intake should result from higher levels of leptin in the blood, this is not always the case for people who are overweight or obese. It is recognized that leptin central resistance in obese people may be the cause of this [32]. Figure 6b,c shows that after FO treatment, leptin and adiponectin mRNA levels steadily increased in the BALB/c obese mice group. The alteration in SREBP-1c expression disrupts the transcription of the SREBP-1 target gene FAS, which controls the synthesis of fatty acids [33]. Our findings showed that FO supplementation drastically lowered SREBP-1c and FAS mRNA levels in HFD- induced obese BALB/c mice (Figure 6d,e). Both LPL and CPT-1 are rate-limiting enzymes, responsible for the conversion of circulating triglycerides within chylomicrons and VLDL, to FFAs [34] and induce the beta-oxidation pathway [35]. Accordingly, we observed a considerable elevation in the mRNA expression of LPL and CPT-1 in both FO- treated groups (Figure 6f,g). IL-10 is an anti-inflammatory cytokine that increases the release of IL-1RA while attenuating inflammation induced by TNF-α, IL-6, and IL-1 [36].

According to a recent study, TNF-α and IL-6 levels are predictive of the onset of type 2 diabetes and are associated with insulin resistance and obesity [37]. The reduction in TNF-α and IL-6 mRNA in the WAT of treated BALB/c obese mice suggests that FO reduces insulin resistance and lowers blood glucose levels, at least in part (Figure 6i,k). However, the expression of IL-1Ra and IL-10 was increased in both the T-I and T-II groups after treatment with *P. paradiseus* oil (Figure 6h,j). Treatment with FO tended to decrease the expression of pro-inflammatory cytokines (TNF-α) and increase the expression of anti-inflammatory cytokines (IL-10) and metabolic regulators (leptin) in WAT (Figure 7). Next, we examined the effects of FO on the expression of proteins linked to hepatic lipogenesis in HFD-fed mice given a HFD. Figure 8 illustrates how dietary supplementation with two distinct dosages of FO markedly increase hepatic PPAR-α protein expression (Figure 8d) and upregulated the expression of the ob (H-5) hormone (Figure 8b). However, the reduced expression of PPAR-γ (Figure 8c) and FAS protein levels in the liver (Figure 8(8e) [38]. Conjugated fatty acids not only offer anti-obesity potential but they also have anti-cancer qualities that can lower inflammation and promote the death of cancer cells. Recent research indicates that novel CFAs called linoelaidic acid, which were extracted from the marine *Opisthopterus tardoore* (Tapra fish), effectively inhibited the growth of human breast cancer cells [39]. Additionally, FO therapy significantly reduces body weight by promoting fatty acid oxidation [29]. Studies have shown that consuming FO reduces body weight and has an anti-obesity effect by preventing the synthesis of lipids in male BALB/C mice. In this study, the results indicated that oil had a protective effect against obesity, but between the two doses of, 400 mg/kg body weight/day (T-II group), *P. paradiseus* crude oil was more effective in reducing adiposity.

5. Conclusion

In conclusion, the primary goal of this study was to examine the biological activities of marine fish (*P. paradiseus*) with significant commercial importance. In the West Bengal coastal region, this fish is extremely plentiful. *P. paradiseus* is a viable choice because it contains a large number of conjugated fatty acids, and its physicochemical characteristics demonstrate that it is suitable for human ingestion. Our study revealed that HFD consumption resulted in elevated weight gain, adiposity, and serum trans-fat levels. By modifying adipocyte cell size, lipolytic rates, and responsiveness to fatty acids and hormones, the inclusion of FO in the diet modifies lipid metabolism. However, *P. paradisus* oil treatment reduced the expression of obesity-related adipokines and increased body metabolism. Hence, we suggest that FO and its PUFAs may be useful substances for preventing metabolic consequences.

Abbreviations

FO	Fish oil
HFD	High-fat diet
C	Control group
OC	Obese control group
T-I	Treatment I group
T-II	Treatment II group
WAT	White adipose tissue
FAs	Fatty acids
BMI	Body mass index
DAB	3,3′,4,4′-Tetraaminobiphenyl tetrahydrochloride
AT	Adipose tissue
n-3 PUFAs	*n*-3 polyunsaturated fatty acids
EPA	Eicosapentaenoic acid
DHA	Docosahexaenoic acid
MUFAs	Monounsaturated fatty acids
GC-MS	Gas chromatography- Mass spectrometry
GC-FID	Gas chromatography- flame ionization detection
sWAT	Subcutaneous white adipose tissue
vWAT	Visceral white adipose tissue
TC	Total cholesterol
HDL-C	High- density lipoprotein cholesterol
LDL-C	Low- density lipoprotein cholesterol
VLDL-C	Very low- density lipoprotein cholesterol
TG	Triglycerides
HE	Haematoxylin and Eosin
GAPDH	Glyceraldehyde 3- phosphate dehydrogenase
SDS-PAGE	Sodium dodecyl- sulphate polyacrylamide gel electrophoresis
SREBP-1c	Sterol regulatory element binding protein 1c
FAS	Fatty acid synthase
LPL	Lipoprotein lipase
CPT-1	Carnitine palmitoyl transfarese 1
TNF-α	Tumour necrosis factor
IL-1ra	Interleukin 1 receptor antagonist
IL-10	Interleukin 10
IL-6	Interleukin 6
CFA	Conjugated fatty acids

Acknowledgments

The authors are thankful to the Director, Dr Pradip Ghosh, Midnapore City College, for providing essential support to carry out this work. The current research study presented in this manuscript is part of the PhD research work of the first author.

Author contributions

CRediT: **Riya Kar:** Data curation, Investigation, Methodology, Resources, Writing – original draft; **Pipika Das:** Formal analysis, Resources; **Titli Panchali:** Methodology, Resources, Software; **Ananya Dutta:** Resources, Software; **Manisha Phoujdar:** Formal analysis, Resources, Software; **Kuntal Ghosh:** Formal analysis; **Shrabani Pradhan:** Conceptualization, Supervision, Validation, Writing – review & editing.

Funding

The authors acknowledge the Indian Council of Medical Research (ICMR), Govt of India for providing financial support as Extramural Adhoc research grant File no. 5/9/1453/22-Nut. dated 02.01.2023.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Consent for publication

No individual person's data has been included in this manuscript.

Data availability statement

The data that support the findings of this study are openly available in https://doi.org/10.6084/m9.figshare.27861081

References

[1] World Health Organization. The double burden of malnutrition: policy brief.Switzerland: World Health Organization; 2016. https://www.who.int/publications/i/item/WHO-NMH-NHD-17.3

[2] Lee MJ, Wu Y, Fried SK. Adipose tissue heterogeneity: implication of depot differences in adipose tissue for obesity complications. Mol Aspects Med. 2013;34(1):1–11. doi: 10.1016/j.mam.2012.10.001

[3] Kadowaki T, Yamauchi T, Kubota N, et al. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J Clin Invest. 2006;116(7):1784–1792. doi: 10.1172/JCI29126

[4] Zhang J, Yi C, Han J, et al. Novel high-docosahexaenoic-acid tuna oil supplementation modulates gut microbiota and alleviates obesity in high-fat diet mice. Food Sci Nutr. 2020;8(12):6513–6527. doi: 10.1002/fsn3.1941

[5] Garaulet M, Hernandez-Morante JJ, Lujan J, et al. Relationship between fat cell size and number and fatty acid composition in adipose tissue from different fat depots in overweight/obese humans. Int J Obes (lond). 2006;30(6):899–905. doi: 10.1038/sj.ijo.0803219

[6] Yang ZH, Miyahara H, Mori T, et al. Beneficial effects of dietary fish-oil-derived monounsaturated fatty acids on metabolic syndrome risk factors and insulin resistance in mice. J Agric Food Chem 13. 2011;59(13):7482–7489. doi: 10.1021/jf201496h

[7] Ondo-Azi AS, Kumulungui BS, Mewono L, et al. Proximate composition and microbiological study of five marine fish species consumed in Gabon. Afr J Food Sci. 2013;7(8):227–231. doi: 10.5897/AJFS12.177

[8] Majumder TH, Chowdhury AJ, Sarwer MG, et al. Proximate composition of estuarine fish paradise threadfin, Polynemus paradiseus (Linnaeus, 1758). American journal of food nutrition and health, 6(4), pp.117–125. 2016

[9] Gopalan C, Sastri BV, Balasubramanian SC. Nutritive value of Indian foods. Hyderabad: National Institute of Nutrition Publications, ICMR; 2004.

[10] AOCS). In D. Firestone, editor. Official methods and recommended practices of the American oil Chemists' society. Champaign, IL: AOCS press; 1994.

[11] Gonçalves RM, Petenuci ME, Maistrovicz FC, et al. Lipid profile and fatty acid composition of marine fish species from northeast coast of Brazil. J Food Sci Technol. 2021;58(3):1177–1189. doi: 10.1007/s13197-020-04631-y

[12] Firestone D. The Association of Official Analytical Chemists. In: Horwitz, W, editors. Official methods of analysis. 13th ed. (Champaign IL USAOAC); 1989. p. 1038.

[13] Miotto PM, Horbatuk M, Proudfoot R, et al. α-linolenic acid supplementation and exercise training reveal independent and additive responses on hepatic lipid accumulation in obese rats. Am J Physiol Endocrinol Metab. 2017;312(6):E461–E470. doi: 10.1152/ajpendo.00438.2016

[14] Yang T, Feng F, Zhan K, et al. Effect of the tea tree oil on growth performance, meat quality, serum biochemical indices, and antioxidant capacity in finishing pigs. Front Vet Sci. 2022;9:916625. doi: 10.3389/fvets.2022.916625

[15] Valenzuela CA, Baker EJ, Miles EA, et al. Eighteencarbon trans fatty acids and inflammation in the context of atherosclerosis. Prog Lipid Res. 2019;76:101009. doi: 10.1016/j.plipres.2019.101009

[16] Kojadinovic MI, Arsic AC, Debeljak-Martacic JD, et al. Consumption of pomegranate juice decreases blood lipid peroxidation and levels of arachidonic acid in women with metabolic syndrome. J Sci Food Agric. 2017;97(6):1798–1804. doi: 10.1002/jsfa.7977

[17] Li L, Spranger L, Stobäus N, et al. Fetuin-B, a potential link of liver-adipose tissue cross talk during diet-induced weight loss–weight maintenance. nutr & Diabetes. 2021;11(1):31. doi: 10.1038/s41387-021-00174-z

[18] Wei W, Hu M, Huang J, et al. Anti-obesity effects of DHA and EPA in high fat-induced insulin resistant mice. Food Funct. 2021;12(4):1614–1625. doi: 10.1039/d0fo02448a

[19] Dranca F, Ursachi F, Oroian M. Bee bread: physicochemical characterization and phenolic content extraction optimization. Foods. 2020;9(10):1358. doi: 10.3390/foods9101358

[20] Waehler R. Fatty acids: facts vs. fiction. Int J Vitam Nutr Res. 2023;93(3):268–288. doi: 10.1024/0300-9831/a000713

[21] Johnston LW, Harris SB, Retnakaran R, et al. Longitudinal associations of Phospholipid and cholesteryl ester fatty acids with disorders underlying diabetes. J Clin Endocrinol Metab. 2016;101(6):2536–2544. doi: 10.1210/jc.2015-4267

[22] Guggisberg D, Burton-Pimentel KJ, Walther B, et al. Molecular effects of the consumption of margarine and butter varying in trans fat composition: a parallel human intervention study. Lipids Health Dis. 2022;21(1):74. doi: 10.1186/s12944-022-01675-1

[23] Delgado GE, Krämer BK, Lorkowski S, et al. Individual omega-9 monounsaturated fatty acids and mortality-the Ludwigshafen risk and cardiovascular Health study. J Clin Lipidol. 2017;11(1):126–135.e5. doi: 10.1016/j.jacl.2016.10.015

[24] Christiansen E, Watterson KR, Stocker CJ, et al. Activity of dietary fatty acids on FFA1 and FFA4 and characterisation of pinolenic acid as a dual FFA1/FFA4 agonist with potential effect against metabolic diseases. Br J Nutr. 2015;113(11):1677–1688. doi: 10.1017/S000711451500118X

[25] Pradhan S, Panchali T, Paul B, et al. Anti-obesity potentiality of tapra fish (opisthopterus tardoore) oil. J Food Biochem. 2020;44(11):e13448. doi: 10.1111/jfbc.13448

[26] Irnawati I, Nadia LO, Windarsih A, et al. Fatty acid composition, biological activity and authentication of marine fish oil. Food Res. 2023;7(6):187–196. doi: 10.26656/fr.2017.7(6).472

[27] Coraça-Huber DC, Steixner S, Wurm A, et al. Antibacterial and anti-biofilm activity of omega-3 polyunsaturated fatty acids against periprosthetic joint infections-isolated multi-drug resistant strains. Biomedicines. 2021;9(4):334. doi: 10.3390/biomedicines9040334

[28] Lan YL, Lou JC, Lyu W, et al. Update on the synergistic effect of HSL and insulin in the treatment of metabolic disorders. Ther Adv Endocrinol Metab. 2019;10:2042018819877300. doi: 10.1177/2042018819877300

[29] Arai T, Kim HJ, Chiba H, et al. Anti-obesity effect of fish oil and fish oil-fenofibrate combination in female KK mice. J Atheroscler Thromb. 2009;16(5):674–683. doi: 10.5551/jat.1313

[30] Zhou M, Huang J, Zhou J, et al. Anti-obesity effect and mechanism of chitooligosaccharides were revealed based on lipidomics in diet-induced obese mice. Molecules. 2023;28(14):5595. doi: 10.3390/molecules28145595

[31] Schwartz MW, Seeley RJ, Zeltser LM, et al. Obesity pathogenesis: an endocrine society scientific statement. Endocr Rev. 2017;38(4):267–296. doi: 10.1210/er.2017-00111

[32] Feng H, Zheng L, Feng Z, et al. The role of leptin in obesity and the potential for leptin replacement therapy. Endocrine. 2013;44(1):33–39. doi: 10.1007/s12020-012-9865-y

[33] Edwards PA, Tabor D, Kast HR, et al. Regulation of gene expression by SREBP and SCAP. Biochim Biophys Acta. 2000;1529(1–3):103–113. doi: 10.1016/s1388-1981(00)00140-2

[34] Chang HR, Josefs T, Scerbo D, et al. Role of LpL (Lipoprotein Lipase) in Macrophage Polarization In Vitro and In Vivo [Published correction appears in arterioscler Thromb vasc biol. 2019 Dec;39(12):e293]. Arterioscler Thromb Vasc Biol. 2019;39(10):1967–1985. doi: 10.1161/ATVBAHA.119.312389

[35] Bonnefont JP, Djouadi F, Prip-Buus C, et al. Carnitine palmitoyltransferases 1 and 2: biochemical, molecular and medical aspects. Mol Aspects Med. 2004;25(5–-6):495–520. doi: 10.1016/j.mam.2004.06.004

[36] Juge-Aubry CE, Somm E, Pernin A, et al. Adipose tissue is a regulated source of interleukin-10. Cytokine. 2005;29(6):270–274. doi: 10.1016/j.cyto.2004.10.017

[37] Pradhan AD, Manson JE, Rifai N, et al. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA. 2001;286(3):327–334. doi: 10.1001/jama.286.3.327

[38] Chiu CY, Chang TC, Liu SH, et al. The regulatory effects of fish oil and chitosan on hepatic lipogenic signals in high-fat diet-induced obese rats. J Food Drug Anal. 2017;25(4):919–930. doi: 10.1016/j.jfda.2016.11.015

[39] Dutta A, Panchali T, Khatun A, et al. Anti-cancer potentiality of linoelaidic acid isolated from marine tapra fish oil (ophisthopterus tardoore) via ROS generation and caspase activation on MCF-7 cell line. Sci Rep. 2023 Aug 29;13(1):14125. doi: 10.1038/s41598-023-34885-3

Applied Biochemistry and Biotechnology
https://doi.org/10.1007/s12010-025-05488-7

ORIGINAL ARTICLE

Mechanistic Insights Into the Anti-Obesity Effects of Gondoic Acid: Regulation of AMPK Signalling and Obesity Related Gene Expression in Obese Mice

Riya Kar[1,2] · Pipika Das[1,2] · Titli Panchali[1,2] · Ananya Dutta[1,2] · Manisha Phoujdar[1,2] · Shrabani Pradhan[2]

Received: 18 August 2025 / Accepted: 10 December 2025
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2026

Abstract

Considered one of the most prevalent monounsaturated fatty acids (MUFA) from marine and plant sources, omega-9 fatty acids (n-9) have gained worldwide recognition since due to its strong anti-obesogenic properties. These findings highlight the anti-obesity efficacy and mechanism of n-9, gondoic acid (GA) for anti-obesity interventions, offering a novel research perspective at recent research field. The animals (BALB/c male mice) were segregated into six study groups: the control group (C) received normal chow diet; the vehicle control group (VC) received normal chow diet + olive oil; high fat diet (HFD) received by the obese control group (OC); and the treatment-I (T-I), treatment-II (T-II), treatment-III (T-III) group co-administered with GA as follows: 1 mg/kg GA + HFD; 2 mg/kg GA + HFD; 4 mg/kg GA + HFD. In this study, GA was delivered orally once daily for six weeks to diet-induced obese mice. According to the findings, administering GA decreased weight gain, blood lipid profile levels, improved inflammation and adipogenesis. The mRNA expression studies and western blot assay found that GA administration may boost glucose metabolism, enhance the process of thermogenesis and β-oxidation in mitochondria via the AMPK mechanism, reducing HFD-induced adiposity. In brief, our findings can point in a broader direction for the use of GA in obesity based on genetic indicators.

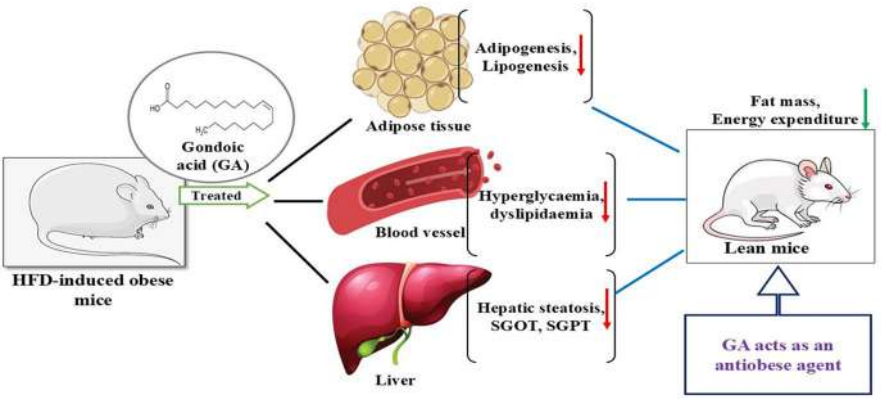
✉ Shrabani Pradhan
shrabanipradhan@mcconline.org.in

[1] Biodiversity and Environmental Studies Research Center, Vidyasagar University, Midnapore City College, Paschim Medinipur, Bhadutala 721129, West Bengal, India

[2] Central Research Laboratory, Department of Paramedical and Allied Health Sciences, Midnapore City College, Paschim Medinipur, Bhadutala 721129, West Bengal, India

Graphical Abstract

Gondoic acid (GA) treatment performs as an anti-obese agent by improving a number of metabolic markers.



Keywords Gondoic acid · Obesity · Metabolic syndrome · Lipid metabolism · Adipokines · AMPK pathway

Introduction

Rising incidence of metabolic disorders, particularly diabetes, cardiovascular disease, hypertension, and non-alcoholic hepatosteatosis, are mostly caused by the present worldwide obesity pandemic. The past 20 years have seen a surge in interest in adipocyte physiology due to efforts to identify treatments for obesity and related conditions. This has resulted in significant advancements in our scientific understanding of adipose tissue (AT) as a vital regulatory organ [1]. The majority of public health organisations currently advise increasing aerobic activity and maintaining a well-balanced diet as strategies to lose weight. Although people are having trouble adjusting to these modifications to their lives, especially over an extended period of time. As a result, there is a fascination with investigating nutritional choices that may aid in controlling obesity. A possible option might involve the usage of marine fish oil (FO), which has a broader spectrum of fatty acids, with a particular emphasis on polyunsaturated and monounsaturated fatty acids (PUFAs and MUFAs) that will be beneficial in or together with our meals [2]. Fish is recognised as a cost-effective source of a variety of essential components, mostly high in fat and protein, and micronutrients such as selenium, iodine, and potassium, all of which are vital elements of an individual's diet [3]. Eicosa pentaenoic acid (EPA) and docosahexaenoic acid (DHA), two dietary sources of long-chain polyunsaturated fatty acids (LC n-3 PUFAs) derived from marine FO, have been demonstrated to have hypotriglyceridemic, anti-inflammatory, and heart-healthy effects [4]. According to the European Food Safety Authority (EFSA), the LC n-3 to LC n-6 fatty acid ratio falls between 1.3 and 21.2. During our life cycle, the ratio of n-6/n-3 LC-PUFAs plays a crucial role in maintaining homeostasis, healthy growth, and mental

state. Thus, increasing fish consumption may greatly enhance the body's capacity of taking in LC n-3 fatty acids and optimise the utilisation of EPA and DHA, both of which will be advantageous for a variety of biological functions [5]. Numerous studies revealed that FO administration enhanced rodent hepatocytes' expression of uncoupling protein 2 (UCP-2), which may account for the reported safeguarding against weight gain [6]. Later, Tsuboyama-Kasaoka et al. [7] demonstrated using a UCP-2 knockout model that eating FO inhibits body weight in mice even when UCP-2 is not present, indicating that multiple pathways may be involved in regulating the impact of FO on weight regulation. One well-known laboratory model for increasing body mass growth and encouraging fat deposition is the management of a high-fat diet (HFD) to rodents [8], whereas FO supplementation has been shown to improve insulin resistance, especially in liver and skeletal muscle, promote lipid oxidation and decrease of body fat in these animals [9–12]. Many studies have hypothesised putative antiobesity pathways in FO, such as enhanced adipocyte death, higher plasma adiponectin levels [13], and improved fat oxidation and calorie consumption [14, 15].

Regarding nutritional therapy for anti-obesity healthy foods generated from natural components are often recommended over medicines that may cause negative effects. The health benefits of LC n-3 and LC n-6 FAs have been widely studied [16]. Furthermore, investigations have indicated that MUFAs have anti-inflammatory properties [17]. However, LC n-9 FAs are currently attracting a lot of interest due to new research and findings about their metabolic significance. LC n-9 FAs are a kind of unsaturated FA with a double bond at 9th place from the methyl end. The most common well known LC n-9 FAs are hypogeic acid, oleic acid, elaidic acid, gondoic acid, mead acid, erucic acid and nervonic acid. Oleic acid received the most attention in research as compared to the other mentioned MUFAs [18]. Gondoic acid (GA, cis-11-Eicosenoic acid, molecular formula $C_{20}H_{38}O_2$, molecular weight 310.51 g/mol), a 20-Carbon MUFA, is contained in a variety of plant oils such as jojoba oil camelina seeds oil and nuts [19]. They are synthesised endogenously in humans, but do not entirely meet al.l physiological demands. Nevertheless, GA can also be produced in vivo via the process of fatty acid metabolism. Camelina sativa seed oil has been shown in studies to lower blood cholesterol, improve the body's defence, prevent senile dementia, cardiovascular disease, and cerebrovascular illness [18]. Few papers were discovered on the health impacts of this specific LC n-9 fatty acid, indicating that additional investigation is necessary to confirm its health advantages or harmful effects. For the first time, our research shows that n-9, GA has an anti-obesity impact. GC-MS and GC-FID analysis indicated that *P. paradiseus* FO contains a total of 25.04% MUFA, while GA accounts for 6.38% of total MUFA [20]. GA's antiobesity efficacy are promising, but underlying mechanisms till unclear. In light of the advantages of MUFA, we sought to investigate how GA's regulation of AMPK signalling and obesity related gene expressions, enhanced the utilisation of lipids in HFD-induced obese mice to exploring new insights into its therapeutic potential.

Materials and Methods

Chemicals

To complete each experiment in this investigation, the following substances and reagents were purchased. Using treatment compound named Gondoic acid or cis-11- Eicosenoic

acid (E3635-100MG), purchased from Merck, Darmstadt, Germany; RNA-Xpress Reagent, Hi-cDNA synthesis kit, 2X PCR mixture, 3,3′,4,4′-Tetraaminobiphenyl tetrahydrochloride (DAB) obtained from Himedia, HiGenoMB, Maharashtra, India. Graded ethanol and paraffin purchased from Histosec, Merck, Darmstadt, Germany; DAPI (4',6-Diamidino-2-Phenylindole) (Cat no. 62248) from Invitrogen; fluorescent mounting medium purchased from Dako, Hamburg, Germany. All of the PCR primers were acquired from Bioserve in India. The primary antibodies: AMPK mouse McAb (Cat No.: 66536-1-Ig), CEBP-α Mouse McAb (Cat No.: 66649-I-Ig/), SREBP-1c Mouse McAb (Cat No.: 66875-1-Ig), β- actin Mouse McAb (Cat No.:66009-1-Ig) were purchased from Proteintech (ChromoTek GmbH & Proteintech Germany) and Ob (H-5) mouse monoclonal IgG (Sc-393043), PPAR-γ mouse monoclonal IgG (Sc-7273), FAS mouse monoclonal IgG (Sc-74540) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA-USA). The secondary antibody: Goat Anti-mouse IgG-HRP (cat# 11–301) was purchased from Abgenex (Odisha, India).

Animal Care

This study was approved by the Institutional Ethics Committee, Midnapore City College (2256/PO/RE/S/23/CCSE, MCC/IAEC-SP/10/23−003) and the management of every experimental protocol agreed with the rules governing the use and care of laboratory animals. The animal data has been submitted in accordance with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) criteria. Six-week-old male BALB/c mice from Saha Enterprise, IEC No: BAEPS7949M (Habra, North 24 Pargana, West Bengal- 743271) were housed and caged individually in a room at 23 ± 1 °C with a 12/12-h light–dark cycle. The animals were given free access to water and a normal chow meal for one week following arrival.

Administration of Diets and Fatty Acid

The mice were fed two different types of diets. Composition of normal chow diet was (wt/wt): 10% fat, 60% carbohydrate, 20.5% protein, 2.9% fibre, 6.6% minerals with water ad libitum. Composition of HFD was (wt/wt): 41.5% fat from lard, 35% carbohydrate, 15% protein, 5.0% fibre, 3.5% mineral with water ad libitum. Composition of treatment compound (100 mg GA/1 ml olive oil): Gondoic acid or cis-11- Eicosenoic acid (E3635-100MG), purchased from Merck was dissolved per 1 ml of olive oil and used as treatment solution. Olive oil was co-administered to the vehicle control group (VC) and HFD-induced mice groups (Treatment-I, Treatment-II, Treatment-III) supplemented with GA by oral gavage during experiment.

Experimental Protocol

Total experiment was continued for 16th weeks. Except for the control and vehicle control groups, mice were fed a HFD for 10 weeks before being administered the test oils. Six groups ($n = 6$ of each group) were randomly divided from these acclimatized mice: (1) Normal chow feeding, control group (C); (2) Normal chow feeding and co-administered with olive oil (4 mg/kg body weight/day), vehicle control group (VC) (3) HFD feeding, obese control group (OC); (4) HFD-fed mice groups supplemented with 1 mg/kg body weight/ day

GA (T-I group); (5) HFD-fed mice groups supplemented with 2 mg GA/kg body weight/ day (T-II group); (6) HFD-fed mice groups supplemented with 4 mg/kg body weight/ day of GA (T-III group) by oral gavage for last the 6th weeks of total experiment.

Collection of Blood and Tissue Samples

After 16 weeks of the experiment, mice were anesthetized with ether as well as euthanized via dislocation of the cervical spine. Blood samples were taken by rupturing the orbital plexus and centrifugation at 3000 rpm for 15 min at 4 °C, with serum kept at -20 °C for biochemical evaluation. The liver, subcutaneous, and visceral adipose tissues were excised, weighed, and promptly preserved in liquid nitrogen at -20 °C for further testing. Adipose fat depots were extracted, weighed, and processed according to the protocol outlined below for protein and gene expression profiling.

Assessment of Daily Food Intake, Somatic Body Weight index, BMI (gm/cm^2) in Different Experimental Group

The food intake was recorded per day and bodyweight was recorded every week for sixteen weeks. Body length was measured from nasal to anal distance using a Central caliper scale (Model no. 6420) to the closest 0.1 mm. Daily food intake was measured by using food weight machine (ASIN, Model no. KS33). The standard approach was used to determine BMI, and the findings were expressed as g/cm^2 [21]. Body mass index (BMI) was calculated using the standard method; by following equation given below and the results were indicated as g/cm^2.

$$BMI\left(g/cm^2\right) = Body\ weight\,(g)\,/Body\ length\left(cm^2\right)$$

Biochemical Analysis

The serum levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides, serum protein level and total serum glucose level were determined using the kit assay techniques (Enzopak, India). We also estimated the level of some toxicity marker such as SGOT, SGPT, uric acid, urea through semiauto analyser following Enzopak kit method [10].

Histological Analysis by HE Staining

After 24 h of oral administration of the experimental diets, liver tissues and ATs (visceral and subcutaneous) were collected from sacrificed mice instantly frozen at -80 °C. Following standard procedure, tissues that were frozen were cryo-sectioned, embedded in paraffin wax, and fixed in a 10% formalin solution before being cut. Haematoxylin and eosin (H&E) were used to stain tissue sections 6 μm thick for histological examination. Histological images were captured using the compound microscope (Olympus, Model No. CX21i) [22].

Morphological Analysis by DAPI (4,6-diaminido-2-phenylindole) Staining

Following experimental animal sacrifice, liver tissues were preserved, paraffin embedded, and dissected into 6 µm slices. On a rotary microtome (Mod. RM2255; Leica-Microsystems, Wetzlar, Germany), fixed specimens were cut into serial 6 μm slices after being dried in graded ethanol and embedded in paraffin (Histosec, Merck, Darmstadt, Germany). Graded ethanol (2 × 30 Sect. 100%, 2 × 30 Sect. 90%, 2 × 30 Sect. 70%, and 2 × 30 Sect. 50%) was added to paraffin-embedded sections, which were subsequently immersed in xylene. Then five minutes of incubation with 5 µg/mL DAPI (Invitrogen) in PBS followed. The slices were then covered with fluorescent mounting material (Dako, Hamburg, Germany) and rinsed three times with PBS. A fluorescent microscope was used to examine the sections (Olympus, Model No. BX43F) [23].

Gene Expression Study Using Semi qPCR

Adipose tissue samples were used to extract whole RNA using the HiPurA Total RNA Miniprep purification kit methodology (Hi-Media Hi-GenoMB, Maharashtra, India). Reverse transcriptase was used to create cDNA, and the NanoDrop-2000 (Eppendorf) spectrophotometer was used to measure the amount of isolated RNA. The mixture for the reverse transcription reaction will be made using the Hi-cDNA synthesis kit (Himedia, HiGenoMB, Maharashtra, India). *Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)* mRNA expression was employed as the house-keeping primer set (*sense:* GGTGAAGGT CGGAGTCAACG, *antisense:* GTGAAGACGCCAGTGGACTC). PCR will function as follows: DNA is first denaturated at 95˚C for 1 min, then denaturated for 30 s, annealed at 65˚C for 45 s, and then extended at 72˚C for 2 min and finally extended for 10 min. Total 42 heat cycles were achieved by repeating the second and third steps. Finally, the PCR tube containing the material was kept at 4˚C. All the primer will go through the same procedure (Table 1).

Protein Analysis by Immunoblotting Assay

Using radioimmunoprecipitation assay (RIPA) buffer, adipose tissue samples were lysed, and the Lowry method was used to measure the protein content. Protein samples weighing 50 µg were boiled for 5 min in loading buffer before being electrophoresed using sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and deposited on a nitrocellulose membrane (Bio-Rad, Model No. 165.8033FC). Then 4% bovine serum albumin (BSA) wasnext applied to block the membrane, and the primary antibody was incubated at 4 °C for the whole night. The primary antibody used in this study included β-actin, Ob (H-5), PPAR-γ, FAS, SREBP-1c, CEBP-α and AMPK at a 1:1000 ratio. The membrane was then incubated for one hour at 4 °C with horseradish peroxidase (HRP) labelled anti-mouse secondary antibody 1:7500 (Abgenex) after being cleaned three times with phosphate buffer saline with tween-20 (PBST). Tetra aminobiphenyl tetrahydrochloride (3,3′,4,4′) was used to identify particular protein bands (DAB). Densitometric examination utilising Image J densitometry software was used to quantify the bands [20].

Table 1 Listed primer sequences used in semi q PCR analysis. Annealing temperature of each primer denoted by Tm (˚C)

Primers	Amplicon size (bp)	Primary Sequences			
		Forward Sequence	Tm (˚C)	Reverse Sequence	Tm (˚C)
AMPK	*105*	*AGGAAGAATCCTGTGAC AAGCAC*	*64.6*	*CCGATCTCTGTGGAGT AGCAGT*	*64.9*
Adiponectin	*152*	*GTCAGTGGATCTGACGA CACCAA*	*57*	*ATGCCTGCCATCCAA CCTG*	*53*
Leptin	*151*	*CAAGCAGTGCCTATCC AGA*	*51*	*AAGCCCAGGAATGAA GTCCA*	*52*
SREBP-1c	*184*	*ACGGAGCCATGGATTG CACA*	*54*	*AAGGGTGCAGGTGTC ACCTT*	*54*
GLUT 4	*173*	*GAGCCTGAATGCTAATG GAG*	*60.2*	*GAGAGAGAGCGTCCA ATGTC*	*61.6*
FAS	*136*	*TGCTCCCAGCTGCAGGC*	*54*	*GCCCGGTAGCTCTGG GTGTA*	*58*
LPL	*134*	*CCACAGCAGCAAGACC TTC*	*53*	*AGGGCGGCCACAAG TTTG*	*53*
CPT-1	*217*	*GTGACTGGTGGGAGGA ATAC*	*54*	*GAGCATCTCCATGGC GTAG*	*53*
Insulin	*189*	*CCCCAGAAAAACCTCTT CAGG*	*54*	*GTCACATTCCCAACAT CGCC*	*54*
PPAR-α	*191*	*CCTGAACATCGAGTGTC GAATAT*	*53*	*GTTCTTCTTCTGAATCT TGCAGCT*	*54*
PPAR-γ	*190*	*AGGCCGAGAAGGAGAAG CTGTTG*	*67.9*	*TGGCCACCTCTTTGCT CTGCTC*	*68.4*
TNF-α	*194*	*TTCTGTCTACTGAACTTC GGGGTGATCGGTCC*	*66*	*GTATGAGATAGCAAATC GGCTGACGGTGTGG*	*66*
IL-10	*196*	*TCTCCGAGATGCCTTCA GCAGA*	*57*	*TCAGACAAGGCTTGGC AACCCA*	*57*
IL-1Ra	*194*	*GCAGCACAGGCTGGTGA ATGAC*	*59*	*TGCCCCCGTGGATGC CCAAG*	*60*

Statistical Analysis

The data (triplicate values) were examined by GraphPad Prism software (San Diego, CA, USA) and expressed as mean ± SEM. One-way ANOVA and Tukey's multiple-comparison tests were used to compare treatment groups to the control group. Significance levels were set at $p < 0.0001$, $p < 0.001$, $p < 0.01$, and $p < 0.05$ [24].

Results

GA Suppresses diet-induced Weight Gain and Fat Formation

To determine that GA has anti-obesity benefits, we employed HFD-fed BALB/c mice as an obese model; mice were provided with the HFD over 16 weeks, except in the C and VC groups. The successfully simulated mice were then given a suitable n-9 fatty acid at various doses (T-I, T-II, and T-III groups treated with 1 mg, 2 mg, and 4 mg GA/kg body weight/day) for 6 sequent weeks. At the start of the experiment, mice had a mean weight of

20.2 ± 0.45 g, with no significant difference between groups. After 16 weeks of HFD, mice in the OC group had an average body weight of 49.12 ± 0.82 g, which was significantly greater than mice in the C and VC groups ($p < 0.0001$). The mice's appearances were illustrated in Fig. 1a, which most likely looks into how various GA dosages affect body weight and fat storage. Curiously, the overall body weight increased by the OC and T-I groups was almost equal. As shown in (Fig. 1c), during administration with GA, the mean body weight gain of the T-II (38 ± 0.32 g) and T-III (35 ± 0.21 g) groups were significantly reduced than those in the OC group ($p < 0.0001$). As shown in (Fig. 1b), the daily caloric intake of each group was the same; however, the OC group ingested a little more calorie than the other GA-treated groups. In contrast to obese mice given a HFD, animals gavaged with GA usually show a considerably reduced BMI (Fig. 1d). So, the BMI (g/cm^2) of mice group was as follows for C: 0.52 ± 0.01, VC: 0.55 ± 0.04, OC: 0.74 ± 0.06, T-I: 0.61 ± 0.03, T-II: 0.55 ± 0.02; T-III: 0.52 ± 0.02 (Fig. 1e, f, g) presents data regarding the weight of peripheral organs in among the experimental groups. OC group tend to have larger WAT depots (2.39 ± 0.34 g) and heavier livers (1.92 ± 0.16 g) than lean mice, while the weight of the kidney (0.65 ± 0.02 g) may not change much or may even slightly increase depending on the study and degree of obesity. Furthermore, mice treated with GA at various doses (1 mg, 2 mg, and 4 mg/kg body weight/day) tend to have lower WAT (1.80 ± 0.04 g, 1.36 ± 0.07 g, 0.75 ± 0.12 g) and liver (1.76 ± 0.05 g, 1.67 ± 0.03 g, 1.38 ± 0.06 g) weights than the OC group that does not received GA. Particularly, the T-III group showed more efficacy; this is because GA can help control lipid metabolism and possibly lessen the buildup of fat in these organs.

Efficacy of GA Administration on the Lipid Metabolism in Obese Mice

Impaired glucose and lipid absorption are frequently linked to obesity induced by an HFD. The red blood cells rapidly utilise carbohydrates as their primary energy source once they are mostly absorbed in the gastrointestinal tract as glucose. But in fat cells, surplus glucose is broken down into glycerol-3-phosphate and fatty acids, which then aggregate to form TGs [25]. As illustrated in Fig. 2a, the total blood glucose readings of mice with HFD-induced obesity were 35.62% higher than those of the animals in the C and VC groups, and they were across the normal blood glucose range. Compared with that the mice treated with GA decreased serum glucose level by 16.40% in T-II and 41.57% in T-III group. Hormone-sensitive lipase (HSL) was the initially discovered lipolytic enzyme found to break down cholesterol and triglycerides both of which are essential for lipolysis. In this investigation the serum triglyceride and cholesterol level were higher in OC group by 32.69% and 23.83% compared with normal fed C group. However, this study explored that GA can maintain serum HSL homeostasis by diminished the serum triglyceride (12.68% -26.31%) and cholesterol (8.17% -20.07%) level in treated groups (Fig. 2b, c**)**. Circulating non-esterified fatty acids are highly sensitive to metabolic abnormalities in obesity. The HFD caused a notable increase in LDL and VLDL levels in the OC group by 32.69%-40.26%, but these levels were dramatically reduced in the GA treated group (T-I &T-II) by 26.32–34.08% (Fig. 2e, f**)**. Furthermore, to the lower quantity of HDL, the functionality of HDL particles may be altered in OC group compare to the other experimental groups. In our study HDL level was elevated gradually by 37.25%- 51.26% in the GA treated groups (Fig. 2d**)**. Subsequently, we investigated the total serum protein and some toxicity markers level in the AT and livers of mice in all diet group. The levels of uric acid, urea, SGOT and SGPT for the OC group were

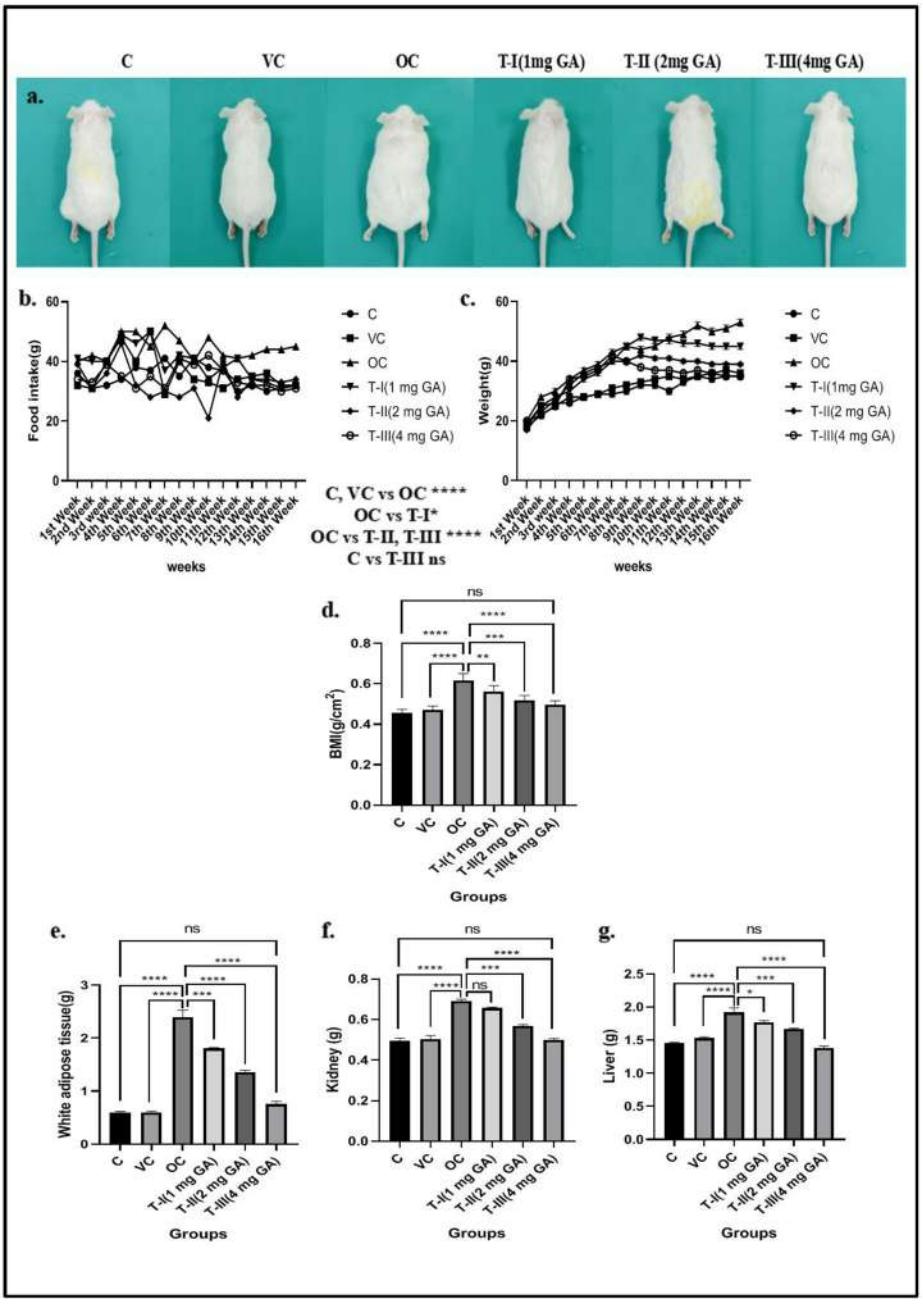


Fig. 1 Impacts of GA on body and peripheral organ weight in mice consuming a high-fat diet. (**a**) Advancement of fat build up; (**b**) measurements of weekly food consumption; (**c**) body weight measurements taken over the study duration; (**d**) BMI (g/cm^2); (**e**) weight of WAT; (**f**) weight of liver; (**g**) weight of kidney in different experimental groups. Values are means ± SEM ($n = 6$). Statistical analysis was performed using one-way ANOVA accompanied by Tukey's multiple comparison test. ****$p < 0.0001$, ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$

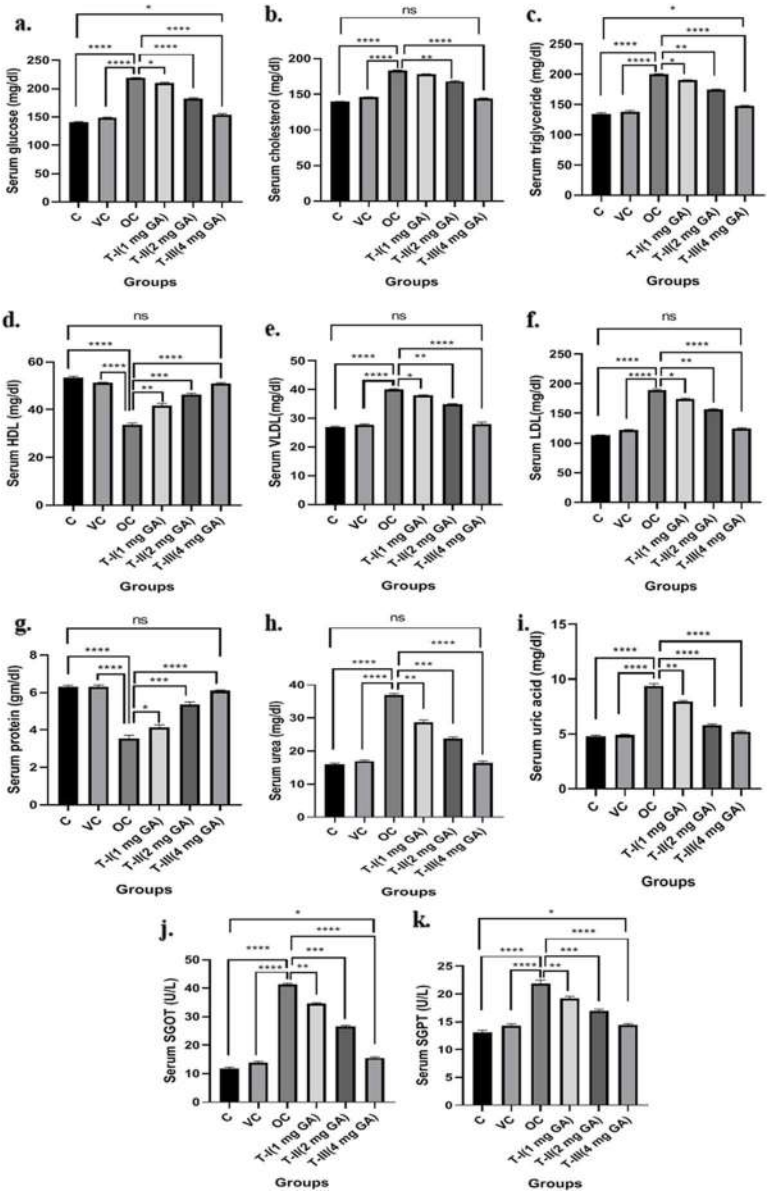


Fig. 2 Efficacy of GA administration on lipid metabolism in obese mice. After the development of obesity, obese mice were segregated in to six groups, normal chow feeding, control group (C); normal chow feeding+olive oil, vehicle control group (VC), HFD feeding, obese control group (OC); HFD+1 mg GA/day (T-I group); HFD+2 mg GA/ day (T-II group); HFD+4 mg GA/ day (T-III group). Blood samples from every mouse were taken on the final day of the experiment. Blood samples were used to separate serum, and its concentrations were determined of (**a**) glucose; (**b**) cholesterol; (**c**) triglyceride; (**d**) HDL; (**e**) VLDL; (**f**) LDL; (**g**) protein; (**h**) urea; (**i**) uric acid; (**j**) SGOT and (**k**) SGPT level in all the experimental group ($n=6$). Values represent the mean±SEM. Statistical analysis was performed using one-way ANOVA accompanied by Tukey's multiple comparison test. ****$p<0.0001$, ***$p<0.001$, **$p<0.01$, *$p<0.05$

comparatively higher (almost 47.34–56.58%) than the C and PC group. However, serum protein level (Fig. 2g**)** was lower (63.56%) in OC group. Mice groups fed HFD combined 1 mg, 2 mg, 4 mg GA/kg body weight/day had a lower serum urea (55.61%; Fig. 2h, uric acid (46.37%; Fig. 2i), SGOT (62.35%; Fig. 2j**)**, and SGPT (34.14%; Fig. 2k**)** levels compared to the OC group. In summary, GA might assist with obesity-related problems involving lipid and glucose metabolism.

Buildup of Lipids in Liver Tissue

Each treatment group's mice liver tissues were examined for the accumulation of lipids by HE staining (Fig. 3b) and DAPI staining (Fig. 3c). Here Fig. 3a represent the morphological images of liver in different experimental group. A major characteristic of steatosis, or fatty liver disease, that frequently arises from an accumulation of fat droplets, is cytoplasmic enlargement, which is the expansion of the liver's cells (hepatocytes). HE staining of the OC group's liver usually showed a considerable increase in big, transparent vacuoles inside hepatocytes, caused by an imbalance between the delivery, synthesis, and export of fat [20, 23]. In contrast, mice treated with GA (T-I group:1 mg, T-II group: 2 mg and T-III group: 4 mg GA/kg body weight/ day) exhibited less liver damage and inflammation, which was frequently accompanied by a more ordered hepatic architecture. In the framework of an obesity investigation, we used DAPI staining to identify liver morphology and nuclear patterns of architecture. Due to the hyperplasia and hypertrophy of the tissue, we can observe that

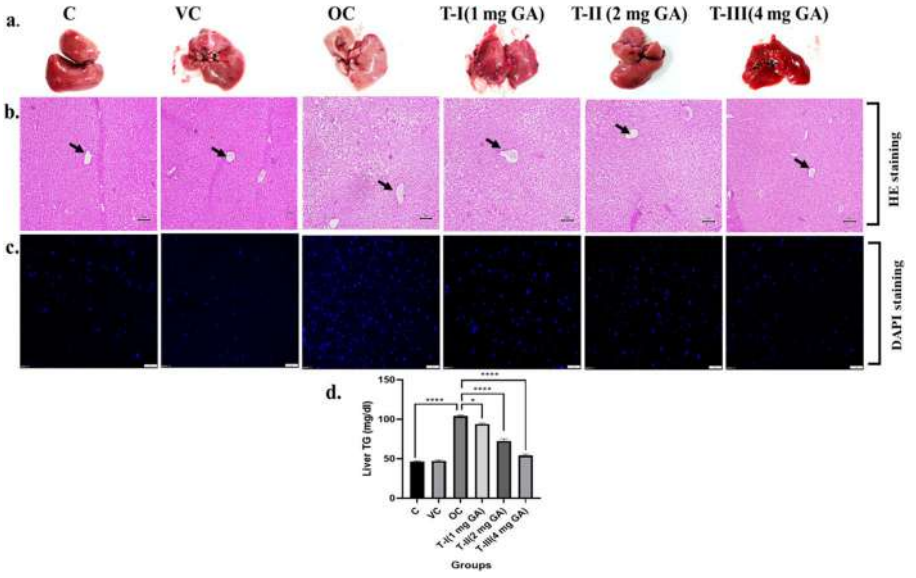


Fig. 3 Histological analysis of liver tissues. Groups are separated as normal chow feeding, control group (C); normal chow feeding + olive oil, vehicle control group (VC), HFD feeding, obese control group (OC); HFD + 1 mg GA/day (T-I group); HFD + 2 mg GA/ day (T-II group); HFD + 4 mg GA/ day (T-III group). (**a**) Represent the image of liver, tissue sections were stained with (**b**) hematoxylin and eosin & (**c**) DAPI, (d) Assessment of liver TG. The results for all groups ($n = 6$) are presented as mean ± SEM. Statistical analysis was performed using one-way ANOVA accompanied by Tukey's multiple comparison test. ****$p < 0.0001$, ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$. In the staining part arrows (→) represent the way of cytoplasm enlargement in several experimental groups. The scale bar correspondence to 100 μm

nuclei may appear large and that the liver tissue may have increased cellular density in the OC group. On the other hand, the liver tissue's normal cellular density appears in the C, VC, and GA treated groups. This suggests that GA with selective dose (T-III group: 4 mg GA/kg body weight/ day) has a protective impact on the liver tissue (Fig. 3).

GA Inhibited AT's Adipocyte Hypertrophy

Increasing fat storage causes fat cells to enlarge. Pathological examination using HE (Fig. 4A, B**)** revealed that the OC group's adipocytes in the visceral white adipose tissue (vWAT) and subcutaneous white adipose tissue (sWAT) were not ordered cleanly because of an abundance of lipid droplets in the cytoplasm, which were typically big and had big inner diameter fat vacuoles. However, the inner diameter of the vacuoles was smaller and the adipocytes in the C and VC groups were grouped neatly and tightly (Fig. 4C). The GA treated groups showed a steady decrease in adiposity in the vWAT and sWAT as compared to the OC group, which more closely resembled findings in the C group.

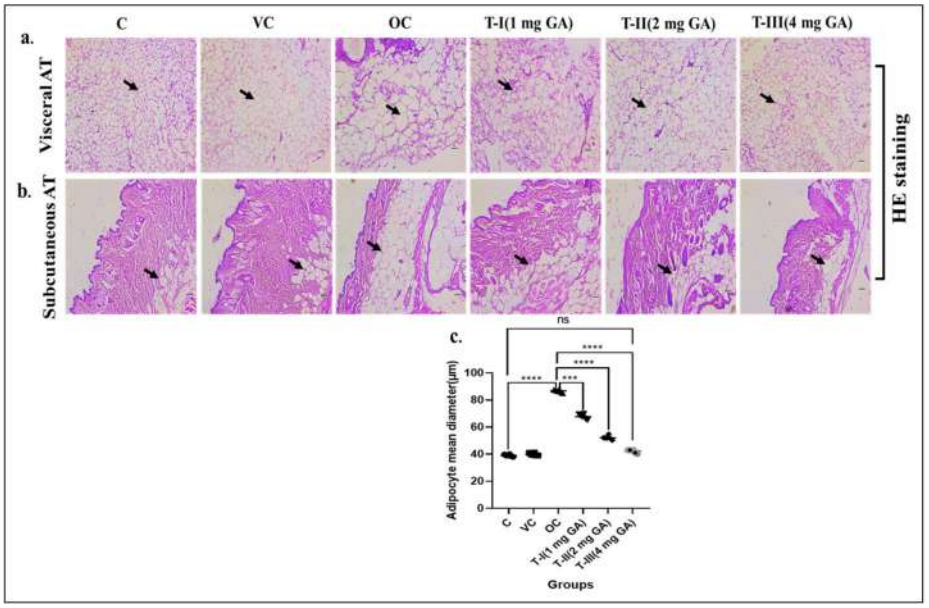


Fig. 4 Effect of GA supplementation on adipose tissue in obese mice. Groups are segregated as normal chow feeding, control group (C); normal chow feeding + olive oil, vehicle control group (VC), HFD feeding, obese control group (OC); HFD + 1 mg GA/day (T-I group); HFD + 2 mg GA/ day (T-II group); HFD + 4 mg GA/ day (T-III group). Mass of sWAT and vWAT sections were stained with hematoxylin and eosin (HE) staining (**a**, **b**). (**c**) Mean adipocyte size diameter. The results for all groups ($n = 6$) are presented as mean ± SEM. Statistical analysis was performed using one-way ANOVA accompanied by Tukey's multiple comparison test. ****$p < 0.0001$, ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$. In the staining part arrows (→) represent the fat cell size (visceral AT) and fat accumulation layer (subcutaneous AT) in several experimental groups. The scale bar correspondence to 100 μm

Gene Expression of Adipogenic Markers

An essential regulatory organ, WAT releases a variety of chemicals known as adipokines that regulate a number of biological functions, such as vascular metabolism (*adiponectin*), and body weight (*leptin*), regulating energy metabolism (*AMPK*), glucose metabolism (*GLUT 4*), insulin sensitivity (*insulin*), fatty acid oxidation (*CPT-1*, *LPL*, *PPAR-α*), adipocyte differentiation (*PPAR-γ*, *SREBP-1c*) and increased secretion of inflammatory cytokines (*TNF-α*, *FAS*), side by side reduced production of anti-inflammatory cytokines, including *IL-10*, *IL-1ra* among others. *Leptin*, *adiponectin* and *AMPK* mRNA in AT was incredibly increased by 1.71-fold,1.10- fold and 1.87- fold in the mice (T-III group) treated with 4 mg/kg body weight/day of GA (Fig. 5d, b, c**)** along with HFD. In this study, HFD induced obese group was increased *SREBP-1c*, *FAS* and *PPAR-γ* expression by 2.23-fold, 3.36-fold and 1.75-fold individually, whereas GA treated group decreased *SREBP-1 C*, *FAS* and *PPAR-γ* expression by 2.29-fold, 3.31-fold and 1.68-fold respectively (Fig. 5e, g, l**)**. *LPL* & *CPT-1* expression was gradually increased in GA treated groups by 1.37-2.25-fold, and 1.06-1.28-fold individually (Fig. 5h, i**)**, on the other hand *GLUT 4* and insulin expression have elevated 1.66-fold and 1.81-fold in T-III group contrasted with HFD induced OC group (Fig. 5f, j**)**. Furthermore, following intervention with GA, the T-III group's mRNA expression of related anti-inflammatory markers, such as *IL-10 and IL-1ra*, increased 1.76 and 1.40 times, respectively (Fig. 5n, o**)**. Although GA treatment dramatically decreased the expression up to 1.12–1.44 times in treated groups (T-I, T-II, and T-III), the pro-inflammatory marker *TNF-α* level was found to be higher in the OC group than in the C group (Fig. 5m**)**.

Protein Analysis in AT

The metabolism of dietary fats and cholesterol is strongly associated with obesity [25]. The control of the utilisation of fat is significantly influenced by AMPK. In Fig. 6, GA substitution significantly activated AMPK phosphorylation and increased PPAR-γ protein expressions in HFD induced obese mice by 1.17-fold, whereas AMPK protein expression levels were upregulated by 1.11-fold and PPAR-γ protein levels were downregulated by 1.15-fold after GA administration (Fig. 6a, e). Similarly, administration with GA markedly elevated the level of expression of Ob(H-5) by 1.12-fold gradually (Fig. 6f). However, in the OC group, CEBP-α, SREBP-1c and FAS protein expression increased, and natural fatty acid production in fat tissues was enhanced. The expression of CEBP-α, SREBP-1c and FAS GA-treated mice was markedly downregulated by 1.11- fold, 1.15-fold, and 1.19-fold (Fig. 6b, c, d). These findings demonstrate that GA treatment can limit de novo fatty acid synthesis and markedly enhance mitochondrial lipid transport and glucose utilisation in fat cells.

Discussion

Numerous in vivo studies have established the credibility of n-9 GA's anti-obesity action. However, it is well established that co-administration of n-3 PUFAs produced an anti-obesity impact by decreasing the formation of fat in animal trials employing obese animals fed HFD [26, 27]. Furthermore, our earlier research discovered that *P. paradiseus* FO was an essential mediator of lipometabolic dysregulation in the liver and AT in HFD-fed mouse

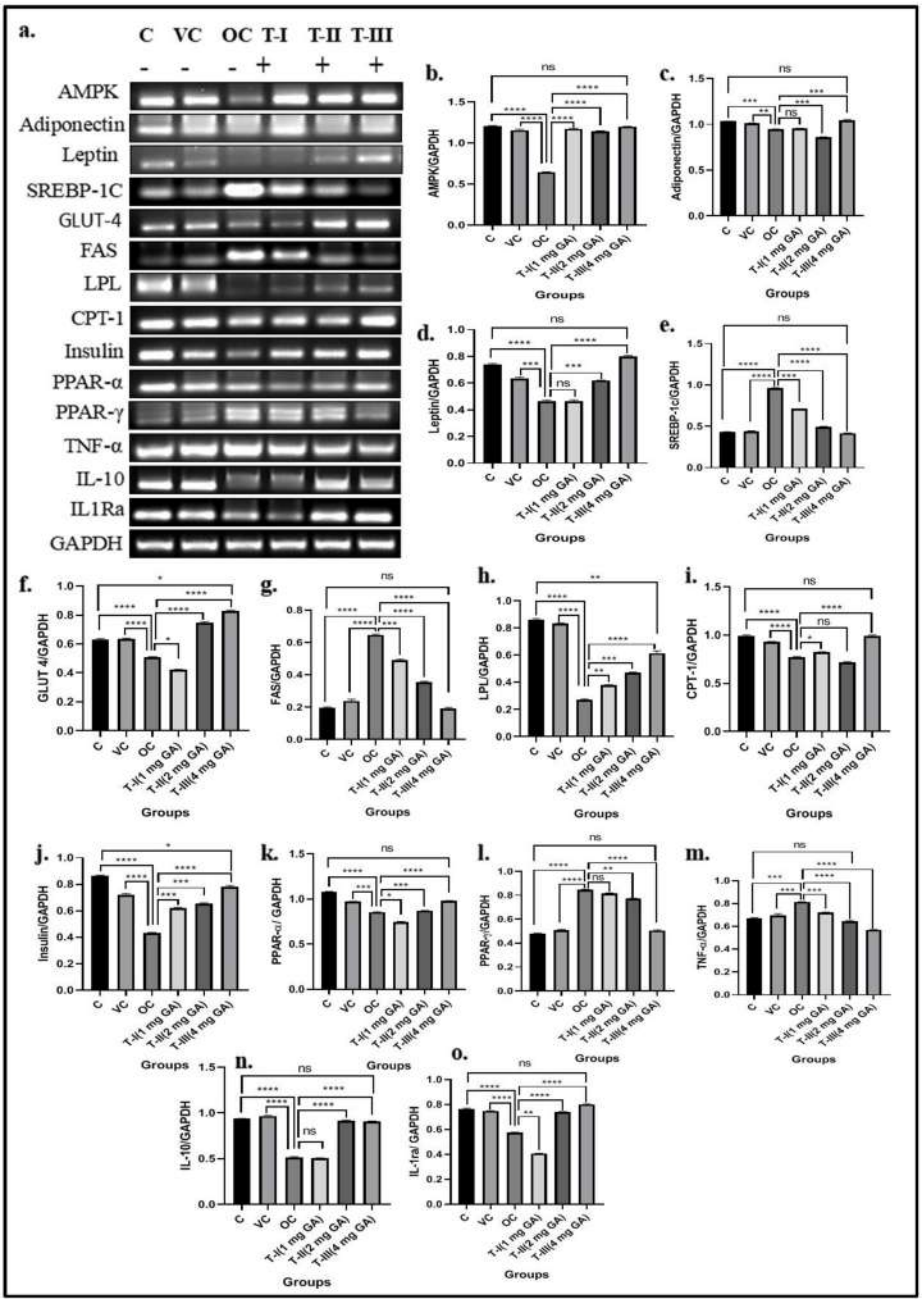


Fig. 5 Impact of GA supplementation on obesity and inflammatory-related adipocytokines using semi-q-PCR (**a**) Relative gene expression level of (**b**) *AMPK*; (**c**) *Adiponectin*; (**d**) *Leptin*; (**e**) *SREBP-1c*; (**f**) *GLUT 4*; (**g**) *FAS*; (**h**) *LPL*; (**i**) *CPT-1*; (**j**) *insulin*; (**k**) *PPAR-α*; (**l**) *PPAR-γ*; (**m**) *TNF-α*; (**n**) *IL-10* and (**o**) *IL-1ra* in AT of male BALB/c obese mice in different experimental groups ($n=6$) were adjusted to control gene expression level (*GAPDH*; b-o). The results for all groups ($n=6$) are presented as mean ± SEM. Statistical analysis was performed using one-way ANOVA accompanied by Tukey's multiple comparison test. ****$p<0.0001$, ***$p<0.001$, **$p<0.01$, *$p<0.05$

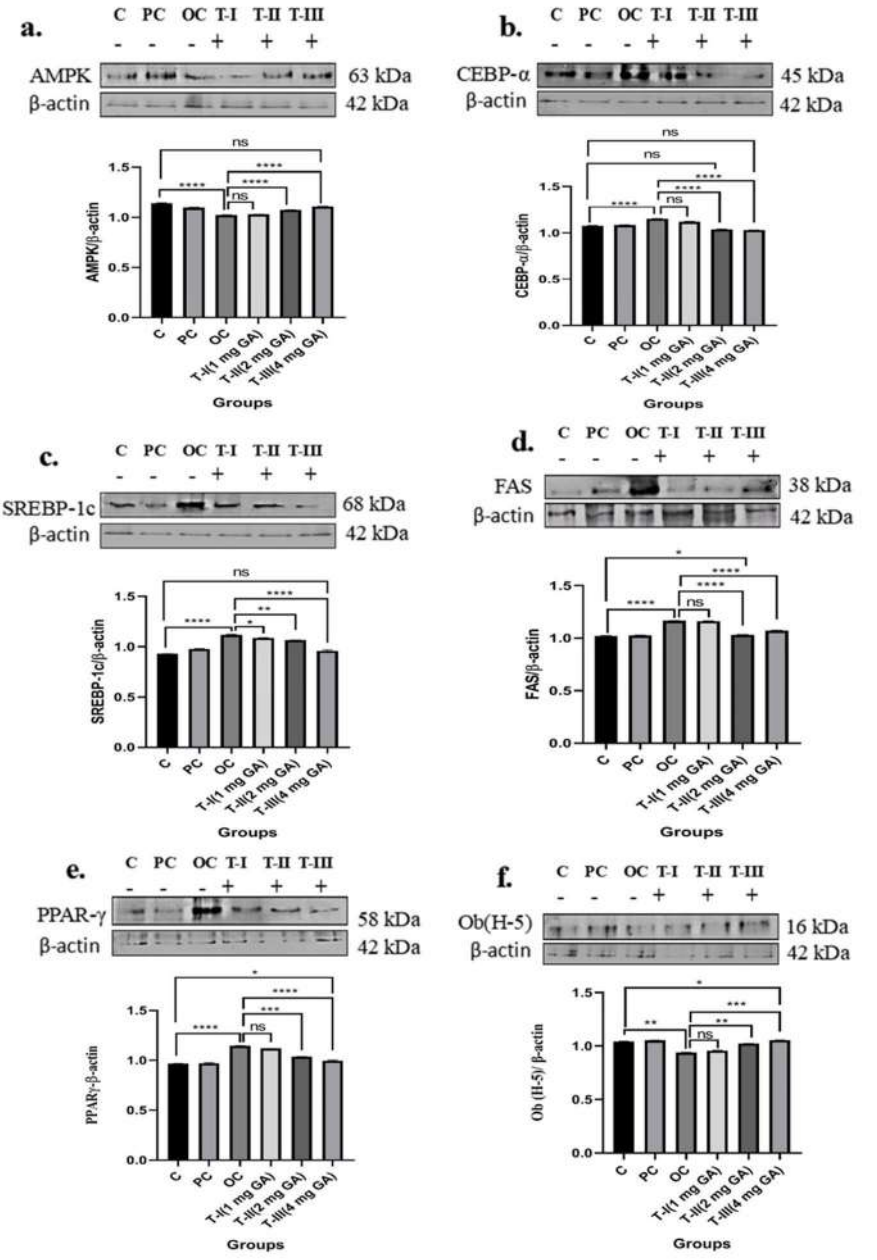


Fig. 6 Efficacy of GA intervention on protein in the WAT of nutritionally obese mice. Groups are segregated as normal chow feeding, control group (C); normal chow feeding + olive oil, vehicle control group (VC), HFD feeding, obese control group (OC); HFD + 1 mg GA/day (T-I group); HFD + 2 mg GA/ day (T-II group); HFD + 4 mg GA/ day (T-III group). β-actin was used in order to normalise the results. (**a**) AMPK; (**b**) CEBP-α; (**c**) SREBP-1c; (**d**) FAS; (**e**) PPAR-γ; (**f**) Ob (H-5). The results for all groups ($n = 6$) are presented as mean ± SEM. Statistical analysis was performed using one-way ANOVA accompanied by Tukey's multiple comparison test. ****$p < 0.0001$, ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$

groups [20]. In this study, we fed the mice a HFD to create a model of physiologically obese animals. In this study, we treated mice with commercial GA (T-I group:1 mg, T-II group: 2 mg and T-III group: 4 mg GA/kg body weight/ day) to investigate its anti-obesity function, and following dosing, the mice's daily biological state was favourable. The mice that received GA treatment had considerably decrease body weight than the animals who did not get GA treatment. Mice in the GA group consumed slightly less food during dose therapy than mice in the HFD group, however the variation was not statistically significant. Effective variation was observed in the peripheral organs (WAT, liver, kidney) among the different experimental treatment groups. By preventing fat cell growth and expansion, GA decreased body fat deposition and weight maintenance throughout the progression of obesity in BALB/c mice **(**Fig. 1**).** Saturated fatty acids are typically regarded as a risk factor for dyslipidaemia, characterised by abnormal lipid levels (e.g., cholesterol and/or fat) in the blood, frequently leading to hyperlipidaemia, it is linked to a higher chance of developing several cardiovascular conditions [28]. Obesity causes chronic inflammation of hypertrophied adipocytes in AT, which disrupts insulin signalling and causes insulin resistance [29]. In our study, GA markedly lower plasma glucose **(**Fig. 2a**)** and higher *insulin* levels, although the parameters did not differ in OC and T-I group. Following results explored that GA treatment can corrected the hyper insulinemic status in male BALB/C mice **(**Fig. 5j**)**. Consuming a high-fat meal caused weight progress, primarily in the form of retroperitoneal fat, whereas FO and its composition reversed these changes and also decreased circulating cholesterol and epididymal fat [22]. Here, we investigated the effects of GA on build up of lipids in the livers of BALB/C mice fed a HFD evaluated with HFD + GA treated experimental groups. Our findings demonstrated that the GA-administered group (mostly the T-III group) had considerably lower liver TC, TG, LDL, and VLDL levels, suggesting that GA could reduce the incidence of lipotoxicity. The efficacy of hyperglycemia and elevated oxidative stress may be the cause of excess urea and uric acid, which are indicators of impaired kidney function **(**Fig. 2b, c, e, f**)**. After the administration of GA, there was significant decrease in serum urea and uric acid level (T-III group) indicates protective effect of GA on kidney (Fig. 2h, i). The current study reveals elevated levels of SGOT and SGPT, indicating histoarchitecture abnormalities that may have resulted from oxidative stress and tissue ischaemia related to this enzyme's leakage into the blood pool, while in our study the GA-treated groups exhibit decreased levels of SGOT and SGPT **(**Fig. 2j, k**).** Present experiment that utilized BALB/c mice receiving HFD, elevated buildup of hepatic lipids in OC group was seen after 8 weeks of HFD intake, whereas no liver alteration noted in C and PC group. Since GA leads to corrected hepatic injury mostly in HFD + GA treated group (T-III: 4 mg/kg body weight/day). The majority of cases of hepatitis are brought on by a viral infection; however, hepatitis can also be brought on by a persistent restriction of bile flow brought on by lipid accumulation. Through the activation of many signalling pathways in hepatic parenchymal cells, studies have shown that MUFAs can aid in preventing oxidative stress, endoplasmic reticulum stress, glucose resistance, inflammatory processes, and mitochondrial breakdown [18]. In our present study, the histological examination revealed that GA had an inhibitory effect on the buildup of lipids through the liver **(**Fig. 3**)**. Using HE staining, we conducted pathological section investigation on both sWAT and vWAT. The size of the lipid droplets in the T-I, T-II, and T-III groups was considerably decreased by GA treatment **(**Fig. 4**)**. In the current study the expression of *AMPK*, *adiponectin*, *leptin*, *SREBP-1c*, *GLUT 4*, *FAS*, *LPL*, *CPT-1*, *Insulin*, *PPAR-α*, *PPAR-γ*, *IL-6*, *TNF-α*, *IL-10* and *IL-1ra*, in AT were directly regulated by

HFD. Additionally, we described that treatment with different doses of GA (specially T-III group treated with 4 mg/kg body weight/day) was able to recover the expression of these genes **(**Fig. 5**)**. According to certain research, n-9 fatty acids may increase leptin sensitivity, which makes the body more sensitive to the *leptin* signal and may result in a more reduced caloric intake and appetite [30]. In the current study, mice given a HFD and treated (T-III) with GA may have higher *leptin* levels because it may help control fat storage and result in a more balanced metabolic state, which increases the production of *leptin* due to an increase in AT mass **(**Fig. 5d**)**.

One of the main antidiabetics and antiatherogenic adipokines is *adiponectin*, a hormone released by adipocytes. Obesity, type 2 diabetes and insulin resistance are all associated in lower levels of plasma *adiponectin*. *Adiponectin's* insulin-sensitizing action appears to be mediated by *AMPK* and *PPAR-α* activation, which inhibits gluconeogenesis and stimulates fatty acid oxidation [31]. Following the above statement our study showed a reduction of *adiponectin* level in OC group which corrected after the treatment of GA (T-III: 4 mg/kg body weight/day) **(**Fig. 5c**)**. Side by side several studies explored that *AMPK* activation increases lipid oxidation through *PPAR-α* [30]. Here, GA was shown to upregulated the expression of *AMPK* level gradually in treated groups **(**Fig. 5b**)**. Although positive energy balance is the primary cause of fat deposition, GA was known to have a significant effect on lipid synthesis. Numerous studies stated that, HFD increases the expression of lipogenic enzymes including *FAS* and acetyl-CoA carboxylase via promoting *SREBP1-c* activation through the induction of hyperinsulinemia [32, 33]. Considering intrahepatic lipid generation rises when it surpasses hepatic lipid production, the mice in experiment most likely ingested a HFD that stimulated lipid formation in the liver, which in turn led to elevated TG synthesis and storage in AT [34]. On the other hand, GA has been demonstrated to promote lipolysis by raising hepatic *PPAR-α* and decrease *FAS* by reducing *SREBP-1c* expression, hence blocking TG production and fat accumulation in treated mouse groups **(**Fig. 5e, g, k**)**. Fatty acids and eicosanoids induce Peroxisome proliferator activated receptors (*PPARs*) are members of a steroid-thyroid hormone receptor superfamily that regulates gene expression in our bodies. Numerous hypothesised biological processes suggested that MUFA could be an edible *PPAR-γ* ligand and that its interaction with *PPAR-γ* could have a therapeutic effect in metabolic disorders [35]. However, WATs from GA-treated animal groups showed a markedly lower mRNA level of *PPAR-γ*, a master transcription element that maintains terminal adipocyte differentiation (Fig. 5l).

GA treatment, suggested that the mechanism of weight reduction may be the modulation of glyceride and cholesterol metabolism by the *AMPK* mechanism. In skeletal muscle, the heart, and adipocytes, *GLUT 4* is the main insulin-regulated glucose transporter. The pathophysiology of insulin resistance and type 2 diabetes begin with the downregulation of *GLUT 4* in the adipocytes of obese or T2D rats and humans [36]. Moreover, GA have been alleviating obesity-induced insulin resistance and hepatic steatosis in treated groups through inducing *GLUT 4* pathway (Fig. 5f). Correspondently, we observed at GA treated group (T-III group) **(**Fig. 5i**)** markedly elevated mRNA levels of the gene encoding *CPT-1*, a rate-limiting enzyme involved in the β-oxidation pathway that transfers fatty acyl coenzymes to the inner mitochondrial membrane [29]. Since *LPL* is the primary enzyme responsible for chylomicron and VLDL-TG lipolysis in AT capillary endothelial cells, it is believed to be a key regulator for adipocyte fatty acid intake from triglyceride-rich lipoprotein [37]. In the HFD + GA-fed mouse groups, the current research observed reduced adipose *LPL* and

fat weight simultaneously with enhanced *LPL* and lowered plasma triacylglycerol, indicating a transition from fat accumulation to oxidation (Fig. 5h). Furthermore, we assessed the expression of the pro-inflammatory marker *TNF-α* in vWAT because chronic inflammation is linked to obesity and may aggravate insulin resistance [38]. The level of inflammation raised after HFD feeding, whereas GA alleviated inflammation in the treated groups (T-I, T-II, T-III) gradually **(**Fig. 5m**)**. On the other hand, anti-inflammatory markers such as *IL-10*, *IL-1ra* genes are responsible for reduce inflammation by supressing the macrophage activity. Here, the expressions of *IL-10* and *IL-1ra* were raised in GA treated groups rather than OC group **(**Fig. 5n, o**)**.

AMPK is a serine/threonine protein kinase that is extensively distributed and plays a crucial role in WAT's energy metabolism and catabolism [38, 39]. In addition, SREBP-1c and CEBP-α are crucial transcription factors in adipogenesis that can be regulated by AMPK [40]. GA reduces endogenous cholesterol production by downregulating the expression of the proteins SREBP-1c and CEBP-α **(**Fig. 6c, d**)**. In addition to being abundantly produced by the liver and adipocytes, PPAR-γ also improves glucose tolerance by lowering adipose TG lipolysis [41]. We found that GA could activate AMPK and supress FAS and PPAR-γ expression, leading to inhibition of hepatic lipogenesis in mice model **(**Fig. 6b, e, f**)**. Taken together, these results demonstrated the potential antiobesity effect of GA for the first time. In summary, all of these experiments showed that in order to improve insulin resistance and control glucose homeostasis, GA triggered the AMPK pathway to combat obesity (Supplementary Fig. 1).

Conclusions

LC n-9 fatty acids have a wide range of pharmacological properties that make them a promising choice for treating a variety of diseases. With an emphasis on their effects on AT and adipocyte metabolism and function, we provide an overview of the current understanding of the advantageous effects of n-9, GA on obesity and related illnesses in this paper. Combined, our interventional trial employing HFD-induced obesity in mice revealed that treatment with GA generated large alterations in the metabolic process of obese mice and demonstrated considerable protective properties through improve fatty acid oxidation and subsequent fatty acid breakdown. The considerable decreased in body weight and diminished fat buildup in the liver in response to GA could be attributed to its high biological activity. Therefore, in addition to lifestyle adjustment, GA may be a useful adjunct to managing obesity.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12010-025-05488-7.

Acknowledgements The authors are thankful to the Director, Dr. Pradip Ghosh, Midnapore City College for provide all essential supports to carry out this work. The current research study presented in this manuscript is a part of the PhD research work of the first author.

Author Contributions All authors contributed to the study conception and design. Conceptualization, methodology, investigation, data curation, writing—original draft preparation by Riya Kar and Shrabani Pradhan. Review writing and editing, software analysis performed by Pipika Das. Validation and visualization of study analysed by Titli Panchali. Formal analysis performed by Ananya Dutta and Monisha Phoujdar. All authors read and approved the final manuscript.

Funding The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Data Availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics Approval The animal data have been reported in adherence with the Animal Research: Reporting of In vivo Experiments (ARRIVE) guidelines. This study was approved by the Institutional Ethics Committee, Midnapore City College (2256/PO/RE/S/23/CCSE, MCC/IAEC-SP/10/23 − 003) and all experimental protocol management was in accordance with the guidelines for the care and use of laboratory animals.

References

1. Cao, H. (2014). Adipocytokines in obesity and metabolic disease. *Journal of Endocrinology, 220*(2), T47-T59. https://doi.org/10.1530/JOE-13-0339
2. Goel, S., Chaudhary, M., Khedkar, R., & Khandelwal, S. (2018). The effect of long chain N-3 PUFA on obese Individuals-Desk review. *Nutri Food Sci Int J*, *7*(3), 555714. https://doi.org/10.19080/NFSIJ.2018.07.555714
3. Kar, R., Panchali, T., Das, P., Dutta, A., Phoujdar, M., & Pradhan, S. (2024). Overview of the therapeutic efficacy of marine fish oil in managing obesity and associated metabolic disorders. *Physiological Reports*, *12*(19), e70019. https://doi.org/10.14814/phy2.70019
4. Carpentier, Y. A., Portois, L., & Malaisse, W. J. (2006). n−3 fatty acids and the metabolic syndrome. *The American Journal of Clinical Nutrition*, *83*(6), 1499S–1504S. https://doi.org/10.1093/ajcn/83.6
5. Ellulu, M. S., Khaza'ai, H., Abed, Y., Rahmat, A., Ismail, P., & Ranneh, Y. (2015). Role of fish oil in human health and possible mechanism to reduce the inflammation. *Inflammopharmacology*, *23*(2–3), 79–89. https://doi.org/10.1007/s10787-015-0228-1
6. Pedersen, M. H., Mølgaard, C., Hellgren, L. I., Matthiessen, J., Holst, J. J., & Lauritzen, L. (2011). The effect of dietary fish oil in addition to lifestyle counselling on lipid oxidation and body composition in slightly overweight teenage boys. *Journal of Nutrition and Metabolism*, *2011(1)*, 348368. https://doi.org/10.1155/2011/348368
7. Tsuboyama-Kasaoka, N., Sano, K., Shozawa, C., Osaka, T., & Ezaki, O. (2008). Studies of UCP2 Transgenic and knockout mice reveal that liver UCP2 is not essential for the antiobesity effects of fish oil. *American Journal of Physiology-Endocrinology and Metabolism*, *294*(3), E600–E606. https://doi.org/10.1152/ajpendo.00551.2007
8. Ramalho, L., Da Jornada, M. N., Antunes, L. D. C., & Hidalgo, M. P. (2017). Metabolic disturbances due to a high-fat diet in a non-insulin-resistant animal model. *Nutrition & Diabetes*, *7*(3), e245–e245. https://doi.org/10.1038/nutd.2016.47
9. Rokling-Andersen, M. H., Rustan, A. C., Wensaas, A. J., Kaalhus, O., Wergedahl, H.,Røst, T. H., … Drevon, C. A. (2009). Marine n-3 fatty acids promote size reduction of visceral adipose depots, without altering body weight and composition, in male Wistar rats fed a high-fat diet. *British Journal of Nutrition*, 102(7), 995–1006. https://doi.org/10.1017/S0007114509353210
10. Hwang, S. M., Kim, Y. U., Kim, J. K., Chun, Y. S., Kwon, Y. S., Ku, S. K., & Song, C. H. (2022). Preventive and therapeutic effects of Krill oil on obesity and obesity-Induced metabolic syndromes in High-Fat Diet-Fed mice. *Marine Drugs*, *20*(8), 483. https://doi.org/10.3390/md20080483
11. Janovska, P., Flachs, P., Kazdova, L., & Kopecky, J. J. P. R. (2013). Anti-obesity effect of n-3 polyunsaturated fatty acids in mice fed high-fat diet is independent of cold-induced thermogenesis. *Physiological Research*, *62*(2), 153–161. https://doi.org/10.33549/physiolres.932464
12. Liu, S. H., Chiu, C. Y., Wang, L. P., & Chiang, M. T. (2019). Omega-3 fatty acids-enriched fish oil activates AMPK/PGC-1α signaling and prevents obesity-related skeletal muscle wasting. *Marine Drugs*, *17*(6), 380. https://doi.org/10.3390/md17060380

13. Wakutsu, M., Tsunoda, N., Mochi, Y., Numajiri, M., Shiba, S., Muraki, E., & Kasono, K. (2012). Improvement in the high-fat diet-induced dyslipidemia and adiponectin levels by fish oil feeding combined with food restriction in obese KKAy mice. *Bioscience Biotechnology and Biochemistry*, *76*(5), 1011–1014. https://doi.org/10.1271/bbb.110743
14. Pérez-Echarri, N., Pérez-Matute, P., Marcos-Gómez, B., Martínez, J. A., & Moreno-Aliaga, M. J. (2008). Effects of eicosapentaenoic acid Ethyl ester on visfatin and Apelin in lean and overweight (cafeteria diet-fed) rats. *British Journal of Nutrition*, *101*(7), 1059–1067. https://doi.org/10.1017/S0007114508048307
15. Haugaard, S. B., Madsbad, S., Høy, C. E., & Vaag, A. (2006). Dietary intervention increases n-3 long-chain polyunsaturated fatty acids in skeletal muscle membrane phospholipids of obese subjects. Implications for insulin sensitivity. *Clinical Endocrinology*, *64*(2), 169–178. https://doi.org/10.1111/j.1365-2265.2006.02444.x
16. D'Angelo, S., Motti, M. L., & Meccariello, R. (2020). ω-3 and ω-6 polyunsaturated fatty acids, obesity and cancer. *Nutrients*, *12*(9), 2751. https://doi.org/10.3390/nu1209275
17. Fan, G., Li, Y., Liu, Y., Suo, X., Jia, Y., & Yang, X. (2022). Gondoic acid alleviates LPS-induced Kupffer cells inflammation by inhibiting ROS production and PKCθ/ERK/STAT3 signaling pathway. *International Immunopharmacology*, *111*, 109171. https://doi.org/10.1016/j.intimp.2022.109171
18. Johnson, M., & B C, A. (2014). C. (Ed.), Omega-3, omega-6 and omega-9 fatty acids: Implications for cardiovascular and other diseases. *J Glycomics Lipidomics* 4 123 2153–0637. https://doi.org/10.4172/2153-0637.1000123
19. Farag, M. A., & Gad, M. Z. (2022). Omega-9 fatty acids: Potential roles in inflammation and cancer management. *Journal of Genetic Engineering and Biotechnology*, *20*(1), 48. https://doi.org/10.1186/s43141-022-00329-0
20. Kar, R., Das, P., Panchali, T., Dutta, A., Phoujdar, M., Ghosh, K., & Pradhan, S. (2025). Anti-obese potentiality of marine Topse (Polynemus paradiseus) fish oil by inhibiting the expression of SREBP-1c & promoting β-oxidation of fat through upregulating PPAR-α. *Adipocyte*, *14*(1), 2524640. https://doi.org/10.1080/21623945.2025.2524640
21. Dasgupta, A., Bandyopadhyay, G. K., Ray, I., Bandyopadhyay, K., Chowdhury, N., De, R. K., & Mahata, S. K. (2020). Catestatin improves insulin sensitivity by attenuating Endoplasmic reticulum stress: In vivo and in Silico validation. *Computational and Structural Biotechnology Journal*, *18*, 464–481. https://doi.org/10.1016/j.csbj.2020.02.005
22. Esteves, G. P., Manca, C. S., Veida-Silva, H. P., Ovidio, P. P., Holland, H., Matsuo,F. S., … Jordao, A. A. (2020). A fish oil-rich diet leads to lower adiposity and serum triglycerides but increases liver lipid peroxidation in fructose-fed rats. *Egyptian Liver Journal*, 10(1), 35. https://doi.org/10.1186/s43066-020-00045-9
23. Koellensperger, E., Niesen, W., Kolbenschlag, J., Gramley, F., Germann, G., & Leimer, U. (2013). Human adipose tissue derived stem cells promote liver regeneration in a rat model of toxic injury. *Stem Cells International*, 2013(1), 534263. https://doi.org/10.1155/2013/534263
24. da Cunha, R. D., Cruz, M. M., de Farias, T. M., da Silva, V. S., de Jesus Simão, J., Telles, M. M., & Alonso-Vale, M. I. C. (2020). Fish oil reverses metabolic syndrome, adipocyte dysfunction, and altered adipokines secretion triggered by high-fat diet-induced obesity. *Physiological Reports*, *8*(4), e14380. https://doi.org/10.14814/phy2.14380
25. Zhou, M., Huang, J., Zhou, J., Zhi, C., Bai, Y., Che, Q., & Su, Z. (2023). Anti-obesity effect and mechanism of chitooligosaccharides were revealed based on lipidomics in diet-induced obese mice. *Molecules*, *28*(14), 5595. https://doi.org/10.3390/molecules28145595
26. Ruzickova, J., Rossmeisl, M., Prazak, T., Flachs, P., Sponarova, J., Vecka, M., & Kopecky, J. (2004). Omega-3 PUFA of marine origin limit diet-induced obesity in mice by reducing cellularity of adipose tissue. *Lipids*, *39*(12), 1177–1185. https://doi.org/10.1007/s11745-004-1345-9
27. Pérez-Matute, P., Pérez-Echarri, N., Martínez, J. A., Marti, A., & Moreno-Aliaga, M. J. (2007). Eicosapentaenoic acid actions on adiposity and insulin resistance in control and high-fat-fed rats: Role of apoptosis, adiponectin and tumour necrosis factor-α. *British Journal of Nutrition*, *97*(2), 389–398. https://doi.org/10.1017/S0007114507207627
28. Go, R. E., Hwang, K. A., Park, G. T., Lee, H. M., Lee, G. A., Kim, C. W., & Choi, K. C. (2016). Effects of microalgal polyunsaturated fatty acid oil on body weight and lipid accumulation in the liver of C57BL/6 mice fed a high fat diet. *Journal of Biomedical Research*, *30*(3), 234. https://doi.org/10.7555/JBR.30.2016K0004
29. Yang, Z. H., Miyahara, H., Mori, T., Doisaki, N., & Hatanaka, A. (2011). Beneficial effects of dietary fish-oil-derived monounsaturated fatty acids on metabolic syndrome risk factors and insulin resistance in mice. *Journal of Agricultural and Food Chemistry*, *59*(13), 7482–7489. https://doi.org/10.1021/jf201496h

30. Gonçalves-de-Albuquerque, C. F., Medeiros-de-Moraes, I. M., Oliveira, F. M. D. J., Burth, P., Bozza, P. T., Castro Faria, M. V., & Castro-Faria-Neto, H. C. D. (2016). Omega-9 oleic acid induces fatty acid oxidation and decreases organ dysfunction and mortality in experimental sepsis. *PLoS One*, *11*(4), e0153607. https://doi.org/10.1371/journal.pone.0153607
31. Yamauchi, T., Nio, Y., Maki, T., Kobayashi, M., Takazawa, T., Iwabu, M., … Kadowaki,T. (2007). Targeted disruption of AdipoR1 and AdipoR2 causes abrogation of adiponectin binding and metabolic actions. Nature medicine, 13(3), 332–339. https://doi.org/10.1038/nm1557.
32. Miyazaki, M., Dobrzyn, A., Man, W. C., Chu, K., Sampath, H., Kim, H. J., & Ntambi, J. M. (2004). Stearoyl-CoA desaturase 1 gene expression is necessary for fructose-mediated induction of lipogenic gene expression by sterol regulatory element-binding protein-1c-dependent and-independent mechanisms. *Journal of Biological Chemistry*, *279*(24), 25164–25171. https://doi.org/10.1074/jbc.M402781200
33. Ferre, P., & Foufelle, F. (2007). SREBP-1c transcription factor and lipid homeostasis: Clinical perspective. *Hormone Research*, *68*(2), 72–82. https://doi.org/10.1159/000100426
34. Ter Horst, K. W., & Serlie, M. J. (2017). Fructose consumption, lipogenesis, and non-alcoholic fatty liver disease. *Nutrients*, *9*(9), 981. https://doi.org/10.3390/nu9090981
35. Kumar, J. S., & Sharma, B. (2022). A review on neuropharmacological role of erucic acid: An omega-9 fatty acid from edible oils. *Nutritional Neuroscience*, *25*(5), 1041–1055. https://doi.org/10.1080/1028415X.2020.1831262
36. Moraes-Vieira, P. M., Saghatelian, A., & Kahn, B. B. (2016). GLUT4 expression in adipocytes regulates de Novo lipogenesis and levels of a novel class of lipids with antidiabetic and anti-inflammatory effects. *Diabetes*, *65*(7), 1808–1815. https://doi.org/10.2337/db16-0221
37. Martínez-Fernández, L., Laiglesia, L. M., Huerta, A. E., Martínez, J. A., & Moreno-Aliaga, M. J. (2015). Omega-3 fatty acids and adipose tissue function in obesity and metabolic syndrome. *Prostaglandins & Other Lipid Mediators*, *121*, 24–41. https://doi.org/10.1016/j.prostaglandins.2015.07.003
38. Xu, M., Xue, H., Kong, L., Lin, L., & Zheng, G. (2023). Smilax China L. polyphenols improves insulin resistance and obesity in high-fat diet-induced mice through IRS/AKT-AMPK and NF-κB signaling pathways. *Plant Foods for Human Nutrition*, *78*(2), 299–306. https://doi.org/10.1007/s11130-023-01052-y
39. Li, J., Wu, K., Zhong, Y., Kuang, J., Huang, N., Guo, X., & Sun, R. (2023). Si–Ni-SAN ameliorates obesity through AKT/AMPK/HSL pathway-mediated lipolysis: Network Pharmacology and experimental validation. *Journal of Ethnopharmacology*, *302*, 115892. https://doi.org/10.1016/j.jep.2022.115892
40. Herzig, S., & Shaw, R. J. (2018). AMPK: Guardian of metabolism and mitochondrial homeostasis. *Nature Reviews Molecular Cell Biology*, *19*(2), 121–135. https://doi.org/10.1038/nrm.2017.95
41. Hsu, W. H., Chen, T. H., Lee, B. H., Hsu, Y. W., & Pan, T. M. (2014). Monascin and Ankaflavin act as natural AMPK activators with PPARα agonist activity to down-regulate nonalcoholic steatohepatitis in high-fat diet-fed C57BL/6 mice. *Food and Chemical Toxicology*, *64*, 94–103. https://doi.org/10.1016/j.fct.2013.11.015

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.

Chapter XII: Conference Proceedings

Conference Proceedings

International

1. **Kar, R.,** Pradhan, S., 2025. Understanding the Molecular Mechanisms Leading Gondoic Acid's Anti-Obesity Effects via AMPK pathway and obesity related gene. Integration of Traditional Medicine on Modern Healthcare System held in Department of Pharmaceutical Science, Jharkhand Rai University. (**1st position**)

National

1. **Kar, R**., Pradhan, S., 2026. "Gondoic acid supresses adipocyte differentiation and lipogenesis-related protein expression in 3T-3L1 cells." 7th Annual National Conference "MCCCON 2026" on "Trends in Synthetic Biology and Sustainable Development" organized by Midnapore City College, West Midnapore, West Bengal**. (1st position)**
2. **Kar, R.,** Pradhan, S., 2025. "Gondoic acid reduces the obesity caused by a high-fat diet in mice via activating the AMPK-GLUT4 pathway and modifying the expression of important adipogenic genes". 57th Annual Conference of the Nutrition Society of India held by ICMR- National Institute of Nutrition, Hyderabad, Telangana, India.
3. **Kar, R**., Pradhan, S., 2025. "Gondoic acid attenuates high-fat diet induced obesity in mice by activating AMPK-GLUT4 pathway". 6th Annual National Conference "MCCCON 2025" on "Biotechnology for Healthier life" organized by Midnapore City College, West Midnapore, West Bengal**. (1st position)**
4. **Kar, R.,** Pradhan, S., 2024. "Examining the characteristics of marine topse (*Polynemus paradiseus*) fish oil and its potential effects". 5th Annual National Conference "MCCCON 2024" on "Functional Food as Dietary Intervention for Chronic Diseases" organized by Midnapore City College, West Midnapore, West Bengal**. (1st position)**
5. **Kar, R.,** Pradhan, S., 2023. "Characterization of marine topse (*Polynemus paradiseus*) fish oil and exploring its impact on high fat diet induced BALB/c mice model to combat obesity". 55th Annual Conference of the Nutrition Society of India held by ICMR-National Institute of Nutrition, Hyderabad, Telangana, India. **(NSI Prize for Best Presentation)**
6. **Kar, R.,** Pradhan, S., 2023. "Anti-obesity effect of marine topse (*Polynemus paradiseus*) fish oil on high fat diet induced BALB/c mice: a novel therapeutic

approach". Trends of Research in Health and Nutritional Sciences and Its Application held by Raja N. L. Khan Women's College, West Midnapore, West Bengal. **(1st position)**

7. **Kar, R**., Pradhan, S., 2022. "Extraction, isolation and characterization of *Polynemus paradiseus* fish oil and its antiobesity potentiality". 4th Annual National Conference "MCCCON 2023" on "Food Informatics: Opportunities and Challenges" organized by Midnapore City College, West Midnapore, West Bengal.

8. **Kar, R**., Pradhan, S., 2022. "Effect of fatty acids (s) extracted from marine topse (*Polynemus paradiseus)* fish oil on obesity and obesity related metabolic disorders". XXXIst Annual Conference of the Physiological Society of India (PHYSICON 2019) organized by Bankura Christian College, West Bengal.