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Original research article

Amelioration of obesity induction by a high-fat diet and related inflammation by Phasa fish (*Setipinna phasa*) oil in BALB/c mice

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Abstract
We have extracted and characterized Phasa fish (*Setipinna phasa*) oil for the first time to evaluate the anti-obesity and related anti-inflammatory effects on obese mice. Inbred male albino BALB/c mice were segregated into three categories: control (C), Obese control group (OC), and Phasa fish oil treated group (TX). To establish the potentiality of *Setipinna phasa* oil for its anti-obesity and anti-inflammatory properties, it was extracted and characterized using GC-MS method. To evaluate the anti-obesity effect, different parameters were considered, such as body weight, lipid composition, obesity, and obesity associated inflammation. The physicochemical characteristics of Phasa fish oil revealed that the oil quality was good because acid value, peroxide value, *p*-anisidine value, Totox value, refractive index, and saponification value were within the standard value range. The GC-MS study explored the presence of fatty acids beneficial to health such as Hexadec-9-enoic acid; Octadec-11-enoic acid; EPA, DHA, Methyl Linolenate, *etc*. The application of *Setipinna phasa* oil on the treated mice group acutely lowered body weight and serum lipid profile compared to the obese group. In connection with this, leptin, FAS, and pro-inflammatory cytokines TNF-α genes expression were downregulated in the treated group compared to the obese group. The Phasa oil treated group had an elevated expression of PPAR-α, adiponectin, LPL gene, and anti-inflammatory markers IL-10 and IL-1Ra compared to the obese group. This study suggests that Phasa fish oil, enriched with essential fatty acid, might be used as an anti-obesity and anti-inflammatory supplement.

Keywords: Adiponectin; Anti-inflammatory; Fatty acid; Leptin; Obesity; Phasa fish

Highlights:
- Phasa fish oil contains different Polyunsaturated Fatty Acids which may combat obesity.
- Phasa fish oil has lipid lowering activity which might minimize the risk of heart disease.
- Phasa fish oil downregulated the expression of inflammatory cytokines such as TNF-α, IL-6, which are associated with obesity-related inflammation.

Introduction

Nowadays, obesity has spread worldwide. It occurs due to an imbalance between energy intake and energy usage, creating an excess of calories that are stored in adipose tissues (Guyenet and Schwartz, 2012). According to WHO estimates, in 2016 over 1.9 billion persons globally (or 39%) were overweight, and over 650 million (or 13%) were obese. According to the Indian National Family Health Survey-4, the percentage of obese women and men between the ages of 15 and 49 increased from 13% to 21% over a 10-year period (from 2005 and 2006, to 2015 and 2016). For men in the same age range, the percentage increased from 9.3% to 19%. Data from numerous epidemiological studies have shown that obesity can be linked to diabetes, insulin resistance, liver disease, an increase of free fatty acids levels, Gastroesophageal Reflux Disease (GERD), high blood pressure, high cholesterol, and a few types of cancer (Blüher, 2013; Todoric et al., 2006). The excessive white adipose tissue is responsible for inflammation and hypertrophy, and has triggered many pro-inflammatory cytokine's secretion, such as TNF-α (Tumour Necrosis Factor-alpha), IL-6 (Interleukin-6), IL-1b (Interleukin 1b), leptin which promotes a low grade of infection in the body resulting in lower secretion of adiponectin, and anti-inflammatory cytokines such as IL-10 (Interleukin-10) and IL1Ra (Interleukin 1 Receptor antagonist) (Maury et al., 2007; McLaughlin et al., 2014). The key to controlling obesity is appetite control because it is relat-

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ed to the balance of energy. Different dietary approaches have been researched to reduce bodyweight and control excess body weight. The FDA has approved a few obesity drugs for long-term use such as orlistat, lorcaserin, phentermine-topiramate, but they have a lot of adverse effects such as steatorrhea, headaches, dizziness, dry mouth, nausea, and constipation. To avoid these complications, recent research has focused on the dietary specific nutrients for reducing obesity.

It has been reported that fish oil (tuna, sardine, mackerel, and tapra) could be used to prevent obesity (Pradhan et al., 2020). There is a mechanistic function of marine fish oil, which contains omega-3 (n-3) polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (20 : 5n-3, EPA) and docosahexaenoic acid (22 : 6n-3, DHA). These reduce the production of inflammatory cytokines and increase anti-inflammatory cytokines production (Bradley et al., 2008). It has also been suggested that monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) play a role in reducing lipid production and lipogenesis, while promoting lipid oxidation and thermogenesis.

Despite being a freshwater fish and going by the name "Phasa" (*Setipinna phasa*), Gangetic Hairfin Anchovy is thought to be able to tolerate some saline in the water. It can be found in the Orissa River and its estuaries, as well as in the Ganges. The fish is easily available, low cost, and popular in West Bengal and Orissa. To the best of our knowledge, no research has been conducted on *Setipinna phasa* oil. Therefore, our aim is to characterize *Setipinna phasa* oil for the first time and evaluate its anti-obesity and anti-inflammatory potentialities in mice.

Materials and methods

Chemicals

All chemicals were acquired from Merck, HiMedia in India, and Sigma Aldrich in the United States.

Extraction of fish oil

The AOCS's standard extraction procedure for fish oil was followed (AOCS and Firestone, 1994). First, 10 g of Phasa fish dust was mixed with hexane and isopropanol (3 : 2), and the mixture was agitated at 140 rpm for 4 h. Next, Soxhlet extraction took place for 12 h. After cooling, the leftover material was centrifuged for 20 min at 5,000 rpm to separate the solid and liquid phases. The supernatants were collected and filtered through Whatman No. 1 filter paper. The fish oil was collected for further investigation, and to evaporate the volatile compounds, the filtrate was retained in a rotary evaporator.

Physicochemical properties

The peroxide value, saponification value, acid value, P-anisidine value, totox value, refractive index, and iodine value of Phasa fish oil were determined according to the AOCS's recommended procedure (AOCS, 1997).

Fish oil characterization using a gas chromatography-mass spectrometer

Setipinna phasa oil was characterized using Thermo Fisher Scientific S.P.A., Milan, Italy, Trace 1300 series, S/N-717100576, GC ultra-gas chromatograph with mass spectrometer (Model: ISQ QD Single Quadrupole Mass Spectrometer). A TG-WAXMS column (30 m 0.25 mm ID 0.25 m film thickness) was used to achieve the separation with stationary phase and 5% of phenyl polysilphenylene siloxane was used. The oven temperature was initially set to 50 °C for 10 min, and the final temperature was 220 °C (5 °C/min) for 10 min. The carrier gas was helium at a flow rate of 1.5 ml/min. The MS was performed in the electron impact mode (EI) at 70 eV while maintaining a temperature of 250 °C. The detector setting was 40–600 D. GC-MS data was analysed using the National Institute of Standards and Technology's database (NIST). XCALIBUR software was used to detect molecular weight of the compounds.

Selection and animal care

The Institutional Ethics Committee at Vidyasagar University gave its approval to each of these experimental protocols (ICE/7-9/0-9/2016). Male, albino, inbred BALB/c mice that were 5 weeks old were acclimated for 1 week in a controlled environment (at 32 ± 2 °C, 50 ± 5% humidity, 12 h light/dark cycle). While adjusting, the mice received a standard meal (Hindustan Lever, Mumbai, India), and sterile water at their leisure. The mice were split into 3 groups (n = 5) and fed with a normal diet (C; 15% fat, 65% carbohydrate, and 20% protein) with sterile water. The OC group were fed with a high-fat diet (HFD) (60% fat from lard, 17.8% carbohydrate, and 22.2% protein) with water ad libitum (Pradhan et al., 2020). The Phasa fish oil treated group was supplemented with high-fat diet (TX) at a dosage of 12.5 mg/kg of the mouse's body weight, with unlimited access to water. In both the OC and TX groups, the mice were fed a high-fat diet for 12 weeks, and the TX group received Phasa fish oil through oral gavage with methylcellulose after 8 weeks for 4 weeks. The other groups (C and OC) only received methylcellulose (0.2 ml 0.5% methylcellulose) by forced feeding. Mice were cervical dislocated to end their lives after 12 weeks of the experiment. The orbital plexus was punctured to obtain blood samples, which were then centrifuged at 3000 rpm for 15 min at 4 °C, while the serum was kept at –20 °C for biochemical analysis. Livers were harvested and weighed. Adipose fat depots were harvested, weighed, and processed (as described below) for protein expression and gene expression analysis.

Somatic body weight index and analysis of food intake

The investigation continued for 12 weeks, and weight changes were monitored once a week in accordance with the protocol. The body's length was calculated using a vernier calliper from the distance between the nasal and anal (Central; Model no. 6420) of mice. The conventional method for calculating body mass index (BMI) was used, and the results were indicated as g/cm^2 (Ray et al., 2018). Food intake was recorded weekly.

Estimation of biochemical parameters

Serum lipid profile (such as total cholesterol, triglyceride, high density lipoprotein, low density lipoprotein, and very low-density lipoprotein in the serum) were ascertained by Enzopak kit (Reckon, India) according to the manufacturer's protocol. Serum glucose level was measured by GODPOD standard protocol using Autospan Glucose (93DP100-74, Surat, India), and total protein was estimated by Biuret method using Coral clinical system kit (1101201150, Goa, India).

Histological examinations

Subcutaneous, visceral WAT and liver were fixed with 10% formalin, embedded in paraffin wax, and sliced through a standard protocol. These sections were then stained with haematoxylin and eosin (Alturkistani et al., 2015) and Oil Red O (Gao et al., 2015), and observed under an optical microscope (Olympus, CX21iLED).

Adipose tissue isolation

After performing cervical dislocation, the mice were laid on their back. The central skin had been elevated near the genital organ and an incision was made with scissors. The skin was unfastened from the abdominal wall. Visceral fat and subcutaneous fat were isolated and weighed individually. Then the adipose tissues were kept in sterilized aluminium foil and stored in a mini liquid nitrogen tank. All the equipment (motor, pestle, spatula) used for isolation and extraction were stored in a mini liquid nitrogen tank. RNase-free screw cap tubes were pre-chilled in dry ice. The frozen samples were ground using a motor pestle until a fine powder was obtained. Samples were then processed for RNA and protein extraction (Tan et al., 2018).

Gene expression analysis

RNA extraction was conducted in accordance with the HiPura Total RNA Miniprep purification kit's instructions (Himedia, India). Total RNA (1 µg) was converted to cDNA according to the Hi-cDNA synthesis kit (Himedia, HiGenoMB, Maharashtra, India). Gene expression was evaluated by quantitative PCR (BioRad, China). The reaction conditions were 95 °C for 1 min, followed by 42 cycles of 95 °C for 30 s, 65 °C for 45 s, 72 °C for 2 min, and a final cycle of 72 °C for 10 min. To confirm the size of the fragment and the specificity of amplification, PCR results were tested on an agarose gel. The primers used were: adiponectin (5′ to 3′ sense: GTCAGTGGATCTGACGACACCAA; 5′- to 3′ anti-sense: ATGCCTGCCATCCAACCTG), Leptin (5′- to 3′ sense: CAAGCAGTGCCTATCCAGA; 5′ to 3′ anti-sense: AAGCCCAGGAATGAAGTCCA), TNF-α (5′ to 3′ sense: TTCTGTCTACTGAACTTCGGGGTGATCGGTTCC; 5′ to 3′ anti-sense: GTATGAGATAGCAAATCGGCTGACGGTGTGGG), IL-1Ra (GCAGCACAGGCTGGTGAATGAC; 5′ to 3′ anti-sense: TGCCCCCGTGGATGCCCAAG), PPAR-α (5′ to 3′ sense: CCTGAACATCGAGTGTCGAATAT; 5′ to 3′ anti-sense: GTTCTTCTTCTGAATCTTGCAGCT), FAS (5′ to 3′ sense: TGCTCCCAGCTGCAGGC; 5′ to 3′ anti-sense: GCCCGGTAGCTCTGGGTGTA), LPL (5′ to 3′ sense: CCACAGCAGCAAGACCTTC; 5′ to 3′ anti-sense: AGGGCGGCCACAAGTTTG) and GAPDH (5′ to 3′ sense: GGTGAAGGTCGGAGTCAACG; 5′ to 3′ anti-sense: GTGAAGACGCCAGTGGACTC). GAPDH was used as housekeeping.

Immunoblotting

Adipose tissue was homogenized and lysed with ice on RIPA buffer which contained Tris-HCL 50 mM, NaCl 150 mm, EDTA 5 mM, and 1% Triton-X100 and cocktail protease inhibitors (Himedia, India). The Lowry procedure was used to measure the protein content (Waterborg, 2009). Using gel loading, a 50 µg protein sample was placed in a well, boiled for 5 min, electrophoresed using SDS-PAGE, and then blotted onto nitrocellulose membrane (BioRad, China). The nitrocellulose membrane was blocked with 5% BSA for 1 hour at room temperature and then incubated with the primary antibody overnight at 4 °C. β-actin (Abgenex, India), IL1Ra, IL10 (Affinity, UK), PPAR-α, Ob (H-5) and FAS (Santa Cruz Biotechnology, Santa Cruz, CA, USA cat no: 398394, 393043) were used as the primary antibody in this study. The membrane was then treated for 1 h with an anti-mouse secondary antibody that was HRP-conjugated (Abgenex, India). Specific protein bands were ferreted out using DAB (Bass et al., 2017).

Real-Time Polymerase Chain Reactions (RT-PCR) from adipose tissue

Total RNA from adipose tissue was extracted from HiPurA Total RNA Miniprep purification kit (HiMedia). Thereafter it was converted to cDNA using cDNA synthesis kit (Himedia, HiGenoMB, Maharashtra, India), followed by the previous standard methodology. The expression of leptin, adiponectin, FAS, LPL, PPAR-α, and TNF-α genes were standardized against the GAPDH gene. All nucleotide sequences are the same as previously mentioned in q-PCR. Melting curve analyses were utilised to separate the particular PCR product from non-specific products and primer dimers. It was possible to differentiate between genuine DNA products and primer dimers because different DNA products melt at various temperatures. SYBR green was employed in RT-PCR (Agilent technologies, Singapore Pvt Ltd.) for quantification.

Statistics

The data was displayed as mean SEM, with $n = 5$. The statistical programme Graphpad Prism8, one-way and two-way ANOVA (Tukey's test) was used to examine the variations in the analytical outcomes. At the levels of 5% and 1% (*i.e.*, p 0.05 and p 0.001), significant variance was accepted.

Results

Physicochemical properties of Phasa fish oil

The acid and peroxide indexes evaluate the oil's quality. The peroxide value was 4.67 ± 0.67 mEq/kg, which is within the range of the maximum of 5 mEq/kg, established by the Codex. The acid number was 2.97 ± 0.48 mg KOH/g, falling within the National Agency of Sanitary Surveillance's limit of 4.0 mg KOH (Muniz et al., 2015). The refractive value was 0.73 ± 0.07, which was lower than the range of standard value (Ferreira et al., 2006). The saponification value was 166.88 ± 0.87 mg KOH/g, which implies that the oil comprised a large proportion of polyunsaturated fatty acids (Ferreira et al., 2006). Iodine number was 122.34 ± 4.22 mg, which was within the range of the National Agency of Sanitary Surveillance (Ferreira et al., 2006) (Table 1).

GC-MS chromatograms of Setipinna phasa *oil*

A total of four different types of monounsaturated fatty acids (MUFA) and seven polyunsaturated fatty ac-

Table 1. Assessment of the physicochemical characteristics of Phasa fish oil

Analysis	Results Mean ± SE
Peroxide value (mEq/kg)	4.67 ± 0.67
Saponification number (mg KOH/g)	166.88 ± 0.87
Acid number (mg KOH/g)	2.97 ± 0.48
P-anisidine value	12.25 ± 1.16
Totox value	22.31 ± 0.52
Refractive index	0.73 ± 0.07
Iodine value (mg)	122.34 ± 4.22

Note: Values are means ± SEM of triplicate determinations.

ids (PUFA) were marked out by GC-MS (Fig. 1 and Suppl. 1). The identified MUFAs were Hexadec-9-enoic acid; Octadec-11-enoic acid; Octadec-7-enoic acid; and Octadec-12-enoic acid. Other PUFAs were Octadeca-9,12-dienoic acid; (5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraenoic acid/ Methyl arachidonate; Methyl cis-7,10,13,16,19-docosapentaenoate; Docosa-4,7,10,13,16,19-hexaenoic acid (DHA); Icosa-5,8,11,14,17-pentaenoic acid/ Methyl cis-5,8,11,14,17-Eicosapentaenoate (EPA); (9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid/ Methyl linolenate; and Icosa-11,14,17-trienoic acid/ Methyl cis-11,14,17-Icosatrienoate (Fig. 1 and Suppl. 1).

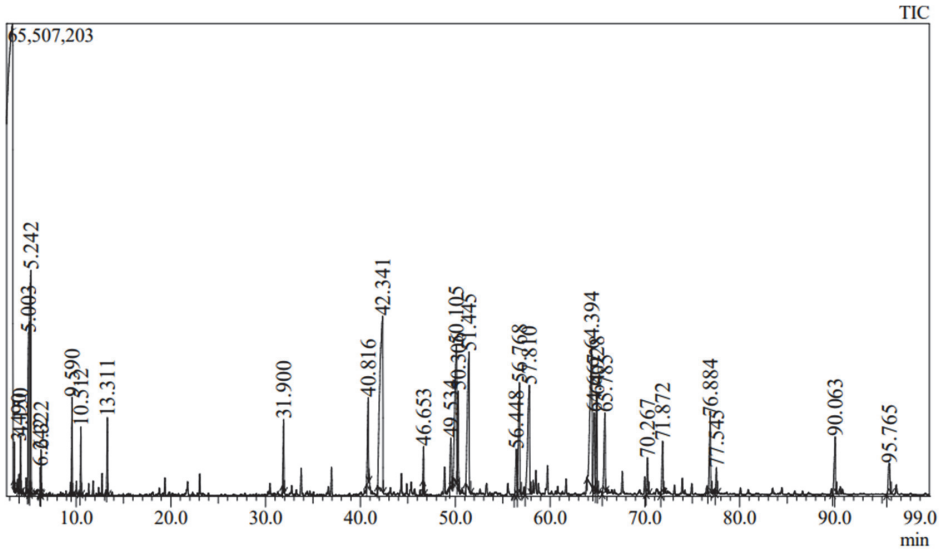

Fig. 1. Fish oil derived from Phasa fish was analysed using Gas Chromatography-Mass Spectrometry (GC-MS) chromatograms

Assessment of the body weight of dietary Phasa fish in high-fat diet induced obese BALB/c mice

We periodically measured the body weight and BMI of the experimental animals to determine how Phasa fish oil affected the obesity produced by a high-fat diet. The starting weights of all of the mice groups were almost the same (C, 15.97 ± 0.33 g; OC, 15.55 ± 0.31 g; TX, 16.08 ± 0.43 g). After 12 weeks of treatment, the mean body weights in C, OC, and TX were 32.74 ± 0.68 g, 42.73 ± 4.54 g, 27.33 ± 2.23 g, respectively (Fig. 2a). The mice in the control group consumed more food on average than the mice in the high-fat diet group, proving that the high-fat diet affected the appetite of mice. However, *Setipinna phasa* oil did not significantly impact the appetite of mice, as seen by the lack of a significant difference between the control group and the treatment group (Fig. 2b). The BMI was significantly elevated in the OC group (83.46%) and decreased in the TX group (48.07%) after the *Setipinna phasa* oil treatment compared to the control (Fig. 3a).

Evaluation of the effect of Setipinna phasa *oil on biochemical parameters in obese BALB/c mice*

The OC had greater lipid profiles than the C, with the exception of HDL cholesterol. However, significant decreases were observed in the serum total cholesterol (40.37% decrease), tryglycerides (55.64%), LDL (52.04%), and VLDL (55.64%) of TX compared to OC (Fig. 3b, 3c, 3e, and 3f), whereas, HDL cholesterol level was significantly increased (82%) after Phasa fish oil feeding (Fig. 3d). Serum glucose level was significantly

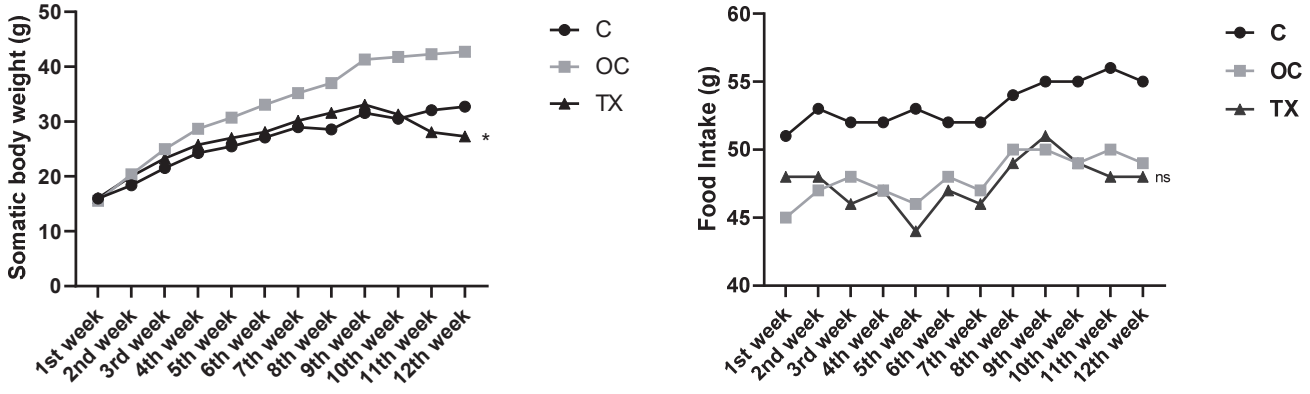

Fig. 2. Analysis of somatic body weight changes and average food intake in different experimental groups. Values are expressed as mean ± SEM (n = 5) followed by two-way ANOVA. Significant changes were observed OC vs. TX. * $p < 0.05$, ns – not significant.

increased in OC group but 27.93% lower in TX compared to OC (Fig. 3g). In comparison to the obese control group, there was a statistically insignificant drop in serum total proteins in the control group. The serum protein levels of the high-fat diet group and the *Setipinna phasa* oil treatment group did not significantly differ (Fig. 3h).

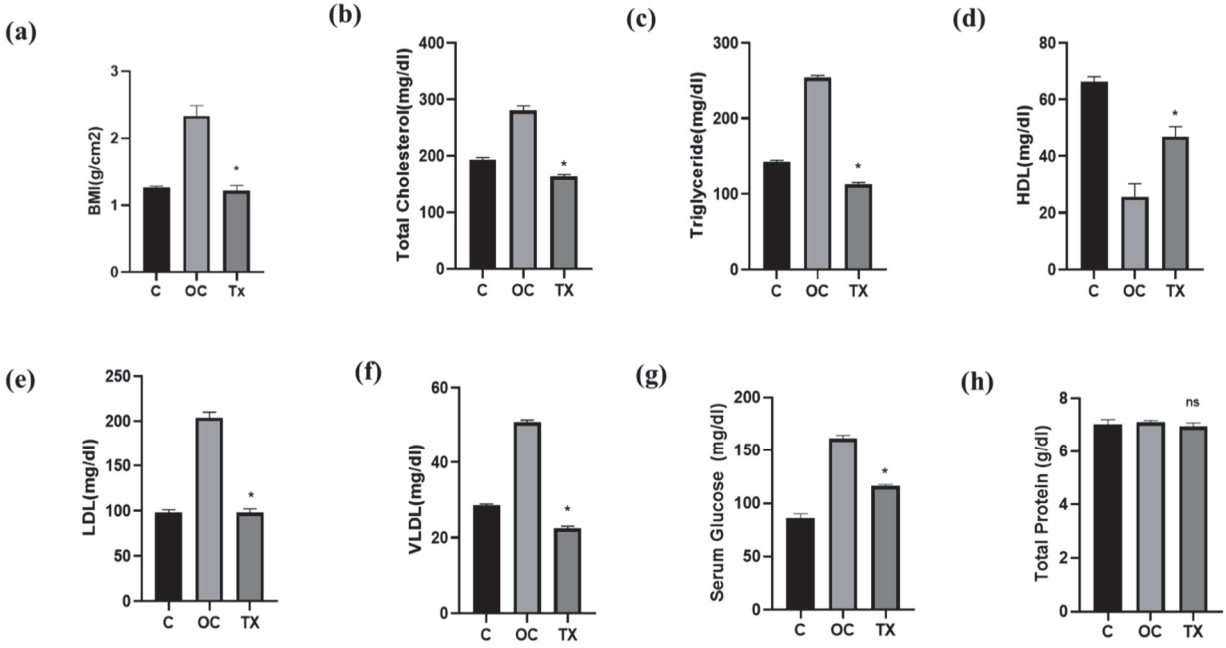

Fig. 3. Analysis of BMI (**a**) and total lipid profiles including total cholesterol (**b**), Triglycerides (**c**), HDL (**d**), LDL (**e**), VLDL (**f**), serum glucose (**g**), Total protein (**h**) in several experimental groups (n = 5). Values are expressed as mean ± SEM followed by ANOVA. Significant changes were observed OC vs. TX. * $p < 0.05$, ns – not significant.

Impact of Phasa fish oil on abdominal fat depots

To observe the effect of fat accumulation in the abdomen, we weighed the adipose tissue and liver. *Setipinna phasa* oil suppressed the weight of WAT and liver compared to the OC group (Table 2). We performed the HE staining of liver sections, subcutaneous white adipose tissue histology by HE staining, as well as Oil Red O and visceral white adipose tissue by Oil Red O staining. Compared to the control group, the OC group had more abdominal fat. Abdominal fat was reduced in the TX group following fish oil administration. Obesity is typically associated with ectopic fat accumulation in the liver. HFD induced progressively enlarged vacuoles in the liver, suggesting hepatic fat deposition, and after supplementation of *Setipinna phasa* oil can reduce hepatic lipid deposition (Fig. 4). Collectively, these results suggest that *Setipinna phasa* oil enriched with PUFA can significantly minimize HFD-associated hepatic steatosis.

Table 2. Weight of White Adipose Tissue (WAT) and liver

Groups	C	OC	TX
WAT (g)	0.61 ± 0.05	3.08 ± 0.18	1.43 ± 0.16
Liver (g)	1.18 ± 0.06	1.54 ± 0.12	1.26 ± 0.07

Note: Effect of High-Fat Diet and *Setipinna phasa* oil on WAT and liver. Control (C), high-fat diet-induced obese (OC), high-fat diet-induced obese experimented with *Setipinna phasa* oil (TX). Values are expressed as mean ± SEM.

Gene expression in adipose tissue

To investigate the reduced fat accumulation in adipose tissues, we have tested different obesity related genes such as leptin, FAS (Fatty Acid Synthase), PPAR-α, adiponectin, and LPL. The proinflamaatory marker, TNF-α, was found to be more increased in the OC group than C, whereas Phasa fish oil treatment significantly reduced TNF-α expression by 1.32-fold (Fig. 5g). The HFD diet obese group was increased leptin, and FAS expression by 1.43-fold and 1.28-fold individually, whereas, Phasa fish oil treated group decreased leptin and FAS expression 1.37-fold and 1.19-fold respectively in comparison to the obese group (Fig. 5b and 5e). We also measured peroxisome proliferator-activated receptor-α (PPAR-α), Interleukin 1 receptor antagonist (IL1Ra), adiponectin, and LPL (Lipoprotein Lipase) expression in adipose tissue. IL1Ra and adiponectin expression was increased in the Phasa fish oil treated group, 1.25-fold and 1.41-fold individually but PPAR-α and LPL expression have marginally elevated *i.e.*, 1.13-fold and 1.14-fold compared with the HFD obese group diet (Fig. 5h, 5d, 5c and 5f).

Protein expression in adipose tissue

The expression of PPAR-α, IL1Ra, and IL10 were decreased by 1.33-fold, 1.22-fold, and 1.17-fold in the obese group, respectively than control. Phasa fish oil treatment advanced the expression by 1.19-fold, 1.16-fold, and 1.18-fold, individually (Fig. 6f–6h) and degraded leptin and FAS expression by 1.1-fold and 1.17-fold (Fig. 6i and 6j).

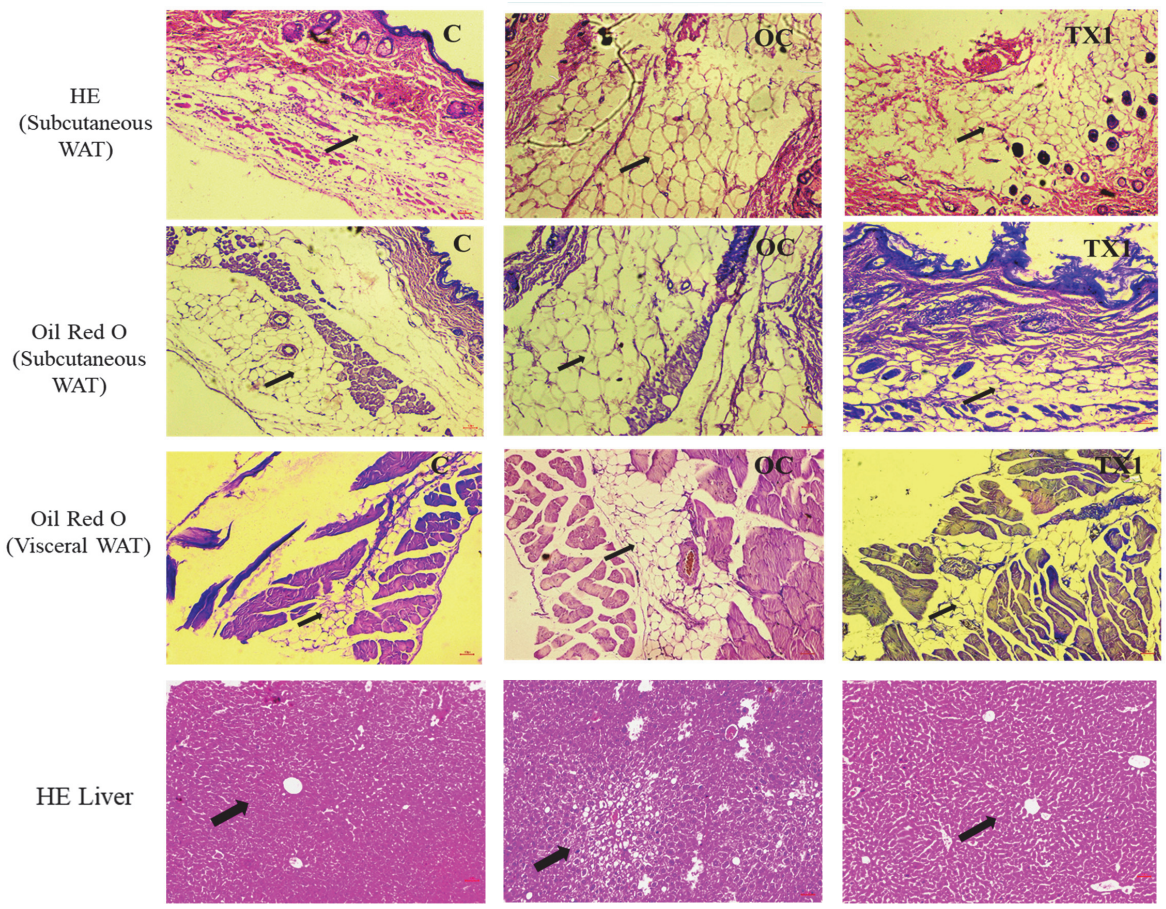

Fig. 4. The effect of Phasa fish oil on subcutaneous and Visceral WAT and liver histology. Control (C), high-fat diet-induced obese (OC), high-fat diet-induced obese experimented with *Setipinna phasa* oil (TX).

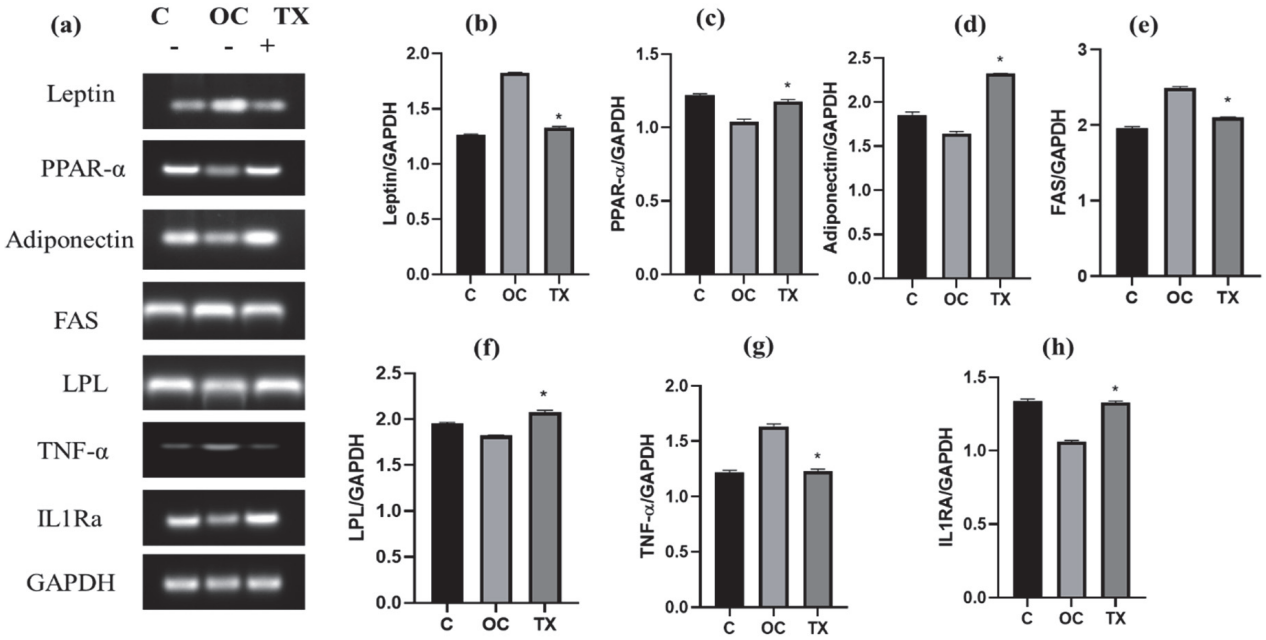

Fig. 5. Expression of obesity-related marker by semi q-PCR (**a**). Relative gene expression level of Leptin, PPAR-α, Adiponectin, FAS, LPL, TNF-α, and Il1Ra in adipose tissue of male mice in different experimental groups (n = 5) were adjusted to control gene expression level (GAPDH; **b–h**). Values are expressed as mean ± SEM, followed by ANOVA. Significant changes were observed in OC vs. TX. * $p < 0.05$.

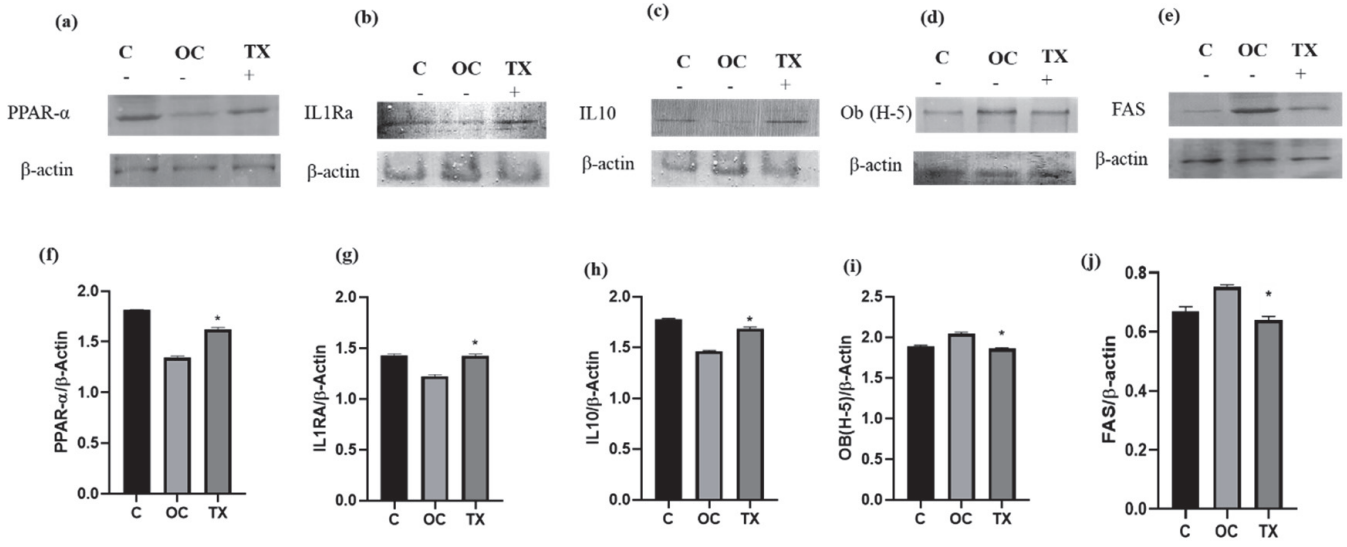

Fig. 6. Phasa fish oil induced expression obesity-related marker by western blot analysis (**a–e**). Relative protein expression level of PPAR-α, Il1Ra, IL10, OB (H-5), and FAS in adipose tissue of male mice in different experimental groups (n = 5) were adjusted to control protein expression level (β-actin; **f–j**). Values are expressed as mean ± SEM followed by ANOVA. Significant changes were observed OC vs. TX. * $p < 0.05$.

RT-PCR reaction in adipose tissue

For further verification of fat accumulation in adipose tissue, we have reiterated the obesity and inflammatory related gene expression by RT-PCR reaction. These results were represented on the basis of CQ value, where CQ value is inversely proportional to the gene expression. CQ values were low for leptin, FAS, and TNF-α in OC group. The HFD diet decreased leptin and FAS CQ value by 1.02-fold and 1.18-fold individually, whereas Phasa fish oil increased leptin and FAS CQ value 1.0-fold and 1.18-fold individually in comparison with the obese group (Fig. 7a and 7d). Peroxisome proliferator-activated receptor-α (PPAR-α), adiponectin, and LPL (Lipoprotein Lipase) CQ value in the OC group were increased by 1.03-fold, 1.15-fold, and 1.02-fold, compared to the C group, but in the TX group they were lowered by 1.14-fold, 1.07-fold, and 1.08-fold respectively in adipose tissue, compared with the OC group (Fig. 7b, 7f, 7e). TNF-α CQ value was lowered in the OC group in comparison to C by 1.07-fold, whereas it increased in the TX group by 1.07-fold (Fig. 7c).

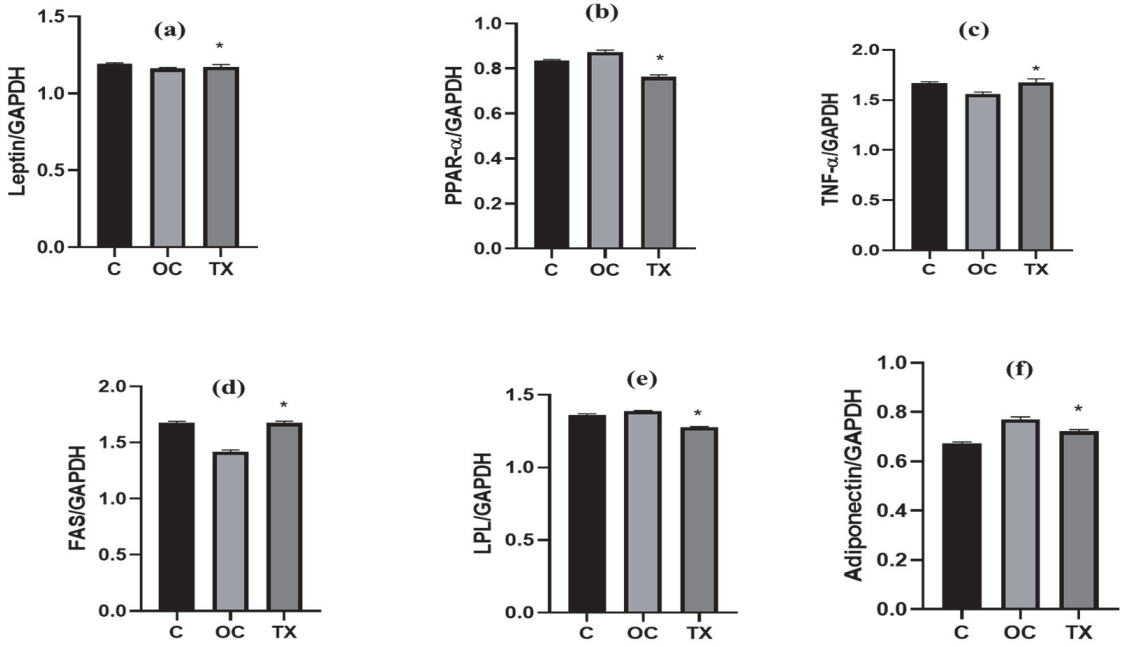

Fig. 7. Expression of obesity-related marker by RT-PCR. Relative gene expression level of Leptin, PPAR-α, TNF-α, FAS, LPL, and Adiponectin in adipose tissue of male mice in different experimental groups (n = 5) were adjusted to control gene expression level (GAPDH; **a–f**). Values are expressed as mean ± SEM followed by ANOVA. * $p < 0.05$.

Discussion

Though the Phasa fish (*Setipinna phasa*) is very popular in West Bengal, India, the fish has not been the subject of significant scientific attention so far, considering the different health benefits of fish oil as suggested earlier (Calder, 2017). In this research, we elicited and specified the *Setipinna phasa* oil and examined its effect on obesity and related inflammation. Physicochemical parameters of fish oil from Phasa were compared to data from the standard oil quality control (Muniz et al., 2015) (Table 1). The lower acid number (2.97 ± 0.48) of *Setipinna phasa* oil proved its good stability, and this result was comparable to that of earlier research (Pradhan et al., 2020) on *Opisthopterus tardoore* oil. The increased saponification number in the Phasa fish oil explained the large proportion of medium-chain fatty acids. The saponification number (a procedure that converts neutral fat in an alkaline environment into glycerol and fatty acids) was 166.88 ± 0.87 mg KOH/g. The quantity of peroxides found in oil is a sign of rancidity and primary oxidation. The peroxide value for *Setipinna phasa* oil was slightly lower than the standard value of 5 meq/kg, indicating the oil's good quality for preservation. The refractive index of the *Setipinna phasa* oil was 0.73, which is lower than that of the standard value for oils (Adeniyi and Bawa, 2006). The iodine number measures the number of reactive double bonds in oil. The iodine value was 122.34 ± 4.22, which is within the standard value range, and signals the presence of unsaturated fatty acid. The totox value was 22.31 ± 0.52, which indicated the oxidation state of oil (Pereiro de Abreu et al., 2010). In addition, GC-MS data suggests that Phasa fish oil is rich with different monounsaturated fatty acids, which might exhibit health beneficial effects such as anti-inflammation, anti-diabetes, and anti-obesity, and can improve digestive health (Suppl. 1).

After supplementing the obese mice with the *Setipinna phasa* oil, we observed significant reductions in body weight, weight of adipose tissue and liver, without affecting daily food intake. Considering this, we suspect that the primary causes of weight loss may be related to metabolism and breakdown, rather than changes in hunger. Changes in BMI were similar to the changes in somatic body weight index (Fig. 3a). Similarly, the skin epidermal fat and total lipid profile in TX mice were significantly decreased compared to the OC group (Fig. 3b–f and Fig. 4). Generally, a high fat diet is typically linked to an increased risk of dyslipidemia, hyperglycemia, *etc.* de Sá et al. (2016) reported that the supplementation of omega-3 fatty acid present in marine fish oil reduces adiposity along with hyperglycemia. In the current study, we confirmed that a diet supplemented with *Setipinna phasa* oil can cut down the serum glucose level compared to the OC group (Fig. 3g). This can be caused by the presence of various fatty acids such as Methyl linolenate, Methyl arachidonate, DHA, EPA, *etc.* (Fig. 1, GC-MS, and Suppl. 1), which might enhance fatty acid β-oxidation and prevent lipid synthesis, and eventually reduce adipocyte cells, and thereby control obesity (de Sá et al., 2016). In the subcutaneous WAT and visceral WAT layer of OC, lipid is deposited due to the aggregation of triglyceride (Vázquez-Vela et al., 2008), but after *Setipinna phasa* oil treatment, triglyceride deposition decreased, as shown in the subcutaneous and visceral part. Hypercholesterolemia results from a diet rich in cholesterol. Because of the lipid imbalance, free fatty acids are released into the serum and converted to triglycerides in the liver. This causes lipolysis. Since the liver is the first organ to process ingested cholesterol, and is thus the major target of the lipoxidative damage, it was also examined in this study. The OC group, which had a high density of lipid accumulation, fatty hepatocyte degeneration, indicated steatohepatitis due to the high accumulation of cholesterol. Mice treated with *Setipinna phasa* oil, the hepatocytes of those treated mice were organized radially around the centrilobular vein, indicating normal cellular morphology (Fig. 4) (Carrera et al., 2023).

Setipinna phasa fish oil treatment increased the mRNA and protein expression level of PPAR-α by 1.13-fold and 1.19-fold, individually (Fig. 5c and Fig. 6f). Moreover, the adiponectin and LPL expressions were individually increased by 1.41-fold and 1.14-fold in TX group (Fig. 5d and 5f). This might be due to the presence of unsaturated fatty acids (Fig. 1, Suppl. 1) which influence adiponectin circulation to control obesity (Gammelmark et al., 2012). Moreover, the elevated expression of LPL in TX might help in the hydrolysis of triglyceride, which may protect the individual from excessive weight gain (Wang and Eckel, 2009). On the other hand, the lower level of adiponectin in obese condition advances the risk of central obesity due to abnormal glucose homeostasis and lipid metabolism (Kassi et al., 2010). In addition, an elevated expression of PPAR-α in TX group might stimulate β-oxidation and decrease tissue lipid content, resulting in the prevention of lipid accumulation as suggested earlier. PPAR-α may also play crucial roles in the clearance of apolipoproteins for triglycerides and cholesterol, which could help to prevent different cardiovascular disease. PPAR-α is expressed in monocytes/macrophages, T lymphocytes, endothelial cells, and vascular smooth muscle cells (VSMCs). Several studies have looked at the anti-inflammatory capabilities of PPAR-α through reducing the production of several cytokines and proteins involved in inflammation, monocyte activation, VSMC proliferation, and other inflammatory processes (Blaschke et al., 2006). Leptin is produced from white adipose tissue and binds to the receptors in the brain. In this way, it sends signals to the brain to regulate food consumption and energy usage (Izquierdo et al., 2019). mRNA and protein expression of leptin were decreased after Phasa fish oil treatment (TX, Fig. 5b and Fig. 6i), which may be correlated with the inhibition of fat accumulation and adipocyte proliferation in TX group mice by disrupting the leptin signalling – as suggested by a previous study (Izquierdo et al., 2019). Moreover, the mRNA and protein expression level of FAS was overexpressed (1.28-fold and 1.17-fold) in adipose tissue in the OC group due to the active lipogenesis. However, *Setipinna phasa* oil downregulated lipogenesis in mice, as evidenced in Fig. 5e and Fig. 6j, which is comparable with the findings of a previous study (Veras et al., 2021) on fish and chia oil.

Generally, adipocyte hypertrophy is associated with an elevated expression of chronic inflammation-related genes. Proinflammatory M1 macrophages create crown-like structures surrounding decomposing adipocytes in the obese WAT, which adds to the low-grade inflammation imposed by obesity. The major source of TNF and interleukin-6 (IL-6) in the obese WAT is the increased number of M1 macrophages. TNF is a cytokine that macrophages create in response to endotoxemia, inflammation, and malignancy that lowers lipoprotein lipase activity (Kern et al., 2018). In this study, we have studied different anti-inflammatory and pro-inflammatory genes and proteins expressions such as IL1Ra, IL-10, and TNF-α. *Setipinna phasa* oil treatment increased the mRNA and protein expression level of IL1Ra by 1.25-fold and 1.16-fold (Fig. 5h and Fig. 6g), which suggest anti-inflammatory activity. It also downregulated TNF-α expression by 1.32-fold (Fig. 5g). IL-10 expression was increased by 1.18-fold in TX group (Fig. 6h), which might activate anti-inflammatory signalling pathways

through its interaction with the IL-10 receptor and thus exert anti-inflammatory effects (Lumeng et al., 2007). In obese condition, M1 macrophages and M2 macrophages are activated to play a crucial role in the prevention of inflammatory activity (Lumeng et al., 2007). M2 macrophages release different anti-inflammatory chemokine such as IL-10, IL1Ra, *etc.* This IL1Ra bind to IL-1 receptors and block pro-inflammatory signalling (Volarevic et al., 2010). For further justification and establishment of our hypothesis, we have done RT-PCR for the same obesity-related markers. Fig. 7 shows that the obesity-related markers were upregulated in the OC group compared to the C group, but after the application of Phasa fish oil FAS and leptin, TNF-α markers were downregulated, and PPAR-α, LPL, and adiponectin were increased compared to the OC group.

Conclusion

This study confirmed that the physicochemical characteristics of Phasa fish oil were acceptable and contained different types of MUFAs and PUFAs that are beneficial to health. Treatment with *Setipinna phasa* oil might lower BMI, total lipid profile, and fat deposition in HFD-induced obese mice and reduce the expression of obesity-associated inflammatory markers via activation of fatty acid β-oxidation and lipolysis. To determine the precise bioactive fatty acids responsible for anti-obesity and associated anti-inflammation, a dose and duration dependent mechanistic research is required.

Author contributions

SP: Conceptualization, Methodology, Data curation, Formal analysis, Writing – original draft, Writing – review and editing. *TP:* Conceptualization, Methodology, Writing and editing. *KG:* Project administration, Writing – review and editing. *SM, KCM:* GC-MS analysis. *AD, AK, & PD:* assembled the samples and carried out the experiments. All authors have reviewed, edited, and approved the final manuscript.

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Ethical aspects and conflict of interest

The authors have no conflict of interest to declare.

References

Adeniyi O, Bawa A (2006). Mackerel (*Scomber scrombrus*) oil extraction and evaluation as raw materials for industrial utilization. Leonardo J Sci 5(8): 33–42.

Alturkistani HA, Tashkandi FM, Mohammedsaleh ZM (2015). Histological Stains: A Literature Review and Case Study. Glob J Health Sci 8(3): 72–79. DOI: 10.5539/gjhs.v8n3p72.

AOCS – American Oil Chemists' Society (1997). Official and Recommended Practices of the AOCS. 7th Ed., AOCS Press Publication, Champaign.

AOCS – American Oil Chemists' Society, Firestone D (1994). Official methods and recommended practices of the American Oil Chemists' Society: AOCS press.

Bass, JJ, Wilkinson DJ, Rankin D, Phillips BE, Szewczyk NJ, Smith K, et al. (2017). An overview of technical considerations for Western blotting applications to physiological research. Scand J Med Sci Sports 27(1): 4–25. DOI: 10.1111/sms.12702.

Biswas P, Datta C, Rathi P, Bhattacharjee A (2022). Fatty acids and their lipid mediators in the induction of cellular apoptosis in cancer cells. Prostaglandins Other Lipid Mediat 160: 106637. DOI: 10.1016/j.prostaglandins.2022.106637.

Blaschke F, Takata Y, Caglayan E, Law RE, Hsueh WA (2006). Obesity, peroxisome proliferator-activated receptor, and atherosclerosis in type 2 diabetes. Arterioscler Thromb Vasc Biol 26(1): 28–40. DOI: 10.1161/01.ATV.0000191663.12164.77.

Blüher M (2013). Adipose tissue dysfunction contributes to obesity related metabolic diseases. Best Pract Res Clin Endocrinol Metab 27(2): 163–177. DOI: 10.1016/j.beem.2013.02.005.

Bradley RL, Fisher FM, Maratos-Flier E (2008). Dietary Fatty Acids Differentially Regulate Production of TNF-α and IL-10 by Murine 3T3-L1 Adipocytes. Obesity 16(5): 938–944. DOI: 10.1038/oby.2008.39.

Calder PC (2017). Omega-3 fatty acids and inflammatory processes: from molecules to man. Biochem Soc Trans 45(5): 1105–1115. DOI: 10.1042/BST20160474.

Carrera I, Corzo L, Naidoo V, Martínez-Iglesias O, Cacabelos R (2023). Cardiovascular and lipid-lowering effects of a marine lipoprotein extract in a high-fat diet-induced obesity mouse model. Int J Med Sci 20(3): 292–306. DOI: 10.7150/ijms.80727.

Carvalho EB, Melo IL, Mancini-Filho J (2010). Chemical and physiological aspects of isomers of conjugated fatty acids. Food Sci Technol 30(2): 295–307. DOI: 10.1590/S0101-20612010000200002.

de Sá RDCC, Crisma AR, Cruz MM, Martins AR, Masi LN, do Amaral CL, et al. (2016). Fish oil prevents changes induced by a high-fat diet on metabolism and adipokine secretion in mice subcutaneous and visceral adipocytes. J Physiol 594(21): 6301–6317. DOI: 10.1113/JP272541.

Duan H, Song W, Zhao J, Yan W (2023). Polyunsaturated Fatty Acids (PUFAs): Sources, Digestion, Absorption, Application and Their Potential Adjunctive Effects on Visual Fatigue. Nutrients 15(11): 2633. DOI: 10.3390/nu15112633.

Farag MA, Gad MZ (2022). Omega-9 fatty acids: Potential roles in inflammation and cancer management. J Genet Eng Biotechnol 20(1): 48. DOI: 10.1186/s43141-022-00329-0.

Ferreira ES, Silveira CS, Lucien VG, Amaral AS (2006). Physico-chemical characterization of almond cake and fatty acid composition of the majority of the crude oil-nut Brazil (*Bertholletia excelsa* H.B.K.). Food Nutr 17(2): 203–208.

Gammelmark A, Madsen T, Varming K, Lundbye-Christensen S, Schmidt EB (2012). Low-dose fish oil supplementation increases serum adiponectin without affecting inflammatory markers in overweight subjects. Nutr Res 32(1): 15–23. DOI: 10.1016/j.nutres.2011.12.007.

Gao M, Ma Y, Liu D (2015). High-fat diet-induced adiposity, adipose inflammation, hepatic steatosis and hyperinsulinemia in outbred CD-1 mice. PloS One 10(3): e0119784. DOI: 10.1371/journal.pone.0119784.

Gashaw A, Getasetegn M (2018). Chemistry and health impacts of trans fatty acids. JChem Technol Metall 53(2): 159–169.

Guyenet SJ, Schwartz MW (2012). Regulation of food intake, energy balance, and body fat mass: implications for the pathogenesis and treatment of obesity. J Clin Endocrinol Metab 97(3): 745–755. DOI: 10.1210/jc.2011-2525.

IIfPSI IC (2017). National family health survey (NFHS-4), 2015–16: india. Mumbai: International Institute for Population Sciences.

Izquierdo AG, Crujeiras AB, Casanueva FF, Carreira MC (2019). Leptin, obesity, and leptin resistance: where are we 25 years later? Nutrients 11(11): 2704. DOI: 10.3390/nu11112704.

Kassi E, Dalamaga M, Hroussalas G, Kazanis K, Merantzi G, Zachari A, et al. (2010). Adipocyte factors, high-sensitive C-reactive protein levels and lipoxidative stress products in overweight postmenopausal women with normal and impaired OGTT. Maturitas 67(1): 72–77. DOI: 10.1016/j.maturitas.2010.05.004.

Kern L, Mittenbühler MJ, Vesting AJ, Ostermann AL, Wunderlich CM, Wunderlich FT (2018). Obesity-induced TNFα and IL-6 signaling: the missing link between obesity and inflammation – driven liver and colorectal cancers. Cancers 11(1): 24. DOI: 10.3390/cancers11010024.

Khan SU, Lone AN, Khan MS, Virani SS, Blumenthal RS, Nasir K, et al. (2021). Effect of omega-3 fatty acids on cardiovascular outcomes: A Systematic Review And Meta-Analysis. EClinicalMedicine 38: 100997. DOI: 10.1016/j.eclinm.2021.100997.

Lumeng CN, Bodzin JL, Saltiel AR (2007). Obesity induces a phenotypic switch in adipose tissue macrophage polarization. J Clin Invest 117(1): 175–184. DOI: 10.1172/JCI29881.

Maulu S, Nawanzi K, Abdel-Tawwab M, Khalil HS (2021). Fish nutritional value as an approach to children's nutrition. Front Nutr 8: 780844. DOI: 10.3389/fnut.2021.780844.

Maury E, Ehala-Aleksejev K, Guiot Y, Detry R, Vandenhooft A, Brichard SM (2007). Adipokines oversecreted by omental adipose tissue in human obesity. Am J Physiol Endocrinol Metab 293(3): E656–E665. DOI: 10.1152/ajpendo.00127.2007.

McLaughlin T, Liu LF, Lamendola C, Shen L, Morton J, Rivas H, et al. (2014). T-cell profile in adipose tissue is associated with insulin resistance and systemic inflammation in humans. Arterioscler Thromb Vasc Biol 34(12): 2637–2643. DOI: 10.1161/ATVBAHA.114.304636.

Muniz MAP, Dos Santos MNF, da Costa CEF, Morais L, Lamarão MLN, Ribeiro-Costa RM, Silva-Júnior JOC (2015). Physicochemical characterization, fatty acid composition, and thermal analysis of *Bertholletia excelsa* HBK oil. Pharmacogn Mag 11(41): 147. DOI: 10.4103/0973-1296.149730.

Organization (2000). World Health WHO factsheet on obesity and overweight: World Health Organization fact sheet.

Pereiro de Abreu DA, Paseiro Losada P, Maroto J, Cruz JM (2010). Evaluation of the effectiveness of a new active packaging film containing natural antioxidants (from barley husks) that retard lipid damage in frozen Atlantic salmon (*Salmo salar* L.). Food Res Int 43(5): 1277–1282. DOI: 10.1016/J.FOODRES.2010.03.019.

Pradhan S, Panchali T, Paul B, Khatun A, Rao Jarapala S, Mondal KC, et al. (2020). Anti-obesity potentiality of Tapra fish (*Opisthopterus tardoore*) oil. J Food Biochem 44(11): e13448. DOI: 10.1111/jfbc.13448.

Ray M, Hor PK, Ojha D, Soren JP, Singh SN, Mondal KC (2018). Bifidobacteria and its rice fermented products on diet induced obese mice: analysis of physical status, serum profile and gene expressions. Benef Microbes 9(3): 441–452. DOI: 10.3920/BM2017.0056.

Reilly NA, Lutgens E, Kuiper J, Heijmans BT, Jukema JW (2021). Effects of fatty acids on T cell function: role in atherosclerosis. Nat Rev Cardiol 18(12): 824–837. DOI: 10.1038/s41569-021-00582-9.

Salama A, Amin MM, Hassan A (2023). Effects of oleic acid and/or exercise on diet-induced thermogenesis and obesity in rats: involvement of beige adipocyte differentiation and macrophage M1 inhibition. Res Pharm Sci 18(2): 219–230. DOI: 10.4103/1735-5362.367800.

Swanson D, Block R, Mousa SA (2012). Omega-3 fatty acids EPA and DHA: health benefits throughout life. Adv Nutr 3(1): 1–7. DOI: 10.3945/an.111.000893.

Tan P, Pepin É, Lavoie JL (2018). Mouse adipose tissue collection and processing for RNA analysis. J Vis Exp (131): 57026. DOI: 10.3791/57026.

Todoric J, Löffler M, Huber J, Bilban M, Reimers M, Kadl A, et al. (2006). Adipose tissue inflammation induced by high-fat diet in obese diabetic mice is prevented by n-3 polyunsaturated fatty acids. Diabetologia 49(9): 2109–2119. DOI: 10.1007/s00125-006-0300-x.

Vázquez-Vela ME, Torres N, Tovar AR (2008). White adipose tissue as endocrine organ and its role in obesity. Arch Med Res 39(8): 715–728. DOI: 10.1016/j.arcmed.2008.09.005.

Veras ASC, Gomes RL, Almeida Tavares ME, Giometti IC, Cardoso APMM, da Costa Aguiar Alves B, et al. (2021). Supplementation of polyunsaturated fatty acids (PUFAs) and aerobic exercise improve functioning, morphology, and redox balance in prostate obese rats. Scientific Reports 11(1): 1–18. DOI: 10.1038/s41598-021-85337-9.

Volarevic V, Al-Qahtani A, Arsenijevic N, Pajovi S, Lukic ML (2010). Interleukin-1 receptor antagonist (IL-1Ra) and IL-1Ra producing mesenchymal stem cells as modulators of diabetogenesis. Autoimmunity 43(4): 255–263. DOI: 10.3109/08916930903305641.

Wang H, Eckel RH (2009). Lipoprotein lipase: from gene to obesity. Am J Physiol Endocrinol Metab 297(2): E271–288. DOI: 10.1152/ajpendo.90920.2008.

Waterborg JH (2009). The Lowry Method for Protein Quantitation. In: Walker JM (Eds). The Protein Protocols Handbook. Springer Protocols Handbooks. Humana Press, Totowa, NJ. DOI: 10.1007/978-1-59745-198-7_2.

Zendedel A, Habib P, Dang J, Lammerding L, Hoffmann S, Beyer C, Slowik A (2015). Omega-3 polyunsaturated fatty acids ameliorate neuroinflammation and mitigate ischemic stroke damage through interactions with astrocytes and microglia. J Neuroimmunol 278: 200–211. DOI: 10.1016/j.jneuroim.2014.11.007.

Zhou Y, Khan H, Xiao J, Cheang WS (2021). Effects of arachidonic acid metabolites on cardiovascular health and disease. Int J Mol Sci 22(21): 12029. DOI: 10.3390/ijms222112029.