

Anti-obesity potentiality of Tapra fish (*Opisthopterus tardoore*) oil

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Abstract

In this present investigation, we have extracted and characterized the Tapra fish oil as well as applied it to evaluate anti-obesity potentiality. The Tapra fish oil had 1.14 ± 0.10 mg KOH/g of acid value, 129.8 ± 5.09 mg KOH/g of saponification number, 2.67 ± 0.67 mEq/kg of peroxide value, 121.9 ± 2.14 mg of iodine value, and 17.67 ± 1.45 totox value. Gas Chromatography-Mass Spectrometric analysis clearly revealed the presence of nine different fatty acids. When the fish oil was applied to high-fat diet-induced obese mice, it showed significant reduction of body weight, Body Mass Index, and serum lipid profiles compared to the high-fat diet-induced obese mice. The levels of leptin and TNF- α were moderately reduced in fish oil treated high-fat diet-induced obese mice than control obese mice. In conclusion, the Tapra fish oil was enriched with essential fatty acids and it could be used as an antiobese food supplement.

Practical applications

Considering the adverse effects of drugs used for the treatment of obesity, there is always a need to find out the alternatives. While the anti-obesity potentialities of different sea fish oil have been documented, the same for the Tapra fish (*Opisthopterus tardoore*) oil has not been studied at all. The extracted Tapra fish oil was found good in quality. Administration of fish oil in the mice exhibited anti-obesity effect in terms of lowering body weight, Body Mass Index, and serum lipid profiles, leptin, and TNF- α in mice model. These findings are fostering new therapeutic approaches to obesity treatment.

KEYWORDS

cholesterol, fish oil, leptin, linoelaidic acid

1 | INTRODUCTION

Obesity is a medical condition where excess body fat has accumulated and is defined by a body mass index (BMI). Obesity is normally caused by excessive food intake, lack of physical activity, genetic susceptibility, sedentary behavior, and adoption of a less physically active lifestyle (Yazdi, Clee, & Meyre, 2015). Another view of obesity

is that people eat little however they gain weight due to a slow metabolism and it is not medically supported (Wilkins, Cross, Megson, & Meredith, 2011). Obesity often contributes to the risks of many life-threatening diseases such as cardiovascular diseases, type 2 diabetes, as well as certain types of cancer (Haslam & James, 2005; Luppino et al., 2010). It was reported that about 5% of the total population (~1.2 billion) in India has been suffering from obesity

(Mohan Reddy & Kumar, 2012). Nowadays several drugs (sibutramine, orlistat, phentermine, etc.) are being used for the treatment of obese patients or overweight patients (Bray & Tartaglia, 2000; Cui et al., 2017; Korner & Aronne, 2004). These drugs mainly act as an appetite suppressant, lipometabolism controller, and restrict energy intake (Albuquerque, Stice, Rodríguez-López, Manco, & Nóbrega, 2015; Silventoinen, Rokholm, Kaprio, & Sørensen, 2010; Torres-Fuentes, Schellekens, Dinan, & Cryan, 2015). However, the abovementioned medicines have unexpected side effects and lower efficacies. Therefore, there is a need for an alternative to these drugs to control obesity without any or little side effects. Several reports showed that fatty acids from natural origin including fish oil could alleviate obesity (Buckley & Howe, 2009; de Sá et al., 2016; Pejín, Bianco, et al., 2012; Pejín, Vujisic, Sabovljevic, Tesevic, & Vajs, 2012). The beneficial effects of marine fish oil might be due to the presence of different fatty acids including polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid and docosahexaenoic acid, etc. (Buckley & Howe, 2009). Generally, PUFAs lower down lipid synthesis, inhibits lipogenesis, enhance lipid oxidation, and thermogenesis (Li, Huang, & Xie, 2008), and thus, reduce the body weight. Tapra fish (*Opisthopterus tardoore*) is one of the majorly consumed sea fishes that are available in the sea coastal area of West Bengal, India. However, Tapra fish has not been achieved significant scientific attention till date. Considering that, for the first time, we have scientifically explored this fish. We extracted and characterized the fish oil from Tapra fish and evaluated its anti-obesity potentiality in a mice model.

2 | MATERIALS AND METHODS

2.1 | Chemicals

All of the chemicals were purchased from Sigma Aldrich (USA), HiMedia (India), and Merck (India).

2.2 | Sample collection

The samples were collected from Digha Mohona sea coastal area, Purba Medinipur district, West Bengal, India, and were immediately transferred to an icebox. Then, it was transported to the laboratory. The samples were washed with water and dried in a hot air oven at 60°C for 24 hr for preparation of the fish dust.

2.3 | Fish oil extraction

The fish oil was extracted according to the protocol of AOCS (1994). Briefly, hexane and isopropanol (3:2) were mixed with fish dust and allowed to shake at 140 rpm for 4 hr followed by Soxhlet extraction for 12 hr. After cooling down the remaining material was centrifuged at 5,000 rpm for 20 min and the supernatants were collected

followed by filtration by Whatman No. 1 filter paper. The filtrate was kept into a Rotary evaporator for evaporation of the volatile compounds and the fish oil was collected for further analysis.

2.4 | Physicochemical properties

The acid value, saponification value, peroxide value, iodine value, and totox value of fish oil were measured according to the method of AOAC (1980) and AOCS (1989).

2.5 | Gas chromatography-mass spectrometer analysis

Fish oil was analyzed in GC ultra-gas chromatograph (Thermo Fisher Scientific S.P.A, Milan- Italy, Model: Trace 1300 series, S/N - 717100576) coupled with Mass spectrometer (Model: ISQ QD Single Quadrupole Mass Spectrometer). The sample was passed through a TG-WAXMS column (30 m × 0.25 mm ID × 0.25 μm film thickness) with stationary phase and 5% of Phenyl polysilphenylene siloxane was used. Helium gas was applied as a carrier gas at a flow rate of 1.5 ml/min. The initial column temperature was 50°C for 10 min, then, was increased to 220°C (5°C/min) and maintained for 10 min. The MS was carried out in the electron impact mode (EI) at 70 eV keeping the temperature at 250°C, the detector was set at 40–600 D. Mass spectrum of gas chromatography-mass spectrometer (GC-MS) was interpreted using the database of National Institute Standard and Technology (NIST). The molecular weights of the detected compounds were determined by using XCALIBUR software.

2.6 | Selection and animal care

Animal experiments were carried out after taking prior approval from the Institutional Ethics Committee, Vidyasagar University (ICE/7-9/0-9/2016). Inbred male albino BALB/c mice (15 ± 3 g) at 5 weeks of age were acclimatized under a controlled environmental atmosphere (at 12 hr light/dark cycle, 32 ± 2°C temperature, 50 ± 5% humidity). They were fed with a standard diet (Hindustan Lever, Mumbai, India) and sterile water *ad libitum*. The mice were randomly assigned into three groups ($n = 5$) according to the type of diet: normal diet (control; 13.5% fat, 64.2% carbohydrate, and 22.3% protein) with water *ad libitum*; high-fat diet (OC; 38.9% fat from lard, 38.9% carbohydrate, and 22.2% protein [total 22.3 KJ/g] with water *ad libitum*, a high-fat diet supplemented with Tapra fish oil (HFO) at the concentration of 12.5 mg/kg body weight of mice and water *ad libitum*. The high-fat diet was fed to OC and HFO group for 12 weeks and the Tapra fish oil was fed to the HFO group after 8 weeks for 4 weeks. Tapra fish oil was administered through oral gavaging with methyl cellulose (0.2 ml 0.5% methyl cellulose) to HFO group. The others groups (control and OC) received only methyl cellulose (0.2 ml 0.5% methyl cellulose) by oral gavaging.

After completion of the experiment, mice were euthanized by cervical dislocation and different organs were excised and weighed. Blood samples were collected by puncturing the orbital plexus. Blood samples were centrifuged at 2,500 rpm for 20 min at 4°C and serum was stored at -20°C for biochemical estimation (Ray et al., 2018). For histological examination, the skin sample was initially fixed in formalin (10%, v/v), embedded in paraffin, and sliced following standard protocol. The thin sections (5 µm) were stained with hematoxylin and eosin and observed under a light microscope (Thompson & Richter, 1960).

2.7 | Analysis of somatic body weight index

A weekly body weight measurement of animals and the final weight was recorded according to standard protocol. Briefly, body length was determined by measuring nasal-to-anal distance to the nearest 0.1 mm using a caliper (Central; Model no. 6420). Body mass index was measured according to the standard method and the values were expressed as g/cm² (Ray et al., 2018).

2.8 | Biochemical analysis of serum lipid profile

The total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), triglyceride levels in the serum were analyzed by kit assay methods (Enzopak, India) as per manufacturer's instruction.

2.9 | Estimation of Leptin and TNF-α expression by ELISA

The concentration of serum leptin and liver TNF-α were measured by using ELISA kit (Wuhan Fine Biotech Co. Ltd, China). The sensitivity of leptin and TNF-α were below 50 and 18.75 pg/ml, respectively.

2.10 | Statistical analysis

All the parameters were repeated at least three times. The data were presented as mean ± SE, n = 5. The variations in different analysis results were examined by one-way ANOVA using SigmaStat 11.0 (San Jose, CA, USA) statistical software. Significant variation was accepted at the level of 5 and 1% (i.e., p < .05 and p < .001).

3 | RESULTS

3.1 | Physicochemical characteristics of Tapra fish (*O. tardoore*) oil

Table 1 shows the results of the physicochemical analysis of Tapra fish (*O. tardoore*) oil. We observed that the iodine value was

TABLE 1 Physicochemical characteristic of Tapra fish oil

Acid value (mg KOH/g)	1.14 ± 0.10
Saponification value (mg KOH/g)	129.8 ± 5.09
Peroxide value (mEq/Kg)	2.67 ± 0.67
Iodine value (mg)	121.9 ± 2.14
Totox value	17.67 ± 1.45

121.9 ± 2.14 mg which is within the expected range of National Agency of Sanitary Surveillance (Pena Muniz et al., 2015). The saponification number was 129.8 ± 5.09 mg KOH/g indicating that the oil contained polyunsaturated fatty acids in a large proportion. The acid and peroxide indexes are parameters that demonstrate the quality of the oil. The acid value was 1.14 ± 0.10 mg KOH/g, which is within the range of 4.0 mg KOH established by the National Agency of Sanitary Surveillance (Pena Muniz et al., 2015). The peroxide value was 2.67 ± 0.67 mEq/kg and above the limit of 10.0 mEq/kg established by the Codex Alimentarius (Pena Muniz et al., 2015).

3.2 | GC-MS chromatograms of oil extracted from Tapra fish

GC-MS was used to identify the composition of fatty acids present in fish oils extracted from Tapra fish (*O. tardoore*). A total of nine different types of fatty acids were identified (Figure 1 and Table 2). Out of five were the derivatives of Octadecenoic acid. The identified compounds were Propanoic acid, 2-oxo-ethyl ester; Butyric acid, 2,3-dioxo; Propanedioic acid; 9-Octadecenoic acid; 9-cis,11-trans-octadecadienoate; Linoelaidic acid; 9,12-Octadecadienoic acid, ethyl, ester; 9-Octadecenoic acid (Z)-pentyl ester; trans-9-Octadecenoic acid pentyl ester.

3.3 | Assessment of the body weight and serum lipid profile of dietary Tapra fish in HFD-induced obese BALB/c mice

To evaluate the anti-obesity effects of Tapra Fish oil, we analyzed the changes in body weight and serum lipid profile of high-fat diet-induced obese mice treated with Tapra fish oil for 12 weeks. The initial body weights of all of the groups of mice were nearly same (Control, 15.81 ± 1.06 g; OC, 15.08 ± 1.07 g; HFO, 14.46 ± 0.80 g). The average body weights of Control, OC, and HFO were 18.76 ± 1.50 g, 34.87 ± 1.75 g, 35.55 ± 2.69 g after 8 weeks when the fish oil was administered to the mice. The final average body weights in Control, OC, and HFO were 19.43 ± 4.35 g, 41.13 ± 5.38 g, 22.51 ± 3.96 g after 4 weeks of the fish oil treatment (Figure S1). The BMI was significantly increased in the OC group (2.43 ± 0.35 g/cm²) compared to control (1.19 ± 0.29 g/cm²) (Figure 2). However, BMI were reduced in the fish oil-treated group, HFO (1.31 ± 0.28 g/cm²) (Figure 2). Tested lipid profiles (except HDL cholesterol) were 40%–80% higher in the OC than in control. However, significant reductions were observed in the lipid profiles of HFO compared to OC. A significant

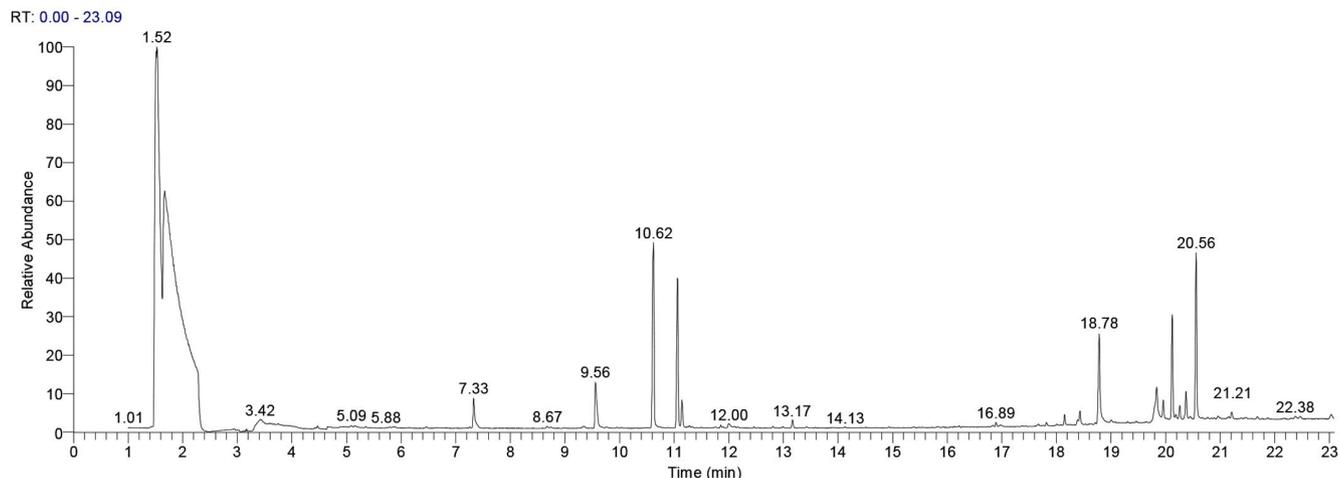


FIGURE 1 Gas chromatography-mass spectrometer (GC-MS) chromatograms of fish oil extracted from Tapra fish (*O. tardoore*)

TABLE 2 Fatty acids profiling of Tapra fish oil by using Gas chromatography-mass spectrometer (GC-MS)

RT	Name of fatty acids	Health beneficial effects	References
7.56	Propanoic acid, 2-oxo-, ethyl, ester	It could significantly lower fatty acids content in the liver and plasma, reduce food intake, and exert immunosuppressive actions. It had been suggested that it might be considered beneficial in the context of the prevention of obesity and diabetes type 2	Al-Lahham, Peppelenbosch, Roelofsen, Vonk, and Venema (2010)
7.78	Butyric acid, 2,3-dioxo	Butyrate supplementation with 5% wt/wt in high-fat diet prevented the development of obesity by increasing the uptake of fatty acid through skeletal muscle, fatty acid β -oxidation, and increasing the energy expenditure promoting physical activity in obese mice than control. Butyric acid also exerted most potential effects as the inhibition of inflammation and carcinogenesis, reinforcing several components of the gut defense barrier, and decreasing oxidative stress injury	Gao et al. (2009)
12.99	Propanedioic acid	Not reported	-
19.96	9-Octadecenoic acid	It helped to reduce the body weight of mice, decrease triglycerides and low-density lipoprotein as well as exert anti-inflammatory effects by reducing the Tumor necrosis factor (TNF- α)	Wang et al. (2015)
20.26	9-cis,11-trans-octadecadienoate	It showed anticancer activities	Białek, Tokarz, Dudek, Kazimierska, and Bielecki (2010)
20.37	Linoelaidic acid	It could modulate cancer, atherosclerosis, obesity, immune function, and diabetes in a variety of experimental models	Miller et al. (2001)
20.56	9,12-Octadecadienoic acid, ethyl, ester	It showed hepatoprotective, antihistaminic, hypocholesterolemic, and anti-eczemic effects	Tyagi and Agarwal (2017)
22.38	9-Octadecenoic acid (Z)-, pentyl ester	Not reported	-
22.38	trans-9-Octadecenoic acid, pentyl ester	Same as 9-Octadecenoic acid	Wang et al. (2015)

decrease (up to 47.43%) in the serum total cholesterol levels in HFO compared to OC (Figure 3a). Besides, serum triglyceride levels (up to 22.21%) were also decreased in the HFO group than OC (Figure 3b).

The marked reduction in serum cholesterol on the Tapra fish oil diets was mainly confined to the VLDL fraction (51.56% decreased in HFO group) (Figure 3d). Moreover, a reduction (58.30%) in LDL

cholesterol was also observed in the HFO group than OC (Figure 3c), while HDL cholesterol levels were selectively increased (79.44%) after Tapra fish oil feeding (Figure 3d).

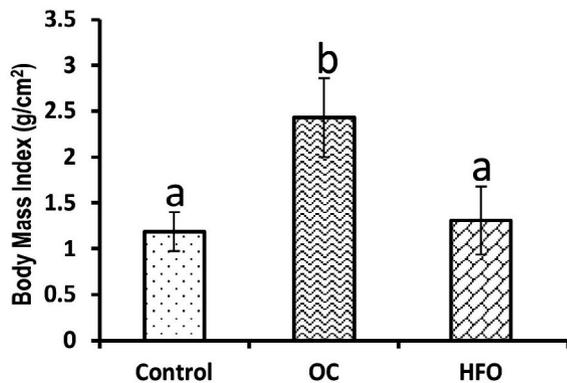


FIGURE 2 Analysis of BMI in different experimental groups ($n = 5$). Values are expressed as mean \pm SE

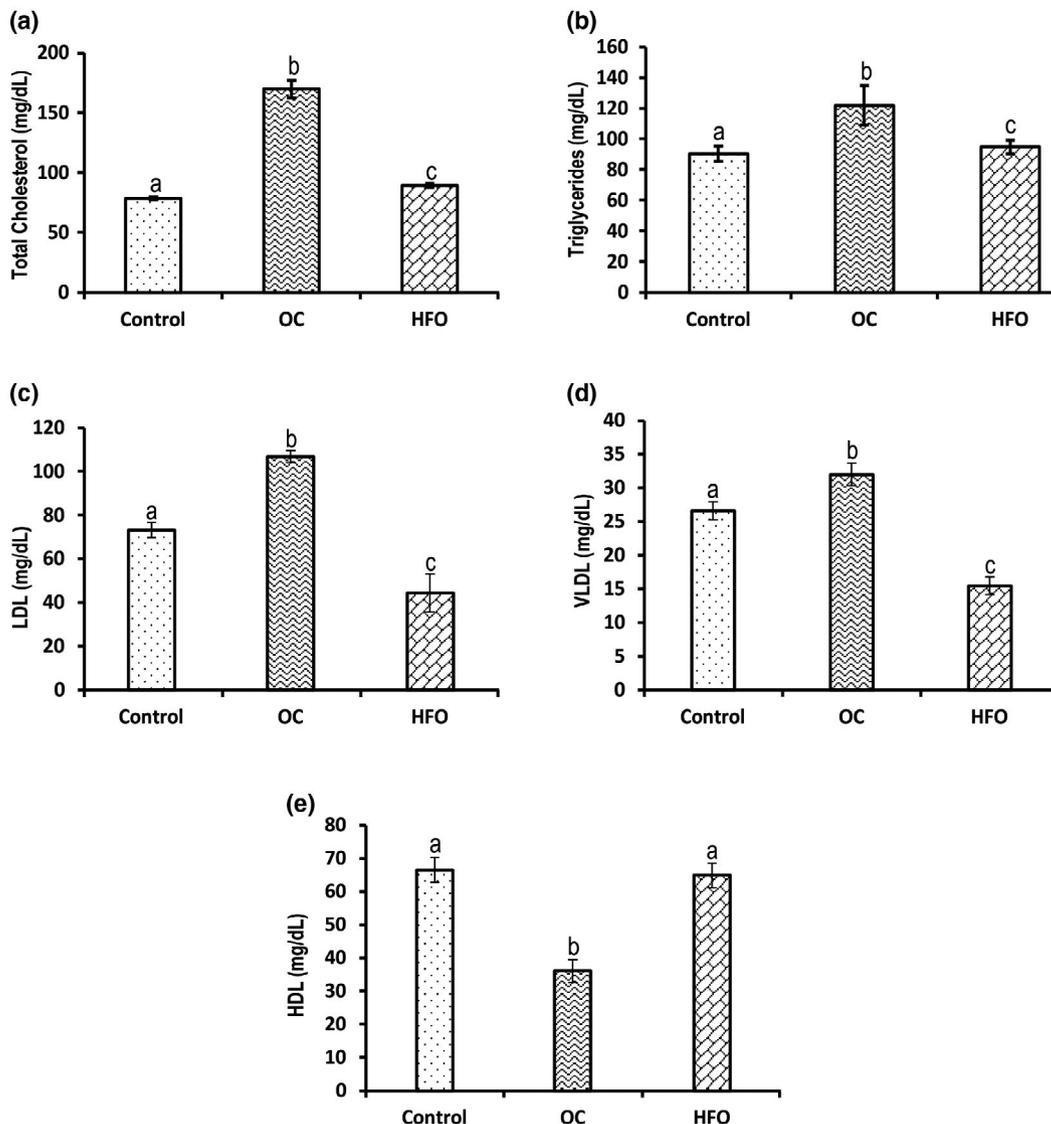


FIGURE 3 Analysis of serum lipid profiles such as total cholesterol (a), triglycerides (b), LDL (c), VLDL (d), HDL (e) in different experimental groups ($n = 5$). Values are expressed as mean \pm SE

3.4 | Effect of Tapra fish oil on abdominal fat accumulation

To investigate the effect of Tapra fish oil on the abdominal section of skin, we performed the skin histology by H&E staining. The OC group had a higher content of abdominal fat as compared to the control group (Figure 4). When fish oil was treated, a reduction of the abdominal fat was observed in the HFO group which is comparable to the control (nonobese).

3.5 | Assessment of serum leptin on Tapra fish oil-treated HFD-induced obese BALB/c mice

The serum leptin level was significantly increased in the OC group (10.2 ng/ml) than in control (0.8 ng/ml). Supplementation with Tapra Fish oil (HFO) significantly reduced serum leptin levels (5.6 ng/ml) compared to OC (Figure 5a).

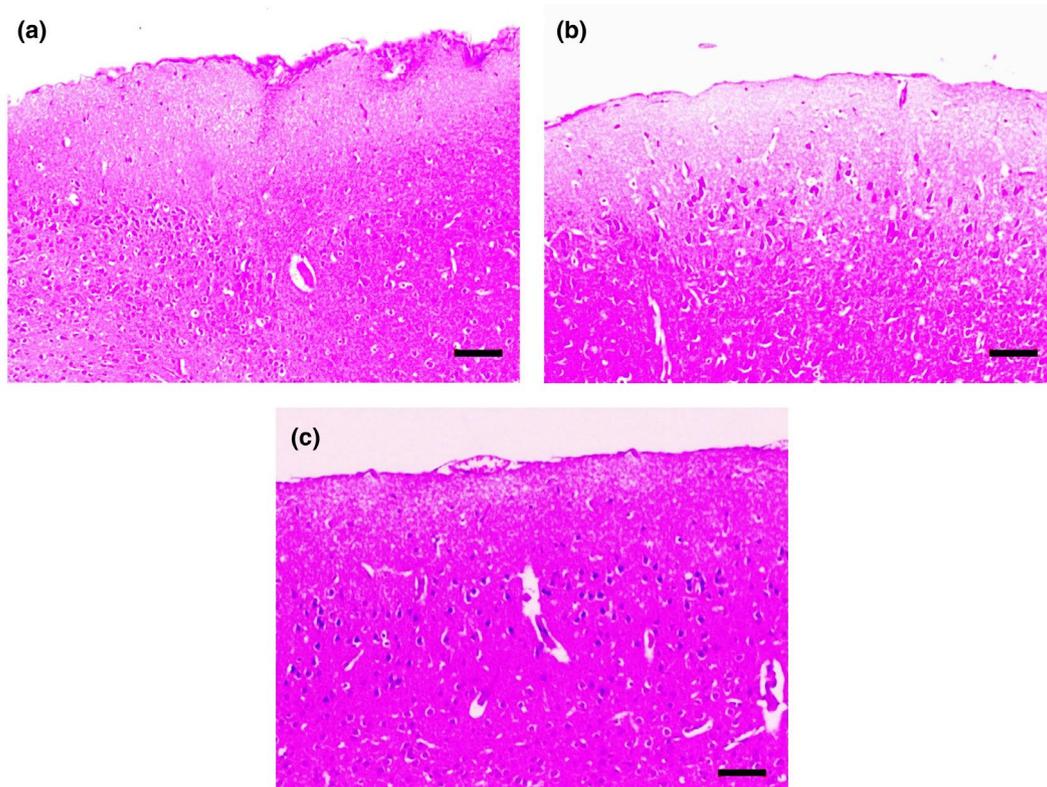


FIGURE 4 The effect of Tapra fish oil on skin histology. Control (a), high-fat diet-induced obese (b), high-fat diet-induced obese treated with Tapra fish oil (c). The skin slices were stained with hematoxylin and eosin. The scale bar correspondence to 40 μm

3.6 | Expression of TNF- α level in HFD-induced obese mice by ELISA

We evaluated the levels of TNF- α which is known to be produced during the acute phase of inflammation. As expected, TNF- α was elevated in the OC group compared with HFO and control. In OC mice, TNF- α expression was significantly increased to 2,557.28 pg/ml ($p < .05$), whereas supplementation of Tapra fish oil (HFO group) alleviated the values as 1,719.37 pg/ml, and these values were restored in the control group of mice 1,302.41 pg/ml ($p < .05$) (Figure 5b).

4 | DISCUSSION

While the bioactive components and health beneficial effects of different sea fish oils have been well established (Chiu, Chang, Liu, & Chiang, 2017), the pharmacological role of Tapra fish (*O. tardoore*) oil has not been studied so far. In this study, we have extracted the Tapra fish oil and evaluated its physicochemical properties and anti-obesity potentiality. Typical analysis of Tapra fish oil such as saponification value, iodine value, peroxide value, acid values, etc. were compared to the standard oil quality control data and appreciated (Ferreira et al., 2011; Pena Muniz et al., 2015) (Table 1). The low acid values determined for Tapra fish oil indicated that the triacylglycerol had not been hydrolyzed, which could indicate good stability. The finding was comparable to the previous report of Ndidiamaka

and Ifeanyi (2018). The higher saponification value in the Tapra fish oil (129.8 ± 5.09 mg KOH/g) indicated the high content of medium-chain fatty acids (Ouilly et al., 2017). The lower peroxide value (2.67 ± 0.67 mEq/Kg) indicated a good quality of oil and a good preservation status (Frankel, Neff, Selke, & Brooks, 1988). Ndidiamaka and Ifeanyi (2018) also found similar saponification and peroxide value of the sea fishes (*Clarias gariepinus* and *Scomber scombrus*). The iodine value of 121.9 ± 2.14 mg suggested that the fish oil had a higher content of unsaturated fatty acid. The TOTOX value was 17.67 ± 1.45 , and it clearly indicated the presence of high primary and secondary oxidative stability (Ixtaina, Nolasco, & Tomás, 2012). GC-MS analysis revealed that the participating fatty acids have different health beneficial effects such as anti-obesity, anti-inflammatory, anticancer, hepatoprotective, antihistaminic, anti-eczemic, etc. (Table 2). Most predominant fatty acids in the Tapra fish oil were Octadecenoic acid and its derivatives which are known to modulate obesity and inflammation (Wang et al., 2015). In spite of the different habitat of Tapra fish (mainly found in Bay of Bengal and Indian Ocean), the fatty acid profiles of Tapra fish were comparable to the other studied sea fishes (salmon, herring, mackerel, anchovies, and sardines) found in Atlantic and Pacific Ocean (Bandarra, Marçalo, Cordeiro, & Pousão-Ferreira, 2018; Jensen, Jacobsen, & Nielsen, 2007; Łuczzyńska, Paszczyk, & Łuczzyński, 2014; Öksüz & Özyılmaz, 2010; Sonavane, Koli, Patange, Naik, & Mohite, 2017). A detailed comparative study is essential to find out the differences in fatty acid contents among the abovementioned fishes. Clearly, the

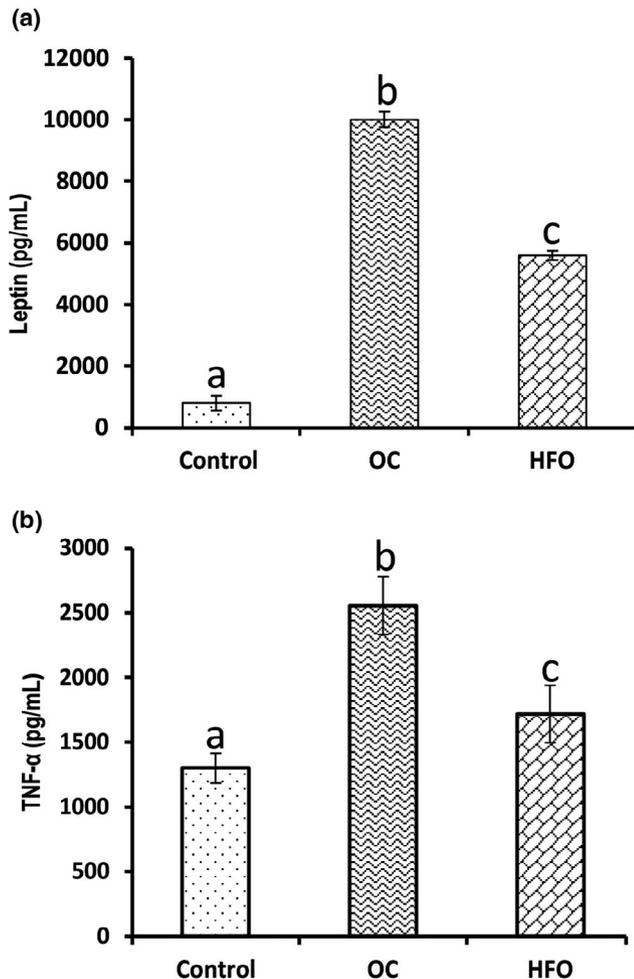


FIGURE 5 Expression of Leptin (a) and TNF- α (b) level in high-fat diet-induced obese mice ($n = 5$). The concentration of Leptin and TNF- α were measured by ELISA. Values are expressed as mean \pm SE

quality of the Tapra fish oil was good and enriched with some health beneficial fatty acids.

The high-fat diet-induced obesity model is an animal model used to study obesity using animals that have obesity triggered by high-fat diets (Chen et al., 2020). Typically mice and other nonhuman primates are used in these models (Chen et al., 2020). Body fat accumulation is a major risk for different chronic diseases including hyperlipidemia, diabetes, cardiovascular disease, and cancer (Lean, 2000). Many studies stated that numerous lipolytic agents, including isoproterenol, norepinephrine, dibutyl- α -cAMP, phosphocholine, and theophylline-induced lipolysis in adipocytes (Morimoto, Kameda, Tsujita, & Okuda, 2001). Yet, these established anti-obesity drugs have serious side effects such as insomnia, vomiting, headache, abdominal pain, constipation, and myocardial infarction (Bray, 2001). Consequently, the development of anti-obesity agents derived from natural foodstuffs is genuine for weight management in defeating and decaying the fat accumulation and having negligible side effects are mandatory (Mayer, Hocht, Puyo, & Taira, 2009).

In our present study, we have analyzed the anti-obesity effect of fish oil extracted from Tapra fish for evaluating the anti-obesity effect in high-fat diet-induced obese mice. At the end of 12 weeks study period, the high-fat diet-induced mice (OC group) had maximum weight gain (Figure S1) and eventually exhibited the highest BMI than the other groups (Figure 2). There was effective weight loss and lower BMI after the Tapra fish oil feeding in high-fat diet-induced obese mice (HFO). This might be due to the presence of different anti-obesity components specifically the fatty acids (Figure 1 and Table 2) in the Tapra fish oil involved in fat metabolism. Chiu et al. (2017) also reported about the body weight loss after consumption of fish oil. In connection with these findings, skin epidermal fat, serum LDL, serum TG, serum TC, and serum VLDL in HFO mice were found lower compared to OC (Figures 3 and 4). These could be the presence of different fatty acids which might enhance lipid oxidation, lower down lipid synthesis, and inhibit lipogenesis (Gao et al., 2009; Li et al., 2008). The effects of fatty acids on obesity were reported by different researchers (Miller, Stanton, & Devery, 2001; Wang et al., 2015). On the contrary, the serum HDL concentration was vividly increased after Tapra fish oil treatment. The deposition of the lipid layer in the skin epidermis of the OC group could be due to the accumulation of triglycerides (Vazquez-Vela, Torres, & Tovar, 2008). As the triglyceride level was decreased in the HFO group, the less deposition of lipid in skin epidermis was observed. A detailed study is needed to find out the bioactive components responsible for anti-obesity in Tapra fish oil.

Leptin is a polypeptide hormone. It is secreted by adipocytes and it signals to the brain to inhibit food intake, and thus, help in decreasing weight (Ahima, 2008). So far, the idea of leptin as an anti-obesity hormone was not clear because obesity is characteristically related to high leptin levels but not to leptin deficiency (Flier, 1998; Heymsfield et al., 1999; Sabol et al., 2020). In this study, we observed the selective downregulation of serum leptin levels in HFO which was supported by the existing literature published by many researchers (Flier, 1998; Gray, Steyn, Davies, & Vitetta, 2013). Besides, elevated leptin concentrations are positively correlated with TNF- α (Corica et al., 1999), which was also reflected in our study (Figure 5). This might lead to the subsequent development of hyperleptinemia (Zhang, Kumar, Barnett, & Eggo, 2000). The exact role of Tapra fish oil and its components in the regulation of leptin and TNF- α expression should be evaluated.

5 | CONCLUSIONS

The present study demonstrates that the physicochemical quality of the Tapra fish oil was good and enriched with health beneficial fatty acids. Besides, the Tapra fish oil supplementation could decrease body weight, BMI, serum total cholesterol, triglycerides, low-density lipoprotein, and very-low-density lipoprotein levels in obese mice. A decrease of leptin and TNF- α was also accounted in the obese mice supplemented with Tapra fish oil. A detailed study is needed to establish the anti-obesity effects of Tapra fish oil.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

AUTHOR CONTRIBUTIONS

SP, KG, and SC have designed the experiments. SP, TP, BP, and AK have collected samples and executed the experiments. KCM has performed the GC-MS analysis. SP, SRJ, KG, and SC have analyzed the data. SP, KG, and SC have written the manuscript. All of the authors have checked and approved the final manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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