Research Square

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Oral supplementation of njansan (Ricinodendron heudelotii Bail) and sardine fillets (Sardina pilchardus) oils alleviate high-fat diet-induced obesity in rats by regulating lipid metabolism and stress oxidative parameters

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Abstract

Background

Obesity has become one of the most common metabolic disorders in the world, characterized by the accumulation of excess fat in the body. Research for natural compounds to improve obesity has become increasingly important. This study aims to explore the effects of *Sardina pilchardus* and *Ricinodendron heudelotii* oils on the management of obesity. *Njansan* (*R. heudelotii*) oil was extracted by cold pressing using an automatic oil press machine and sardine fish oil by a cooking method. To evaluate the anti-obesity effect of these oils, 6 groups of 6 male Wistar rats were fed different diets: C0 group received a normal diet; HFD was fed a high-fat diet; N1, N2, F1and F2 followed by a high-fat diet supplemented with *njansan* and sardine oils at 1g/kg body weight/day and 2g/kg body weight/day, respectively.

Results

It appears that regardless of the type of oil, the acid and peroxide values meet the standards prescribed by the Codex Alimentarius. There was an increase in body weight, relative abdominal fat, and liver weight in the HFD group compared to the control group. There was also a decrease in anthropometric parameters such as the Lee index of the HFD group treated with *njansan* and fish oils, regardless of the concentration. The hyperlipidemic state in the HFD-fed rats was then normalized after treatment with both oils as well as hyperglycemia compared to the control group. Besides, fish and *njansan* oils attenuated HFD-induced oxidative stress, as indicated by a significant increase CA and SOD.

Conclusion

This study demonstrated that *njansan* and sardine oils at a low daily dose (1g/kg body weight) can be helpful in managing obesity and also reduce the risk of developing coronary heart diseases.

Background

Obesity is a metabolic disorder characterized by excessive accumulation of fat in the body that has reached pandemic proportions as defined by the World Health Organization [1]. Its prevalence worldwide has almost tripled since 1975. Currently, around one third of the world's overweight population is obese [1, 2]. In Africa, the prevalence of obesity is estimated at one in five adults and one in ten children and adolescents. In Cameroon, there has been an increase in the percentage of obese adults, from 4.9–15.1% [3]. Obesity is associated with nearly 200 comorbidities, including cardio-metabolic disorders such as type 2 diabetes [4], cardiovascular diseases [5], hypertension [6], dyslipidemia [7], gastrointestinal disorders [8], mechanical disorders (osteoarthritis of weight-bearing joints, hypoventilation), numerous cancers and mental health problems (depression), as well as functional limitations and a reduction in health-related quality of life [1, 6]. It has also been reported to weaken COVID-19 [9]. The cause of obesity

includes the high consumption of energy-dense foods as well as high-sugar diets with little or no physical activity [10].

Surgical interventions (bariatric surgery) are used to manage obesity, but due to unfavorable surgical consequences and their costly nature, many people find it difficult to comply [11, 12]. Medications have also been used to manage obesity, but there are side effects and weight gain when medication is stopped [13, 14]. Other ways have been examined in recent years to treat and prevent obesity with few or no side effects. Thus, eating habits have become an important factor in preventing a large part of overweight and obesity [15]. Among them, different types of fatty acids have received much attention for health [16]. Previous studies have shown that the chain length, the degree of unsaturation, and the position and stereoisomeric configurations of fatty acid double bonds could affect the rate of fatty acid oxidation, thereby influencing body weight [17]. A high intake of fatty fish (sardines) rich in long-chain omega-3 fatty acids (eicosapentaenoic acid, EPA (20:5n-3) and docosahexaenoic acid DHA (22:6n-3)), present in the oil of fish, was associated with an improvement of the lipid profile in humans and rats [18, 19].

In Cameroon, due to their proliferation in the rivers and lakes of the Far North Region, the sardine (*Sardina pulchardus*) is valued and traditionally processed to extract oil. This oil is used for stomach aches and joint diseases. The oil is in high demand locally and even in neighboring countries [20]. The oil is also used for cooking. In addition, due to its richness in long-chain polyunsaturated fatty acids (EPA and DHA), it can be an asset in the management of metabolic diseases for this population.

Results of recent studies have also revealed that several other uncommon fatty acids, such as conjugated fatty acids including Conjugated Linolenic Acids (CLNA) and in particular eleostearic acid (cis-9, trans-11, trans-13-18:3) also have favorable effects on cardiometabolic health [21, 22]. *Ricinodendron heudelotii* also known as *njansan* is a plant of the Euphorbiaceae family. The almonds are used to thicken and season sauces in Cameroon and in many other countries in Africa [23, 24]. These almonds contain about 45–55% oil which is rich in eleostearic acid (47–52%) [23, 25]. They are also rich in antioxidants such as tocopherol (178.3 ± 0.4 mg/100g) [26]. Given its interesting composition, *njansan* oil has demonstrated its pharmacological effects in several studies. It reduces several risk factors for cardiovascular diseases [19, 27]. Moreover, previous work also showed that in rats and in Caco-2 cells, this fatty acid was metabolized to rumenic acid [28, 29].

Oils extracted from *njansan* kernels and sardines were found to be healthy and nutritionally adequate. The aim of this study was therefore to compare the effects of *njansan* oil rich in CLNA and sardine oil rich in omega-3 on rats with HFD-induced obesity. The biochemistry and physiological indicators of obesity-associated disorders were examined to assess the impact of the oils. The results of this study could contribute to the exploration and development of safe and effective dietary supplements for the treatment of obesity.

Materials and Methods

Material

R. heudelotii kernels (picture 1a) used for this study were purchased from the Douala Central Market (Littoral, Cameroon), which is one of the major renowned marketing areas in Cameroon.

S. pilchardus with a pink tail used for the extraction of sardine fish oil was purchased on the shores of Lake Maga located in the Far North region of the Mayo Danay Division (Cameroon) (picture 1b)

Oil extraction

Njansan oil extraction

The oil from *R. heudelotii* kernels was extracted by cold pressing using a CGOLDENWALL automatic oil press machine, model number X5, made in Glendale, California. The *R. heudelotii* almonds were put through the inlet and pressing was done at 50°C. The pressed oils were protected from light. They were stored in a dark environment for 48 hours before being filtered.

Sardine fish oil extraction

The oil from the flesh of sardine fish was extracted at the Bioprocess Laboratory of the University Institute of Technology of the University of Ngaoundéré by the improved traditional cooking process described by the *Musgums* [20].

Physicochemical properties

The oils were characterized in order to see the impact of the extraction method on the quality of the oils. So, the acid value, peroxide value and iodine value of fish oil and *njansan* oil were measured according to the method of AOCS [30].

Animals

In this study, healthy male Wistar rats (11–12 weeks old, 270–290 g weight) were provided by the animal house of the WISTAR Institute Saint Louis Bamenda, Cameroon. The rats were acclimatized for a week in the Biochemistry animal farm house of the University of Bamenda (North West, Cameroon). The animals were maintained in a standard environmental atmosphere (at a 12-hour light/dark cycle, 32 ± 2°C temperature, 50 ± 5% humidity) with a normal diet and water *ad libitum* in a simple cage. The rats were randomly assigned into six groups, 6 rats per group. This study was approved by the institutional animal research committee of the Faculty of Sciences of the University of Bamenda.

Induction of hyperlipidemia using high-fat diet

The diets were prepared in the COLTECH laboratory (College of Technology, University of Bamenda). The control diet (group C0) was formulated based on the modified AIN-93 protocol [31].

Induction of hyperlipidemia in the rats was achieved by feeding HFD formulated according to the protocol described by Aissatou et al. [32], with some modifications. The detailed formulations of the different diets

are given in Table 1. All diets were given to rats *ad libitum.*

Table 1
Detailed composition of experimental rat diets [31, 32]

	Ingredients	Control Diet (%)	High Fat Diet (%)
Proteins	Fish meal	20	10
Carbohydrates	Corn starch	59	29
	Sucrose	5	5
Lipids	**Soya bean oil**	**5**	**5**
	Egg yolk*	**0**	**25**
	Palm oil**	**0**	**20**
Others	Cellulose	5	0
	Vitamin mix	1	1
	Mineral mix	5	5
		100	**100**
* Egg yolk: 300 g			
** Coconut oil: 25 g			

Study design

The rats having received HFD were gavaged with fish oils and *njansan* oils. The rats were split into six groups as follows:

Group 1: Normal (CO); received control diet during the whole experimental period (8 weeks).

Group 2: Hyper (HFD); received HFD all over the experimental period (8weeks).

Group 3 (N1); received HFD all over the experimental period (8 weeks) and gavaged (1g/kg body weight/day) of *njansan* oil for the last 4 weeks.

Group 4 (N2); received HFD all over the experimental period (8 weeks) and gavaged (2g/kg body weight/day) of *njansan* oil for the last 4 weeks.

Group 5 (F1); received HFD all over the experimental period (8 weeks) and gavaged (1g/kg body weight/day) of fish oil for the last 4 weeks.

Group 6 (F2); received HFD all over the experimental period (8 weeks) and gavaged (2g/kg body weight/day) of fish oil for the last 4 weeks.

Measurement of body weight and food intake

The individual body weight of the rats was measured weekly using a scale. Percentage weight gain (%) was calculated as follows: (body weight a specific week (g) − initial body weight) / initial body weight × 100. Food prices were recorded each day (over 24 hours) based on the weight of leftover food per 200 g given.

Analysis of the Lee index

The weights of the rats were taken daily using an electronic balance and their naso-anal lengths were measured at the end of every week using a measuring tape in order to determine their obesity status. The Lee index for each rat was calculated using the formula of Lee [33]:

$$\text{Lee index} = \frac{\sqrt[3]{Weight}(g)}{Naso-anallength(cm)}$$

Blood and samples collection

At the end of the experiment, the animals were fasted for 12 hours but had free access to drinking water. The rats were then anesthetized using diazepam (10mg/Kg) and sacrificed by decapitation. Whole blood was drawn into tubes and stabilized for between 15–30 min to ensure it was able to clot at room temperature. The serum was prepared from the blood using a centrifugation at 3000 rpm for 10 min. The latter was used to determine the hepatosomatic index, the viscerosomatic index and the adiposity index respectively.

Biochemical analyses of serum lipid profiles

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

Serum low density lipoprotein cholesterol (LDL-c) concentration and very low density lipoprotein cholesterol (VLDL-c) were determined using Friedwald formula, where LDL-c = TC– (HDL-c + VLDL-c) and VLDL-c = TG/5[34]. Atherogenic index (AI) and coronary risk index (CRI) were calculated using the Castelli formula, where AI = LDL-C $\div HDL-C$ and CRI = TC $\div HDL-C$ [35].

Analysis of the antioxidant activity

Two different methods were used to evaluate the antioxidant activity in the serum of rats.

Superoxide dismutase (SOD) activity

The activity of SOD was evaluated by the method described by Misra & Fridovish [36]. An aliquot of 200 µL of blood serum was introduced into 1600 µL of carbonate buffer (50 mM, pH 10.2). The reaction was initiated by adding 200 µL of adrenaline (0.6 mg/L) to each tube, and after homogenization, the ODs were

read after 20 seconds and 80 seconds at 480 nm. The specific activity of SOD was expressed in units/mg of blood serum used.

Catalase (CAT) activity

The catalase activity was determined by a previously described method [37]. 300 µL of H_2O_2 (9%) was introduced into tube followed by 1400 µL of the test samples while 1700 µL of phosphate buffer (0.1M pH 7.2) was introduced into the tube for the blank. 300 µL of blood sample from each rat were then added to the tubes and after homogenising, the ODs were read at 240 nm at 30, 60, 90 seconds. The enzymatic activity of catalase was expressed in IU/ mg of blood sampled.

Estimation of Fasting Blood glucose

Before animal sacrifice, the tail of each rat was injured using a single-use lancet and blood was applied to the strip and blood glucose concentrations (mg/dl) were determined using the glucometer.

Statistical analysis

The data reported in the tables and figures were carried out in triplicate or several replicate determinations. All data were expressed as mean ± standard deviation and statistically analyzed using one-way ANOVA. When statistical differences were noted, Duncan's Multiple Range test was applied to classify the samples at a significant level of 5%. The Statgraphics Centurion version XVI.I software-package was used for statistical analysis.

Results

Physicochemical analyses of R. heudelotii almond and S. pilchardus fillets oils

The acid value, iodine value and peroxide value (Table 2) were performed on oils that were used for the treatment of obesity to evaluate their quality at the moment they were used. It appears that fish oil has the highest acid (1.35 mg KOH/g oil) and peroxide values (3.20 meq O_2 per kg of oil**)**. But the values found are lower than those recommended by the Codex Alimentarius [38]. The iodine index shows that *njansan* oil, with an index of 103 g I_2/100 g oil is richer in unsaturated fatty acids than sardine fillet oil.

Table 2
Some physicochemical parameters of *R. heudelotii* almond oils and *S. pilchardus* fillets

	R. heudelotii	Fish oil	Codex Standard[38]
Acid value (mg KOH/g oil)	1.01 ± 0.05^b	1.35 ± 0.01^a	3
Iodine value (g I_2/100 g oil)	103 ± 1^a	87 ± 2^b	
Peroxide value (meq O_2 per kg of oil)	2.43 ± 0.1^a	3.20 ± 0.01^b	5

Values are means ± SD of three determinations. Values on the same line with different superscripts are significantly different at p < 0.05 (Duncan's test)

Effect of High Fat Diet and different oil treatments on anthropometrical parameters

During the first four weeks of the protocol, the animals received food without treatment. In Fig. 1a, all animals gained weight. For the control (CO) the evolution of the weight is constant; the weight gain is therefore 11 g. For the groups that received the high-fat diet, weight gain is visible with an average of around 26 g (Fig. 1b). During the second experimental period (5 to 8 weeks), weight gains were significantly reduced (P 0.05) in groups N1, N2, F1 and F2 compared to the HFD control (Fig. 1b). The body mass of the N1 group showed the highest significant decrease (P 0.05), but there was no significant difference between N1 and N2 and between F1 and F2.

Lee index

The lee index is a good parameter used in animals to assess the induction of obesity by diet [39, 40]. Table 3 shows the variation of this index during the induction of obesity and during treatment with oils. Lee considered values greater than 310 as an indicator of obesity [39]. Before treatment, the five groups excluding the control received an HFD which led to a significant increase in the Lee index during the study, rendering the rats obese before treatment with oils. The Lee index of the control group however had a steady increase throughout the study. In the second phase of the study (treatment with oils), the control group maintained a steady increase in the Lee index which was not relative to the other groups which experienced a decrease in the Lee index. Sardine flesh oils and *njansan* almonds, whatever the quantity, do not significantly influence the decrease in the Lee index ($p < 0.05$).

Table 3
Variation of Lee index of rats before and after treatment

Groups	Before treatment		After treatment
	Initial	End	End
C0	296 ± 1^{b}	297 ± 2 ^{b}	303 ± 3^{c}
HFD	290 ± 1^{b}	324 ± 3^{a}	332 ± 4 ^{a}
N1	305 ± 3^{a}	325 ± 5 ^{a}	318 ± 2^{b}
N2	301 ± 5 ^{ab}	335 ± 4 ^{a}	323 ± 2 ^{b}
F1	302 ± 11^{ab}	332 ± 1 ^{a}	319 ± 3^{b}
F2	295 ± 3 ^{ab}	320 ± 1 ^{a}	318 ± 3^{b}

Means ± SD (n = 6) followed by different letters in the same column are significantly different (P < 0.05) as determined by Duncan's multiple range test. C0: normal control; HFD: Hyperlipidemic group; N1: 1g/kg body weight *of Njansan oil group, N2: 2g/kg* body weight *of Njansan oil group; F1: 1g/kg* body weight *of Sardine fish oil, F2: 2g/kg* body weight *of Sardine fish oil*

Effect of njansan oil and sardine fish oil on food intake and adiposity, hepatosomatic viscerosomatic indices

The adiposity, viscerosomatic and hepatosomatic indices (Table 4) were determined to evaluate the effect of the HFD and different oil treatments on the organs of rats.

Table 4
Effect of *njansan* and sardine oils oral administration on food intake and adiposity, hepatosomatic, viscerosomatic indices of rats fed with high fat diet

	Parameters				
Group	Food intake/g /day/rat	Adiposity index	Liver	Heart	Kidney
CO	30.01 ± 1.62^{a}	1.65 ± 0.47^{d}	2.69 ± 0.68 ^{c}	0.93 ± 0.15^{b}	2.25 ± 0.38 ^{b}
HFD	26.15 ± 2.01^{b}	3.69 ± 0.87 ^{a}	4.68 ± 0.76 ^{a}	1.14 ± 0.19^{a}	2.70 ± 0.30 ^{a}
N1	22.08 ± 1.81^{c}	2.78 ± 0.79^{b}	2.98 ± 0.18 ^{bc}	1.03 ± 0.04^{a}	2.45 ± 0.26^{ab}
N2	21.10 ± 2.11^{c}	2.78 ± 0.75^{b}	3.17 ± 0.42 ^{b}	1.07 ± 0.13^{a}	2.57 ± 0.16^{ab}
F1	22.20 ± 1.60^{bc}	2.40 ± 1.1 ^{bc}	2.98 ± 0.17 ^{bc}	1.04 ± 0.11^{a}	2.2617 ± 0.12^{b}
F2	21.55 ± 1.75^{c}	2.25 ± 0.36^{c}	2.97 ± 0.16 ^{bc}	1.06 ± 0.05^{a}	2.46 ± 0.21^{ab}

Means ± SD (n = 6) followed by different letters in the same column are significantly different (P < 0.05) as determined by Duncan's multiple range test. C0: normal control; HFD: Hyperlipidaemic group; N1: 1g/kg body weight *of Njansan oil group, N2: 2g/kg* body weight *of Njansan oil group; F1: 1g/kg* body weight *of Sardine fish oil, F2: 2g/kg* body weight *of Sardine fish oil*

In terms of food intake, rats fed a normal diet consumed more food (30 g) but had a lower weight gain than the HFD group, which only consumed about 26 g and had a higher adiposomatic index (3.69). Oral administration of the oils in all test groups significantly decreased food intake and adiposomatic index compared to the HFD group (P < 0.05). Eating foods rich in oil leads to a feeling of fullness.

The liver mass ratio of rats with fed the HFD increased significantly compared to that of rats in the control group (p < 0.05). However, this was neutralized after treatment with oils, regardless of the concentrations used. But this reduction remains high compared to the normal group.

The viscerosomatic index (heart, kidney) of the control group was significantly lower as compared to that of the rats that received HFD (p < 0.05) (Table 4). There was nevertheless no significant difference between the groups that received treatment with both oils, irrespective of the concentrations used.

Effect of sardine fish oil and njansan oil on the serum lipid profile

Triglyceride level

During obesity induction, there was a significant increase in the TG level (p < 0.05) (292.80 ± 17.40mg/dL) caused by the HFD with respect to the control group (69.60 ± 10 mg/dL). Nevertheless, this situation was counteracted following treatment with the oils of *njansan* and fish regardless of the concentrations used (p < 0.05). The greatest reductive effect was seen in rats fed with sardine fish oil. For F1, the decrease was from 292.8 mg/dL to 93.45 ± 10.81 mg/dL a drop of 199 mg/dL after 4 weeks (Fig. 2).

Total Cholesterol, HDL-C, LDL-C and VLDL-C

The serum lipid profile of rats that were fed different diets is shown in Fig. 3. Among the circulating lipid fractions, the total cholesterol, the LDL-C and the VLDL-C levels were found to be significantly increased in the HFD group as compared to the control group (p < 0.05) after treatment with obesity .

Nonetheless, all groups that received treatment with oils had a significant reduction in the concentration of these parameters with increasing concentrations of the oils (p < 0.05). Sardine fish oil had the greatest reductive effect as compared to *njansan* oil (Fig. 3).

The HFD significantly reduced the concentration of serum HDL-C (22.01 ± 2.01mg/dL) as compared to the control group (43.35 ± 3.46mg/dL). After treatments with both oils, the serum HDL-C level significantly increased compared to the control group, regardless of the concentration of the oils used (p < 0.05). The difference between fish oil and sardine oil is not significant (Fig. 3).

Effect of sardine fish oil and njansan oil on atherogenic and coronary risk indices

The results of HFD and oil treatment on the atherogenic index (AI) and coronary risk index (CRI) of rats are shown on Fig. 4. HFD significantly increased the AI and CRI of these rats in the obesity induction phase ($p < 0.05$). Both indices were greatly reduced following treatment with both oils, irrespective of the concentration used, as compared to the control group. A significant reduction in the AI of the rats was seen, especially in rats fed with *njansan* (N2) and sardine fish oils (F2) ($p < 0.05$). For the CRI, this decrease is not significant, whatever the concentration or type of oil.

Effect of sardine fish oil and njansan oil on oxidative stress parameters (SOD and catalase)

Figure 5 shows the changes in catalase and SOD in rats fed with HFD and after treatment with oils. For the HFD group, the catalase and SOD levels were 25 and 10, respectively. After treatment with oils, there was a significant increase ($p < 0.05$) regardless, of the type of oil. This increase is proportional to the quantity of oil. *Njansan* oil has a higher protective effect for these two enzymes compared to sardine oil.

Effect of sardine fish oil and njansan oil on serum blood glucose level

The normal blood glucose level in rats is 50-135mg/dL [41]. The results of the effect of sardine fish oil and *njansan* oil on the serum blood glucose level of obese Wistar rats are shown in Fig. 6. During the 8 weeks of obesity induction in HFD rats groups, their blood sugar levels increased significantly compared to those of the control group ($p < 0.05$). There was, however, a significant resolution effect after treatment with both oils, regardless of the concentration used. The greatest decrease in blood glucose was observed in rats treated with N2 ($p < 0.05$). This finding supports the use of *njansan* oil (2 g/kg) as a potential curative food for people suffering from diabetes mellitus caused by obesity, compared to sardine oil at both concentrations.

Discussion

A diet containing more than 30% of energy intake in the form of lipids leads to the development of obesity in rats, mice, dogs and primates due to increased caloric intake [42]. But this public health problem finds a solution thanks to dietary interventions such as the consumption of foods rich in polyunsaturated fatty acids (n-3 PUFA and CLNA). The development of obesity in this study was marked by an increase in anthropometric parameters such as body mass and Lee index in the HFD groups compared to the control group. This is due to accumulated fats, which cause hypertrophy and hyperplasia of adipose tissue (Fig. 1). The reduction in body mass following oil treatment confirms the beneficial effects of *njansan* and sardine oil in the pathogenesis of obesity. *Njansan* oil (plant-based) at 2 g/kg body weight had the highest reducing effect on rat body mass compared to sardine oil (animal-based). The results are consistent with previous studies that showed similar results following oil supplementation in high-fat diet-induced obesity [19, 43, 44, 45]. The majority of studies use high doses of between 10 and 50% oil. In our study, 1 and 2 g oil/kg body weight of *njansan* and/or fish oil were used, which is a relatively low dosage reflecting the benefit for humans. The decrease in Lee index (tableau 3) following treatment with oils this may likely be due to a decrease in adipose tissue associated with accumulated energy expenditure via thermogenic pathways to prevent excessive accumulation of

fat [46]. n-3 PUFAs have been shown to improve lipolysis and suppress lipogenesis, thereby decreasing lipid storage and ultimately leading to a reduction in adipocyte size[43]. Sharma & Agnihotri [43] reported similar effects, with Lee index values higher than those obtained in this study due to the duration of obesity induction.

The significant reduction in the adiposity index of obese rats following treatment with oils (Table 4) is due to the presence of polyunsaturated fatty acids. The mechanism for this effect of n-3 PUFA lies in the modulation of lipid metabolism by promoting fatty acid oxidation rather than their storage, possibly via Sterol Response Element-binding Protein 1c (SREBP1c), a master regulator of lipid homeostasis that prevents the accumulation of hepatic and serum TG. Fish oil supplementation significantly induces phosphorylation and activation of Adenosine Monophosphate Kinase (AMPK) in depots of adipose tissue and therefore increases thermogenesis [46]. This is consistent with the data reported by Haimeur [47] using fish oil and argan oil; the same is true of the work of Yuan et al. [22], Machado et al. [48] using pomegranate seed oil rich in punicic acid (9c, 11t, 13c-CLNA). This finding suggests that fish oils from the Far North of Cameroon and *njansan* oil, even at low concentrations, have a significant effect on reducing body fat. They could have anti-obesity properties. This is the first time that this observation has been reported on *njansan* oil.

The liver is the main organ of detoxification and lipid metabolism in the body. According to the results in Table 4 the most significant liver reducing effect was observed in rats treated with *njansan* oil. Supplementation of n-3 PUFA from oils reduced lipidemia and prevented hepatic steatosis by lowering lipid metabolism in the liver [49]. Chiu et al [50] also reported similar results with 5% fish oil. But the work of Arao et al. [51] showed that supplementation with pomegranate seed oil rich in punicic acid acids had no effect on the liver after 2 weeks.

Triglycerides are defined as an independent risk factor for coronary heart disease [51]. The differences in TG levels among rats that received treatment with both oils (Fig. 2) can be explained by the differences in metabolism of various lipids according to their fatty acid content. An increase in ω-3 fatty acids mainly decreases serum TG [53]. This may be due to decreased hepatic lipogenesis, pancreatic lipase inhibition and suppression of the expression of sterol regulatory element-binding protein-1c. As a result, there is a decrease in the ability to increase β-oxidation of fatty acids through regulation of the hepatic gene involved in lipid metabolism (Stearoyl-CoA desaturase). We can therefore conclude that α-eleostearic acid (a conjugated linolenic fatty acid) from *njansan* oil as well as ω-3 fatty acids from sardine fish oil might have a reductive effect on the TG level. Similar results were obtained by Leudeu et al. [27] with *R. heudelotii* incorporated at 5% in the rats' diet. Haimeur et al. [47] and Lima et al [54] also had similar results in a comparative study using fish oil and another plant based oil. The same is true of the work of Arao et al. [51], Yuan et al. [22] using punicic acid.

A diet rich in saturated fatty acids leads to an increase in plasma levels of total cholesterol and LDL-c), and increases the risk of developing three symptoms of syndrome-X: low HDL-cholesterol, high total cholesterol and high triglycerides [55, 56]. All this increases the risk factors for premature atherosclerotic

cardiovascular disease. In this study, the rats which received a HFD had a significant increase in the serum concentrations of TC, TG, LDL-C, and VLDL-C and a decrease in HDL-C concentration (Fig. 3). The normalization of the hyperlipidemic state observed after treatment of the rats with sardine and *R. heudelotii* oils at different concentrations may be due to the ability of the oils rich in polyunsaturated fatty acids to enhance lipolysis, lower lipogenesis, suppress appetite, reduce lipid absorption and inhibit Peroxisome proliferator-activated receptor gamma (PPARγ) expression [57]. Franczyk-Żarów et al. [58] showed that plasma levels of total cholesterol and LDL + VLDL cholesterol were significantly decreased in animals fed CLNA and CLA compared to the control. A number of lipid parameters have been employed in predicting the risk of coronary atherosclerosis and cardiovascular diseases. However, AI and CRI are more accurate predictors of cardiovascular risk than lipid parameters. In this study, HFD significantly increased AI and CRI (Fig. 4). Treatment with both oils caused a profound reduction in both AI and CRI indices due to the hypolipidemic effect of both oils. Atherogenic dyslipidemia is characterized by a combination of increased levels of TG, LDL-C and AI and a decreased level of HDL as seen in the rats fed with HFD. This result is in concurrence with that of Sharma & Agnihortri [45] and Da Cunha de Sá et al. [18].

Obesity is closely associated with oxidative stress. It was reported that SOD and catalase are the two most important enzymes of the enzymatic antioxidant defense system [(59)]. SOD catalyzes the dismutation reaction of superoxide anions radicalized into molecular oxygen and hydrogen peroxide, which are in turn removed by the other antioxidant enzyme CAT to prevent the body from peroxidative damage [60]. The result of this study suggests effective protection of *R. heudelotii* and sardine oils against the generation of free radicals. This protection is proportional to the oil concentration and is more marked with *njansan* oil (Fig. 5). This could be attributed to their rich omega-3 fatty acid content (for fish oil) and CLNA (47–52%), vitamin E (178.3 ± 0.4 mg/100g) and phytosterol content (for *njansan* oil), which have scavenging capacity free radicals and reduce lipid peroxidation [61, 62, 63]. These results corroborate those of Lima et al. [54] who showed an increase in superoxide dismutase activity after fish oil supplementation. The same is true for the results of Yuan et al. [22] who found CAT and Glutathione peroxidase (GSH-Px) activity levels significantly increased in the liver of mice after punicic acid supplementation for 8 weeks with a CLNA.

Obesity is characterized by hyperglycemia. Boukhari et al. [64] confirmed that in Wistar rats, a high-lipid diet consumed for two months leads to an increase in food intake, body weight and lipid accumulation in adipose tissue, dyslipidemia and hyperglycemia. This is evidenced by the significantly high glucose level in obese rats before treatment with both oils (Fig. 6). This may be due to increased free fatty acids in plasma, which reduces insulin-regulated glucose metabolism [65]. This is similar to a study done by Roza et al [66]. It is also similar to the results of Daidj & Lamri-Senhadji [67] who showed that oils from sardine fillets and viscera lead to a reduction in blood sugar levels in rats given an HFD. The significant decrease in plasma glucose level may probably be due to the PUFAs (CLNA, omega-3) present in both oils, known to activate PPARγ and significantly decrease glucose levels in serum [68, 69] Saha & Ghosh [70] observed that administration of CLNA isomers significantly reduced the blood sugar level of STZ-induced diabetic

rats compared to the diabetic control group. This result is contradictory with that of Lima et al. [54], Franczyk-Żarów et al. [58] who showed no significant changes in serum glucose after feeding rats with HFD and supplementing with fish oil and pomegranate seed oil, respectively.

Conclusion

In conclusion, supplementation with *S. pulchardus* oil and especially *R. heudelotii* oil for 8 weeks effectively attenuated HFD-induced lipid, blood glucose parameters and body weight gain and fat accumulation in rats. They also help fight free radicals.

This provides preliminary evidence that *njansan* oil at a low daily dose (1g/kg body weight) may be useful for the management of obesity and also reduce the risk of developing coronary heart disease.

Abbreviations

AI: Atherogenic Index

ANOVA: Analysis of variance

AOCS: American Oil Chemists' Society

CLNA: Conjugated Linolenic Acids

CRI: Coronary Risk Index

CAT: Catalase

CLA: Conjugated Linoleic Acids

COVID-19: Corona Virus Disease 2019

DHA: docosahexaenoic acid

EPA: eicosapentaenoic acid

PUFA: Polyunsaturated Fatty Acid

SOD: Superoxide Dismutase

Declarations

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provided the reagents for the conduct of this study

Ethics approval and consent to participate

The experimental procedures described below were approved by the institutional animal research committee of the Faculty of Sciences of the University of Bamenda (Cameroon).

Consent for publication

Not applicable.

Data Availability

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

Conflict of interest

The authors declare that they have no conflicts of interest related to the publication of this study

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Authors' contributions

SOH NDE Florent: Investigation, Formal analysis. **GHOMDIM NZALI Horliane**: Conceptualization, Methodology, Writing – review & editing. **EJOH ABA Richard**: Supervision. All authors read and approved the final manuscript.

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Figures

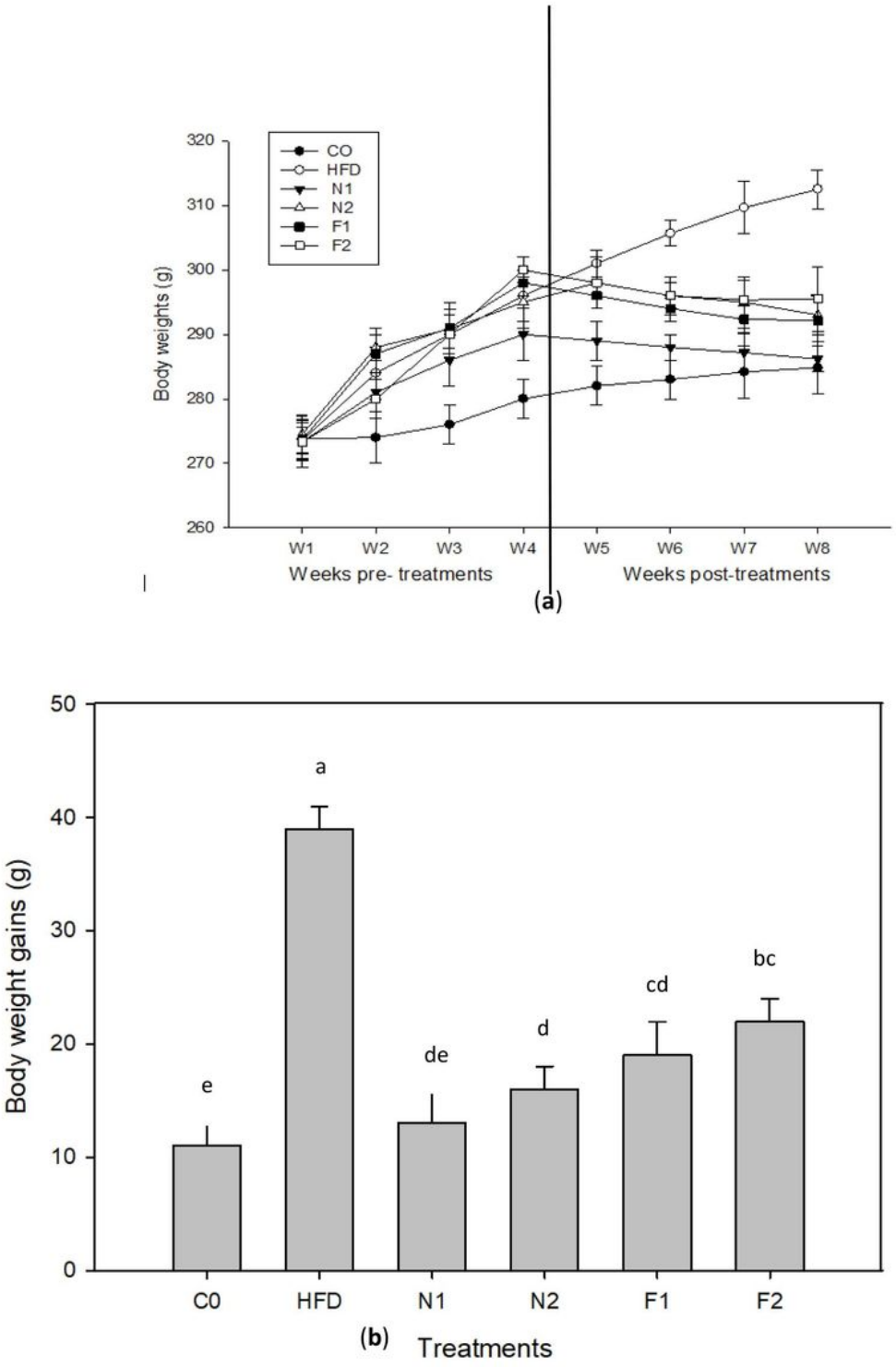


Figure 1

Body weight changes: (a) kinetic weight changes; (b) total weight gains for 8 weeks of rats after gavage with njansan and sardine fillets oils

Means ±SD (n=6) followed by different letters are significantly different ($P < 0.05$) as determined by Duncan's multiple range test. C0: normal control; HFD: Hyperlipidaemic group; N1: 1g/kg body weight of

Njansan oil group, N2: 2g/kg body weight of Njansan oil group; F1: 1g/kg body weight of Sardine fish oil, F2: 2g/kg body weight of Sardine fish oil

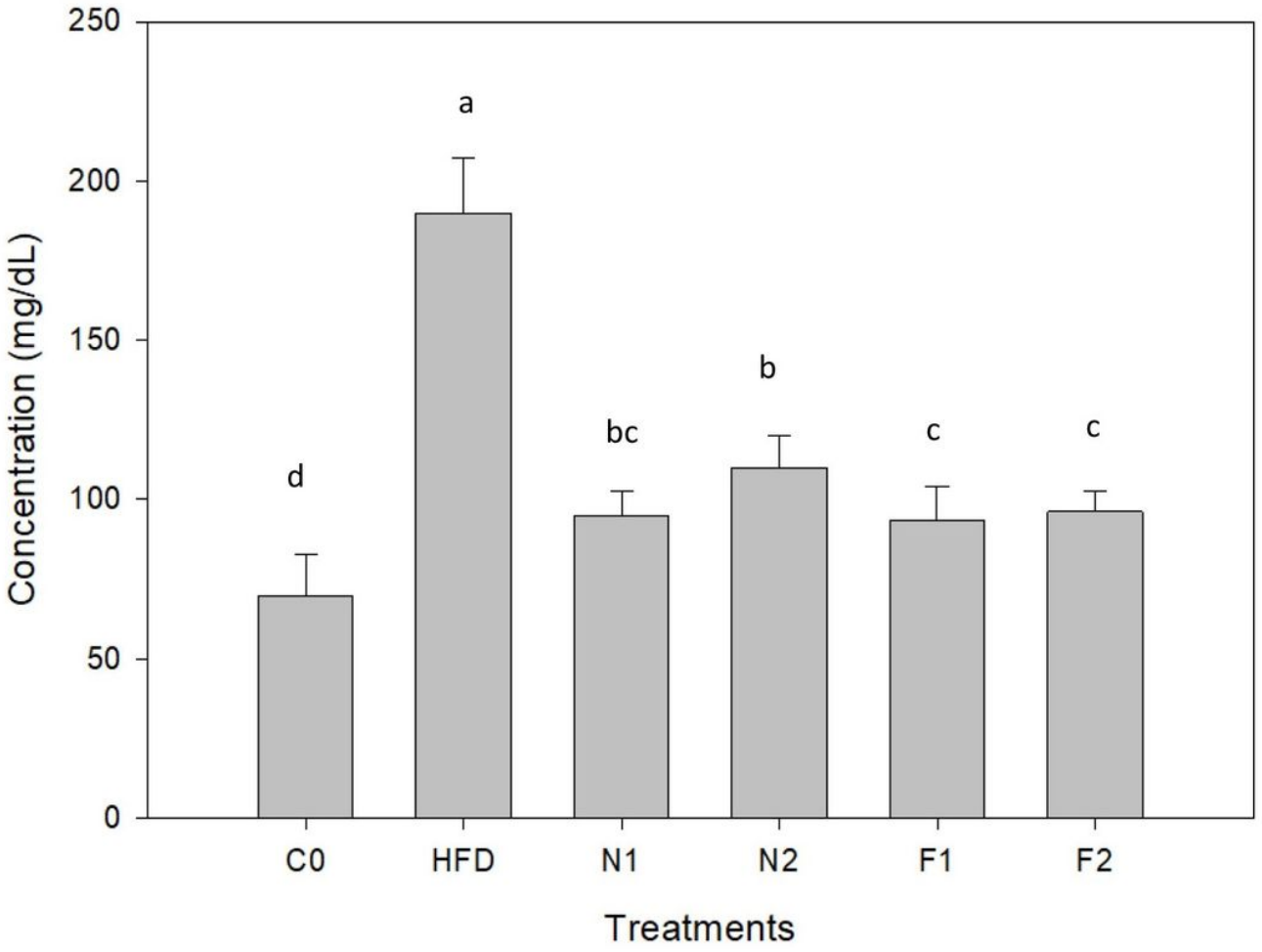


Figure 2

The effect of njansan oil and sardine fish oil on the triglyceride level of obese rats

Means ±SD (n=6) followed by different letters are significantly different ($P < 0.05$) as determined by Duncan's multiple range test. C0: normal control; HFD: Hyperlipidemic group; N1: 1g/kg body weight of Njansan oil group, N2: 2g/kg body weight of Njansan oil group; F1: 1g/kg body weight of Sardine fish oil, F2: 2g/kg body weight of Sardine fish oil

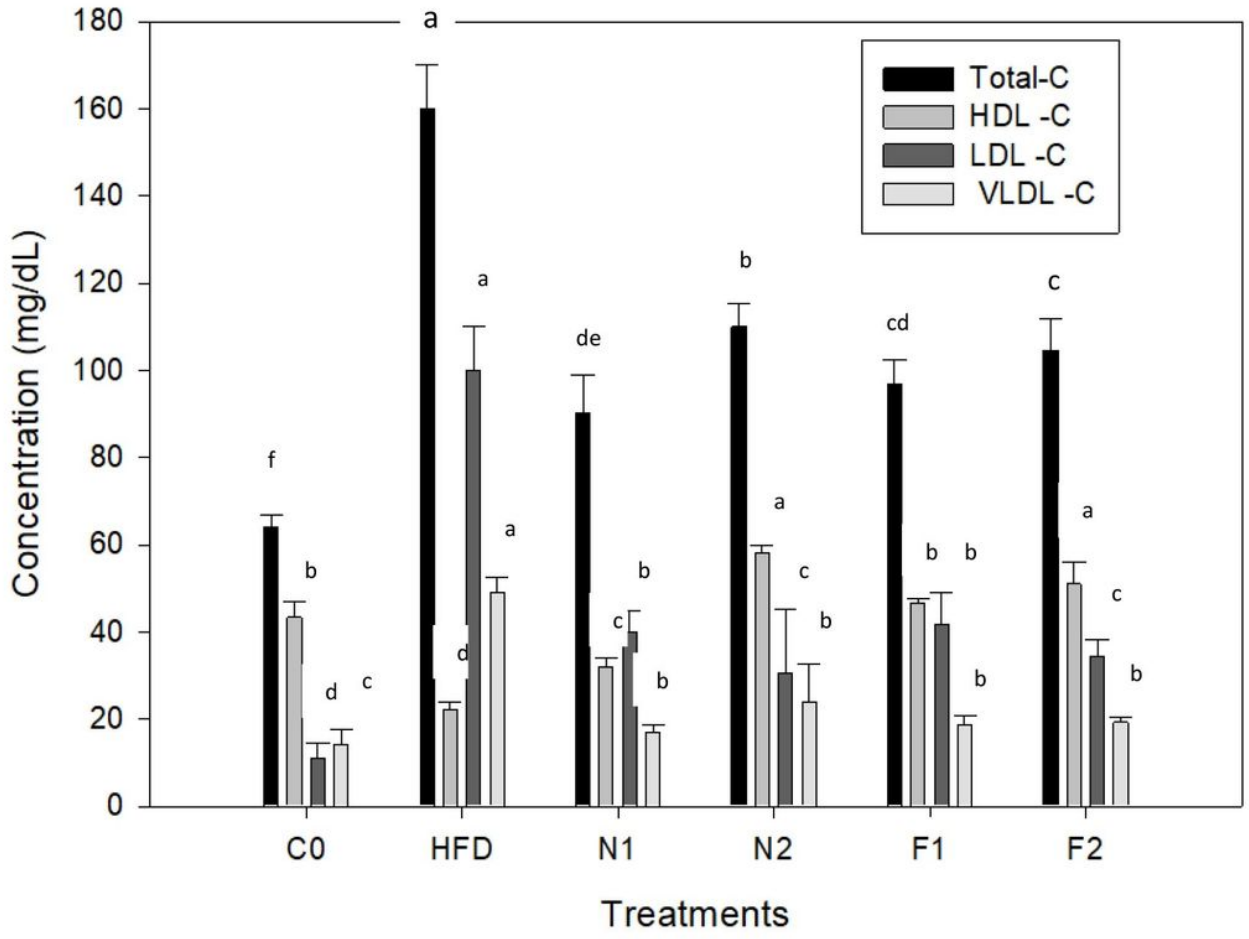


Figure 3

Effect of njansan oil and sardine fish oil on Total cholesterol, HDL, LDL and VLDL levels in the blood serum of hyperlipidemic rats fed a high-fat diet.

Means ±SD (n=6) for histograms with the same color, followed by different letters are significantly different ($P < 0.05$) as determined by Duncan's multiple range test. C0: normal control; HFD: Hyperlipidemic group; N1: 1g/kg body weight of Njansan oil group, N2: 2g/kg body weight of Njansan oil group; F1: 1g/kg body weight of Sardine fish oil, F2: 2g/kg body weight of Sardine fish oil

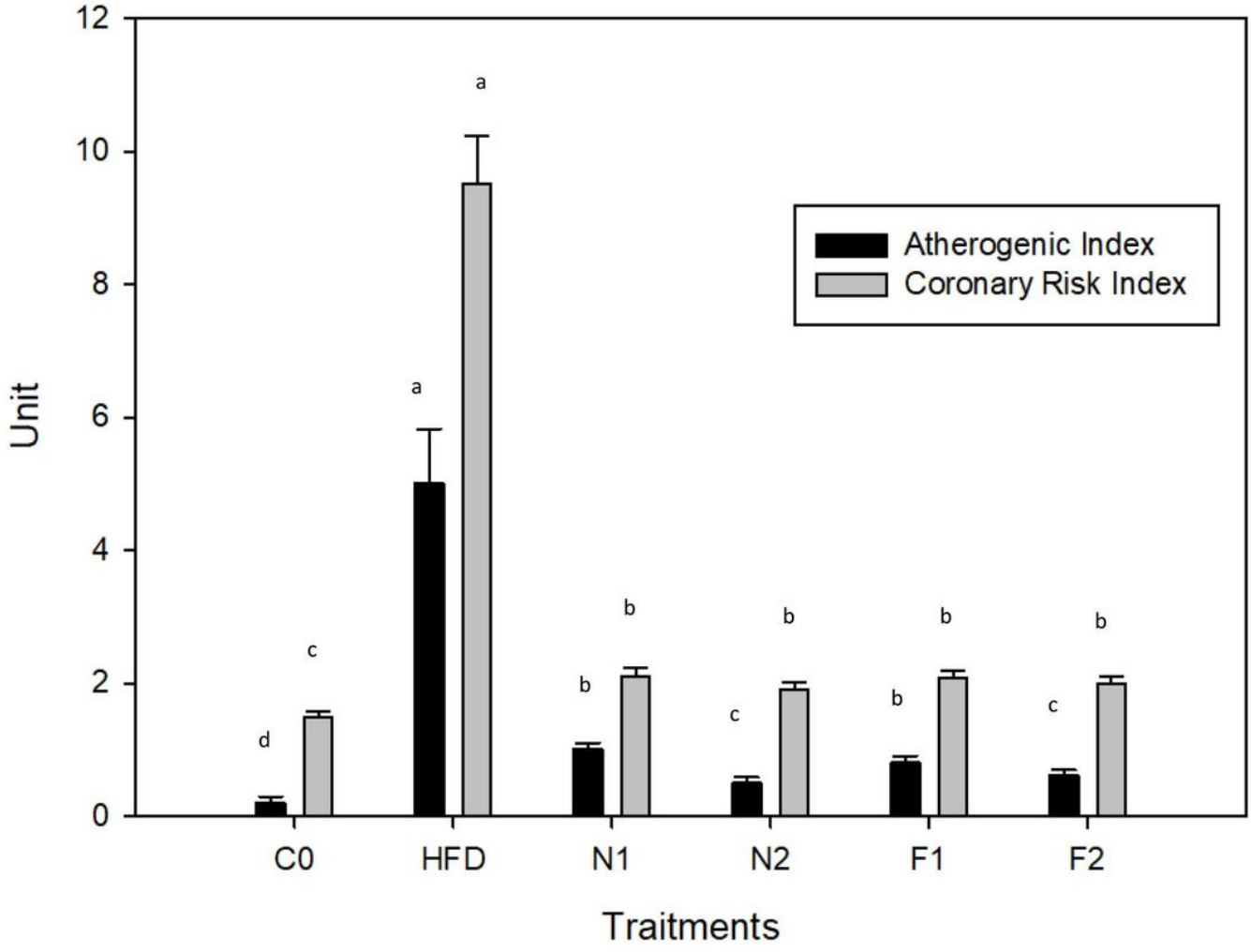


Figure 4

Effect of sardine fish oil and njansan oil consumption on the AI and CRI of obese rats

Means ±SD (n=6) for histograms with the same color, followed by different letters are significantly different ($P < 0.05$) as determined by Duncan's multiple range test. C0: normal control; HFD: Hyperlipidemic group; N1: 1g/kg body weight of Njansan oil group, N2: 2g/kg body weight of Njansan oil group; F1: 1g/kg body weight of Sardine fish oil, F2: 2g/kg body weight of Sardine fish oil

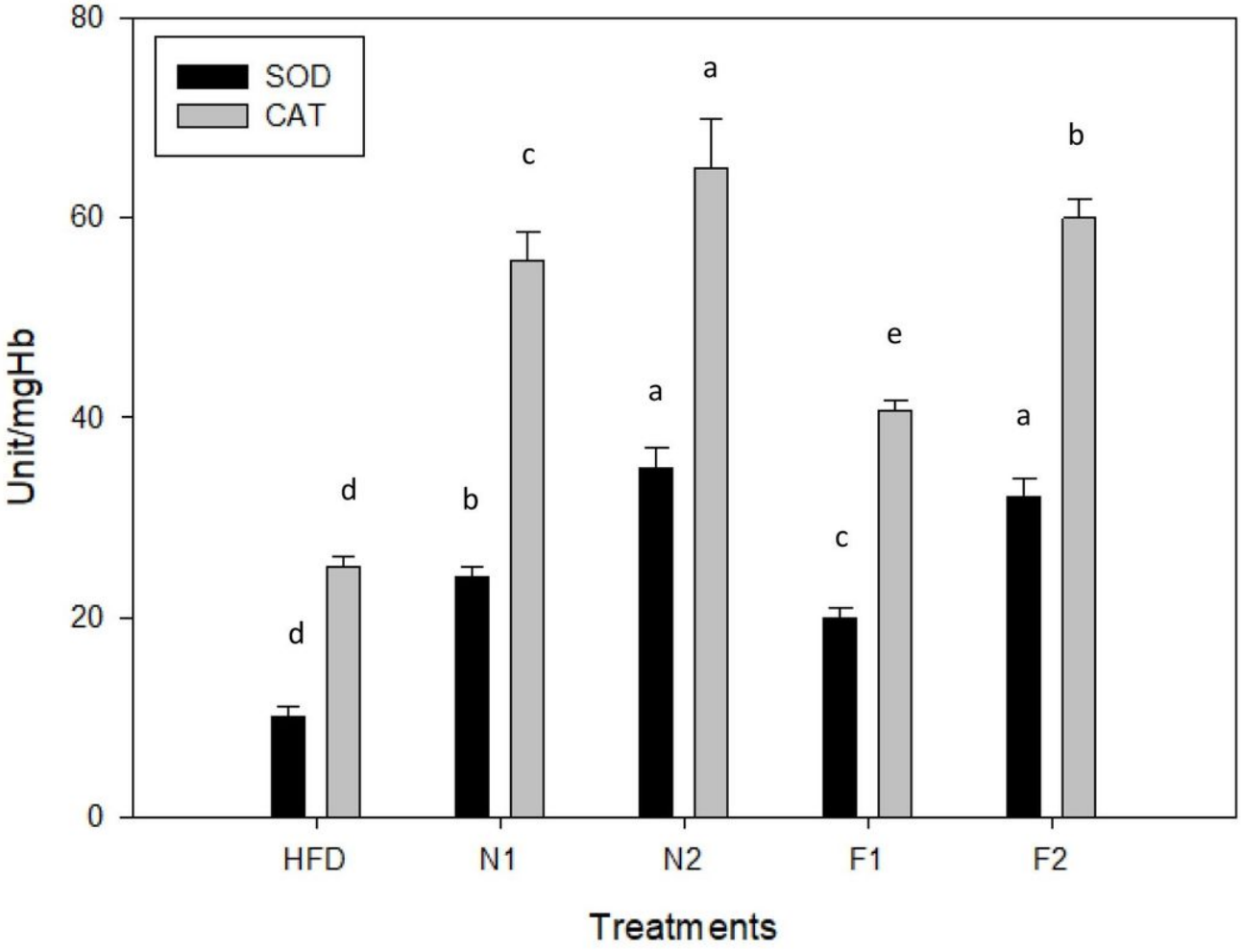


Figure 5

Consumption of njansan and fish oils affects catalase and superoxide dismutase activities in the serum of hyperlipidemic rats fed a high-fat diet.

Means ±SD (n=6) for histograms with the same color, followed by different letters are significantly different ($P < 0.05$) as determined by Duncan's multiple range test. C0: normal control; HFD: Hyperlipidemic group; N1: 1g/kg body weight of Njansan oil group, N2: 2g/kg body weight of Njansan oil group; F1: 1g/kg body weight of Sardine fish oil, F2: 2g/kg body weight of Sardine fish oil

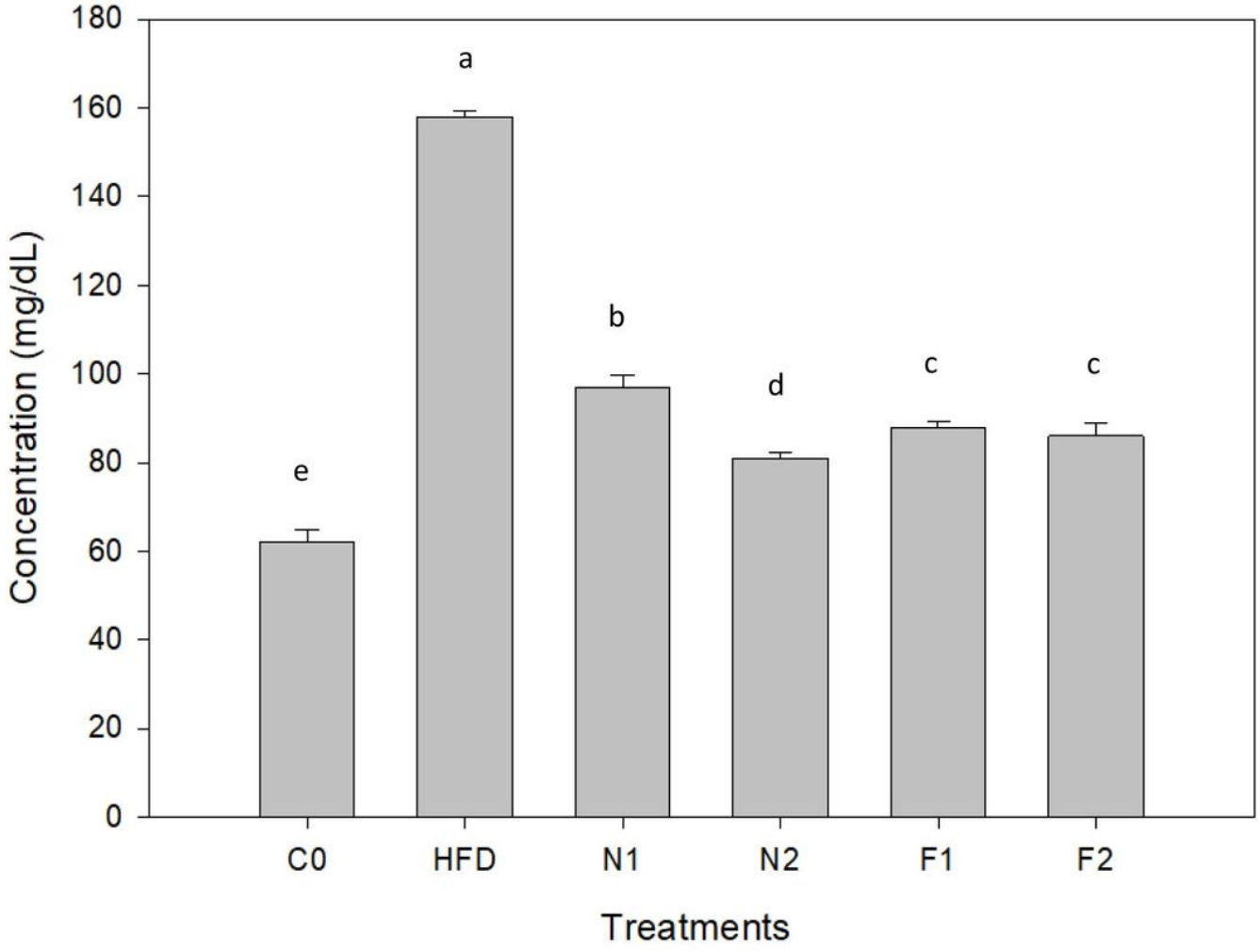


Figure 6

The effect of njansan and fish oils on the blood glucose of obese rats

Means ±SD (n=6) followed by different letters are significantly different ($P < 0.05$) as determined by Duncan's multiple range test. C0: normal control; HFD: Hyperlipidemic group; N1: 1g/kg body weight of Njansan oil group, N2: 2g/kg body weight of Njansan oil group; F1: 1g/kg body weight of Sardine fish oil, F2: 2g/kg body weight of Sardine fish oil