INTRODUCTION

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. Its prevalence worldwide has almost tripled since 1975. Currently, around one-third of the world’s overweight population is obese. 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% to 15.1%. Obesity is associated with nearly 200 adverse health outcomes, including type 2 diabetes, cardiovascular disease, and some cancers.

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

Soh Nde Florent¹, Ghomdim Nzali Horliane², Ejoh Aba Richard³, Tchiegang Clergé⁴

¹ Student, ² Professor, Department of Nutrition, Food and Bioresource Technology, College of Technology, The University of Bamenda, Bambili, Cameroon; ³ Assistant Professor, ⁴ Professor, Department of Food Process and Quality Control, Bioprocess Laboratory, University Institute of Technology (UIT), University of Ngaoundere, Adamaoua, Cameroon

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 into natural compounds to improve obesity has become increasingly important. Aims and Objectives: The aim of the study was to explore the effects of Sardina pilchardus and Ricinodendron heudelotii oils on the management of obesity. Materials and Methods: 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, six groups of six male Wistar rats were fed different diets: C0 group received a normal diet; HFD was fed a high-fat diet; N1, N2, F1 and F2 followed by a high-fat diet supplemented with njansan and sardine oils at 1 g/kg body weight/day and 2 g/kg body weight/day, respectively. Results: 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 superoxide dismutase. Conclusion: This study demonstrated that njansan oils at a low daily dose (1 g/kg body weight) can be helpful in managing obesity and also reduce the risk of developing coronary heart diseases.

Key words: Obesity; High-fat diet; Njansan oil; Sardine oil; Lipid profile; Antioxidant parameters
comorbidities, including cardio-metabolic disorders such as Type 2 diabetes, cardiovascular diseases, hypertension, dyslipidemia, gastrointestinal disorders, 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. It has also been reported to weaken COVID-19. The cause of obesity includes the high consumption of energy-dense foods as well as high-sugar diets with little or no physical activity.

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. Medications have also been used to manage obesity, but there are side effects and weight gain when medication is stopped. 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. Among them, different types of fatty acids have received much attention for health. 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. A high intake of fatty fish (sardines) rich in long-chain omega-3 fatty acids (eicosapentaenoic acid [EPA] [20:5 n-3] and docosahexaenoic acid [DHA] [22:6 n-3]), present in the oil of fish, was associated with an improvement of the lipid profile in humans and rats.

In Cameroon, due to their proliferation in the rivers and lakes of the Far North Region, the sardine (Sardina pilchardus) 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. 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, and trans-13-18:3) also have favorable effects on cardiometabolic health. 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. These almonds contain about 45–55% oil which is rich in eleostearic acid (47–52%). They are also rich in antioxidants such as tocopherol (178.3 ± 0.4 mg/100 g). Given its interesting composition, njansan oil has demonstrated its pharmacological effects in several studies. It reduces several risk factors for cardiovascular diseases. Moreover, previous work also showed that in rats and in Caco-2 cells, this fatty acid was metabolized to rumenic acid.

Oils extracted from njansan kernels and sardines were found to be healthy and nutritionally adequate.

**Aims and objectives**

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.

*Sardina 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 ×5, 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 h 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.  

Physicochemical properties
The oils were characterized to see the impact of the extraction method on the quality of the oils. Hence, the acid value, peroxide value, and iodine value of fish oil and njansan oil were measured according to the method of AOCS.

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 farmhouse of the University of Bamenda (North West, Cameroon). The animals were maintained in a standard environmental atmosphere (at a 12-h 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, six 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 hyper 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.

Induction of hyperlipidemia in the rats was achieved by feeding HFD formulated according to the protocol described by Aïssatou et al., with some modifications. The detailed formulations of the different diets are given in Table 1. All diets were given to rats ad libitum.

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) and gavaged (1 g/kg body weight/day) of water for the past 4 weeks
- Group 2: Hyper (HFD); received HFD all over the experimental period (8 weeks) and gavaged (1 g/kg body weight/day) of water for the past 4 weeks
- Group 3 (N1); received HFD all over the experimental period (8 weeks) and gavaged (1 g/kg body weight/day) of njansan oil for the past 4 weeks
- Group 4 (N2); received HFD all over the experimental period (8 weeks) and gavaged (2 g/kg body weight/day) of njansan oil for the past 4 weeks
- Group 5 (F1); received HFD all over the experimental period (8 weeks) and gavaged (1 g/kg body weight/day) of fish oil for the past 4 weeks
- Group 6 (F2); received HFD all over the experimental period (8 weeks) and gavaged (2 g/kg body weight/day) of fish oil for the past 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 h) 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 to determine their obesity status. The Lee index for each rat was calculated using the formula of Lee:

\[ \text{Lee index} = \sqrt{\frac{\text{Weight (g)}}{\text{Naso-anal length (cm)}}} \]

Blood and sample collection
At the end of the experiment, the animals were fasted for 12 h but had free access to drinking water. The rats were then anesthetized using diazepam (10 mg/kg) and sacrificed by decapitation. Whole blood was drawn into tubes and stabilized for between 15 and 30 min to ensure that 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

---

Table 1: Detailed composition of experimental rat diets

<table>
<thead>
<tr>
<th>Food components</th>
<th>Ingredients</th>
<th>Control diet (%)</th>
<th>High fat diet (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteins</td>
<td>Fish meal</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>Corn starch</td>
<td>59</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Sucrose</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Lipids</td>
<td>Soya bean oil</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Egg yolk*</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Palm oil**</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Others</td>
<td>Cellulose</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Vitamin mix</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Mineral mix</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

*Egg yolk: 300 g, **Coconut oil: 25 g
were analyzed by kit assay methods (Enzopak, India) as per the manufacturer's instruction. Low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein (VLDL-C) were calculated using the formula:

\[
\text{VLDL-C (mg/dL)} = \text{TG} \div 5^{34}
\]

\[
\text{LDL-C} = \text{TC} - (\text{HDL-C} + \text{VLDL-C})^{34}
\]

Atherogenic index (AI) and coronary risk index (CRI) were calculated using the formula:

\[
\text{AI} = \text{LDL-C} \div \text{HDL-C}, \text{CRI} = \text{TC} \div \text{HDL-C}^{35}
\]

**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 and Fridovich.\(^{36}\) 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 s and 80 s at 480 nm. The specific activity of SOD was expressed in units/mg of blood serum used.

**Catalase (CAT) activity**

The CAT activity was determined by a previously described method.\(^{37}\) 300 µL of H\(_2\)O\(_2\) (0.9%) 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 homogenizing, the ODs were read at 240 nm at 30, 60, and 90 s. The enzymatic activity of CAT 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, 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 analysis of variance. 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\(_2\)/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.

**Effect of high fat diet and different oil treatments on anthropometrical parameters**

During the first 4 weeks of the protocol, the animals received food without treatment. In Figure 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 (Figure 1b). During the second experimental period (5–8 weeks), weight gains were significantly reduced (P<0.05) in groups N1, N2, F1, and F2 compared to the HFD control (Figure 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 >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

<table>
<thead>
<tr>
<th>Table 2: Some physicochemical parameters of <em>Ricinodendron heudelotii</em> almond oils and <em>Sardina pilchardus</em> fillets oils</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Some parameters</strong></td>
</tr>
<tr>
<td><strong>R. heudelotii</strong></td>
</tr>
<tr>
<td>Acid value (mg KOH/g oil)</td>
</tr>
<tr>
<td>Iodine value (g I(_2)/100 g oil)</td>
</tr>
<tr>
<td>Peroxide value (meq O(_2)/kg of oil)</td>
</tr>
</tbody>
</table>

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

**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.

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. However, 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.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>End</td>
</tr>
<tr>
<td></td>
<td>End</td>
<td></td>
</tr>
<tr>
<td>C0</td>
<td>2964±1b</td>
<td>2972±a</td>
</tr>
<tr>
<td>HFD</td>
<td>2964±1b</td>
<td>3243±a</td>
</tr>
<tr>
<td>N1</td>
<td>305±3a</td>
<td>325±a</td>
</tr>
<tr>
<td>N2</td>
<td>301±5b</td>
<td>335±a</td>
</tr>
<tr>
<td>F1</td>
<td>302±11ab</td>
<td>332±a</td>
</tr>
<tr>
<td>F2</td>
<td>295±3a</td>
<td>320±1b</td>
</tr>
</tbody>
</table>

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

| Table 4: Effect of njansan and sardine oils oral administration on food intake and adiposity, hepatosomatic, and viscerosomatic indices of rats fed with high fat diet |
|----------|------------------|-----------------|
| Group    | Food intake/g/day/rat | Adiposity index | Parameters |
|          |                   |                 | Liver       | Heart       | Kidney    |
| CO       | 30.0±1.6         | 1.65±0.47      | 2.69±0.68a  | 0.93±0.15a  | 2.25±0.38a |
| HFD      | 26.15±2.01       | 3.69±0.87a     | 4.68±0.76a  | 1.14±0.19a  | 2.70±0.30a |
| N1       | 22.08±1.81       | 2.78±0.79a     | 2.98±0.18a  | 1.03±0.04a  | 2.45±0.26a |
| N2       | 21.10±2.11       | 2.78±0.75a     | 3.17±0.42a  | 1.07±0.13a  | 2.57±0.16a |
| F1       | 22.24±1.6b       | 2.40±1.1b      | 2.98±0.17b  | 1.04±0.11b  | 2.26±0.12b |
| F2       | 21.58±1.75       | 2.25±0.36b     | 2.97±0.16b  | 1.06±0.05b  | 2.46±0.21b |

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. Co: Normal control; HFD: Hyperlipidaemic group; N1: 1 g/kg body weight of Njansan oil group, N2: 2 g/kg body weight of Njansan oil group; F1: 1 g/kg body weight of Sardine fish oil, F2: 2 g/kg body weight of Sardine fish oil.
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±17.40 mg/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 (Figure 2).

**TC, HDL-C, LDL-C, and VLDL-C**

The serum lipid profile of rats that were fed different diets is shown in Figure 3. Among the circulating lipid fractions, the TC, 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 (Figure 3).

The HFD significantly reduced the concentration of serum HDL-C (22.01±2.01 mg/dL) as compared to the control group (43.35±3.46 mg/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 (Figure 3).

**Effect of sardine fish oil and *Njansan* oil on atherogenic and coronary risk indices**

The results of HFD and oil treatment on the AI and CRI of rats are shown in Figure 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 CAT)**

Figure 5 shows the changes in CAT and SOD in rats fed with HFD and after treatment with oils. For the HFD group, the CAT 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 50–135 mg/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 Figure 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 However, 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 (Figure 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.19,43-45 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 (Table 3)
following treatment with oils this may likely be due to a decrease in adipose tissue associated with accumulated energy expenditure through thermogenic pathways to prevent excessive accumulation of fat.\textsuperscript{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.\textsuperscript{43} Sharma and Agnihotri\textsuperscript{45} 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 through Sterol Response Element-binding Protein 1c, 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 in depots of adipose tissue and therefore increases thermogenesis.\textsuperscript{46} This is consistent with the data reported by Haimeur\textsuperscript{47} using fish oil and argan oil; the same is true of the work of Yuan et al.,\textsuperscript{22} Machado et al.,\textsuperscript{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 1st 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 lipemia and prevented hepatic steatosis by lowering lipid metabolism in the liver.\textsuperscript{49} Chiu et al.\textsuperscript{50} also reported similar results with 5% fish oil. However, the work of Arao et al.,\textsuperscript{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.\textsuperscript{22} The differences in TG levels among rats that received treatment with both oils (Figure 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.\textsuperscript{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 ω-3 eicosatetraenoic 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.,\textsuperscript{27} with R. bendelotii incorporated at 5% in the rats’ diet. Haimeur et al.,\textsuperscript{47} and Lima et al.,\textsuperscript{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.,\textsuperscript{51} Yuan et al.\textsuperscript{22} using punicic acid. A diet rich in saturated fatty acids leads to an increase in plasma levels of TC and LDL-c and increases the risk of developing three symptoms of syndrome-X: low HDL-cholesterol, high TC, and high triglycerides.\textsuperscript{35,36} 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 (Figure 3). The normalization of the hyperlipidemic state observed after treatment of the rats with sardine and R. bendelotii 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.\textsuperscript{57} Franczyk-Zarów et al.,\textsuperscript{58} showed that plasma levels of TC 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 (Figure 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 and Agnihotri\textsuperscript{45} and Da Cunha de Sá et al.\textsuperscript{18}

Obesity is closely associated with oxidative stress. It was reported that SOD and CAT are the two most important enzymes of the enzymatic antioxidant defense system.\textsuperscript{39} 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.\textsuperscript{40} The result of this study suggests effective protection of R. bendelotii and sardine oils
against the generation of free radicals. This protection is proportional to the oil concentration and is more marked with njansan oil (Figure 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/100 g) and phytosterol content (for njansan oil), which have scavenging capacity free radicals and reduce lipid peroxidation.\textsuperscript{61-63} These results corroborate those of Lima et al.,\textsuperscript{64} who showed an increase in SOD activity after fish oil supplementation. The same is true for the results of Yuan et al.,\textsuperscript{65} who found CAT and glutathione peroxidase 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.,\textsuperscript{66} confirmed that in Wistar rats, a high-lipid diet consumed for 2 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 (Figure 6). This may be due to increased free fatty acids in plasma, which reduces insulin-regulated glucose metabolism.\textsuperscript{67} This is similar to a study done by Roza et al.,\textsuperscript{68} It is also similar to the results of Daidj and Lamri-Senhadji\textsuperscript{69}, 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 PPAR\textgamma and significantly decrease glucose levels in serum.\textsuperscript{68,69} Saha and Ghosh\textsuperscript{21} 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.,\textsuperscript{51} Franczyk-Zarów et al.,\textsuperscript{70} who showed no significant changes in serum glucose after feeding rats with HFD and supplementing with fish oil and pomegranate seed oil, respectively.

**Limitations of the study**

The limitation of this work is that the experimentation time was short (8 weeks).

**CONCLUSION**

Supplementation with *S. pulchardus* oil and especially *R. bendelotii* 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 (1 g/kg body weight) may be useful for the management of obesity and also reduce the risk of developing coronary heart disease.

**ACKNOWLEDGMENT**

The authors wish to thank Bioprocess Laboratory of IUT (University of Ngaoundere) and the Department of Nutrition, Food and Bioresource Technology of College of Technology (University of Bamenda) which provided the reagents for the conduct of this study.

**REFERENCES**


33. Lee MO. Determination of the surface area of the white rat with its application to the expression of metabolic results. Am J Physiol Content. 1929;89(1):24-33. https://doi.org/10.1152/ajplegacy.1929.89.1.24


https://doi.org/10.1093/ajcn/67.3.505S


68. Oh DY, Talukdar S, Bae EJ, Imamura T, Morinaga H, Fan W, et al. GPR120 is an omega-3 fatty acid receptor mediating...


Authors' Contributions:
SNF- Investigation and formal analysis; GNH- Conceptualization, methodology, and writing - review and editing. EAR and TC- Supervision and writing - review and editing. All authors read and approved the final manuscript.

Work attributed to:
Department of Food Process and Quality Control, Bioprocess Laboratory, University Institute of Technology (UIT), University of Ngaoundere, Adamaua, Cameroon.

Orcid ID:
Soh Nde Florent- https://orcid.org/0009-0001-8255-1281
Ghomdim Nzali Horliane- https://orcid.org/0000-0002-7079-426X
Ejoh Aba Richard- https://orcid.org/0000-0002-6852-2285
Tchiegang Clerge- https://orcid.org/0000-0001-9528-7718

Source of Funding: None, Conflicts of Interest: None.