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Research Article

In vivo Antihyperlipidemic and Antioxidant Effect of Oil Extracted from *Sardinella maderensis* (Lowe, 1838) on Strain *Wistar* Rats

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Abstract

Cardiovascular diseases (CVD) are one of the leading causes of death and disability, the main cause of which is hyperlipidemia. This work aimed to evaluate the antihyperlipidemic and antioxidant *in vivo* potential of oil extracted from *Sardinella maderensis* on rats. The oil was extracted according to the method of Bligh and Dyer and underwent chemical analysis prior to in vivo assays. After studying *in vivo* the acute toxicity of *S. maderensis* oil, their antihyperlipidemic was assessed. Twenty-four male *Wistar* adult rats were randomly divided into four groups of 6 rats each. During a three-week experiment, group 1 was fed with standard laboratory diet (SLD); group 2 received SLD supplemented with boiled egg yolk (5 g/day/rat); group 3 was fed with SLD supplemented with *S. maderensis* oil (1 g/day/kg of body mass) and group 4 was fed with SLD supplemented with boiled egg yolk and *S. maderensis* oil. Liver, lung, kidney, adipose tissues and heart were later removed, weighted and analyzed. Some blood biochemical and oxidative stress parameters were also measured. Results showed that *S. maderensis* oil was siccative, good quality with a lethal dose greater than 5000 mg/Kg of CP and no signs of toxicity were observed. Hyperlipidemic diet increased significantly ($p < 0.05$) lipid profil, glycemia, uremia, activity of transaminase and γGT, oxidative stress in group 2 compared to other groups. Supplementation with *S. maderensis* oil significantly ($p<0.05$) reduced Lee's index, weight gain and BMI by 8.12%, 26.33% and 19.11% respectively in group 4 compared with group 2. Supplementation with *S. maderensis* oil decreased significantly ($p < 0.05$) total-cholesterol, LDL-cholesterol, triglyceride, glycemia, proteinemia and increased levels of HDL-cholesterol in group 4 compared to group 2. Supplementation with *S. maderensis* oil significantly ($p < 0.05$) reduced TBARS levels by 54.36% and significantly ($p < 0.05$) increased GSH levels, SOD, CAT and PON-1 activity by 64.90%, 20.76%, 48.70% and 7.47 respectively in group 4 compared to group 2. This study shows that *S. maderensis* oil can be used in prevention of hyperlipidemia.

Keywords

Sardinella maderensis, *Wistar* Rats, Antihyperlipidemia, Oxidative Stress

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1. Introduction

Eating is a natural part of life. However, poor eating habits have led to unbalanced diets, resulting in a variety of nutritional pathologies, including obesity, which has attracted our attention. Obesity has become a pandemic that affects both emotional well-being and the general physique [1]. The prevalence of obesity has continued to rise worldwide. In 2016, more than 1.9 billion adults were overweight. Of these, 650 million were obese. Obesity affects 13% of the world's adult population, and this rate could rise to 20% by 2025. 124 million children under the age of 5 are obese worldwide. In Africa, the number of overweight or obese children has risen by almost 50% since 2000. Overweight among children aged 0-59 months was estimated at 6.7% in Cameroon, compared with 4.1% in Africa as a whole. The prevalence of obesity in Cameroon rose from 5.6% in 2005 to 8.6% in 2014 and 9.6 in 2016 [2]. Obesity is the fifth leading cause of death in the world. Hyperlipidaemia and hyperglycaemia are responsible for the overproduction of reactive oxygen species (ROS) due to the saturation of the electron transport chain, which can lead to insulin resistance [3]. Oxidative stress (OS) is one of the main risk factors for metabolic diseases. OS is defined as an imbalance in the pro-oxidant/antioxidant balance in favour of the oxidants, leading to significant cellular damage [4]. ROS are key elements in the obesogenic process by stimulating adipogenesis. Several studies have shown the relationship between obesity, arteriosclerosis, SO and cardiovascular diseases (CVD) [5-7]. Cardiovascular diseases (CVD) are one of the leading causes of death and disability, the main 36 cause of which is hyperlipidemia [8]. CVD were the cause of 17.7 million deaths worldwide in 37 2017. It's expected that by 2030, they will be the leading cause of death and disability 38 worldwide. In Cameroon, 4 million people suffered from CVD in 2011, one of the main causes 39 being dyslipidemia and obesity [2]. Various treatments are used to remedy these pathologies, including chemotherapy, phytotherapy and surgery. However, these treatments are expensive and have local or systemic side effects [9]. Research into treatments is now focusing on natural substances. Nutritherapy, based on the use of food in treatment or prevention, could be one of the best strategies for combating obesity and oxidative stress. Nutritherapy is accessible to the vast majority of people, is less expensive and less toxic, and consists of a healthy, balanced diet with therapeutic properties. The use of fish oils in the treatment and prevention of certain diseases has been the subject of several studies [10-13]. Fish is particularly sought after for its richness in proteins, minerals, vitamins and lipids, with polyunsaturated fatty acids, particularly omega-3s, predominating. These have therapeutic properties, including anti-inflammatory and antihyperlipidaemic effects, cognitive development, hepatoprotective effects, prevention and treatment of cardiovascular disease, and anti-diabetic effects [14-16]. World fish production is estimated at 178 million tonnes, with a consumption of 20.5 kg/inhabitant/year [17]. Cameroon has a coastline of 402 km along which artisanal and industrial fisheries are practised. Annual fish production in Cameroon was estimated at 252,764 tonnes in 2016, with an average consumption of 19.4 kg/year/inhabitant [18]. A variety of products are derived from these fisheries, such as *Sardinella maderensis*, commonly known as "Strong Kanda" and locally known as Belolo. *Sardinella maderensis* belongs to the *Clupeidae* family. The *Clupeidae* are pelagic species native to the coasts, beaches, lagoons and estuaries of West Africa. They are distributed along the tropical zone of the Atlantic Ocean. Some prefer shallow water. They feed on phytoplankton, benthic invertebrates, fish, detritus and fish eggs [19]. These fish are easily accessible to all social classes and play an important role in the fishing catch and diet of Cameroonians. The therapeutic properties of *Sardinella maderensis* oil have not yet been investigated. This study contributes to the valorization of fishery products from the Cameroonian coasts by evaluating the antihyperlipidemic effect of oil extracted from *Sardinella maderensis* on rats.

2. Material and Methods

2.1. Fish Sample Collection

The fresh fish was bought at the fishing port of Douala as soon as the fishermen returned from the sea. Identification of fish was done by the veterinary service of the Ministry of Livestock, Fisheries and Animal Industries of Cameroon, using FAO fish identification sheets. After purchase, the fish were transported to the laboratory in a cooler containing ice at a fish / ice ratio of 1/2. At the laboratory fish were rinsed with distilled water. An ichtyometer and a precision balance were used respectively to measure the length and weight of each fish. Average weight and length of the forty fish used in this study were 216.72 ± 3.05 g and 24.50 ± 2.37 cm respectively.

2.2. Fish Oil Extraction and Chemical Indexes Analyses

Fish oil was extracted from the fish fillets according to the method of Bligh and Dyer [20]. Iodine, acid, peroxide, anisidine and thiobarbiturate values were then determined using AOAC method [21]. The total oxidation (TOTOX) values of oil samples were determined using the equation (1) according to Shahidi and Wanasundara [22].

$$TOTOX = [2(\text{peroxyde index}) + (\text{Anisidin index})] \quad (1)$$

2.3. Experimental Animals

Female *Wistar* rats were used for acute toxicity because they are more sensitive than males. To avoid female hormonal influences, analysis on antihyperlipidemic was done on 2.5 months old rats. The animal room was lit for 12 h a day with a temperature of 25 ± 3 ℃. The initial morphometric parameter of rats were: body weight (BW) (151.49 ±2.63 g), body mass index (BMI) (0.50 ±0.02 g/cm^2), Lee index (LI) (308.14 ±3.27 g/cm^3) and naso-anal length (NAL) (17.30 ±1.40 cm). All the experiments were conducted in accordance with the internationally accepted guidelines for experimental animals used and the study was approved by University of Douala Institutional Ethics Committee (N ° 2714 CEI-Udo/06/2021/M).

2.4. Acute Toxicity

The acute toxicity test was performed using the protocol described by OECD No. 425 [23]. The study was conducted on twenty *Wistar*-bred female rats aged 2.5 months and weighing 174.82 ±6.81 g. This protocol recommends a single dose of 2000 mg/kg body weight. The twenty female rats were divided into 4 groups either negative control that received 1ml of distilled water. Test 1 received oil extracted from *Sardinella maderensis* at dose of 2000 mg/kg. Test 2 received oil extracted from *Sardinella maderensis* at dose of 4000 mg/kg. Test 3 received oil extracted from *Sardinella maderensis* at dose of 5000 mg/kg. During this period, signs of toxicity including change in coat, motility, tremors, grooming, breathing, sensitivity to noise, appearance of faeces, mobility as well as the number of deaths were documented. At the end of the study, after 12 hours of fasting, the female rats were weighed, anaesthetized with ketamine (50mg/Kg body weigh) and then sacrificed. The blood collected were recovered in dry tubes and centrifuged at 3000 rpm for 15 min. The serum obtained allowed us to measure the biochemical parameters. The levels of Total proteins, Creatinine, Alanine Aminotransferase (ALT) and Aspartate aminotransferase (AST) were determined using SGM italia kits.

2.5. Experimental Design

Twenty-four male rats *Wistar* albinos were used for animal experiments. They were divided into 4 groups of 6 rats each and housed at an ambient temperature of 25 ± 3 ℃. Throughout the experiment they were given food and water ad libitum and were displayed as follows: group 1 received only standard laboratory diet (SLD). Group 2 were fed with SLD supplemented with boiled egg yolk (BEY) at a dose of 5 g/day/rat. Groups 3 received SLD supplemented with fish oil at a dose of 1 g/day/kg of body weight. Groups 4 received SLD supplemented with BEY at a dose of 5 g/day/rat and fish oil at the dose of 1 g/day/kg of body weight [11]. The experiments lasted for 21 days during which body weight and food intake were recorded weekly. SLD consisted of 67.8 g of corn flour, 20 g of soy flour, 10 g of fishmeal, 0.01 g of corn oil, 1 g of shellfish flour, 1 g of kitchen salt and 0.01 g of vitamin complex. At the end of the study, the rats were subjected to a fasting period of 12 h, weighed, anesthetized with ketamine (50 mg/kg body weight) and then killed. Blood samples were collected in either dry or EDTA containing tubes. Thirty minutes later, blood was centrifuged at 3000 rpm for 15 min. Plasma and sera collected were used for various biochemical analysis. Liver, visceral and abdominal adipose tissues, kidneys, lungs and heart were removed, rinsed in physiological saline solution, weighed, observed and their relative.

2.6. Food and Water Intake

The evaluation of the average of food and water intake was recorded daily by subtracting the quantity of remaining food every day from the initial quantity provided the previous day.

2.7. Determination of Body Mass Index, Lee Index, and Metabolic Efficiency Index

During the experiment, body weight and naso-anal length of each animal was measured once per week in order to document growth. BMI and LI were obtained by equation (2) and equation (3) respectively. Metabolic efficiency index was calculated as equation (4) [23].

$$\text{Body mass index (g/cm}^2\text{)} = \text{Body mass} / (\text{Naso-anal length})^2 \quad (2)$$

$$\text{Lee index (g/cm}^3\text{)} = [\sqrt[3]{\text{Body mass}} / \text{Naso-anal length}] \times 1000 \quad (3)$$

$$\text{Metabolic efficiency index} = \text{Body weight gain} / \text{Food intake} \quad (4)$$

2.8. Analysis of Biochemical Parameters

All assays were performed in triplicate. Sera underwent determination of total cholesterol (TC), HDL cholesterol (HDL-C), LDL cholesterol (LDL-C), triglycerides (TG), total protein, alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, creatinine, alkaline phosphatase (ALP), Gamma glutamyltranspeptidase (γGT) using SGM italia kits. Plasma glucose was measured using SGM italia kit [12].

2.9. Analysis of Oxidative Stress Markers

The determination of oxidative stress was done by measurement of thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD) and paraoxonase 1 (PON-1) by the method reported by Amal [25].

2.10. Statistical Analysis

Data were expressed as mean ± standard deviation. One-way ANOVA was performed to test the differences between species. Significance was established at $P < 0.05$. Fisher's PLSD (Protected Least Significant Difference) (post hoc comparison test) was used to make comparisons between the different groups when the ANOVA p-value was significant. Statistical analyses were performed using SPSS 16.0 for windows (SPSS, Chicago, IL, USA).

3. Results

3.1. Chemical Indexes of Oil Extracted in Fresh Fish

Table 1 shows iodine value and oil degradation indexes such as acid, peroxide, anisidine, thiobarbituric acid and total oxidation index numbers.

Table 1. *Chemical indexes of fish oil Sardinella maderensis.*

Chemical indexes	*Sardinella maderensis* oil	Tolerated value
Iodine value (g I2/100g of oil)	154.83 ±2.05	
Acid value (mg KOH/g of oil)	1.26 ±0.07	3*
Peroxide value (meq O_2/Kg of oil)	0.94 ±0.02	5*
Anisidin value	0.13 ±0.05	20*
TBARS (mg of MDA/Kg of oil)	0.10 ±0.02	10*
Total oxidation value	2.02 ±0.03	26*

Meq: milli-equivalent; TBA: thiobarbituric acid number; MDA: malondialdehyde. The values are the means ± the standard deviation, n=3. (*): tolerated value defined by Codex AlimentarIus [26].

Table 2. *Variations of some biochemical markers after toxicity study.*

	NC	Test 1	Test 2	Test 3
ALAT (UI/L)	12.75 ±0.33^a	13.00 ±0.85^a	12.65 ±0.71^a	11.53 ±1.28^a
ASAT (UI/L)	11.65 ±0.49^a	12.23 ±0.68^a	11.59 ±0.85^a	10.77 ±0.82^a
Creatinine (mg/L)	3.22 ±0.18^a	3.06 ±0.28^a	2.86 ±0.36^a	3.31 ±0.54^a
Uremia (mg/dl)	20.11 ±0.90^a	20.13 ±1.12^a	20.30 ±1.27^a	22.51 ±0.73^a
Proteinemia (g/l)	50.08 ±2.26^a	53.17 ±1.81^a	49.73 ±2.60^a	52.49 ±1.04^a

NC: rats received 1ml of distilled water; Test 1: rats received oil extracted from *Sardinella maderensis* at dose of 2000 mg/kg; Test 2: rats received oil extracted from *Sardinella maderensis* at dose of 4000 mg/kg; Test 3: rats received oil extracted from *Sardinella maderensis* at dose of 5000 mg/kg. ASAT: aspartate aminotransferase; ALAT: alanine aminotransferase. The values are the means ± the standard deviation, n=6. Means assigned with different letters in same line are significant at $p < 0.05$.

3.2. Study of Acute Toxicity of Oil Extracted on Rats

Oral administration of a single dose of 2000, 4000 and 5000 mg/kg body weight of *Sardinella maderensis* oil did not cause any significant changes in the rat. No signs of toxicity such as respiratory difficulties, reduced sensitivity to pain or noise or locomotion were observed. After 14 days of observation, no deaths were observed in the rats fed the

fish oils, which meant that the LD_{50} could not be determined. Table 2 shows some biochemical markers after the acute toxicity study. The table shows that transaminase activity, serum creatinine, urea and protein levels did not vary significantly ($p > 0.05$) between the different groups.

3.3. Morphometric Parameters of Rats in Different Groups

The morphological parameters of the animals are shown in Table 3. This shows that food intake and naso-anal length, metabolic efficiency and relative weights of liver, kidney, heart and lung did not vary significantly ($p>0.05$) between the different groups. However, there was a significant increase ($p<0.05$) in weight gain, Lee index, BMI and relative adipose tissue weight in group 2 rats compared with rats in the other groups. *S. maderensis* oil supplementation significantly ($p<0.05$) decreased Lee index, weight gain and BMI by 8.12%, 26.33% and 19.11% respectively in group 4 compared with group 2.

Table 3. *Morphometric parameters on rats.*

	Group 1	Group 2	Group 3	Group 4
IBW (g)	151.66 ±1.52[a]	150.33 ±1.51[a]	151.33 ±2.08[a]	152.66 ±1.52[a]
FBW (g)	171 ±1[a]	175.33 ±2.04[a]	171.66 ±3.51[a]	171.33 ±1. 42[a]
GW (g)	19.33 ±1.18[a]	24.27 ±1.43[b]	19.38 ±2.24[a]	18.66 ±1.38[a]
NAL (cm)	17.9 ±1.45[a]	17.2 ±0.78[a]	17.81 ±0.39[a]	17.98 ±0.66[a]
LI (g/cm^3)	313.88 ±7.54[a]	339.36 ±3.31[b]	312.87 ±3.54[a]	311.78 ±5.05[a]
BMI (g/cm^2)	0.55 ±0.01[a]	0.68 ±0.01[b]	0.55 ±0.01[a]	0.55 ±0.02[a]
FI (g/j)	134.66 ±16.44[a]	140.33 ±15.63[a]	127 ±6.08[a]	128 ±14.73[a]
ME	0.14 ±0.02[a]	0.18 ±0.02[a]	0.14 ±0.03[a]	0.14 ±0.02[a]
Relative weight				
Liver	2.68 ±0.34[a]	2.50 ±0.25[a]	2.54 ±0.29[a]	2.76 ±0.20[a]
Adipose tissue	0.89 ±0.09[a]	2.38 ±0.18[b]	0.84 ±0.10[a]	0.94 ±0.07[a]
Lungs	0.82 ±0.15[a]	0.81 ±0.09[a]	0.74 ±0.05[a]	0.79 ±0.03[a]
Heart	0.27 ±0.04[a]	0.27 ±0.00[a]	0.28 ±0.02[a]	0.28 ±0.01[a]
Kidney	0.50 ±0.04 [a]	0.55 ±0.03[a]	0.55 ±0.03[a]	0.55 ±0.01[a]

Group 1: rats fed SLD. Group 2: rats fed SLD supplemented with BEY. Group 3: rats fed with the diet supplemented with *Sardinella maderensis* oil. Group 4: Rats fed SLD supplemented with BEY and *Sardinella maderensis* oil. IBW: initial body weight; FBW: final body weigh; FI: food intake; NAL: naso-anal length; LI: Lee index; BMI: body mass index; WG: weight gain; ME: metabolic efficiency. The values are the means ± the standard deviation, n=6. Means in the same line assigned with different letters are significant at $p < 0.05$

3.4. Effect of Oil Fish Supplementation on Some Serum Biochemical Parameters

Some of the biochemical parameters of the different groups of animals are shown in Table 4. The table shows that consumption of boiled egg yolk (BOY) significantly ($p<0.05$) increased TC, LDL-C, TG, blood glucose, blood protein, creatinine and transaminase activity (AST and ALT) in group 2 compared with the other groups. Consumption of egg yolk caused a significant reduction ($p<0.05$) in HDL-C in group 2 compared with the other groups. Administration of oil extracted from *Sardinella maderensis* significantly ($p<0.05$) reduced TC, LDL-C, TG, glycaemia, uraemia and the activity of transaminases (ASAT and ALAT) and γGT in group 4 compared with group 2. Administration of *S. maderensis* oil significantly ($p<0.05$) increased HDL-C in group 4 compared with group 2.

Table 4. *Values of some serum biochemical parameters of rats fed with the different diets.*

	Group 1	Group 2	Group 3	Group 4
TC (mg/dl)	84.42 ±3.93^{b}	112.68 ±3.28^{c}	67.99 ±5.40^{a}	87.57 ±1.81^{b}
TG (mg/dl)	56.56 ±4.25^{a}	90.26 ±3.84^{b}	54.85 ±5.75^{a}	58.49 ±5.89^{a}
HDL (mg/dl)	38.35 ±0.18^{b}	35.36 ±1.07^{c}	43.22 ±0.46^{a}	39.37 ±0.66^{b}
LDL (mg/dl)	34.75 ±4.48^{b}	59.27 ±3.83^{c}	13.80 ±5.15^{a}	36.50 ±2.36^{b}
Glycemia (mg/dl)	67.89 ±1.49^{a}	95.49 ±4.84^{b}	55.75 ±3.65^{c}	75.22 ±3.10^{d}
Proteinemia (g/l)	43.92 ±1.21^{a}	47.29 ±1.94^{bc}	45.56 ±1.10^{ab}	48.70 ±2.52^{c}
ALAT (UI/L)	15.81 ±1.24^{a}	20.46 ±1.78^{b}	13.48 ±2.06^{a}	13.67 ±1.97^{a}
ASAT (UI/L)	15.85 ±2.25^{a}	20.98 ±0.97^{b}	14.11 ±2.50^{a}	13.41 ±1.77^{a}
PAL (UI/L)	23.36 ±0.48^{a}	24.19 ±1.18^{a}	25.06 ±2.02^{a}	23.61 ±1.74^{a}
γGT (UI/L)	39.73 ±0.69^{a}	43.85 ±1.53^{b}	38.72 ±2.39^{a}	38.04 ±1.06^{a}
Creatinine (mg/L)	3.40 ±0.36^{a}	3.69 ±0.85^{a}	3.43 ±0.29^{a}	3.50 ±0.27^{a}
Uremia (mg/dl)	45.45 ±0.40^{a}	48.68 ±1.32^{b}	43.83 ±1.95^{a}	45.01 ±1.02^{a}

Group 1: rats fed SLD. Group 2: rats fed SLD supplemented with BEY. Group 3: rats fed with the diet supplemented with *Sardinella maderensis* oil. Group 4: Rats fed SLD supplemented with BEY and *Sardinella maderensis* oil. TC: total cholesterol; TG: triglycerides; HDL: high density lipoprotein; LDL: low density lipoprotein. ASAT: aspartate aminotransferase; ALAT: alanine aminotransferase; ALP: phosphatase alcaline; γGT: Gamma glutamyl transferase. The values are the means ± the standard deviation, n=6. Means assigned with different letters in same line are significant at $p < 0.05$.

3.5. Effect of *S. maderensis* Oil Supplementation on Some Markers of Oxidative Stress

Table 5 shows some markers of oxidative stress in rats at the end of the study. The table shows a significant increase ($p < 0.05$) in TBARS and a significant decrease ($p < 0.05$) in GSH levels, SOD activity, CAT and PON-1 in group 2 compared with the other groups. Administration of Sardinella maderensis oil significantly ($p < 0.05$) increased GSH concentration, SOD, CAT and PON-1 activity and significantly ($p < 0.05$) reduced TBARS concentration in group 3 compared with group 2. Supplementation with *S. maderensis* oil significantly ($p < 0.05$) reduced TBARS levels by 54.36% and significantly ($p < 0.05$) increased GSH concentration, SOD, CAT and PON-1 activity by 64.90%, 20.76%, 48.70% and 7.47 respectively in group 4 compared with group 2.3.5. Effet de la supplémentation en huile de *S. maderensis* sur quelques marqueurs du stress oxydatif.

Table 5. *Markers of oxidative stress.*

Groups	TBARS (nM MDA/mg protein)	GSH (µM/mg protein)	SOD (unit/mg protein)	CAT (µMH_2O_2/min/mg protéin)	PON-1 (U/mL)
Group 1	1.24 ±0.08^{b}	5.88 ±0.18^{d}	34.70 ±0.60^{b}	194.85 ±7.72^{bc}	0.251 ±0.003^{d}
Group 2	2.98 ±0.76^{a}	3.39 ±0.10^{a}	28.46 ±0.52^{a}	124.74 ±3.35^{a}	0.214 ±0.002^{a}
Group 3	1.22 ±0.14^{b}	6.73 ±0.13^{c}	34.83 ±0.47^{b}	198.24 ±9.53^{c}	0.293 ±0.009^{c}
Group 4	1.36 ±0.11^{b}	5.59 ±0.10^{b}	34.37 ±0.63^{b}	185.50 ±6.90^{b}	0.230 ±0.004^{b}

Group 1: rats fed SLD. Group 2: rats fed SLD supplemented with BEY. Group 3: rats fed with the diet supplemented with *Sardinella maderensis* oil. Group 4: Rats fed SLD supplemented with BEY and *Sardinella maderensis* oil. TBARS: thiobarbituric acid reactive substances. MDA: malondialdehyde. GSH: glutathione. SOD: superoxide dismutase. CAT: catalase. The values are the means ± the standard deviation, n=6. Means assigned with different letters in same column are significant at $p < 0.05$.

4. Discussion

Regarding chemical indexes of oils, iodine number provides information on the degree of unsaturation of the oil and makes it possible to classify them into non-drying oils (II<100), semi-drying oils (100<II<130) and drying oils (II>130). Therefore, the oils extracted from *Sardinella maderensis* are drying. The higher iodine index of *Sardinella maderensis* oil could be explained by their high level of highly unsaturated fatty acids such as eicosapentaenoic, docosahexaenoic and arachidonic acids. According to Gao et al [16], they have anti-inflammatory, antioxidant, antidiabetic and antihyperlipidaemic properties. Manz et al [27] obtained different results on *Ilisha africana* (154.04) and *Sardinella maderensis* (148.73). Iodine value obtained are lower than those obtained by Tenyang et *al*. [28] on *Polypterus bichir bichir* oil (260), Saher et *al*. [29] on *Labeo rohita* (204). These iodine indices are higher than those obtained by Tenyang et *al*. [30] on *Oreochromis niloticus* (39.65). The fishing period, species, diet and environment could explain this difference. Acid value measures the amount of free fatty acid in a fatty substance. These results are below the tolerated value (3) recommended by the Codex Alimentarius [26]. Similar results were obtained by Pradhan et *al*. [31] on *Opisthopterus tardoore* oil (1.14 mg KOH/g of oil). This could be explained by a low hydrolytic activity of triglyceride lipase [32]. Acid value obtained are lower than those obtained by Manz et *al*. [33] on *Ilisha africana* (2.15 mg KOH/g of oil), Simo et *al*. [34] on *Fontitrygon margarita* Liver oil (2.30). The extraction method of oil, the tissue, the fishing period, species, diet and environment could explain this difference. The peroxide value is generally used to measure the primary oxidation products (hydroperoxides) in an oil. This difference could be due to the extraction method and the nature of the species. The higher the peroxide value, the more the fat is oxidised. However, the peroxide value is only an indicator of the start of oxidation, increasing to reach a peak and then decreasing with the state of advanced oxidation [36]. The anisidine index measures the secondary products of oil oxidation and takes into account non-volatile aldehyde compounds. These results are lower than those obtained by Manz et al. [27] on *Ethmalosa fimbriata* (0.76) and the standard set (20) by the Codex alimentarius [26]. The low anisdine value indicates the best quality of oil. Tenyang et al [37] reported different results on smoked *Liza falcipins* (16.20). Increase in this index would reflect a conversion of hydroperoxides and peroxides into secondary oxidation products stimulated by high temperatures [34]. The Thiobarbituric acid reactive substances (TBARS) quantifies the secondary oxidation compounds of lipids, particularly Malondialdehyde (MDA). This latter is an indicator of lipid peroxidation in tissues and oils. Bao et *al*. [38] obtained similar result in filet of *Micropterus salmoides* (0.2). Results reported by El-Lahamy et *al*. [39] in *Oreochromis niloticus* (0.55) and *Mugil cephalous* (0.95) oil, Manz et *al*. [27] in *Cyprinus carpio* (0.87) are higher than those obtained in our study. Different results were obtained by Tenyang et *al*. [30] on smoked *Oreochromis niloticus* (12.46). Exposure of oil to high temperatures during smoking could justify this difference [40]. High levels of TBARS are thought to play a role in oxidative stress and the pathogenicity of atherosclerosis [41]. To estimate the quality of oil, the total oxidation (TOTOX) value may be used. This index makes it possible to better assess the oxidation state of the fat, taking into account the different forms of fatty acid oxidation. TOTOX reflect the initial and later stages of the oil oxidation. These results are below the standards set by the Codex Alimentarius [27] which recommends a value below 26 for virgin fats and oils. These results are lower than those obtained by Manz et *al*. [27] on *S. maderensis* (4.52) and *A. parkii* oil (3.59). The lower value of total oxidation indicates a higher quality of the oil. According to ours results, the TOTOX value of *Sardinella maderensis* oil was in acceptable limits and suggest the good quality of this fish oil.

Administration of *Sardinella maderensis* oil at doses of 2000, 4000 and 5000 mg/Kg body weight (bw) had no adverse effects on the behavioural parameters of the rats and caused no deaths. The lethal dose could be higher than 5000 mg/Kg of bw so this oil could be classified as almost non-toxic according to the Gosselin et al. [42] scale. In addition, values of ASAT, ALAT, total protein, urea and creatinine were not significantly in different group of rats indicating the condition of the heart, liver, muscles and kidneys was not significantly degraded.

The administration of *Sardinella maderensis* oil had no effect on food intake, metabolic efficiency (ME) or naso-anal length. Boukhari et al. [43] showed that consumption of a high-fat diet supplemented with fish oil had no effect on food intake. ME provides information about the body's capacity to mobilise or store energy. The higher or lower the ME, the more energy the body stores or mobilises respectively [24]. Different results were obtained by Gaspar et al. [44] who showed that rats consuming a hyperfat diet were significantly shorter than those consuming only the standard laboratory diet. This difference could be due to the duration of the study and the composition of the obesogenic diet [45]. Consumption of boiled egg yolk significantly increased BMI, LI and relative adipose tissue weight in group 2 compared with the other groups. Buyukdere et al. [46] showed that rats consuming a hyperlipidic diet were overweight. For a BMI greater than 0.45 ± 0.02 - 0.68 ± 0.05 g/cm2 for males rat is considered to be obese. Indeed, if Lee's index is greater than 300, the animal grows in thickness rather than in length, whereas if this index is less than 300, the animal grows in length rather than in thickness [24].

The relative weight of an organ provides information about the growth of the organ in relation to the whole organism. The greater the relative weight, the faster the organ grows compared with the whole body, and vice versa. Activation of

the protein SPAK kinase promoting adipogenesis could explain the state of obesity [47]. The significant reduction in weight gain, BMI, LI and relative adipose tissue weight in rats in groups 3 and 4 compared with group 2 is evidence of the anti-obesity properties and ability of *S. maderensis* oil to prevent overweight. Madani et al. [48] showed that rats consuming a fatty diet supplemented with fish oil were not obese and had a better weight development. Manz et al. [11] showed that administration of Ilisha africana oil significantly reduced weight gain, BMI and LI in rats fed boiled egg yolk. In fact, the omega-3s contained in fish oils have an anti-adipogenic effect due to the increase in lipolysis [12]. This consumption of boiled egg yolk negatively alters the lipid profile. Milic et al. [49] showed that the administration of a high-calorie diet adversely affected the lipid profile. Inhibition of lecithin cholesterol acyl transferase (LCAT) activity could explain the hyperlipidaemia. Insulin resistance, characterised by dysfunction of the beta cells of the pancreas, could explain the hyperglycaemia [3]. Endogenous synthesis stimulated by external nutritional factors and the conversion of excess lipids and carbohydrates into proteins would explain the hyperproteinemia. According to Amal [25], rats consuming a hyperlipidaemic diet showed hepato-renal damage. Indeed, the rise in transaminase activity and serum creatinine reflected hepatocellular cytolysis and renal dysfunction respectively [50]. This result testifies to the antihyperlipidaemic nature of *S. maderensis* oil. Ferguson et al. [51] have shown that the omega-3 polyunsaturated fatty acids contained in fish oils help combat hyperlipidaemia. Manz et al. [11] showed that administration of Ilisha africana oil significantly reduced TC, LDL and TG and significantly increased HDL in rats fed boiled egg yolk. Pradhan et al. [31] showed that administration of *Opisthopterus tardoore* oil reduced obesity in mice. The increase in HDL is thought to provide protection against cardiovascular disease. The significant reduction in glycaemia following administration of *S. maderensis* oil demonstrates its hypoglycaemic properties. Pinel et al. [52] showed that omega-3 supplementation in rats as a preventive measure improved blood glucose levels. In fact, omega-3s are converted into 3-series eicosanoids, which improve insulin sensitivity [47]. Supplementation with *S. maderensis* oil significantly reduced the activity of transaminases, γGT and uraemia in groups 3 and 4 compared with group 2. This result demonstrates the hepato-renoprotective properties of this oil. Fergusson et al. [51] showed that fish oils reduced transaminase activity and elevated creatinine levels in rats fed an obesogenic diet. In fact, omega-3s have the ability to inhibit lipooxygenase and cyclooxygenase, which are enzymes involved in the synthesis of pro-inflammatory molecules [53]. Consumption of boiled egg yolk for 21 days induces oxidative stress in rats. Amal [25] has shown that consumption of a hyperlipidic diet adversely alters the oxidant/antioxidant balance via an increase in pro-oxidants in Wistar rats. The increased accumulation of reactive oxygen species (ROS) resulting from lipid auto-oxidation further stimulates lipid peroxidation and or reduces the activity of antioxidant enzymes [6]. The decrease in GSH levels could be explained by its involvement in the elimination of various toxic metabolites resulting from the degradation of xenobiotics responsible for tissue damage [54]. It has been reported that PON-1 activity is inversely correlated with obesity. PON-1 prevents the oxidation of LDL. Inactivation by S-glutathionylation or radical attack stimulated by oxidising molecules could account for the decrease in PON-1 [13]. The results obtained show that oil extracted from *Sardinella maderensis* has an antioxidant effect. Hamza-Reguig et al. [3] showed that fish oil supplementation significantly reduces oxidative stress by increasing the activity of enzymatic and non-enzymatic antioxidants. Boukhari et al. [13] showed that supplementation with *Sardina pilchardus* oil significantly reduced oxidative stress. This result may be due to the richness of these oils in bioactive compounds (n-3 PUFAs) that inhibit lipoperoxidation and damage caused by the excessive production of free radicals. The ability of n-3 PUFAs to capture the single electron from free radicals could also explain this antioxidant property [55].

5. Conclusion

This study shows that *Sardinella maderensis* oil is of good quality and could be used for human nutrition. It protects from weight gain and has no effect on food intake. Used as nutraceutic, it improves, blood sugar, and serum protein. It therefore shows an antihyperlipidemic potential. Hence, this oil can be used to combat some risk factors of metabolic syndrome.

Data Availability

The data used in this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

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