Acta Scientific NUTRITIONAL HEALTH (ISSN:2582-1423)

Volume 9 Issue 10 October 2025

Research Article

Evaluation of the Level of Pro and Anti-Inflammatory Cytokines and Haematological Parameters in/Polyunsaturated Rich Fish-Oil-Treated Rats after Induction of Cardiac Ischemia with Isoprenaline

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Received: August 25, 2025

Published: September 29, 2025

Abstract

Cardiovascular diseases (CVD), including cardiac ischemia; an imbalance between coronary blood supply and myocardial demand have emerged as a major public health problem and impose an escalating burden on the health care system in Cameroon. Moreover, anti-ischaemic medications only eliminate or reduce cardiac ischemia by decreasing myocardial oxygen demand, increasing myocardial oxygen supply, or both, but do not correct the underlying cause of ischemia (atherosclerosis). But dietary n-3 polyunsaturated fatty acids have positive effects on cardiovascular diseases, with mackerel fish being one of its rich source, due to the EPA and DHA it contains. The present study investigated the effects of pre-treatment with mackerel fish oil (FO), on the heart of the rat which is associated with the isoprenaline (ISO)-induced cardiac ischemia, since it is a rich source of n-3 polyunsaturated fatty acids. The study was conducted on 42 Wistar rats which were divided into seven groups of 6 rats each, and pre-treated as follows. The normal control (NO) received distilled water, negative control (NC), was only induced with isoprenaline, test groups 1 and 2 (8C and 10C), received cooked fish oil at doses of 8 and 10 ml/kg body weight, test groups 3 and 4 (8R and10R), received raw fish oil at doses of 8 and 10 ml/kg body weight, orally for 4 weeks and the positive control (PC) received propranolol (standard drug) 10 days before sacrifice. Acute myocardial ischemia was induced by isoprenaline (ISO, 85 mg/kg) twice, 48 and 24 hours before sacrifice. Eventually, the animals were sacrificed on day 29, after an overnight fast and samples collected for plasma cytokine levels and hematological parameter evaluation. Moreover, their growth response and exploratory behavior were also examined during the assay. Based on the results, ISO administration significantly increased the plasma levels of inflammatory cytokines and decreased haematological parameters compared to the normal control group. In addition, rats were less explorative in the open field, as their number of crossing and rearing decreased significantly from 67.3 to 19.7 and from 26.2 to 3.3 with a decreased weight due to loss of appetite following ISO administration. However, a remarkable decrease in plasma levels of TNF-α, IL-6, IL-1β, IL-10, and INF-γ was observed. Additionally, pre-treatment showed a significant restoration in the plasma levels of hematological parameters, including WBC count and its subtypes compared to the ISO group. In general, pre-treatment with FO significantly decreased plasma levels of inflammatory cytokines compared to the standard drug, with raw FO showing a better effect. Therefore, FO, especially oil from raw *Scomber scrombrus* results in preventing cardiac ischemia, and thus implies mackerel fish oil should be used as supplements source of omega fatty acids.

Keywords: Cardiac Ischemia; Mackerel Fish Oil; Isoprenaline; Hematological; *Scomber Scrombrus*; Exploratory Behavior; Cytokine

Introduction

Over the last decade, cardiovascular diseases have become one of the leading causes of death worldwide and in many high-income countries during the past century; nowadays, low- and middle-income countries are seeing an alarming and accelerating increase in cardiovascular disease rates [1]. Coronary heart diseases often occur at a lower prevalence rate than stroke and account for 10% to 35% of deaths, but still, in 2020, they caused an estimated 17.9 million deaths and led to 293 million disability-adjusted life years lost [2]. Acute myocardial infarction is defined as the necrosis of cardiomyocytes owing to prolonged myocardial ischemia due to an imbalance between coronary blood supply and myocardial demand [3]. It is associated with an inflammatory response, and alteration of the extracellular matrix due to the release of free radicals and proteolytic enzymes, which progresses towards remodeled myocardium [4]. The inflammatory process can influence the extent of myocardial lesions, [5]. Anti-inflammatory therapies in myocardial ischemia may reduce the extent of ischemic lesions [6].

Inflammation is a normal defense mechanism that protects the host from infection and other insults; it initiates pathogen killing as well as tissue repair processes and helps to restore homeostasis at infected or damaged sites [7]. It is typified by redness, swelling, heat, pain, and loss of function, and involves interactions amongst many cell types and the production of, and responses to, several chemical mediators [8]. Where an inflammatory response does occur, it is normally well regulated so that it does not cause excessive damage to the host, is self-limiting, and resolves rapidly. This self-regulation involves the activation of negative feedback mechanisms such as the secretion of anti-inflammatory mediators, inhibition of pro-inflammatory signaling cascades, shedding of receptors for inflammatory mediators, and activation of regulatory cells [9]. As such, when controlled properly, regulated inflammatory responses are essential to remain healthy and maintain homeostasis. Pathological inflammation involves a loss of tolerance and/or of regulatory processes [10]. Where this becomes excessive, irreparable damage to host tissues and disease can occur. The inflammatory response involves four major events: An increased blood supply to the site of inflammation; increased capillary permeability caused by retraction of endothelial cells. This permits larger molecules, not normally capable of traversing the endothelium, to do so and thus deliver soluble mediators to the site of inflammation; leukocyte migration from the capillaries into the surrounding tissue. This is promoted by the release of chemo-attractants from the site of inflammation and by the up-regulation of adhesion molecules on the endothelium. Once in the tissue, the leukocytes move to the site of inflammation and Release of mediators from leukocytes at the site of inflammation. These may include lipid mediators (e.g., prostaglandins (PGs), leukotrienes (LTs), peptide mediators (e.g., cytokines), reactive oxygen species (e.g., superoxide), amino acid derivatives (e.g., histamine), and enzymes (e.g., matrix proteases) depending upon the cell type involved, the nature of the inflammatory stimulus, the anatomical site involved, and the stage during the inflammatory response [11]. These mediators normally would play a role in host defense, but when produced inappropriately or in an unregulated fashion they can cause damage to host tissues, leading to disease [12]. Several of these mediators may act to amplify the inflammatory process acting, for example, as chemoattractant [13]. Some of the inflammatory mediators may escape the inflammatory site into the circulation and from there they can exert systemic effects. For example, interleukin (IL)-6 induces hepatic synthesis of the acute phase protein C-reactive protein, while tumor necrosis factor (TNF)- elicits metabolic effects within skeletal muscle, adipose tissue, and bone [14]. Pathological inflammation, therefore, leads to cardiovascular diseases.

Cardiovascular diseases (CVD) have emerged as a major health problem and impose an escalating burden on the health care system in Cameroon [15]. On an annual basis, 13.2% of all deaths are attributed to coronary artery disease (CAD), which makes CAD-with 17.9 million deaths-the leading cause of death in the world [16]. The pathophysiology of atherosclerosis with its progression to stable CAD and its destabilization and complication with thrombus formation most often results in myocardial infarction (MI) [17]. n-3 polyunsaturated fatty acids aid in reducing the risk of having cardiovascular diseases by modification of the cell membrane milieu on incorporation [18]. Also, omega-3 PUFAs have shown anti-inflammatory effects by decreasing the circulatory concentrations of inflammatory cytokines and ameliorating left ventricular functional capacity [19]. Unfortunately, mammals cannot synthesis either linoleic acid (LA; 18:2n-6) nor α-linolenic acid (ALA; 18:3n-3), which are precursors of polyunsaturated fatty acids, the n-6 and n-3 fatty acids (eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) de novo, since they do not produce

delta-12 and delta-15 desaturase enzymes [19]. Nonetheless, fish oil contains important omega-3 fatty acids that can be absorbed easily, though, there is evidence of oxidation of these molecules during the cooking process owing to high temperatures [20]. Additionally, it is still undetermined how fatty acid from mackerel fish can prevent the myocardium from ischemia-reperfusion injury, as more work has been done on the effects of pre-treatment with plant extracts on MI –ISO induced mice and rats [21]. Isoprenaline (ISO) can lead to an induction of MI in low, moderate, and high doses, therefore, the present study made MI model in the rats using ISO [22]. Thus, the study verified the effect of mackerel fish (*Scomber scrumbrus*) oil rich in DHA/EPA extracted from raw and cooked fish on the plasma levels of haematological parameters and inflammatory cytokines in myocardial ischemia induced rats.

Materials and Methods

Study design and ethical issues

An ethical clearance with permit number: UB-IACUC Nº 05/2021 was obtained from the University of Buea-Institutional Animal Care and use committee (UB-IACUC), in order to carry out this cross sectional study. This study is a cross sectional and laboratory based study, conducted on fresh mackerel fish consumed by the people of Buea community.

Sample collection

Fresh mackerel fish (*Scomber scrombrus*) was purchased from Congelcam, Great Soppo Buea, packaged, and carried to the Biochemistry Laboratory, University of Buea. Mackerel was selected on the criteria that it is readily available, higher oil content, and it is moderately inexpensive, unlike the other fishes.

Sample preparation

The fish was divided into two parts; both parts were cleaned and deboned. One part was cooked (boiled at 100°C) for 10 minutes and oil was extracted from it (cooked fish oil), while oil was extracted from the other part uncooked (raw fish oil).

Oil extraction

The oil was extracted by Bligh and Dyer method [23]. The fish was ground and mixed with water, methanol, and chloroform in solvent ratios 100ml: 100ml: 400ml. The mixture was blended, squeezed using a clean linen cloth, and filtered using a coffee filter paper, within 10 to 15 minutes. Decanting funnel was used to separate the non - polar phase (chloroform phase). The chloroform was separated from oil by evaporation at 45°C using a rotary evaporator and the residual solvent eliminated by ambient exposition in a dark containers.

Animal bioassay; to measure the preventive effect of mackerel fish oil on cardiac ischemia

Animal grouping, induction and experimental phase

A total of 42 Wistar albino rats aged three months, weighting 150 - 180g were used in this study. The rats were purchased at the animal house of the Department of Zoology and Animal Physiology, Faculty of Science, University of Buea. They were kept in cages in the lab where they acclimatised at standard environmental conditions of 25 ± 2°C, 50 ± 15%humidity and a natural light-dark cycle. Animals had free access to standard pellets and water *ad libitum* for a week. The Wistar rats were evenly distributed into seven groups (numbered 1-7) of six animals 3 males and 3 females each. Daily animal weight and food consumed were recorded. Table 1 summarizes the treatment administered to each group during the 28days of the study. The normal group received distilled water (10ml/kgbw) orally, the negative control group received only isoprenaline (ISO), test groups one and two received cooked fish oil 8ml/kg and 10ml/kg respectively plus ISO and test groups three and four received raw fish oil 8ml/kg and 10ml/kg respectively plus ISO. The positive control group received propanolol as drug 10ml/kg plus isoprenaline (ISO) 85mg/kg,

After 26 days' rats were induced orally with 85 mg/kg isoprenaline (ISO) 48 and 24 hours before sacrifice, the animals were fasted for 24 hours after the last dose of induction, and the Evaluation of exploratory behavior, locomotion, and motor coordination in the open field conducted. And on day 29, they were anesthetized with ketamine and diazepam (dose was weight dependent) and sacrified by decapitation and blood collected in an anticoagulant (ethylene diamine tetra acetate) test tubes for plasma preparation to be used for hematological and inflammatory cytokines studies.

Groups	Group name	Treatment
1	Normal Control (NOC)	Distilled water
2	Negative control (NC)	No pre-treatment with fish oil, induced with cardiac ischemia by (isoprenaline) ISO, 85 mg/kg body weight, for 48hours and 24hours before sacrifice.
3	Test group one (8C)	Pre-treated with 8 ml/kg body weight cooked fish oil for 26 days, and induced cardiac ischemia by ISO, 85 mg/kg body weight, for 48hours and 24hours before sacrifice
4	Test group two (10C)	Pre-treated with 10 ml/kg body weight cooked fish oil for 26 days, and induced cardiac ischemia by ISO, 85 mg/kg body weight, for 48hours and 24hours before sacrifice
5	Test group three (8R)	Pre-treated with 8 ml/kg body weight raw fish oil for 26 days, and induced cardiac ischemia by ISO, 85 mg/kg body weight, for 48hours and 24hours before sacrifice
6	Test group four (10R)	Pre-treated with 10 ml/kg body weight raw fish oil for 26 days, and induced cardiac ischemia by ISO, 85 mg/kg body weight, for 48hours and 24hours before sacrifice
7	Positive control (PC)	Pre-treatment with 8 mL/kg propranolol (for ten days before sacrifice), and induced cardiac ischemia by ISO, 85 mg/kg body weight, for 48hours and 24hours before sacrifice

Table 1: Animal grouping and treatment.

Evaluation of exploratory behavior, locomotion and motor coordination in the open field

The open-field test was used to evaluate locomotory activity, level of exploration, and emotional reaction of animals [24]. The open field consisted of a surrounding square (40 cm x 40 cm) divided into 16 small square and 1 center field (10 cm x 10 cm) wall of 19 cm high [25]. One hour after the second ISO administration, each rat was placed individually in the center of the arena and the time spent at the center, the number of crossing, grooming, rearing, and fecal boli weight were recorded for 5 minutes duration.

Evaluation of hematological parameters

Hematological analysis was performed using an automatic hematological analyzer (Sysmex KX-21N) according to the manufacturer's instructions. The parameters included: red blood cell (RBC) count, leukocyte (WBC) count, hemoglobin (Hb), hematocrit (HCt), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count, lymphocyte, monocyte, neutrophil, basophil and eosinophil counts.

Estimation of cytokine levels

Determination of the plasma concentration of TNF-α

The plasma levels of TNF-α were quantified using Quantikine ELISA kit (R and D Systems, USA), following instructions provided by the manufacturer. This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mice TNF-α was pre-coated onto a 96 wells of a microplate. One hundred microliters (100 µL) of standards, or samples were pipetted into the wells and any TNF-α (100 µL) present was bound by the immobilized antibody. They were incubated for 2 hours at room temperature. After washing away using a wash buffer (400 µL) of any unbound substances, an enzyme-linked polyclonal antibody specific for mice TNF-α (100 µL) was added to the wells [26]. They were later incubated at room temperature for duration of 2 hours. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution (100 µL) was added to the wells. In addition, the mixtures were protected from light, and then incubated for 30 minutes at room temperature. The enzyme reaction yielded a blue product that turned yellow when the Stop Solution (50 µL) was added. The optical density of each well was determined within 30 minutes, using an ELISA plate reader set to 450 nm. To obtain the standard curve, which was used to determine the concentration of the cytokines, serial dilutions of protein standard were made [27]. The analysis modelled protein concentration as a function of the optical density (OD), judged by an R^2 value greater than 0.98. A standard curve for the determination of TNF-α concentration was then used to calculate the other concentrations. The intensity of the colour measured is in proportion to the amount of TNF-α bound in the initial step.

Determination of the plasma concentration of INF-γ

The quantity of INF-γ in the plasma was evaluated in accordance to Quantikine ELISA kit (R&D Systems, USA), following instructions provided by the manufacturer. This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mice IFN-γ was pre-coated onto a 96 wells of a microplate. One hundred microliters (100 µL) of standards or samples were pipetted into the wells and any IFN-γ present was bounded by the immobilized antibody. They were incubated for 2 hours at room temperature. After washing away (wash buffer; (400 µL) any unbound substances, an enzyme-linked polyclonal antibody specific for mice IFN-γ (100 µL) was added to the wells. They were later incubated at room temperature for a duration of 2 hours. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution (100 µL) was added to the wells. Furthermore, the mixtures were protected from light, and then incubated for 30 minutes at room temperature. The enzyme reaction yielded a blue product that turned yellow when the Stop Solution (100 µL) was added. The optical density of each well was determined within 30 minutes, using an ELISA plate reader set to 450 nm. A standard curve for the determination of INF-γ concentration was used to calculate the other concentrations. The intensity of the colour measured is in proportion to the amount of IFN-γ bound in the initial step [28].

Determination of the plasma concentration of IL1-β

The plasma levels of IL-1β were quantified by Quantikine ELISA kit (R and D Systems, USA), following instructions provided by the manufacturer. The used assay is the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mice IL-1β was pre-coated onto a microplate (96 wells of a microplate). Standards (50 µL) or samples (50 µL) were pipetted into the wells and any IL-1β present was bounded by the immobilized antibody. They were incubated for 2 hours at room temperature. After washing away using a wash buffer (400 µL) any unbound substances, an enzyme-linked polyclonal antibody specific for mice IL-1β (100 µL) was added to the wells. They were again incubated at room temperature for a duration of 2 hours. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution (100 µL) was added to the wells. The enzyme reaction yielded a blue product that turned yellow when the Stop Solution (100 µL) was added. Thereafter, the mixtures were protected from light, and then incubated for 30 minutes at room temperature. Following the stop of the reaction the optical density of each well was evaluated within 30 minutes, using an ELISA plate reader set to 450 nm. A standard curve for the determination of IL-1β concentration was used to calculate the other concentrations. The intensity of the colour measured is in proportion to the amount of IL-1β bound in the initial step [29].

Determination of plasma concentration of IL-6

The quantity of IL-6 in the plasma was evaluated in accordance to Quantikine ELISA kit (R and D Systems, USA). This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mice IL-6 was pre-coated onto a microplate. One hundred microliters (100 µL) of standards or samples were pipetted into the wells and any IL-6 present was bounded by the immobilized antibody. They were incubated for 2 hours at room temperature. After washing away (wash buffer; 400 µL) any unbound substances, an enzyme-linked polyclonal antibody specific for mice IL-6 (100 µL) was added to the wells. They were again incubated at room temperature for duration of 2 hours. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution (100 µL) is added to the wells. Thereafter, the mixtures were protected from light, and then incubated for 30 minutes at room temperature. The enzyme reaction yielded a blue product that turned yellow when the Stop Solution (100 µL) was added. The intensity of the colour measured is in proportion to the amount of mice IL-6 bound in the initial step. The optical density of each well was determined within 30 minutes, using an ELISA plate reader set to 450 nm. A standard curve for the determination of IL-6 concentration was used to calculate the other concentrations. Then Optical density values (in picograms per millilitre) above 54.6 pg/ml were considered abnormal [29].

Determination of plasma concentration of IL-10

The quantity of IL-10 in the plasma was evaluated in accordance to Quantikine ELISA kit (R and D Systems, USA). This assay employs the quantitative sandwich enzyme immunoassay technique.

A monoclonal antibody specific for mice IL-10 was pre-coated onto a microplate. One hundred microliters (100 µL) of standards or samples were pipetted into the wells and any IL-10 present was bounded by the immobilized antibody. They were incubated for 2 hours at room temperature. After washing away (wash buffer; 400 µL) any unbound substances, an enzyme-linked polyclonal antibody specific for mice IL-10 (100 µL) was added to the wells. They were again incubated at room temperature for a duration of 2 hours. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution (100 µL) was added to the wells. Thereafter, the mixtures were protected from light, and then incubated for 30 minutes at room temperature. The enzyme reaction yielded a blue product that turned yellow when the Stop Solution (100 µL) was added. The intensity of the colour measured is in proportion to the amount of mice IL-10 bound in the initial step. The optical density of each well was determined within 30 minutes, using an ELISA plate reader set to 450 nm. A standard curve for the determination of IL-10 concentration was used to calculate the other concentrations [30] .

Data analysis

The statistical analysis of the data (continuous quantitative data) was performed by one-way analysis of variance (ANOVA) followed by Dunnet's multiple comparison test, (where the different treatment groups were all compared to the negative control group) using Graph Pad Prism (version 4.00, Graph Pad Software Inc., San Diego, CA, USA). The results were presented as mean ± S.E.M. Comparisons of the results were performed using computerized linear regression analysis, in all cases; differences were considered significant at $p \leq 0.05$.

Results

Following the induction with ISO, 3 rats were lost from the negative control group that was not pre-treated, but was induced with ISO, 2 from experimental group one that received 8 mL/Kg body weight cooked FO and ISO, 2 from experimental group two (10 mL/kg cooked oil). One from experimental group that received 8 mL/Kg body raw fish Oil and ISO and one from positive that isoproterenol and ISO. MI was successfully induced after ISO administration, demonstrated by the general inactivity in the open field.

Evaluation of growth response

Growth response (animal's weight) for across all the groups' rats is summarized in Figure 1. The daily growth response for the negative control group was lower than that of all the groups. However, across all the groups there was an increase in growth for the first till the fourth week except in boiled mackerel fish 8ml/Kg oil treated group in which there was a decrease in growth during the fourth week. Following the induction of cardiac ischemia by ISO, there was a decrease in the weights of the rats on the 27^{th} and 28^{th} day, (Figure 1), as a result of decreased food intake (Figure 2) in the induced rats due to decrease appetite. The food intake in the propanolone treated group (positive control) during the first week was highest.

Evaluation of exploratory behavior, locomotion, and motor coordination in an open field

The number of crossing significantly decreased ($p \leq 0.05$) after the administration of isoprenaline comparing to the normal rats and increase compare to the negative control group of rats (Table 2). Crossing decreased significantly from 67.3 to 9.8 at $p \leq 0.05$ and rearing also significantly reduced from 26.2 (normal Group) to 3.3 at $p \leq 0.01$. Pre-treatment with cooked fish oil at a dose of 8 ml/kg body weight significantly increased the number of rearing from 3.3 to 7.0 at $p \leq 0.01$ compare to negative control group. Cooked oil at a dose of 10 ml/kg body weight increased rearing, from 3.3 to 6.0 at $p \leq 0.05$. Raw fish oil (FO) at all doses significantly boosted crossing and rearing from 19.7 to 30.2 ($p \leq 0.01$) and 3.3 to 5.8 ($p \leq 0.01$), respectively. Propranolol also boosted rearing from 3.3 to 4.0 at $p \geq 0.05$. Fecal boli and centre time were not affected significantly $P > 0.05$.

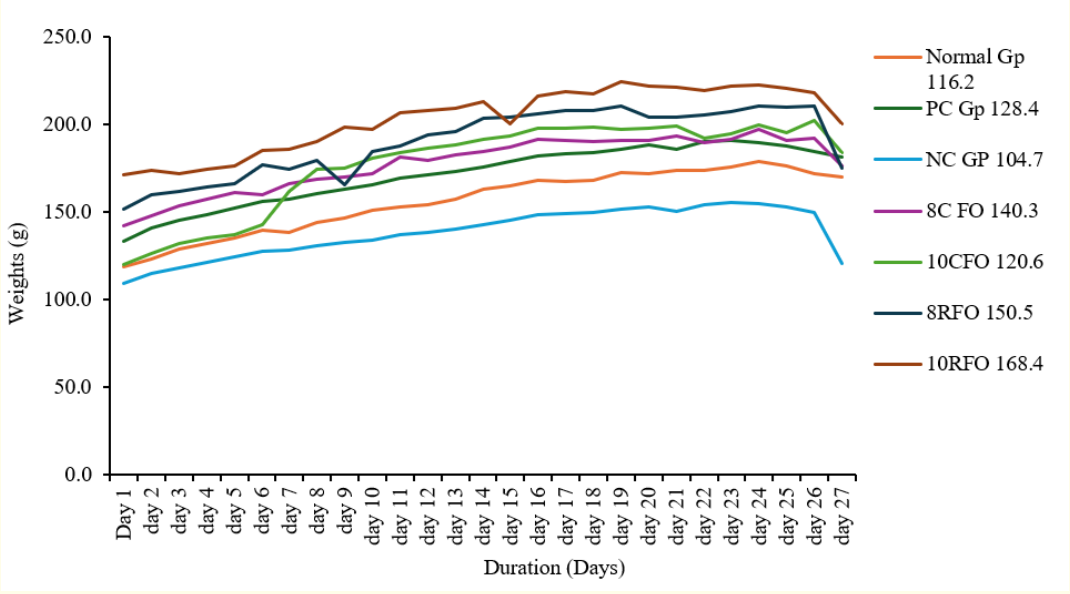

Figure 1: Animal growth response.

Normal Gp = Normal control group; NC Gp = negative control group that received isoprenaline (2 mL/200g) without any pretreatment with fish oil; 8CFO 10CFO = cooked FO, in doses of 8 mg/kg body weight (8C) and 10 mg/kg body weight (10C); 8RFO and 10RFO = raw (virgin) FO, in doses of 8 mg/kg body weight (8R) and 10 mg/kg body weight (10R), PC Gp = positive control group receiving proprano-lol in doses of 2 mL/200g body weight.

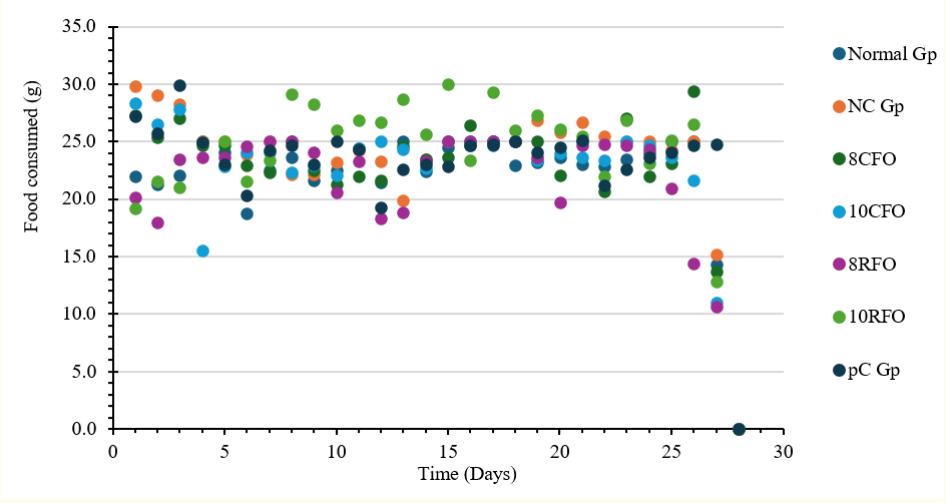

Figure 2: Daily food consumption by the rats.

Normal Gp = control group; Normal Gp= control group; NC = negative control group that received isoprenaline (85 mg/kg) without any pretreatment; 8C FO and 10C FO = cooked FO, in doses of 8 mg/kg body weight (8C) and 10 mg/kg body weight (10C), and 8R and 10R = raw (virgin) FO, in doses of 8 mg/kg body weight (8C) and 10 mg/kg body weight (10C), pC = positive control group receiving proprano-lol in doses of 2 mL/200g body weight.

Dose of FO (mL/kg)	NormalGp (n = 6)	NC Gp (n = 3)	8CFO (n = 4)	10CFO (n = 4)	8RFO (n = 5)	10RFO (n = 6)	pC Gp (n = 5)
Centre time (s)	1.8 ± 0.8[a]	7.3 ± 6.1[a]	3.5 ± 0.7[a]	6.25 ± 3.3[a]	10.75 ± 9.5[a]	16.0 ± 4.8[a]	6.5 ± 2.1[a]
Crossing	67.3 ± 1.7[a**]	9.8 ± 2.8[b]	15.0 ± 1.3[c]	16.5 ±6.3[c]	19.7 ± 1.8[c]	27.3 ± 7.7[d*]	30.2 ± 7.2[d*]
Rearing	26.2 ± 3.0[a*]	3.3 ± 1.1 [b]	7.0 ± 0.0* [c]	6.0 ± 1.8[c]	3.8 ± 2.8 [b]	5.8 ± 4.8 [b]	4.0 ± 0.0 [b]
Grooming	7.8 ± 1.1[a]	4.0 ± 1.1[b]	0.0 ± 0.0	0.5 ± 0.3 [c]	3.0 ± 1.6[b]	0.6 ± 0.4 [c]	2.5 ± 1.1[b]
Feacal boli (g)	0.4 ± 0.4[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.2 ± 0.2[a]

Table 2: Exploratory behavior, locomotion, and motor coordination in an open field.

a, b, c, d: values carrying different superscript letters on the same line are significantly different between experimental group and NC Gp at p-value less than 0.05 and 0.01*. Normal Gp = Normal control group; NC Gp = negative control group that received isoprenaline (2 mL/200g) without any pretreatment with fish oil; 8CFO and 10CFO = cooked FO, in doses of 8 mg/kg body weight (8C) and 10 mg/kg body weight (10C); 8RFO and 10RFO = raw (virgin) FO, in doses of 8 mg/kg body weight (8R) and 10 mg/kg body weight (10R), PC Gp = positive control group receiving propranolol in doses of 2 mL/200g body weight.

Evaluation of plasma cytokine levels

The plasma levels of TNF-α, IL-6, IL-1β, IL-10, and INF-γ increased after the induction of cardiac ischemia (Table 3). All doses of raw and cooked Scomber *scombrus* oil and propranolol prevented the increase in TNF-α, IL-1β, IL-6 IL-10, and INF-γ, by decreasing their plasma levels for cooked fish oil at a dose of 10 ml/kg body weight, and for raw fish oil at a dose of 10 ml/kg body weight (Table 3).

The plasma level of TNF-α increased after the induction of cardiac ischemia by isoprenaline, as seen in the negative control group compared to the normal group (Table 3). All doses of raw and cooked *Scomber scombrus* oil and propranolol used significantly prevented the increase in TNF-α, for groups 8C and 10C ($p < 0.05$) and 8R and 10R ($p < 0.01$), respectively. Oil from raw *Scomber scrombrus* (mackerel fish) and propranolol showed a greater effect compared to oil from cooked *Scomber scrombrus*.

The plasma level of INF-γ increased after the induction of cardiac ischemia by isoprenaline (Table 4). All doses of raw and cooked *Scomber scombrus* oil and propranolol used prevented the increase in INF-γ ($p < 0.05$). Oil from raw Scomber scrombrus and propranolol also showed a greater effect compared to oil from cooked Scomber scrombrus ($p < 0.01$). The effect of raw oil showed a direct relationship with dose, where pre-treatment with 10 mL/kg body weight significantly reduced plasma level of INF-γ ($p < 0.001$ and $p < 0.01$).

The plasma level of IL-1β also increased after the induction of cardiac ischemia by isoprenaline as seen in the negative control group that received no pre-treatment, yet was induced with cardiac ischemia by ISO (Figure 3). All doses of raw and cooked *Scomber scombrus* oil and propranolol used prevented the increase in IL-1β ($p < 0.05$). There was no significant difference in the effects between groups pre-treated with raw *Scomber scrombrus* oil and those pre-treated with cooked oil, as well as propranolol ($p < 0.05$).

The plasma level of IL-6 increased after the induction of cardiac ischemia by isoprenaline (Tableau 3). All doses of raw and cooked *Scomber scombrus* oil and propranolol used prevented the increase in IL-6. There was no significant difference in the effects exerted by the different treatments ($p < 0.05$).

The Plasma level of IL-10 increased after the induction of cardiac ischemia by isoprenaline (Table 3 3). All doses of raw and cooked *Scomber scombrus* oil and propranolol used prevented the increase in IL-10 ($p < 0.05$). Though the difference between the effects of the raw and cooked fish oil was not significant, raw oil had a greater effect in lowering the plasma IL-10 levels, bringing it to normalcy, same as the standard drug, propranolol.

(Pg/ml)	Normal Gp (n = 6)	NC Gp (n = 3)	8CFO (n = 4)	10CFO (n = 4)	8RFO (n = 5)	10RFO (n = 6)	PC Gp (n = 5)
TNF-α	170.5 ± 18.43c	352.69 ± 15.32a	233.5 ± 13.48b	209.96 ± 10.4b	150.97 ± 6.65c	156.52 ± 5.26c	161.74 ± 4.99c
INF-δ	124.86 ± 11.62d*	351.85 ± 6.76a	265.97 ± 8.87b	227.94 ± 12.52c	131.90 ± 13.18d*	117.83 ± 14.90d*	133.69 ± 12.68d*
IL-1β	153.42 ± 7.42c	332.55 ± 19.32a	255.30 ± 6.86b	215.58 ± 24.91b	169.59 ± 12.31c	144.78 ± 10.28c	152.21 ± 7.49c
IL-6	295.06 ± 3.20c	467.54 ± 11.46a	341.66 ± 22.27b	310.83 ± 8.80b	310.11 ± 12.74b	295.91 ± 5.67c	294.78 ± 3.95c
IL-10	200.31 ± 7.57c	378.45 ± 19.77a	299.58 ± 8.54b	220.85 ± 21.73c	204.70 ± 14.38c	192.97 ± 10.43c	197.84 ± 6.73c
(Pg/ml)	Normal Gp (n = 6)	NC Gp (n = 3)	8CFO (n = 4)	10CFO (n = 4)	8RFO (n = 5)	10RFO (n = 6)	PC Gp (n = 5)
TNF-α	170.5 ± 18.43c	352.69 ± 15.32a	233.5 ± 13.48b	209.96 ± 10.4b	150.97 ± 6.65c	156.52 ± 5.26c	161.74 ± 4.99c
INF-δ	124.86 ± 11.62d*	351.85 ± 6.76a	265.97 ± 8.87b	227.94 ± 12.52c	131.90 ± 13.18d*	117.83 ± 14.90d*	133.69 ± 12.68d*
IL-1β	153.42 ± 7.42c	332.55 ± 19.32a	255.30 ± 6.86b	215.58 ± 24.91b	169.59 ± 12.31c	144.78 ± 10.28c	152.21 ± 7.49c
IL-6	295.06 ± 3.20c	467.54 ± 11.46a	341.66 ± 22.27b	310.83 ± 8.80b	310.11 ± 12.74b	295.91 ± 5.67c	294.78 ± 3.95c
IL-10	200.31 ± 7.57c	378.45 ± 19.77a	299.58 ± 8.54b	220.85 ± 21.73c	204.70 ± 14.38c	192.97 ± 10.43c	197.84 ± 6.73c

Table 3: Plasma levels of cytokines per group (values expressed as mean ± standard deviation.

a, b, c, d: values carrying different superscript letters on the same line are significantly different between experimental group and NC Gp at p-value less than 0.05 and 0.01*. TNF-***α*** = tumor necrosis factor alpha; IL-6 = interleukin 6; IL-1***α*** = interleukin 1 ***α***; IL-1***β*** = interleukin 1***β***; IL-10 = interleukin 10; INF-δ = interferon gamma; a, b, c, d: values carrying different superscript letters on the same line are significantly different between experimental group and NC Gp at p-value less than 0.05* and 0.01** . Normal Gp = Normal control group; NC Gp = negative control group that received isoprenaline (2 mL/200g) without any pretreatment with fish oil; 8CFO and 10CFO = cooked FO, in doses of 8 mg/kg body weight (8C) and 10 mg/kg body weight (10C); 8RFO and 10RFO = raw (virgin) FO, in doses of 8 mg/kg body weight (8R) and 10 mg/kg body weight (10R), PC Gp = positive control group receiving propranolol in doses of 2 mL/200g body weight.

Evaluation of hematological parameters

There was a decrease in white blood cell count from 11.41 in the normal animals to 11.14 in the negative control group and its sub-types at $p < 0.05$, following MI administration (Table 4). Nonetheless, pre-treatment with fish oil significantly increased white blood cell count from 11.14 in the negative control group to 12.94 in the test group that was pre-treated with 8 mL/kg body weight of cooked fish oil, 12.37 in the test group pre-treated with 10 mL/kg body weight of cooked fish oil, 13.31 in the test group that was pre-treated with 8 mL/kg body weight of raw fish oil, 12.56 in the test group pre-treated with 10 mL/kg body weight of raw fish oil, 11.76 in the positive control group and its sub-types at $p < 0.01$, using the turkeys' test comparison of the normal group and the pretreated groups. Comparison using the t-test between the pre-treated groups, the different oil types (raw and cooked), and different administered doses significantly increased the white blood cell count and its subtypes.

Discussion

Food consumption and animal growth response

An increase in the body weight of the rats was observed in all the groups from day 0 to day 28 can be attributed to the nutritive components received throughout the experiment There was no significant variation in in the food and water intake amongst all the groups, as such no significant variation was seen in the mean weight gain. The dropped in food consumption was because fish

Parameters	NOG (n = 6)	NC Gp (n= 3)	8C FO (n = 2)	10C FO (n = 3)	8R FO (n = 4)	10R FO (n = 6)	PC Gp (n = 2)
Wbc (×10^3 µL^{-1})	11.41 ± 0.14	11.14 ± 0.61a*	12.94 ± 0.14b	12.37 ± 0.47 b	13.31 ± 0.65b	12.56 ± 0.03 b	11.76 ± 0.61
Rbc (x10^6 µL^{-1})	6.05 ± 0.35	5.77 ± 0.55	6.23 ± 0.16	6.18 ± 0.13	6.35 ± 0.24	6.77 ± 0.67a	6.45 ± 0.22
Haemoglobin (g/dL)	11.67 ± 0.28	11.96 ± 0.40	12.99 ± 0.34 a	12.11 ± 0.42	12.47 ± 0.47	12.39 ± 0.69	12.99 ± 0.40
Haematocrit (%)	35.54 ± 1.05	34.94 ± 0.49	35.82 ± 0.63	35.41 ± 1.08	35.39 ± 1.08	35.11 ± 0.97	37.49 ± 0.83 a
Platelets (×10^3 µL^{-1})	462.67 ± 29.11	438.17 ± 8.11	462.17 ± 8.5	467.67 ± 42.56	462.17 ± 36.56	471.33 ± 22.11	474.33 ± 30.11
Lymphocytes (%)	72.00 ± 6.91	68.89 ± 7.83	70.60 ± 3.57	72.57 ± 5.02	70.40 ± 8.24	70.95 ± 5.04	70.43 ± 7.84
Monocytes (%)	6.85 ± 0.55	5.62 ± 0.20a*	5.93 ± 0.38	6.45 ± 0.24a	6.55 ± 0.35a	6.39 ± 0.27a	6.09 ± 0.65
Granulocytes (%)	19.59 ± 0.49	18.6 ± 0.17a*	20.09 ± 0.98	20.99 ± 0.96 b	20.93 ± 0.64 a	20.78 ± 0.11b	20.69 ± 0.49a
Neutrophils (%)	11.38 ± 0.31	9.44 ± 0.28 a*	11.13 ± 0.47b	9.17 ± 0.20	11.46 ± 0.24b	11.23 ± 0.54b	11.06 ± 0.39b
Eosinophils (%)	2.41 ± 0.23	2.32 ± 0.08	2.38 ± 0.15	2.60 ± 0.14	2.57 ± 0.13	2.66 ± 0.33	2.48 ± 0.15
Basophils (%)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
MCV (fL)	55.36 ± 0.38	54.87 ± 1.19	55.79 ± 0.53b	55.81 ± 0.36	56.24 ± 0.38 a	56.09 ± 0.59b	55.41 ± 0.32
MCH (pg)	16.73 ± 0.13	15.89 ± 0.48	16.81 ± 0.22	16.49 ± 0.36	16.77 ± 0.17	16.87 ± 0.15	16.59 ± 0.18
MCHC (g/dL)	29.08 ± 0.28	29.53 ± 0.69	29.89 ± 0.21	29.69 ± 0.26	29.52 ± 0.63	29.69 ± 0.39	29.88 ± 0.87

Table 4: Effects of *Scomber scombrus* oil on haematological parameters in rats challenged with isoprenaline.

a*, b* = Significant difference between normal group and NC Gp at p-value less than 0.05 and 0.01 respectively.

a, b = Significance difference between the pre-treated groups and the NC Gp at p-value less than 0.05 and 0.01 respectively.

RBC = red blood cell count, WBC = leukocyte count, MCV = mean corpuscular volume, MCH = mean corpuscular haemoglobin, Normal Gp= control group; NC Gp = negative control group receiving isoprenaline (2 mL/200g) without any pretreatment; 8C and 10C = cooked FO, in doses of 8 mg/kg body weight (8C) and 10 mg/kg body weight (10C), and 8R and 10R = raw (virgin) FO, in doses of 8 mg/kg body weight (8C) and 10 mg/kg body weight (10C), pC Gp = positive control group receiving propranolol in doses of 2 mL/200g body weight.

oils can trigger side effects, which are usually minor gastrointestinal problems such as belching and indigestion that would lead to reduce hunger and appetite [31] working on fish oils and omega-3 oils health benefit. This was in conformity with Laiglesia-González [32] results which conformed that omega-3 fish oil reduces fat and loss of appetite. The significant drop in the test groups was because the test groups were fed with fish oils that turn to reduce their taste for water. This study is in line with that of Osborne, Radhakrishnan [33] working on the effects of supplementing fish oil in the drinking water of dairy cows on production performance and milk fatty acid composition. There was a decrease in appetite after the induction of MI by isoprenaline as seen in the groups that were induced with ISO. Unfortunately, FO had little or no effect on appetite regulation, given that all the animal's weight decreased with ISO induction as their food intake dropped. This is in confirmation with the report of Gan, Ettinger [34], who stated that bodyweight loss after myocardial infarction in rats is a marker of early heart failure development.

Exploratory behaviour, locomotion and motor coordination

The present study evaluated the effects of raw and cooked S*comber scrombrus* oils of doses 8 and 10 mL/kg body weight on MI induced by the ISO in the rats. The administration of ISO to rats caused cardiac ischemia, which was proven by the general inactivity of the rats in the open field, compared to the normal rat, increased plasma levels of inflammatory cytokines, and other evaluated parameters.

The open-field test is often used as a test for anxiety, exploration, and locomotion in animals [35]. Results from this study reveal that cardiac ischemia reduces locomotion, exploratory ability, and anxiety, comparing the normal group and the negative control group. The turkey test comparison of the negative control group and the fish oil pre-treated groups showed a significant increase in the number of crossing and rearing in the groups that received fish oil. The results showed that there was no significant difference between the groups pre-treated with raw and cooked oils, neither was there a significant difference in the different doses administered. Omega-3 fats have little or no effect on anxiety and depression [36], his could be the reason why there was no significant increase in grooming in the groups that received fish oil. Also, rodents (rats) typically prefer not to be in the center, lit area of the apparatus and tend to walk close to the wall (thigmotaxis) since this is a novel, and presumably, stressful environment to the animal. In general, the open field test may cause anxiety to animals due to social isolation and fear of a novel environment and open space, as social isolation has been linked to indicators of stress by several studies [37]. This explains why there was no significant difference in the center times of the animals as all the animals (both the healthy ones and ischemic ones) moved to the wall of the field, though the rats in the normal group spent a relatively lesser time in the center of the field.

Cytokine levels

TNF-*α* and IL-6 are proinflammatory cytokines involved in the synthesis of collagen and scar formation after acute myocardial infarction [38]. TNF-*α* is not expressed in normal cardiomyocytes, but after myocardial infarction, the ischemia and anoxia activate cardiomyocytes and myocardial mononuclear macrophages, which will produce large amounts of TNF- *α* in the myocardium in the infarcted zone and the infarction border zone. In myocardial ischemia, plasma levels of IL-1*β* are increased, and they cause the activation of the myofibroblasts involved in cardiac remodeling and the alteration of systolic function after acute myocardial infarction [39]. The expression of IL-6, TNF-*α*, and IL-1*β* cytokines is stimulated by interleukin-1*α* [40] whose release from myocardial cells is stimulated by hypoxia and the acidosis accompanying ischemia [39]. IL-1*α*, released from necrotic cardiomyocytes, may serve as a signal, implicated in the activation of the post-infarction inflammatory response that contributes to adverse cardiac remodeling [40]. It has been suggested that the release of constitutive IL-1*α* may extend ischemic myocardial injury by increasing the apoptosis of cardiomyocytes. Based on the results, pre-treatment with FO significantly improved myocardial injury induced by the ISO. Higher incorporation of DHA into the myocardial membrane phospholipid has been regarded as one of the other benefits of taking the FO [41]. Additionally, the modulation of the membrane fatty acid has varied impacts on intracellular and membrane events such as intracellular lipid-based secondary messengers and receptor function [42]. In the present study, daily consumption of FO decreased the plasma level of inflammatory cytokine and prevented the development of chronic myocardial injury. Although the mechanisms of the function of ω3-PUFAs are not completely known, it can decrease arrhythmias of the heart by decreasing the blood pressure, platelet aggregation, and heart rate, as well as by ionic remodeling in the heart [43]. In humans, improving myocardial resistance to the injuries caused by the ischemia-reperfusion is considered one of the protective impacts of the dietary n-3 PUFA against congenital heart disease complications. Disturbance in levels of the key pro-inflammatory cytokines, such as IL-6, IL-1β, and TNF-α, and anti-inflammatory cytokines, such as IL-10, IL-4, and TGF-β, are mainly responsible for the development of chronic inflammation (Ciubotaru *et al*., 2003) and patients with CVD [44]. Mackerel fish oils are rich in omega fatty acids, and Omega-3 polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid and docosahexaenoic acid, are widely regarded as cardioprotective. Several large-scale, randomized clinical trials have shown that dietary intake of omega-3 PUFAs improves the prognosis of patients with symptomatic heart failure or recent myocardial infarction [45]. Therefore, dietary consumption of omega-3 PUFA is recommended in international guidelines for the general population to prevent the occurrence of cardiovascular diseases (CVDs). However, the precise mechanisms underlying the cardioprotective effects of omega-3 PUFAs are not fully understood. Omega-3 PUFAs can be incorporated into the phospholipid bilayer of cell membranes and can affect membrane fluidity, lipid microdomain formation, and signaling across membranes. Omega-3 PUFAs also modulate the function of membrane ion channels, such as Na and L-type Ca channels, to prevent lethal arrhythmias [46]. The plasma levels of INF- γ was significantly decreased in rats that received raw oil, but the 10 mg/kg dose behaved better. These

results confirm the cardioprotective effects of fish oil on cardiac myocytes in both healthy people [47] Endo and Arita [18] reported that omega-3 PUFAs also prevent the conversion of arachidonic acid into pro-inflammatory eicosanoids by serving as an alternative substrate for cyclooxygenase or lipoxygenase, resulting in the production of less potent products [48]. In addition, a number of enzymatically oxygenated metabolites derived from omega-3 PUFAs were recently identified as anti-inflammatory mediators (Jin and Makoto, 2016). It is widely believed that the antiinflammatory properties of omega-3 PUFAs contribute to their cardioprotective effects [18]. In fact, dietary intake of omega-3 PUFAs was reported to decrease the circulating concentrations of inflammatory cytokines such as tumor necrosis factor (TNF), IL1b, and IL-6, and ameliorate left ventricular functional capacity in non-ischemic dilated cardiomyopathy [49]. EPA and DHA downregulate the expression of inflammationrelated genes through inhibition of NF-kB signaling by blocking IkB phosphorylation [50].

Hematological parameters

Hematological parameters are valuable tools for addressing cardiac problems. The association between hematological parameters and metabolic syndrome are well known [51]. The parameters included: red blood cell (RBC) count, leukocyte (WBC) count, hemoglobin (Hb), hematocrit (HCt), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count, lymphocyte, monocyte, neutrophil, basophil, and eosinophil counts. White blood cells and its sub-types are made in the bone marrow [52] and are part of the immune system. They play vital roles in the body such as; defending the body against foreign materials, recognizing intruders, killing harmful bacteria, creating antibodies to protect the body against future exposure to same bacteria and virus, therefore helping to fight against infection [53].

High white blood cell count is a strong and independent predictor of coronary risk in patients of both sexes, with and without coronary heart disease. A high number of white blood cells and their subtypes (for example, neutrophils, monocytes, lymphocytes, and eosinophils) are associated with the presence of coronary heart disease, peripheral arterial disease, and stroke. The coronary heart disease risk ratios associated with a high white blood cell count are comparable to those of other inflammatory markers, including C-reactive protein. Omega-3 index is directly associated with a healthy red blood cell distribution width [54]. In addition, other components of the complete blood count, such as hematocrit and the erythrocyte sedimentation rate, also are associated with coronary heart disease, and the combination of the complete blood count with the white blood cell count can improve the ability to predict coronary heart disease risk.

In the current study, there was a decrease in white blood cell count and its sub-types following MI induction. This is because ISO, the MI inducer acts on the bone marrow in a virus-like manner causing a decrease in hematopoiesis. Nonetheless, pre-treatment with fish oil significantly increased white blood cell count and its sub-types, using the t-test comparison of the normal group and the negative control group. Comparing the pre-treated groups and the negative control group, there was a significant increase in the white blood cell count and its sub-types including monocytes, granulocytes, and neutrophils. The different oil types (raw and cooked) at different administered doses showed no significant difference in their ability to increase the white blood cell count and its subtypes. This result is in accordance with the findings of [55] who reported that fish - oil-rich diet promotes hematopoiesis in the bone marrow and spleen and Omega-3 index is directly associated with a healthy red blood cell distribution width [56].

Conclusions

The study showed that cardiac ischemia impairs growth, exploratory ability, locomotion, and motor coordination in the induced rats, but FO ameliorated these effects, enhancing exploratory ability in the ischemic rats. The present study also showed that FO could reduce cardiac ischemia caused by isoprenalinein rats, by improving on hematological parameters. It also improved the anti-inflammatory capacity of the body. Interestingly, the raw oil sample showed a greater effect, with results showing a direct relationship with omega-3 index, the relative proportion of DHA and EPA in the cell membrane is dependent on the consumption of omega-3 fatty acids. Therefore, FO can serve as an important component in preventing ischemic heart disease Sea fishes such as mackerel which

are the optimal sources of *n*-3 FA be consumed adequately, or the use of fish oil supplements in order to obtain the maximal protective effect towards the development of CVD.

Data Availability

The data used to support the findings of this study are included in this article.

Conflicts of Interest

The authors declare that they have no conflict of interest regarding the publication of this paper.

Acknowledgments

We thank the management and staff of the Agroecology laboratory, University of Buea for the technical support in the realization of most analyses.

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