



## Oxidative Stress and Histopathological Studies of the Effects of Mackerel Fish Oils Against Isoproterenol-Induced Cardiac Ischemia in Rats

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### Abstract

Cardiovascular Diseases (CVDs) remains the leading cause of death worldwide accounting for about 18 million deaths yearly. The high prevalence of cardiac diseases (14%) has emerged as a major public health problem and imposes an escalating burden on the health care system in Cameroon. Fish oils are rich in Eicosatetraenoic acid/Docosahexaenoic acid EPA/DHA respectively and these molecules have been reported to have positive effect on the body. This work therefore aimed to study the protective effect of oils originated from raw and cooked mackerel fish (*Scomber scombrus*) on the development of Cardiac ischemia induced in albino rats as well as their anti-oxidant activities and histopathological effects on some organs. This was done by extracting fish oils from raw and cooked (boiled) Horse Mackerel fish (*Scomber scombrus*) using Bligh and dyer method, followed by oil quality analysis. The effect of oil was studied in vivo using 42 healthy albino rats divided into 7 groups of rats, randomly and evenly distributed with each having 6 rats. The normal group received distilled water orally, the positive control group received propranolol plus Isoproterenol (ISO), the negative control group received only ISO, test groups one and two received cooked fish oil plus ISO and test groups three and four received raw fish oil plus ISO. Body weight, water intake and food consumption were recorded daily. After 28 days, the rats were anaesthetized, blood collected and centrifuged for 10 minutes. Serum obtained was used for oxidative stress analysis. Rats were dissected and organs (heart, liver, lung and kidney) were isolated and used. Heart homogenates were prepared and the level of different oxidative stress markers (catalase, glutathione, and Malondialdehyde) were measured. Liver, lung and kidney were used for histopathological studies. The results obtained reveal that raw fish oil showed an increasing effect in HDL levels as compared to cooked fish oil which showed a milder effect and this resulted in increasing the level of glutathione (3700umol µl /g of tissues) and catalase (3.625UI/mg protein of tissues) while reducing the level of Malondialdehyde (0.00001µl/g of tissue). Findings revealed a decrease in the degree of necrosis and inflammation of the lungs, liver and kidney following pretreatment with raw and cooked fish oil. This results then suggest that pretreatment with both cooked raw fish oil, prevented cardiac Ischemia disease in rats by reducing oxidative damage and necrosis in organs.

**Keywords:** Cardiovascular; Disease; Fish; Oils; Histopathological; Ischemia; Oil quality; Oxidative stress; *Scomber scombrus*

## Introduction

Cardiovascular diseases are multifactorial disorders of the heart and blood vessels, they include: hypertension, coronary heart disease, peripheral vascular disease, abnormal heart rhythms, aorta disease, deep vein thrombosis and pulmonary embolism are considered as the major cause of morbidity and death worldwide, in both developed and developing countries Konstantinides, Barco [1] without living out Cameroon.

In 2020, CVDs represents globally 31% of all deaths, causing approximately 17.9 million deaths per year, with South Sahara Africa and low middle-income countries having about 80% of the deaths [2]. In the 21<sup>st</sup> century, there is a constant effort to incorporate complementary and alternative medications into the practice of conventional medicine, for the management of cardiovascular diseases which are the foremost cause of morbidity and mortality in the world [3]. A large difference in the consumption of eicosatetraenoic acid (EPA) and docosahexaenoic acid (DHA) was considered by Horrocks and Faroqui [4] to be the principal underlying factor. This study led to epidemiological study of cardiovascular disease prevention with n-3 fatty acids. However, some authors [5, 6] have questioned this assessment, with the main criticisms related to the systems used for health monitoring and for registration of the cause of death in Greenland.

Nevertheless, many epidemiological and clinical studies have provided evidence that the polyunsaturated n-3 FAs in fish oils provides cardiovascular disease protection [7]. Some studies shown by Al-Okbi, El-Qousy [7] that fatty liver with inflammation, which is increasing worldwide, is accused as being major player in induction of cardiovascular diseases. Fish oil is rich in long chain polyunsaturated fatty acids and was reported by Al-Okbi, El-Qousy [7] to possess the aforementioned health effects as well as hepatoprotective effect.

It was also found out by Cervantes Gracia, Llanas-Cornejo [8] that there is a link between cardiovascular diseases and oxidative stress and that oxidative stress should be considered

as a primary or a secondary cause for many CVDs. Oxidative stress refers to an imbalance between the systemic manifestation of reactive oxygen species (ROS) and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage.

When ROS production increases, they start showing harmful effects on important cellular structures like proteins and lipids [9]. A large body of evidences shows that oxidative stress can be responsible, with different degrees of importance, in the onset and /or progression of several diseases (atherosclerosis, and cardiovascular diseases [10]. Cardiovascular diseases are clinical entities with a multifactorial etiology, generally associated with a very large amount of risk factors, the most broadly recognized of which are hypercholesterolemia, hypertension, chemical inducers, diabetes, unbalanced diet, stress, and sedentary life [11]. Oxidative stress acts mainly as a trigger of atherosclerosis. It is well known that atheromatous plaque formation results from an early endothelial inflammation, which in turn leads to reactive oxygen species generation by macrophages recruited in situ [12].

Therefore, a new therapeutic approach holds the potential to ameliorate endothelial dysfunction and as such prevent CVDs development [13]. Cardiovascular disease is a public health problem in Cameroon with increase mortality every decade (10years). Despite the available treatment and dietetic management, people are still suffering from cardiovascular diseases. Mackerel fish is rich in oil (35-50 % conc) and is highly consumed in Cameroon due to its availability [14]. However, insufficient data is available about the state of organs after consuming fish oils and less attention have also been paid on the effects of fish oil on histology/stress in Cameroon. This work aimed at evaluating the protective effects of mackerel fish oils against isoproterenol induced cardiac ischemia in rats.

## Materials and Methods

### Materials

The following materials were used: 42 Male and female Wistar rats, 30 kg of Sea Horse Mackerel Atlantic Fish, animal metabolic

cages, hand grinding machine and an electric blender, Organic solvents (11Ls Chloroform and 10Ls Methanol), 1g of Isoproterenol, 3 tablets of 40 mg each of propranolol, Saline water, Syringes of different volumes, gavage needle of length (inches) 2-3in, ball diameter 2.25mm and shape curved, tubes of different sizes and volumes, Pipettes of different sizes and volumes, Eliza plates, Oxidative stress and Lipid profile reagents (CHRON LAB), Weighing balances scale, Centrifuge (Labnet-C0226R) etc.

## Methods

### Study design

This study was a laboratory-based study carried out in the Medical Research and Applied Biochemistry Laboratory in the Faculty of Health Science, Molecular and Cell Biology Laboratory, the Animal Physiology laboratory of the university of Yaoundé 1 and the Life Science Laboratory of the Faculty of Science of the University of Buea.

### Sample collection

Thirty kg of Atlantic Ocean horse mackerel fish (*Scomber scombrus*) was freshly purchased, in CONGELCAM Great Soppo Buea directly on the arrival of the refrigerated truck, they were packed in ice polystyrene bags and transferred in a flask to the laboratory for oil extraction.

### Sample preparation

*Scomber scombrus* were thawed in running water, scales, fins, spines, digestive excretory system and bones were removed and the tissues along with the head (omega-3 is mostly concentrated in the brain) was taken to obtain maximum amount of flesh. They were divided into two batches of 10 kg each. One batch boiled at 100° for 35 minutes with 500 cm<sup>3</sup> of tap water using gas cooker. They were cut into fillet, sliced, ground and blended using hand and electrical blender respectively for oil extraction.

### Extraction and characterization of mackerel (*Scomber scombrus*) fish oils.

#### Oil Extraction

Extraction of the fish oils was done according to the method of Bligh and Dyer [15] with some modification by Kinsella [16] on

the cooked and raw fish separately. 200g of fish out of the 10kg was first roughly ground in a hand machine and finely ground and homogenized in the electric blender for 2 minutes with a mixture of methanol (400ml) and chloroform (200ml) in ratio 2:1V/V and the mixture was thoroughly homogenized for another 2 minutes. 200ml of chloroform was added again to the mixture. After blending for an additional 30 seconds, 200ml distilled water was added (Whenever 200g of fish tissue was not available, the solvent volumes used was adjusted to the same ratio). The homogenate mixture was stirred with a glass rod, poured in a clean spreaded out cotton tissue in a bowl. It was pressed to separate the solvent from the chaffs and then filtered through a Whatman no.1 filter paper using a Buchner funnel. It was then poured on a separating funnel under vacuum suction to separate the water from the organic solvent that contained the oil. 100ml chloroform was used to rinse the container that contained the mixture. The filtrate was allowed to settle for 6 to 24 hours for it to totally separate into the organic and aqueous layers. The chloroform layer containing the lipids was transferred into another beaker. Chloroform was evaporated to obtain oil using a rotary evaporator at 45°C. The evaporated oils were weighed and the Percentage Oil Yield calculated as:

$$\text{Oil yield} = \frac{\text{Weight of extracted oil (g)} \times 100}{\text{Weight of the initial sample (g)}}$$

The obtained oils were stored at 25°C in clean non-transparent bottles for further analysis.

### Quality evaluation of extracted fish oils

#### Free fatty acid

The determination of free fatty acid (FFA) of fish oil samples was made according to the method described by AFNOR [17]. The results were expressed as % oleic acid (% FFA).

#### Iodine value

The iodine value (IV) of fish oil samples was determined using the Wijs method, as described in the AOAC official method [18]. The IV was expressed as g I<sub>2</sub> per 100 g of sample.

**Peroxide value**

The peroxide value was determined by referring to the IDF standard method, 74A: 1991 [19]. The results were expressed as milliequivalents of O<sub>2</sub> per kg sample.

**P-Anisidine value**

Anisidine value was determined by the standard AOCS Cd 18-90 « p-anisidine value » using a Perkin Elmer UV-Visible Spectrophotometer (Norwalk CT, USA) [18].

**Total oxidation value**

Total oxidation (TOTOX) values of oil samples were determined using the equation TOTOX = 2PV + p- AnV according to [20].

**Rats bioassay**

**Ethical clearance**

All procedures were carried out in accordance with the ethical clearance obtained from the University of Buea Institutional Animal Care and Use Committee with ref (2021/05/UB/IACUC/BTU/FS) of the 16/07/2021 date.

**Standard feed preparation**

Standard feed was composed according to the American National Academy of Science/Nutrition Research Council using the method reported by Aning, Ologun [21] and Aletor [22]. The composition of all ingredients was as follows (Table 1).

Ingredient	Composition (g)
Maize flour	680
Soya beans flour	200
Fish bones flour	100
Animal bone flour	10
Soya beans oil or maize oil	1
Multi vitamin	1

**Table 1:** Standard feed composition.

**Experimental phase**

Forty-two albino rats, *Rattus norvegicus* aged between 12 and 14 weeks, and weighing 160-180g, were purchased from the animal house of the University of Dschang and transferred to the animal house of the Medical Research and Applied Biochemistry Laboratory, University of Buea. They were kept in cages in the lab where they were allowed to acclimatized to the laboratory conditions (temperature 25-30°C and maintained a 12hours light/12hours darkness cycle) with free access to water before the beginning of the experiment proper. Solid pellet diet was delivered *ad libitum* for the first two weeks and then, at 25 g/rat/day for the rest of the experiment, in order to avoid overweight.

The forty-two rats were randomly and orderly assigned into seven experimental groups, with each group made up of six rats (3 males and 3 females) and fed till the 28<sup>th</sup> day on a semi-synthetic and isoenergetic diet according to the AIN93 (American Institute of Nutrition) criteria modified in relation to the fat source (balance diet or fish oil). Their daily food and water intake were taken.

**Animals grouping**

Group 1 normal control group (untreated rats) were fed by standard laboratory chow, water receive 10ml/kg of saline water orally for 26 days.

Group 2 (positive control rats) were given standard laboratory chow, water plus 10ml/kg of saline water for 18days and 10days prior to sacrifice, propranolol (10ml/kg) was given orally.

Group 3 (negative control groups) were given standard laboratory chow, water plus 10ml/kg of saline water orally for 26 days.

Group 4 (Test1 R08: raw fish oil 8ml/kg body weight) and Group 5 (Test2 R010: Raw fish oil 10ml/kg body weight) were given standard laboratory chow, water plus raw fish oil 8ml/kg and 10ml/kgbw respectively for 26 days by gavage.

Group 6 (Test3 C08: Cooked fish oil 8ml/kg body weight) and Group 7 (Test4 C010: Cooked fish oil 10ml/kg body weight) were given standard laboratory chow, water plus cooked fish oil, 8ml/kg, 10ml/kgbw respectively for 26days by gavage.

On the 27 and 28days, cardia ischemia rats from group 2 to group 7 were all induced with 85mg/kg body weight of isoproterenol using subcutaneous parenteral routes of administration and on the 28<sup>th</sup>, all the rats were allowed to fast for 24 hours' prior for sacrifice. On the 29<sup>th</sup> day, the animals were anesthesia (ketamine/diazepam diluted with distilled water in ratio 2:1:2) and scarified by decapitation. Blood was collected in a dry tube and serum was prepared. The organs (liver, lung, heart, brain, spleen and the kidney) removed, rinsed in normal saline solution, weighted and relative organs weight calculated (mass organ/mass of the animal). The isolated heart tissue divided into two, one of them was homogenized (20% homogenate) in cold Tris-HCl buffer (50 mM, pH 7.4) for oxidative stress analysis such as: Malondialdehyde (MDA), glutathione (GSH) and Catalase (CAT) and the other used for histopathological studies.

#### Evaluation of oxidative stress markers in the heart and serum Malondialdehyde (MDA) determination

MDA as an indicator of lipid peroxidation in tissues, was determined in the heart and serum of rats by the method described by Mas-Bargues, Escriva [23] Tissue homogenate (1 ml) was added to 0.5 ml of trichloroacetic acid (20%) and 1 ml of thiobarbituric acid (0.67%). The mixture was allowed to react for 10 min at high temperature (90°C, water bath). The mixture was centrifuged and the absorbance of the supernatant measured at 530 nm. The concentration of MDA was quantified with the extinction coefficient of  $1.56 \times 10^5 \text{ M}^{-1} \cdot \text{cm}^{-1}$  and expressed as  $\mu\text{M}$  of MDA per g of protein.

#### Reduced glutathione level

The assay of reduced glutathione was performed following the protocol described by Ellman [24]. Heart tissue homogenates and serum (10  $\mu\text{L}$ ) were added to Ellman's reagent (1500  $\mu\text{L}$ ). The mix-

ture was then incubated for 1 h at room temperature and the absorbance was determined at 412 nm. The amount of reduced glutathione (mol/mg of protein) was calculated with molar extinction coefficient ( $13,600/\text{M} \times \text{cm}$ ).

#### Catalase activity

Catalase activity was evaluated according to the protocol of Sinha [25]. Briefly, 25  $\mu\text{L}$  of homogenate and 375  $\mu\text{L}$  of phosphate buffer (0.1 M, pH 7.5) were mixed. Hydrogen peroxide solution (100  $\mu\text{L}$ , 50 mM) was introduced in the mixture and the reaction was stopped one minute later by adding 1 mL of dichromate/pure glacial acetic acid. All tubes were heated (100°C) for 10 minutes. After cooling, the absorbance was read at 620 nm, catalase activity was determined using the calibration curve and expressed as mmol of  $\text{H}_2\text{O}_2/\text{min}/\text{mg}$  of protein.

#### Histopathological analysis

The organ samples previously kept in buffered formol (10%) were subjected to the following histological techniques. After dehydration, liver samples were embedded in paraffin (melting point:  $56^\circ\text{C} \pm 2^\circ\text{C}$ ) to form blocks, 5  $\mu\text{m}$  thick sections from each sample were stained with haematoxylin-eosin and observed under light microscope (Olympus, GHBS, Japan) [26].

#### Statistical analysis

Values are presented as mean  $\pm$  standard error of mean (SEM). Statistical differences between control and treated groups were calculated by analysis of variance (ANOVA) and Bonferroni's post hoc test was also applied using GraphPad InStat Software. Values were considered significantly different if  $p < 0.05$ .

## Results

#### Extraction yield and quality of fish oils

There is great significant difference in percentages yield between the raw fish oil and cooked fish oil. Oil quality indices are present in table 2. The table showed that raw fish had lower oil yield of 3.02% as compared to cooked fish with 5.6% yield.

Parameter	Raw Mackerel fish oil	Cooked Mackerel fish oil	Standard CXS 329-2017 WHO/FAO
Oil yield (%)	3.02	5.6	2-5
% Free fatty acid value	4.02 ± 0.02 <sup>a</sup>	11.5 ± 0.45 <sup>b</sup>	1-2.5
Saponification value (mgKOH/g)	168.30 ± 1.12 <sup>a</sup>	246.3 ± 1.57 <sup>b</sup>	165-195
Iodine value (gI <sub>2</sub> /100g)	159.5 ± 0.00 <sup>a</sup>	34.47 ± 0.070 <sup>b</sup>	135-190
Peroxide value (meqO <sub>2</sub> /Kg)	12.5 ± 0,00 <sup>a</sup>	14.6 ± 0,57 <sup>b</sup>	5-20
P-Anisidine value	0.49 ± 0.05 <sup>a</sup>	16.75 ± 4.65 <sup>b</sup>	≤ 20
TOTOX Value	25.50 ± 0.06 <sup>a</sup>	44.95 ± 2.08 <sup>b</sup>	≤ 26

**Table 2:** Characterization of mackerel fish oils physicochemical properties.

The free fatty acids (FFA) value in the raw fish oil was found to be 4.02%, which was far better than that in cooked fish oil (11.5%). On the other hand, higher standard FFA value in fish oil according to the Codex Alimentarius [27] and International Association of Fish Meal and Oil Manufacturers (IFOMA) [28] ranges from 1-2.5% oleic acid.

The saponification values obtained from this study were 168.3mgKOH/g for raw fish and 246.3mg KOH/g for cooked fish. The highest value was observed in cooked fish.

The iodine value obtained ranged from 159.5 to 34.47/100g of sample.

The standard peroxide value of fish oil is 3-20Meq/kg [29]. The boiling significantly increased the peroxide value compare to raw fish oil.

Boiling significantly affected p-anisidine value of the oil. The lowest p-anisidine value was found in raw fish oil extracted at ambient temperature < 40°C.

The Totox values of oil from boiled was higher than value of raw fish oil and was within the standard (25.5meq/kg).

### Animal bio-assay

#### Animal growth response

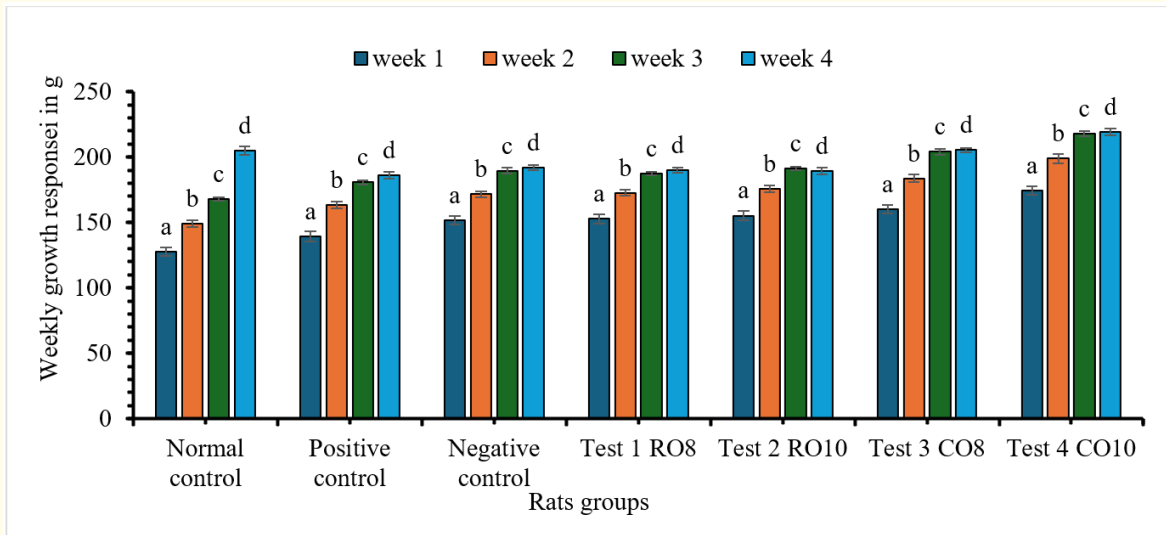
Change in total body weight of rats in grams throughout this study period is shown in figure 1. There was a general increase in body weight in all the groups from week 1 to week 4, with week 4 showing greater significant increase ( $p < 0.05$ ) in growth response as compared to the other weeks.

#### Food consumption in rats

The weekly food consumption by the different groups of animals is presented in figure 2. The results reveal that there was a general decrease in food consumption from week 1 to week 4 with most groups consuming 20 to 34g of food a week. The positive control group consumed the highest with 34 g of food in week1; while food consumption in all groups were not significantly different.

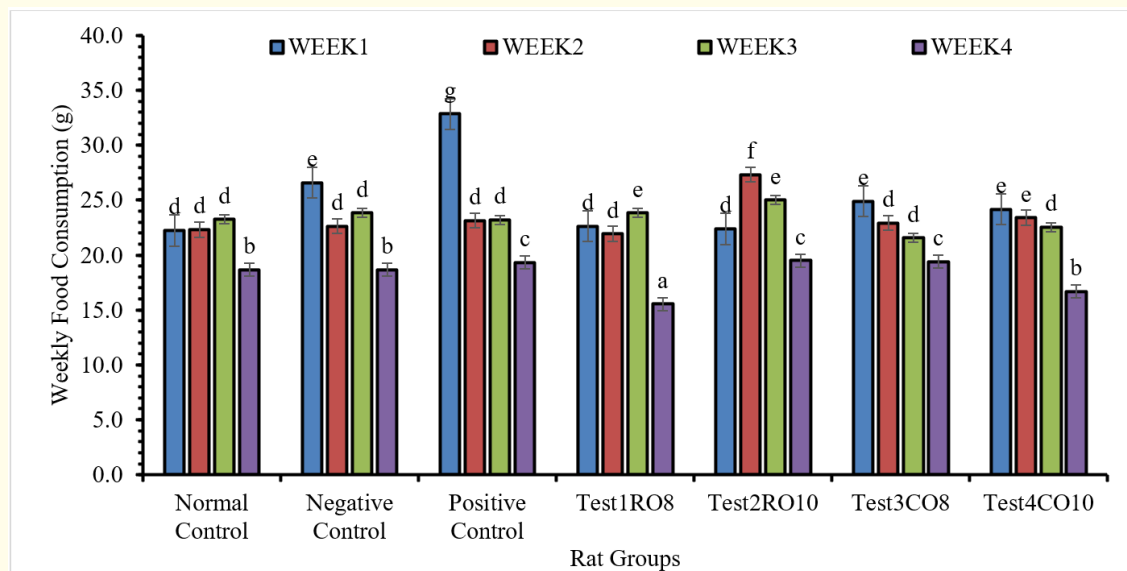
#### Water intake by rats

The figure 3, represents weekly water intake by rat groups from week 1 to week 4. Each bar represents the mean ± SEM, (n = 6). There was fluctuation in water intake for all the groups from week1 to week4 with the test groups having a significant drop of water intake in week4 as compare to the control groups.



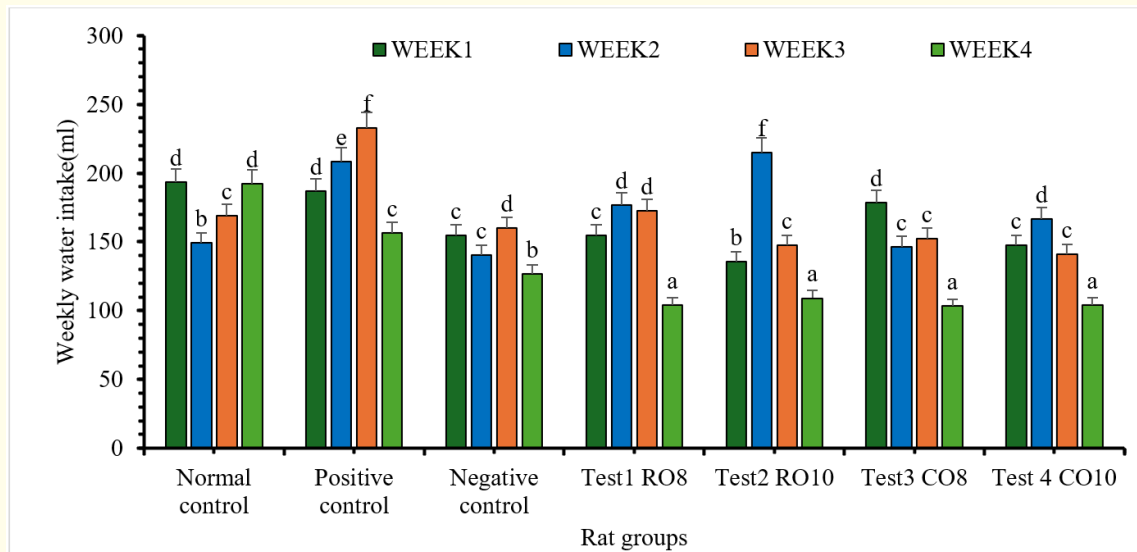
**Figure 1:** Effect of fish oils on body weight gain of rats

n = 6; a, b, c and d peaks carrying the same letter for a group or different group of rats are not significantly different at  $p > 0.05$ .



**Figure 2:** Effect of fish oils on food consumption in rats

n = 6; a, b, c, d, e, f, and g peaks carrying the same letter for a group or different group of rats are non-significant ( $p > 0.05$ ).



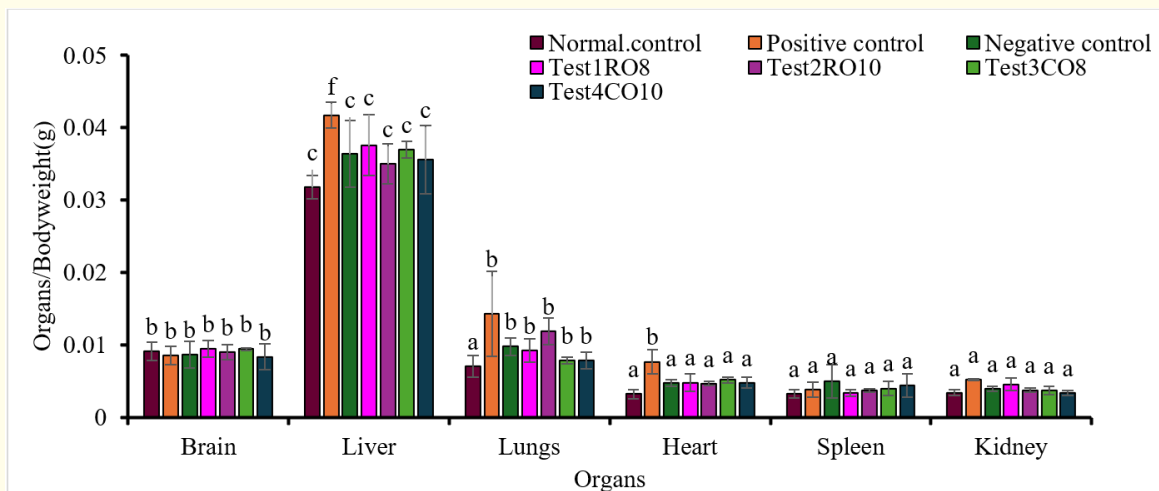
**Figure 3:** Effect of fish oils on water intake in rat.

n = 6; a, b, c and d peaks carrying the same letter for a group or different group of rats are not significant (p > 0.05).

**Effect of fish oils on the organs weight**

The changes in the relative organs weight (organs weight/body-weight ratio (%)) are represented in figure 4. The liver/body weight ratio of rats in all the groups were significantly high as compared to other organs. No significant difference (p > 0.05) was observed in the brain and spleen/body weight ratio in all the groups. Mean-

while, there was a significant drop in the liver, lung, heart and kidney/body weight ratio for normal control group as compared to the other groups. Isoproterenol turns and inflected the organs of the induced rats. On the other hand, there was a small decrease in the liver, heart and kidney body weight ratio fed with high concentration of raw fish oil (10ml/kg).



**Figure 4:** Effect of fish oils and isoproterenol on the weight of organs

n = 6; a, b, c, d, e, and f peaks carrying the same letter for a group or different group of rats are non-significant (p > 0.05). Values represents mean ± SD. (n = 6) One-way ANOVA followed by Student’s t-test.

**Effect of fish oils on oxidative stress biomarkers in the heart and serum.**

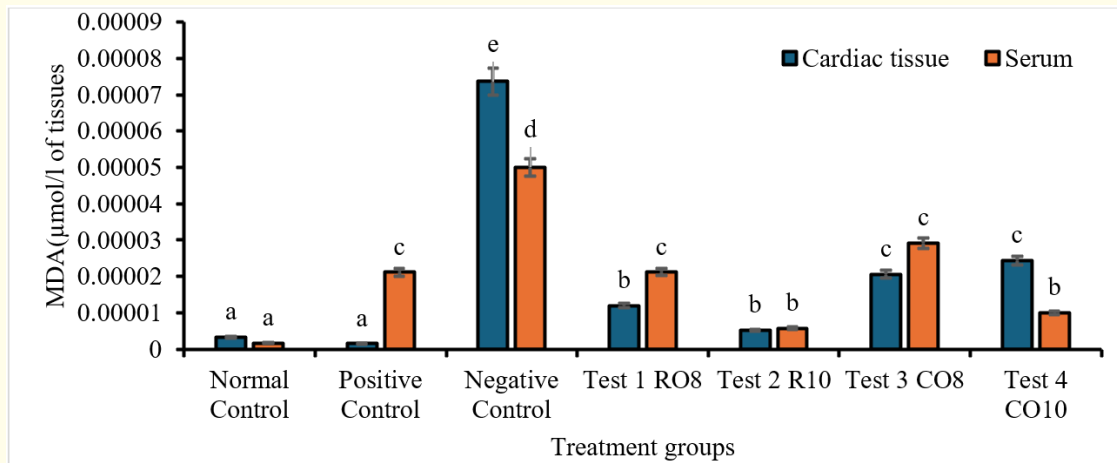
**Effect of fish oils on MDA levels in the heart and serum.**

Heart and serum MDA values (Figure 5) were significantly ( $p < 0.05$ ) higher in the negative control group compared to all other groups, while the positive control groups, normal control group

and raw mackerel fish oil groups had a significant ( $p < 0.05$ ) lower MDA value in the heart and serum.

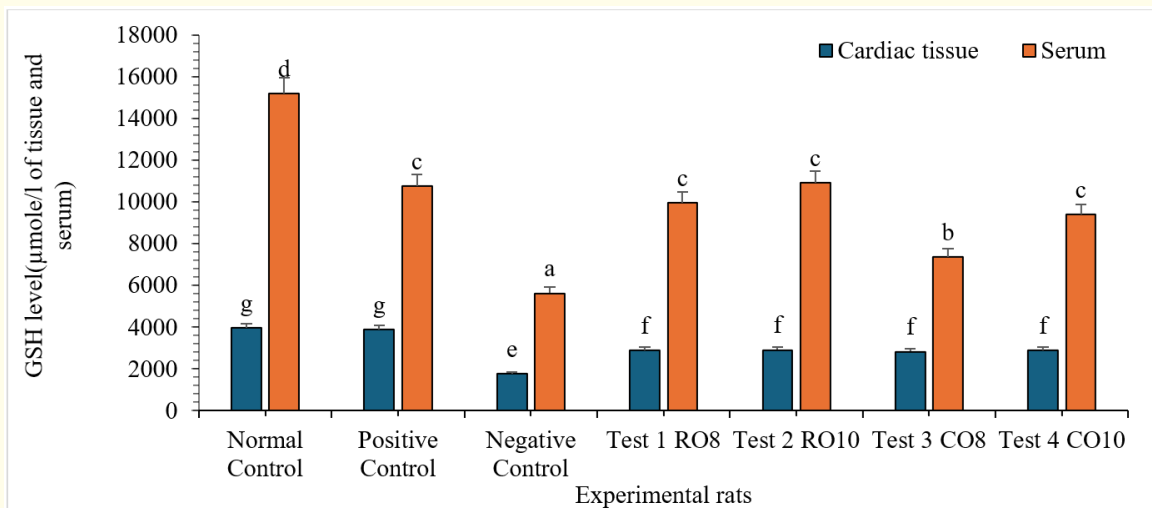
**Effect of fish oils on GSH levels in the heart and serum**

The level of reduced glutathione (Figure 6) was significantly high in the heart and serum of the normal control, positive control and Test groups ( $P < 0.05$ ) as compared with the negative control.



**Figure 5:** Effect of fish oils on MDA level of the heart and serum

n = 6; a, b, c, d, and e peaks carrying the same letter are not significantly different in the cardiac tissue and serum ( $p > 0.05$ ).



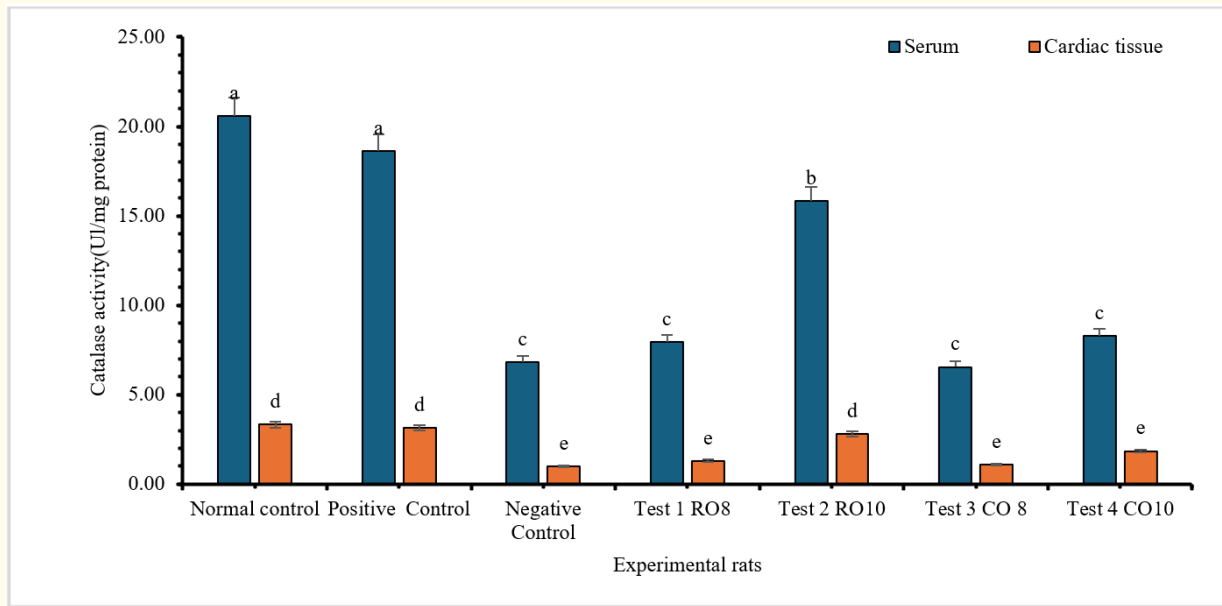
**Figure 6:** Effect of fish oils on GSH levels in the heart and serum.

n = 6; a, b, c, d and f, peaks carrying the same letter are non-significant different at  $p > 0.05$  compare to normal control.

**Effects of fish oils on catalase levels in the heart and serum**

Figure 7 presents the serum and tissue levels of catalase (CAT). CAT levels of serum and cardiac tissue homogenate, were significantly reduced in isoproterenol-treated rats as compared with nor-

mal and positive control rats. The altered levels in inducer-treated rats were normalized by raw fish oil pretreatment in test 2 RO10 rats compared with that of normal and positive control group, with the Negative control having the lowest value.



**Figure 7:** Effects of fish oils on Catalase levels in the heart and serum.

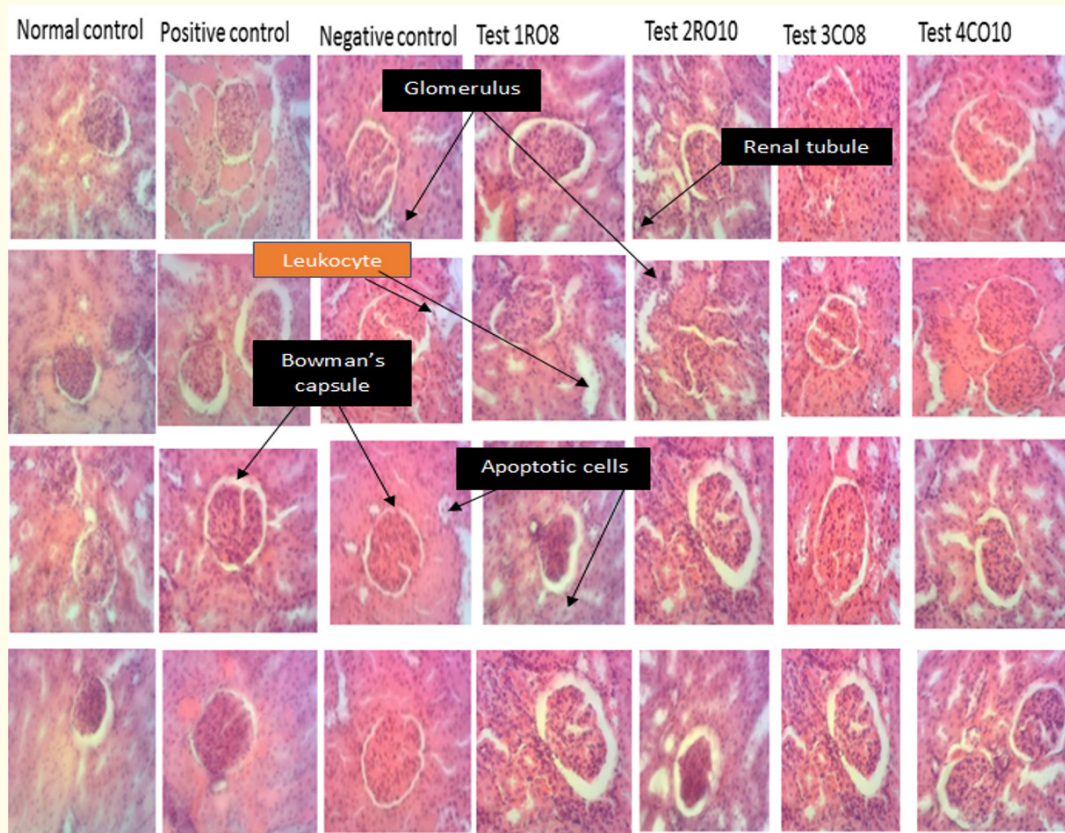
n = 6; a, b, c, d and f peaks carrying different letter are significantly different at (p < 0.05) for serum.

**Histology of organs (Kidney, Liver and Lung)**

**The kidney**

In kidney tissue, as seen in figure 8, section of Normal control rats, a normal structure of glomeruli, renal cortex as well as proximal and distal tubule was observed. In contrast, the kidney sections of the Positive control rats showed signs of morphological and pathological changes. In the Negative control the glomerular region of the kidney, showed several glomeruli atrophic changes, widening of Bowman’s capsule with obvious degeneration of cells and losing the prominent glomerular structure, suggesting apoptotic cell death. The proximal convoluted tubule also showed atrophic changes including epithelial shedding and loss of brush bor-

der. There was also the presented of leukocyte infiltration, edema exudate, and necrotic foci. As compared to normal controls and positive control. Tubular lumen was found to be sometimes obliterated due to degeneration and swelling of lining epithelial cells. Test1RO8, showed relatively healthy glomeruli and tubules, Test2RO10 showed relatively healthy glomerulus with abundant Bowman’s capsular, renal cortex as well as proximal and distal tubule was observed. Test3CO8 diminished and distorted glomeruli and dilated tubules with some edema exudate, and necrotic foci. Test4CO10 showed some infiltration of inflammatory cells surrounding the distorted glomeruli and tubules with some healthy glomerulus.



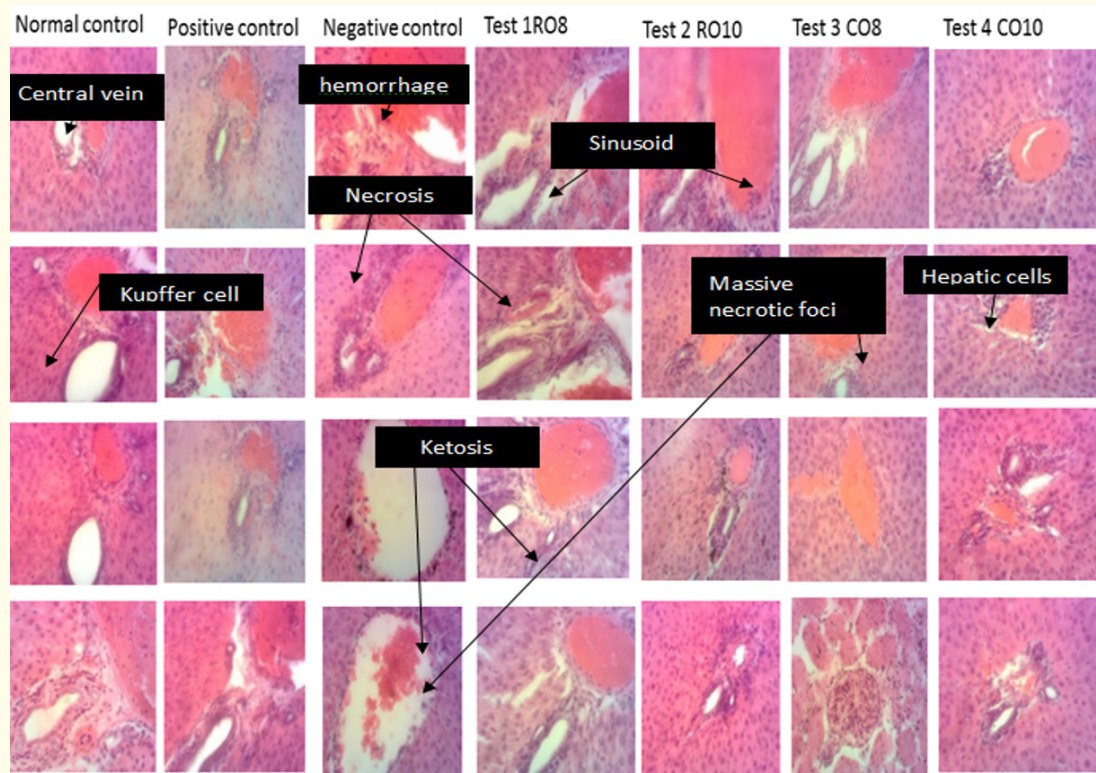
**Figure 8:** Histopathology of the kidney. Light micrographs of the kidney sections from the different treatment group. (Magnification 200×).

**The liver**

Light micrographs of the liver sections from different treatment groups.

It could be noticed that in figure 9, Normal control group showed normal liver structure, the hepatic cords radiating from central vein separated by sinusoid. Positive control group demonstrated focal area of hepatic necrosis infiltrated with inflammatory cells and most of hepatocyte showing fatty degeneration. Negative control group also showed fatty degeneration with the hepatocytes taking signet ring appearance, cytoplasmic degeneration and some aggregation of cells also the presence of infiltrative cells. Test1R08 group treated with raw fish oil of less concentration

(8ml/kg of body weight) contained multi focal areas of fatty degeneration and focal aggregation of inflammatory cells and looked healthy with normal hepatocytes while some bi-nucleated cells refer to regeneration. Test2R010 group treated with raw fish oil of high concentration (10ml/kg of body weight) showed a high mark improvement of histopathology changes in their pictures, looked healthy with bi-nucleated cells and the activation of Kuepfer cells. Liver from the majority of rats treated with cooked fish oil showed marked improvement in the histopathology changes also but not as effective like that of raw fish Test3C08 and Test4C010. Liver of rats received cooked fish oil of high concentration revealed marked improvement in histopathological changes, looks healthy with mild activation of Kuepfer cells, Test4 C010 while only one rat from this group showed focal area of vacuolar degeneration.



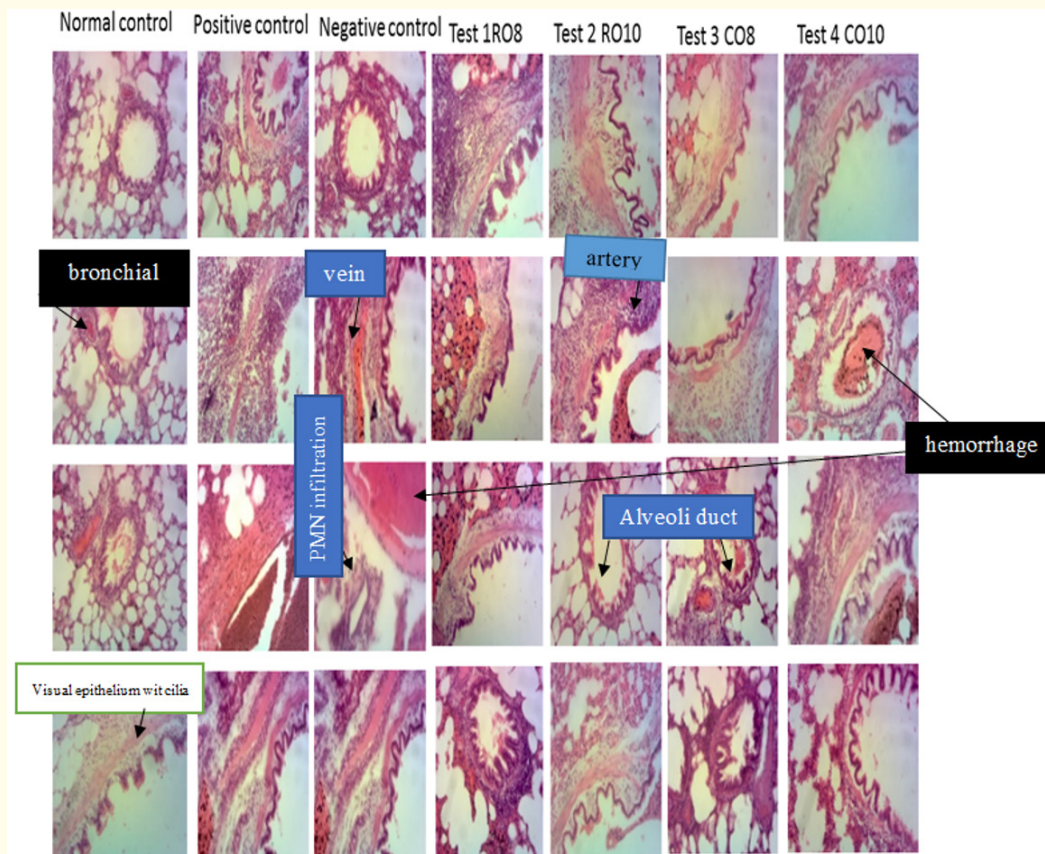
**Figure 9:** Histopathology of the liver. Light micrographs of the liver sections from different treatment group (Magnification 200×).

**The lungs**

Figure 10 illustrates the light micrographs of the lung sections. The Normal control group showed the normal visual epithelium, alveoli, pulmonary artery, and bronchial epithelium structure in the lung tissues. The microscopic examination of the Normal control animal group yielded no pathological findings. However, the lung tissues of the Positive control group that were given standard drug showed a nearly-normal appearance except for slight thickening, dilatation, and congestion in the vessels and mild alveolar edema.

Significant polymorph nuclear neutrophil (PNL) infiltration, severe hemorrhage, alveolar destruction, and edema were

observed in the lung tissue of the Negative control group. The animal group treated with TestRO8 shows significant polymorph nuclear neutrophil infiltration few hemorrhage, alveolar destruction, and edema in the lungs. The vascular wall of TestRO10 treated lung tissue was seen as normal, inflammation appeared lessened, except for slight thickening, dilatation, congestion and mild alveolar edema. The group treated with (TestCO8) induced severe hemorrhage, inflammatory damage, alveolar destruction, few edemas in the lungs. The group treated with (TestCO10) induced some few inflammatory damage, represented as infiltration of inflammatory cells macrophages and lymphocytes in to airway and surrounding tissues.



**Figure 10:** Histopathology of the lung. Light micrographs of the liver sections from different treatment group (Magnification 200×).

### Discussion

The epidemiological studies of cardiovascular diseases indicated that there is a relation that exists between CVDs and omega-3 fatty acids (EPA/DHA), which shows a low incidence of cardiac diseases in populations that consumed large amounts of seafood in Inuit of Greenland [6, 30]. The rat’s organs, oxidative stress biomarkers and histology in some organs were evaluated in Albino Wistar rats. The rats were fed with raw and cooked Mackerel fish oils for 26 days prior to isoproterenol treatment on the 27<sup>th</sup> and 28<sup>th</sup> day.

### Oils yields

Bligh and Dyer [15] was performed using chemical solvents (methanol and chloroform to extract the total fat in fish. Polar solvent penetrated into the cells and extract the lipids from the cell membrane to be converted into phospholipid material. Percentage yield from raw and cooked mackerel fish oils was semi fatty (3.02% and 5.6%). Based on Linder, Belhaj [31] Linder *et al.*, (2010), Zhang, Ning [32], Tenyang, Womeni [33], they can therefore be classified as semi fatty fish (oil content 5 -10%). Oil contents less than 5% are lean fish (low fatty fish e.g. salmon) while those with oil contents

above 10% are fatty fishes [31]. However, cooking improves the extraction yield of mackerel fish oil. This was also in confirmation with Szparaga, Kocira [34] that at 95°C, the oil yield was 20% far more than that obtained in the study. Yasin, Ahmad [35] showed that the yield of extracted fish oil increased from 60°C to 80°C, but decreased as extraction temperature increases to 100 °C. Suseno, Nurjanah [36] noted that proteins usually undergo irreversible denaturation when heated at 70-100 °C and denatured proteins forming a dense structure can cause inhibition of oil release. The high temperature was more effective to split fatty cells in cooked fish which contain lipids, so the yield of extracted lipid is great [36]. Extraction which was performed at low temperature produced a lower yield, due to incompletely split of fatty cells [36].

### Oils qualities

In other to test the safety quality of oil before the *in vivo* test, oil quality indices were evaluated compared with CODEX oil standard 2017. According to Tenyang, Womeni [37] acid value is a measure of the percentage of free fatty acids present in fat. The higher the value, the more oil is prone for oxidation and hence have shorter shelf life. The free fatty acid value of the raw oil was found to be 4.02 % not far from the recommended fish oil value ( $1 \leq 2.5$  %FFA) [38] which show a considerable reduction from that of cooked (11.5 %FFA). The hydrolysis of triglycerides brought about by high temperatures during cooking may account for the significant variation in acid value for boiled mackerel fish oil. High extraction temperature could trigger the onset of FFA impairment, in which free fatty acids were degraded into volatile components [36]. The saponification value has been reported to be inversely related to the average molecular weight of the fatty acids in oils, it helps to know the fatty acid length [39]. Raw oil was reduced to 168.3KOH/g as compared to 246.3mg/KOH/g in cooked oil. Oils with saponification values less than 200mg KOH/g have high molecular weight fatty acids [40]. Raw mackerel fish oil had 168.3KOH/g therefore showing that the oil is rich in long chain fatty acids (PUFA). The high saponification value of boiled Mackerel fish oil revealed destruction of the double bonds in cooked oil. The standard value for cooked fish oil (180- 200mgKOH/g) set by WHO/FAO [41]. The values are however comparable with 190-200mgKOH/g obtained by authors such as Adeniyi and Bawa [42].

The iodine value of the raw oil was found to be 159.5 iodine/100g and the standard value is 135-190/100g [43] as compared to that of the cooked oil 34.7iodine/100g of the sample, which implies that few of the double bonds in the raw oil has been destroyed, hence, giving the oil a different colour. While the cooked fish oil value revealed great destruction of the double bonds because the value is significantly lower than the value obtained by Tenyang, Tiencheu [44]. This also suggests that Mackerel fish oil is particularly rich in polyunsaturated fatty acids as reported by Mostafa, Abouzied [45]. Similar observation of iodine value was obtained by Tenyang, Womeni [33], [46, 47] during processing of fish, edible insects and larvae respectively. A higher iodine value indicates more double bonds in the sample and therefore greater care will be needed to slow down oxidation. The iodine value obtained is higher than that obtained by Oladapo and Awojide [48] for raw fish (130/100g). It is however comparable with that proposed by the Codex Alimentarius Commission [41].

The peroxide value of the raw oil was also found to be 12.5MEq/kg and that of cooked oil 14.6MEq/kg both are within the limit (<20 milliequivalent of active oxygen/kg oil) prescribed by the WHO/FAO for edible oils showing low deterioration for raw oils, however, greater than the threshold for fish oils prescribed by the CODEX ( $\leq 5$ ) milliequivalent of active oxygen/kg oil). The peroxide value is a measure of primary oxidation product present in fat sample. The higher peroxide value in cooked fish oil suggest its deterioration caused by the alteration of unsaturated fatty acids probably during boiling. It has been shown that polyunsaturated fatty acids molecules are destroyed by high temperatures and even at room temperature due to the presence of a lot of pro-oxidant (oxygen, heat, metal) in fish oil, when studying the effects of cooking and smoking on cat fish [37,44,49]. Rancidity protracted peroxides value and decrease the quality of the fish [36]. The unpleasant rancid odour was due to the formation of aldehydes or ketones as derived products of hydro peroxides. The change in peroxide value was an indicator of lipid oxidation (oxidation increases the peroxide value) [50]. As oil goes rancid, triacylglycerides is converted to fatty acid and glycerol which increases acid number [51]. Rancidity in oil causes undesirable chemical changes in flavour, odour and nutritional value

of oil. The low acid and peroxide values of the oil was an indicator of the oils ability to resist lipolytic hydrolysis and oxidative deterioration [52]. These reductions in acid value and peroxide value of raw oil imply that there is an improvement in the quality of the raw oil, as it reduces the susceptibility of the oil to rancidity and improve stability.

According to International Fish Oil Standard (IFOS)[36] p-anisidine value should be less than 15 meq/kg. While WHO/FAO (2017) standard value was  $\leq 20$  meq/kg. Secondary oxidation product was measured as p-anisidine value, the higher the extraction temperature, the higher the p-anisidine value and the maximum decomposition of hydroperoxides that generated secondary oxidation products [53].

The TOTOX value was applicable for raw oils and not for cooked oil because its value was above the limits. Raw fish oil 25.5 and cooked fish oil 44.95 respectively, that of the standard value ( $\leq 26$ ) by WHO/FAO. This study clearly demonstrates that raw fish oil examined meet the n-3 label content claims, and were not heavily oxidized based on CODEX Alimentations [41] for recommended standards for fish oils.

#### **In vivo assay**

The organ body/weight ratio gives a proportional size of the organ to body weight. It has been suggested that the use of organ-body weight ratios may be valuable in evaluating the relationship between certain experimental situations and the biological response of a test organism. The liver being the major organ carrying out metabolic and detoxification processes is unique among the body's vital organs in that it can be regenerated. Nirogi, Goyal [54] said that organs weight can be the most sensitive indicator of an effect of an experimental compound, as significant differences ( $p < 0.05$ ) in organs weight between treated and untreated (control) animals to show the absence or present of any morphological changes. Thus the organ-body weight ratio observed a better ( $p < 0.05$ ) performance in animals fed on the raw fish oil in the brain, liver, heart and kidney compared to those placed with the cooked oil and negative control.

This healthy general increased in growth response, was due to increase in the rat's lean mass, bone mass and fat mass but not obesity and this was in line with Garcia Cruz, González Azpeitia [55] who says fish oil can improve early child growth.

The drop in food consumption was because fish oils can trigger side effects, which are usually minor gastrointestinal problems such as belching and indigestion that would lead to reduce hunger and appetite [56]. This was in conformity with Emanuel, Krampitz [57] 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 reduced their taste for water. This study is in line with that of Osborne, Radhakrishnan [58] working on the effects of supplementing fish oil in the drinking water of dairy cows on production performance and milk fatty acid composition.

The significant drop in the liver, lung, heart and kidney/body weight ratio for normal control rats was because the normal control group was the standard group, it was not induced with the isoproterenol. Isoproterenol turns and inflected the organs of the induced rats. On the other hand, there was a decrease in the liver, heart and kidney body weight ratio fed with high concentration of raw fish oil RO10 (10ml/kg). This was in accordance with the results of Garcia Cruz, González Azpeitia [55] working on beneficial effect of fish oil and omega-3.

#### **Oxidative stress**

The increased generation of reactive oxygen metabolites, such as hydrogen peroxide, has been shown to occur in various diseased conditions [59]. Free radicals are formed in the body as part of the normal metabolic process. Lipid peroxidation of the cell membrane is associated with a number of pathologic phenomena, such as increased membrane rigidity, decreased cellular deformability, and lipid fluidity in erythrocytes [60]. On the other hand, the human body possesses several defenses against oxidative stress, mediated by endogenous or exogenous antioxidant substances [61].

Among several important functions of proteins is that of anti-oxidant scavenging capacity. Isoproterenol administration induces oxidative stress by generation of free oxygen radicals. The decrease in serum total protein in oxidative stress can also be due to, the increased degradation of plasma proteins by free oxygen radical [62]. The effects of this study suggest that the increase in total serum protein may be due to the involvement of isoproterenol in the defense against oxidative stress-related with acute cardiac infarction.

Endogenous antioxidant enzymes such as CAT, and GSH are the first-line cellular and myocardial defense against oxidative stress, decomposing oxygen, and hydrogen peroxide before they interact to form the more reactive hydroxyl radical. The equilibrium between these enzymes is a significant process for the effective elimination of oxygen stress in intracellular organelles. CAT is an important antioxidant enzyme in alleviating free radical-induced cell injury. A decrease in the activity of CAT can result in the decreased removal of hydrogen peroxide radicals that brings about a number of reaction which are harmful to the myocardium [63].

In this study, the levels of endogenous antioxidant parameters (CAT, and GSH) in heart tissue and serum, decreased significantly in the negative control groups as compared with normal, positive and test control rat groups. In the treatment groups, raw fish oils antagonized the decrease in endogenous antioxidant parameters levels and produced beneficial effects.

Lipid peroxidation, a class of oxidative deterioration of polyunsaturated fatty acids has been associated with changed membrane structure and enzymes inactivation. Enhanced lipid peroxidation appears to be the early stage of the tissue making it more susceptible to oxidative damage. This is responsible for the observed membrane damage as evidenced by the high level of lipid peroxidation [64]. Lipid peroxidation (MDA released) increases under mild stress and causes an alteration in the membrane structure, leading to its damage and enzyme inactivation. In this study pretreatment with raw oil significantly restored the levels indicating maintained integrity of cardiac diseases this was in conformity with Mahesh, Shrivastava [65] results working lipid peroxidation in MDA level.

Regarding to the parameters of oxidative stress, our study observed significant increase in MDA level in negative control group in both the serum and tissue and a comparable increase in cooked fish oil as compared to the normal, positive and raw fish oils treated groups. This come in agreement with [66] who said that this elevation could be attributed to the fact that cooked fish oils can be responsible for increase in the production of reactive oxygen species (ROS) in cells, which in turn result in increased lipid peroxidation levels and oxidative stress. Wang, Xu [67] reported that raw fish oil inhibited the production of MDA, which is the end product of lipid peroxidation. In addition to lipid peroxidation, raw fish oil suppressed ROS production and increased the amount of GSH this was in agreement with Zhang, Zhao [68]. The protective effect of raw oil on oxidative stress was reported to be due to the multiple active compounds present in raw fish oil [69].

### Histopathology

The Kidney tissue sections showed light microscopic alterations such as congestion, glomerular atrophy with widening of Bowman's capsule, edema with epithelial shedding in tubular structures, pyknic (broadness) nuclei, desquamated cells, and hemorrhages suggesting apoptotic events. The heart performance and kidney function are closely interconnected, and a close link exists between these organs [70]. Dysfunction of one organ often leads to a deterioration of function of the other one such as the kidney [71]. Investigation on organ crosstalk in acute renal injury reported that cell death, inflammation, cytokine and caspase-mediated apoptotic mechanisms, and oxidative stress might induce distant organ dysfunction [72]. Our histological investigation revealed that the glomeruli and tubuli in the kidneys of heart failure negative control rats showed morphological and pathological changes such as mitochondrial condensation and nuclear degeneration suggesting apoptotic cell death as compared to the controls groups. Our findings are in agreement with Aboryag, Mohamed [73] study, showing that the microvascular endothelial permeability, inflammation, and tubular cell apoptosis significantly increased in rat kidneys following left coronary artery-induced myocardial infarction.

Proinflammatory cytokine levels may increase in the lung tissues of isoproterenol treated animals. Inflammatory cytokines produced by monocytes and macrophages in response to a range of stimuli, including various microbial products, activated T cells, immune complexes, and the combined action of other cytokines. They cause leucocytes to move out of capillaries and accumulate at sites of injury or infection [74]. In the tissues of the Test2R010, the proinflammatory and anti-inflammatory cytokines can be supposed to be at equilibrium. Our biochemical test results showed that raw oil of high concentration (Test2R010) suppressed isoproterenol induced oxidative stress and inflammation in the lung tissue. This was in support with Melo, de Barros Silva [75] that protective effect of raw oil on tissues was due to its antioxidant and anti-inflammatory properties. The results of these biochemical tests obtained from all groups are consistent with the histopathological findings. Furthermore, PNL infiltration, severe hemorrhage, alveolar destruction, and edema were observed. Raw oil prevented the explicit isoproterenol induced PNL infiltration in lung tissue, severe hemorrhage, alveolar destruction, and alleviated edema. Arpag, Gül [74] reported similar pathological findings in the lung tissues of the isoproterenol group, in which oxidant and inflammatory markers were high and antioxidants were low. In another study, carried out by Topal, Bilgin [76] observed similar pathological findings in isoproterenol induced lung oxidative damage.

As a result, isoproterenol has been shown to be biochemically and histopathologically responsible for the damage and inflammation in the lung tissue of animals. We found that raw oil suppressed isoproterenol induced inflammation in the liver tissue. Therefore, this effect of isoproterenol was significantly blocked by supplementation of rats with raw fish oil ( $p < 0.05$ ), indicating that raw fish oil markedly ameliorated isoproterenol induced lung and liver damage. In contrast, cooked fish oil did not show significant inhibition on isoproterenol induced lung and liver damage.

## Conclusion

The present work was undertaken to study the protective effects of mackerel fish oils against isoproterenol induced cardiac ischemia in rats. The oils quality analysis showed high level of confidence in raw fish oil since most of the parameters were creatable and acceptable within the limit. Fish oils decreased MDA level in the heart and serum while increased GSH and catalase levels in the heart and serum in a similar manner, hence showing antioxidant properties. The histology results, revealed that raw fish oils have a positive effect on cardiovascular diseases by maintaining the cells of the organs to normal compared to the negative control. Liver from the majority of rats treated with cooked fish oil showed marked improvement in the histopathology changes also but not as effective like that of raw fish Test3C08 and Test4C010. Liver of rats received cooked fish oil of high concentration revealed marked improvement in histopathological changes, looks healthy with mild activation of Kuepfer cells, Test4 C010 These results suggested that both cooked and raw fish oil protected and prevented damage to the plasma membranes of not only the liver and heart, but also the kidney and the lungs with cooked fish oil showing mild effects as compared to raw fish oil. This reveal that mackerel fish oil has protective effect against cardiac ischemia and may be used in traditional medicine for its cardio protective effect and may also be used in food supplements.

## Conflict of Interest Statement

The author declare no conflict of interest.

## Bibliography

1. Konstantinides SV, *et al.* "Management of pulmonary embolism: an update". *Journal of the American College of Cardiology* 67.8 (2016): 976-990.
2. Reddy KS and MR Mathur. "Global Burden of CVD: Prevalence, Pattern, and Trends". in *Handbook of Global Health* (2021): 423-437.

3. Kavita, *et al.* "Task shifting of cardiovascular risk assessment and communication by nurses for primary and secondary prevention of cardiovascular diseases in a tertiary health care setting of Northern India". *BMC Health Services Research* 20 (2020): 1-12.
4. Horrocks LA and AA Farooqui. "Docosahexaenoic acid in the diet: its importance in maintenance and restoration of neural membrane function". *Prostaglandins, Leukotrienes and Essential Fatty Acids* 70.4 (2004): 361-372.
5. Yates CM, *et al.* "Pharmacology and therapeutics of omega-3 polyunsaturated fatty acids in chronic inflammatory disease". *Pharmacology and Therapeutics* 141.3 (2014): 272-282.
6. Fodor JG, *et al.* "Fishing" for the origins of the "Eskimos and heart disease" story: facts or wishful thinking?" *Canadian Journal of Cardiology* 30.8 (2014): 864-868.
7. Al-Okbi SY, *et al.* "Role of borage seed oil and fish oil with or without turmeric and alpha-tocopherol in prevention of cardiovascular disease and fatty liver in rats". *Journal of Oleo Science* 67.12 (2018): 1551-1562.
8. Cervantes Gracia K, *et al.* "CVD and oxidative stress". *Journal of Clinical Medicine* 6.2 (2017): 22.
9. Wu JQ, *et al.* "Free radicals, antioxidant defense systems, and schizophrenia". *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 46 (2013): 200-206.
10. Senoner T and W Dichtl. "Oxidative stress in cardiovascular diseases: still a therapeutic target?" *Nutrients* 11.9 (2019): 2090.
11. Bays HE, *et al.* "Ten things to know about ten cardiovascular disease risk factors". *American Journal of Preventive Cardiology* 5 (2021): 100149.
12. V Goncharov, *et al.* "Reactive oxygen species in pathogenesis of atherosclerosis". *Current Pharmaceutical Design* 21.9 (2015): 1134-1146.
13. Balakumar P, *et al.* "Pharmacological interventions to prevent vascular endothelial dysfunction: future directions". *Journal of Health Science* 54.1 (2008): 1-16.
14. Njinkoue J, *et al.* "Proximate composition, mineral content and fatty acid profile of two marine fishes from Cameroonian coast: *Pseudotolithus typus* (Bleeker, 1863) and *Pseudotolithus elongatus* (Bowdich, 1825)". *NFS Journal* 4 (2016): 27-31.
15. Bligh EG and WJ Dyer. "A rapid method of total lipid extraction and purification". *Canadian Journal of Biochemistry and Physiology* 37.8 (1959): 911-917.
16. Kinsella J, *et al.* "Fatty acid content and composition of freshwater finfish". *Journal of the American Oil Chemists' Society* 54 (1977): 424-429.
17. AFNOR A, *et al.* "Recueil des normes françaises des corps gras, graines oléagineuses et produits dérivés. Association Française de Normalisation eds, Paris" (1984): 95.
18. Chemists AoOA and AoOA Chemists, "Official methods of analysis of the Association of Official Analytical Chemists". *Association of Official Analytical Chemists* 2 (1921).
19. Shantha NC and EA Decker. "Rapid, sensitive, iron-based spectrophotometric methods for determination of peroxide values of food lipids". *Journal of AOAC international* 77.2 (1994): 421-424.
20. Shahidi F and UN Wanasundara. "14 Methods Oxidative for Rancidity Measuring". *Food Lipids: Chemistry, Nutrition, and Biotechnology* (2008): 387.
21. Aning, K, *et al.* "Effect of replacing dried brewer's grain with sorghum rootlets' on growth, nutrient utilisation and some blood constituents in the rat". *Animal Feed Science and Technology* 71.1-2 (1998): 185-190.
22. Aletor V. "Allelochemicals in plant food and feedstuffs". *Nutritional* (1993).

23. Mas-Bargues C., *et al.* "Lipid peroxidation as measured by chromatographic determination of malondialdehyde. Human plasma reference values in health and disease". *Archives of Biochemistry and Biophysics* 709 (2021): 108941.
24. Ellman GL. "Tissue sulfhydryl groups". *Archives of Biochemistry and Biophysics* 82.1 (1959): 70-77.
25. Sinha AK. "Colorimetric assay of catalase". *Analytical Biochemistry* 47.2 (1972): 389-394.
26. Temdie RJG., *et al.* "Protective activity of Markhamia tomentosa (benth.) K. schum.(Bignoniaceae) methanol leaves extract against D-galactosamine/lipopolysaccharide-induced acute liver injury in mice". *Journal of Biosciences and Medicines* 8.10 (2020): 74.
27. Organization WH. "Food and Agriculture Organization. Codex Alimentarius Commission. Food Additives and Contaminants. Joint FAO/WHO Food Standards Programme. No. ALINORM (2001): 1.
28. Bako T., *et al.* "Criteria for the extraction of fish oil". *Agricultural Engineering International: CIGR Journal* 19.3 (2017): 120-132.
29. Rizliya V and E Mendis. "Biological, physical, and chemical properties of fish oil and industrial applications". in *Seafood processing by-products: Trends and applications* (2013): 285-313.
30. Bang H., *et al.* "The composition of food consumed by Greenland Eskimos". *Acta Medica Scandinavica* 200.1-6 (1976): 69-73.
31. Linder M., *et al.* "From Krill to Whale: an overview of marine fatty acids and lipid compositions". *Oléagineux, Corps gras, Lipides* 17.4 (2010): 194-204.
32. Zhang X., *et al.* "Fatty acid composition analyses of commercially important fish species from the Pearl River Estuary, China". *PLoS One* 15.1 (2020): e0228276.
33. Tenyang N., *et al.* "The chemical composition, fatty acid, amino acid profiles and mineral content of six fish species commercialized on the Wouri river coast in Cameroon" (2014).
34. Szparaga A., *et al.* "Solid-liquid extraction of bioactive compounds as a green alternative for developing novel biostimulant from *Linum usitatissimum* L". *Chemical and Biological Technologies in Agriculture* 10.1 (2023): 108.
35. Yasin NM., *et al.* "Extraction of FAME from fish waste by using modified soxhlet method". in *IOP Conference Series: Materials Science and Engineering*. IOP Publishing (2021).
36. Suseno SH., *et al.* "Determination of Extraction Temperature and Period of Fish Oil from Tilapia (*Oreochromis niloticus*) By Product Using Wet Rendering Method". *KnE Life Sciences* (2015): 125-135.
37. Tenyang N., *et al.* "Lipid oxidation of catfish (*Arius maculatus*) after cooking and smoking by different methods applied in Cameroon" (2013).
38. Commission JFWCA. "Codex alimentarius". Food and Agriculture Org (1992).
39. Ornella TTJ., *et al.* "Chemical and antibacterial properties of lipids extracted from some plant seeds and fruits commonly used in cosmetics". *American Journal of Food Science and Technology* 10.1 (2022): 10-19.
40. Obi C., *et al.* "Oil obtained from *C. Lepidota* seeds: Extraction and characterization". *Scientia Africana* 18.1 (2019): 47-58.
41. Commission JFWCA. "Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission, Twenty-first Session, Rome, Report of the Fourteenth Session of the Codex Committee on Fats and Oils, London, United Kingdom, Food and Agriculture Organization of the United Nations (1993).
42. Adeniyi O and A Bawa. "Mackerel (*Scomber scombrus*) oil extraction and evaluation as raw materials for industrial utilization". *Leonardo Journal of Sciences* 5.8 (2006): 33-42.

43. Alimentarius C. "Codex standard for named vegetable oils". *Codex stan* 210 (1990): 1-13.
44. Tenyang N., *et al.* "Alteration of the lipid of red carp (*Cyprinus carpio*) during frozen storage". *Food Science and Nutrition* 7.4 (2019): 1371-1378.
45. Mostafa MM., *et al.* "Effect of hot smoking conditions on nutritional quality of Indian Mackerel and *Pangasius* fish Fillets". *Aquatic Science and Fish Resources (ASFR)* 4.1 (2023): 13-28.
46. Tiencheu B., *et al.* "Changes of lipids in insect (*Rhynchophorus phoenicis*) during cooking and storage". *European Journal of Lipid Science and Technology* 115.2 (2013): 186-195.
47. Womeni HM., *et al.* "Oils of insects and larvae consumed in Africa: potential sources of polyunsaturated fatty acids". *Oléagineux, Corps gras, Lipides* 16.4-5-6 (2009): 230-235.
48. Oladapo AO and SH Awojide. "Quality evaluation of oil extracted from catfish and mackerel as compared with commercial cod liver oil". *Journal of Food Chemistry and Nutrition* 3.1 (2015): 13-18.
49. Womeni HM., *et al.* "Oxidative stabilization of RBD palm olein under forced storage conditions by old Cameroonian green tea leaves methanolic extract". *NFS Journal* 3 (2016): 33-40.
50. Ahmed J and N Mahendrakar. "Autolysis and rancidity development in tropical freshwater fish viscera during fermentation". *Bioresource Technology* 58.3 (1996): 247-251.
51. Ndidiamaka NC and OE Ifeanyi. "Proximate and physicochemical analysis of oil obtained from two fish species (fresh and frozen)". *International Journal of Advanced Research in Biological Sciences* 5.4 (2018): 167-177.
52. Atasi V., *et al.* "Proximate analysis and physico-chemical properties of groundnut (*Arachis hypogaea* L.)". *Pakistan journal of Nutrition* 8.2 (2009): 194-197.
53. Li X., *et al.* "Kinetic models to understand the coexistence of formation and decomposition of hydroperoxide during lipid oxidation". *Food Research International* 136 (2020): 109314.
54. Nirogi R., *et al.* "What suits best for organ weight analysis: review of relationship between organ weight and body/brain weight for rodent toxicity studies". *International Journal of Pharmaceutical Sciences Review and Research* 5.4 (2014): 1525-1532.
55. Garcia Cruz LM., *et al.* "Factors associated with stunting among children aged 0 to 59 months from the central region of Mozambique". *Nutrients* 9.5 (2017): 491.
56. Arowosola TA., *et al.* "The Role of Food in the Health Management of Geriatrics". *Food Security and Safety African Perspectives* 2 (2022): 59-81.
57. Emanuel A., *et al.* "Nutritional interventions in pancreatic cancer: a systematic review". *Cancers* 14.9 (2022): 2212.
58. Osborne V., *et al.* "Effects of supplementing fish oil in the drinking water of dairy cows on production performance and milk fatty acid composition". *Journal of Animal Science* 86.3 (2008): 720-729.
59. Halliwell B. "The role of oxygen radicals in human disease, with particular reference to the vascular system". *Haemostasis* 23 (1993): 118.
60. Maruyama T., *et al.* "Rheological abnormalities in human erythrocytes subjected to oxidative inflammation". *Frontiers in Physiology* 13 (2022): 837926.
61. Adwas AA., *et al.* "Oxidative stress and antioxidant mechanisms in human body". *Journal of Applied Biotechnology and Bioengineering* 6.1 (2019): 43-47.
62. Tetik Ş., *et al.* "Oxidative stress causes plasma protein modification" (2015).
63. Aguilar TAF, *et al.* "Endogenous antioxidants: a review of their role in oxidative stress. A master regulator of oxidative stress-the transcription factor nrf2" (2016): 3-20.

64. Recknagel RO, *et al.* "Free radical damage and lipid peroxidation". in *Hepatotoxicology*. CRC press (2020): 401-436.
65. Mahesh BU, *et al.* "Ethanol extract of *Boswellia ovalifoliolata* bark and leaf attenuates doxorubicin-induced cardiotoxicity in mice". *Environmental Toxicology and Pharmacology* 36.3 (2013): 840-849.
66. Banerjee A, *et al.* "Altered composition of high-lipid diet may generate reactive oxygen species by disturbing the balance of antioxidant and free radicals". *Journal of Basic and Clinical Physiology and Pharmacology* 31.3 (2020): 20190141.
67. Wang J, *et al.* "Effects of oxidised dietary fish oil and high-dose vitamin E supplementation on growth performance, feed utilisation and antioxidant defence enzyme activities of juvenile large yellow croaker (*Larimichthys crocea*)". *British Journal of Nutrition* 115.9 (2016): 1531-1538.
68. Zhang DG, *et al.* "Oxidized fish oils increased lipid deposition via oxidative stress-mediated mitochondrial dysfunction and the CREB1-Bcl2-Beclin1 pathway in the liver tissues and hepatocytes of yellow catfish". *Food Chemistry* 360 (2021): 129814.
69. Yang J, *et al.* "Oxidative stress and non-alcoholic fatty liver disease: effects of omega-3 fatty acid supplementation". *Nutrients* 11.4 (2019): 872.
70. Virzì GM, *et al.* "Heart–kidney crosstalk and role of humoral signaling in critical illness". *Critical Care* 18 (2014): 1-11.
71. Schefold JC, *et al.* "Heart failure and kidney dysfunction: epidemiology, mechanisms and management". *Nature Reviews Nephrology* 12.10 (2016): 610-623.
72. Ronco C, *et al.* "Cardiorenal syndrome: refining the definition of a complex symbiosis gone wrong". *Intensive Care Medicine* 34 (2008): 957-962.
73. Aboryag NB, *et al.* "Histopathological changes in the kidney following congestive heart failure by volume overload in rats". *Oxidative Medicine and Cellular Longevity* (2017): 2017.
74. Arpag H, *et al.* "Protective effects of alpha-lipoic acid on methotrexate-induced oxidative lung injury in rats". *Journal of Investigative Surgery* 31.2 (2018): 107-113.
75. Melo RB, *et al.* "Anti-inflammatory effect of a fatty acid mixture with high  $\omega$ -9:  $\omega$ -6 ratio and low  $\omega$ -6:  $\omega$ -3 ratio on rats submitted to dental extraction". *Archives of Oral Biology* 74 (2017): 63-68.
76. Topal İ, *et al.* "The effect of rutin on cisplatin-induced oxidative cardiac damage in rats". *Anatolian Journal of Cardiology* 20.3 (2018).