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Fish Oil Rich in Omega-3 Fatty Acids Ameliorates Nephrotoxicity and Oxidative Stress Induced by Lead Exposure in Adult Male Wistar Rats

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ABSTRACT

The kidneys serve as the initial storage for lead, and as such are exposed to the deleterious effect of lead. Chelators utilized in lead induced toxicity are costly and have been related with adverse side effects. Therefore, the present study evaluated the effect of fish oil rich in omega-3 fatty acids on nephrotoxicity and oxidative stress induced by lead exposure in adult male Wistar rats. Twenty adult Wistar rats were divided into four groups of five rats each. Group I served as control and received 1 ml/kg of distilled water, Group II received 5 mg/kg bwt of lead acetate only, Group III received 5 mg/kg bwt of lead acetate and 112.5 mg/kg bwt of fish oil while Group IV received 5 mg/kg bwt of lead acetate and 225 mg/kg bwt of fish oil. Body weights were taken throughout the experiment. At the end of the administration, the kidneys of the Wistar rats were excised and weighed, oxidative stress markers quantified and the kidneys were fixed in buffered formal saline, processed and stained for histological studies. The results revealed significant decrease ($p<0.05$) in body weight gain and antioxidants and distortion of the cytoarchitecture of the kidneys of Wistar rats in lead only treated groups when compared to the Control and the Groups co-administered lead acetate and increasing doses of fish oil. Fish oil was able to ameliorate nephrotoxicity and oxidative stress by scavenging for free radical, increasing body weight gain and protecting against distortion of the cytoarchitecture of the kidney.

Keywords: Fish oil, Omega 3 fatty acids, lead acetate, nephrotoxicity, antioxidants

INTRODUCTION

The harmful nature, non-biodegradability, environmental persistence and very long biological half-life of heavy metals make them a significant ecological and occupational hazard [1]. These metals include mercury, lead, arsenic, cadmium and Aluminium amongst others [2]. Lead one of these heavy metals is the fifth most frequently used metal worldwide and its use can be traced back to a few centuries[3]. Due to its wide usage and applications, exposure of humans and animals to lead is unavoidable [4]. Lead exposure can occur via contact, inhalation ingestion of tainted food and water among others. Exposure to lead has been reported to cause adverse effects on the nervous system, renal system, circulatory system and hematopoietic system etc [5]. This is compounded by reports that lead-associated effects and complications may even occur with lower levels of exposure, thus there is no defined safe threshold for lead toxicity [6].

Albeit several occupational and public health safety measures have been carried out to reduce the cases of lead exposure to the negligible level, yet, a few instances of lead poisoning are still recorded [7]. Lead is still in use in several products in many developing countries [8]. Lead induced toxicity is mediated via two major mechanisms which include causing an imbalance between the generation of free radical and the production of antioxidants also known as oxidative stress [9] or via ionic mechanism where divalent cations such as Zn^{2+}, Fe^{2+} and Cu^{2+} which participate in the regulation of numerous physiological functions are displaced by lead ion thus distorting normal function of the cell [10]. The ability of lead to cause oxidative stress has been suggested to be due to its ability to bind to the sulfhydryl groups of the antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) as well as its ability to displace zinc ions which serve as vital co-factors for these enzymes [7, 11].

Since the kidneys and livers represent the initial storage for lead, lead exposure can cause disruption and distortion of the activity of these organs [4, 12]. The kidney represent less than 1% of the body mass and are important excretory organs that regulate the bodily fluids by regulating the osmotic pressure of the plasma, electrolyte and acid-base homeostasis [13, 14]. Lead exposure may cause acute or chronic nephropathy with several health implications such as deficit in tubular absorption and reabsorption, degenerative changes in tubular epithelium, renal dysfunction, renal failure, and hyperuricemia [15, 16]. Rastogi [16] and Steenland and Barry [17] observed chronic kidney disease (CKD) in workers occupationally exposed to lead. Environmental lead exposure has also been associated with diminished kidney function in subjects with no history of long-term occupational exposure [18].

Heavy metal chelators currently utilized as therapy in heavy metal induced toxicity such as lead toxicity have been associated with mild to severe side effects, which may include fever, headache, nausea and vomiting, seizures, brain damage, permanent kidney and liver diseases etc [19]. They are also costly and have been reported to chelate essential metals necessary for normal physiologic activities, therefore the need to look for natural remedies that are cheap, readily available and capable of ameliorating the effects of lead toxicity.

Omega-3 fatty acids are considered to be essential fatty acids because mammals do not have the ability to synthesize them in vivo [20]. Fish oil is a derivative from the tissues of oily fishes and contains eicosapentaenoic and docosahexaenoic acid which are omega 3 fatty acids [21]. Omega-3 fatty acids have been reported to possess anti-inflammatory potential, protect against oxidative stress as well as play protective

roles in the liver, cardiovascular system, and kidney and they have been widely used in clinical preoperative total parenteral nutrition [22, 23]. They also form integral elements of phospholipid and cell membranes, and have the ability to affect steroidogenesis and some transcription factors controlling gene expression [24]. Therefore, this study investigated the ameliorative potential of Fish oil rich in omega 3 fatty acid on nephrotoxicity and oxidative stress induced by lead exposure in the kidney of adult male Wistar rats.

MATERIALS AND METHODS

Ethical Approval: Ethical approval was obtained from the Ahmadu Bello University committee on Animal use and care with approval number ABUCAUC

Experimental Animals: Twenty (20) apparently healthy adult male Wistar rats weighing between 100-120g were obtained from the Animal Resource Center, Department of Pharmacology, Ahmadu Bello University, Zaria, Kaduna, Nigeria. The rats were kept in plastic confines in the Animal House, Department of Human Anatomy, Faculty of Basic Medical Sciences, Collage of Medical Sciences, Ahmadu Bello University, Zaria. The rats were fed with standard pellets and water ad libitum for a period of two weeks to adapt them prior to the commencement of the experiment.

Acquisition of Lead Acetate and Fish Oil: Lead acetate manufactured by British Drug Houses (BDH) Laboratory Chemicals Division, Poole, England and Fish oil manufactured by US Vitamins, was used in this study.

Experimental Protocol: Based on the oral LD_{50} of lead acetate which is 600 mg/kg body weight for Wistar rats Karamala *et al.* [25], 0.8% of the LD50 was used which is equivalent to 5 mg/kg of lead acetate [26] and 15% and 30 % of the LD_{50} of fish oil (750 mg/kg) [27] was used in this study.

Experimental Design: The twenty (20) adult male Wistar rats were distributed randomly into four groups with five rats each. Group I which served as control and received 1ml/kg bwt of distilled water. Group II received 5 mg/kg bwt of lead acetate only, Group III received 5 mg/kg bwt of lead acetate and 112.5 mg/kg bwt of fish oil while Group IV received 5 mg/kg bwt of lead acetate and 225 mg/kg bwt of fish oil. All administration was done once daily for 28 days via oral gavage using an oropharyngeal cannula and 1ml syringe.

Body Weight Assessment: In each group, the body weight of each rats was taken at the beginning, weekly during the study and at the end of the study before sacrifice using a digital weighing balance. Weight change of the rats in each group was calculated as:

Body weight change (g) = Final body weight (g) – Initial body weight (g).

Euthanasia and Kidney Isolation: 24 hours after last the administration, the rats in each group were weighed and anaesthetized by intraperitoneal administration of 75 mg/kg bwt of Ketamine [28] . Afterwards, the rats were cut open through a midline incision from the tip of the xiphoid process down to the lowest part of the abdomen to expose the abdominal cavity. The kidneys were then isolated from the posterior abdominal wall.

Kidney Weight and Kidney Body Weight Ratio Determination: After isolation of the kidneys, pre nephric fat was removed from the kidneys. Afterwards, each kidney isolated from each rat was immediately weighed using a weighing scale (Tronix organ scale). Kidney body weight ratio was then calculated using the formula;

$$\text{Kidney body weight ratio} = \frac{\text{Kidney weight (g)}}{\text{Bodyweight (g) at the end of experiment}}$$

Biochemical Analysis: The left kidney was homogenized with freshly prepared neutral phosphate buffer saline five times its weight in mls. The resultant homogenate was centrifuged at 4000rpm for 10 minutes using a centrifuge machine (Made in England/Serial No: 846307) at the Department of Human Anatomy, ABU, Zaria. The resultant supernatant was then stored in plain bottles for estimation of concentration of oxidative stress markers.

Catalase Activity Assay : Using a spectrophotometric test, catalase (CAT) enzyme activity was evaluated based on formation of a yellow complex with molybdate and hydrogen peroxide in plasma which was expressed with 1, 1, 2, 2,-tetramethoxypropane [29].

Glutathione Activity Assay: Glutathione activity was assessed following the method of Ellman [30] as described by Rajagopalan et al. [31] . This assay was based on the reaction of reduced glutathione (GSH) with 5, 5'-dithiobis nitrobenzoic acid (DNTB).

Superoxide Dismutase Activity Assay: Activity of superoxide dismutase (SOD) was determined by the technique of Fridovich [32]. This assay was based on the ability of superoxide dismutase to inhibit auto oxidation of adrenaline at pH 10.2.

Malondialdehyde Assay: Lipid peroxidation was determined following the method of Ohkawa et al. [33]. Upon cleavage, lipids release peroxide intermediates such as malondialdehyde (MDA). MDA reacts with thiobarbituric acid to form thiobarbituric-acid reactive substance (TBARS). Hence using a spectrophotometer, MDA was determined by measuring TBARS at 532nm.

Histology: The right kidney from each rat was then fixed in formol saline for 48 hours to allow for proper fixation. Afterwards, they were routinely processed and stained for histological studies at the Human Anatomy Department, Ahmadu Bello University, Zaria. After staining with H and E [34], microscopic slides were viewed under the microscope using X250 magnifications and photomicrographs were taken using MD900 Amscope® digital camera.

Data Analysis: Data obtained from this study was expressed as mean±SEM and analyzed using statistical package for social science (SPSS version 23). One way analysis of variance (ANOVA) was used to compare the mean differences between groups followed by least significant difference (LSD) post–hoc test where significant differences were observed. P-value < 0.05 was considered significant.

RESULTS

Change in Body Weight: The result showed a significant decrease ($p < 0.05$) in body weight gain of Wistar rats in all experimental groups when compared with the control. The result also revealed a significant decrease ($p<0.05$) in weight gain of Wistar rats in Group II treated with lead acetate only when compared with Groups III and IV administered lead acetate and increasing doses of omega 3 rich Fish oil. A significant increase ($p<0.05$) in weight gain was also seen in Wistar rats in Group IV when matched with Wistar rats in Group III (Table 1).

Table 1: Mean Change in Body Weights of Adult Male Wistar Rats Co-administered Lead Acetate and Fish Oil.

Groups	Initial Weight (g)	Final Weight (g)	Body Weight Change (g)
Control 1 ml/kg water	134.29 ± 2.9	161.43 ± 2.06^{b}	27.14 ± 0.59^{d}
5 mg/kg of lead	134.86 ± 2.14	143.86 ± 1.88^{a}	9.00 ± 0.53^{a}
Pb + 112.5mg/kg FO	136.43 ± 6.32	151.57 ± 5.92^{ab}	15.14 ± 0.71^{b}
Pb + 225mg/kg FO	138.86 ± 7.14	159.00 ± 6.66^{b}	20.14 ± 0.91^{c}
F	0.165	3.886	120.221
p	0.92	0.045	<0.001

Values reported as mean ± SEM. One way ANOVA followed by LSD post hoc test. Group I (Control) 1 ml/kg water, Group II (5 mg/kg of lead), Group III (Pb + 112.5 mg/kg fish oil); Group IV (Pb + 225 mg//kg fish oil). Cells carrying different superscripts "$^{a, b, c, d}$" in the same column are significantly different ($p < 0.05$). n=5, FO - Fish Oil, Pb – Lead.

Mean Kidney Weight and Kidney Body Weight Ratio: The result revealed a significant decrease ($p < 0.05$) in mean kidney weight of Wistar rats in Group II when compared with control and other experimental groups. Similar organ weights was observed in Groups III and IV when contrasted with control. There was also a significant decrease ($p < 0.05$) in kidney body weight ratio in Group II when compared with control and other experimental groups. However, comparable kidney body weight ratio was observed in Groups III and IV when contrasted with control (Table 2).

Table 2: Mean Kidney Weight and Kidney Body Weight ratio of Adult Male Wistar rats Co-administered Lead Acetate and Fish Oil.

Groups	Kidney Weight (g)	Kidney Body Weight Ratio (%)
Control 1 ml/kg water	0.48 ± 0.02^{a}	0.29 ± 0.01^{b}
5 mg/kg of Pb	0.36 ± 0.01^{b}	0.25 ± 0.01^{a}
Pb + 112.5mg/kg FO	0.44 ± 0.01^{a}	0.28 ± 0.01^{b}
Pb + 225mg/kg FO	0.44 ± 0.02^{a}	0.29 ± 0.015^{b}
F	8.775	3.107
p	<0.001	0.045

Values reported as mean ± SEM. One way ANOVA followed by LSD post hoc test. Group I (Control) 1 ml/kg water, Group II (5 mg/kg of lead), Group III (Pb + 112.5 mg/kg fish oil); Group IV (Pb + 225 mg//kg fish oil). Cells carrying different superscripts "$^{a, b}$" in the same column are significantly different ($p < 0.05$). n=5, FO - Fish Oil, Pb – Lead.

Oxidative Stress Markers: There was a significant reduction ($p<0.05$) in the level of CAT, SOD and GSH in all experimental groups when compared with control group. A significant decrease ($p<0.05$) in the level of CAT, SOD and GSH was also observed in Wistar rats in group II when compared with Wistar rats in Groups III and IV. Significant increase ($p<0.05$) in the level of SOD and GSH was also observed in Group IV when compared to Group III. The result also showed a significant increase ($p<0.05$) in the level of MDA in all experimental groups when compared with control group. MDA level was also significantly higher ($p<0.05$) in Wistar rats in Group II when compared to Groups III and IV. A significant increase in MDA level was also observed in Wistar rats in Group III when compared to Group IV.

Table 3: Mean Concentration of MDA, CAT, SOD and GSH of Adult Male Wistar rats Co-administered Lead Acetate and Fish Oil.

Group	MDA (ɥmol/mg protein)	CAT (U/mg)	SOD (U/ml)	GSH (**ug/ml)**
Control 1 ml/kg water	36.14 ± 0.59^{a}	16.42 ± 0.23^{c}	24.90 ± 0.36^{d}	40.92 ± 0.20^{d}
5 mg/kg of Pb	51.96 ± 0.81^{d}	11.06 ± 0.34^{a}	11.42 ± 0.38^{a}	21.48 ± 0.34^{a}
Pb + 112.5mg/kg FO	47.10 ± 0.28^{c}	14.58 ± 0.12^{b}	16.32 ± 0.79^{b}	30.44 ± 0.38^{b}
Pb + 225mg/kg FO	41.58 ± 0.41^{b}	15.30 ± 0.16^{b}	21.40 ± 0.46^{c}	36.26 ± 0.71^{c}
F	147.506	104.278	123.247	343.139
p	<0.001	<0.001	<0.001	<0.001

Values reported as mean ± SEM. One way ANOVA followed by LSD post hoc test. Group I (Control) 1 ml/kg water, Group II (5 mg/kg of lead), Group III (Pb + 112.5 mg/kg fish oil); Group IV (Pb + 225 mg//kg fish oil). Cells carrying different superscripts "$^{a, b, c, d}$" in the same column are significantly different ($p < 0.05$). n=5, FO - Fish Oil, Pb – Lead. MDA – Malondialdehyde, CAT – Catalase, SOD – Superoxide Dismutase, GSH – Glutathione.

Histological Observation: Results from H & E staining of the kidney of Wistar rats in Group I (control) revealed normal cytoarchitecture of the kidney with intact glomerulus, normal mesanguim within the capillary tuft of the glomerulus, basement membrane and its normal endothelial lining and normal outlines of all the tubules with well-preserved cytoplasm, nuclei and nucleoli (figure 1). Kidney sections of Wistar rats in Group II treated with lead acetate only showed marked distortion of the glomerulus with spontaneous focal lipid vacuolation of the glomerulus and severe dilation of the renal tubules (figure 1). Photomicrograph of kidney sections of Wistar rats in Group III treated lead acetate and fish oil (112.5 mg/kg) showed mild degenerative features in the cytoarchitecture of the kidney with mild spontaneous obliteration of the glomerulus, mild dilatation of Bowman Space and the renal tubules (figure 1). The kidney sections of Wistar rats in Group IV administered lead acetate and fish oil (225 mg/kg) showed kidney architecture similar to control (figure 1).

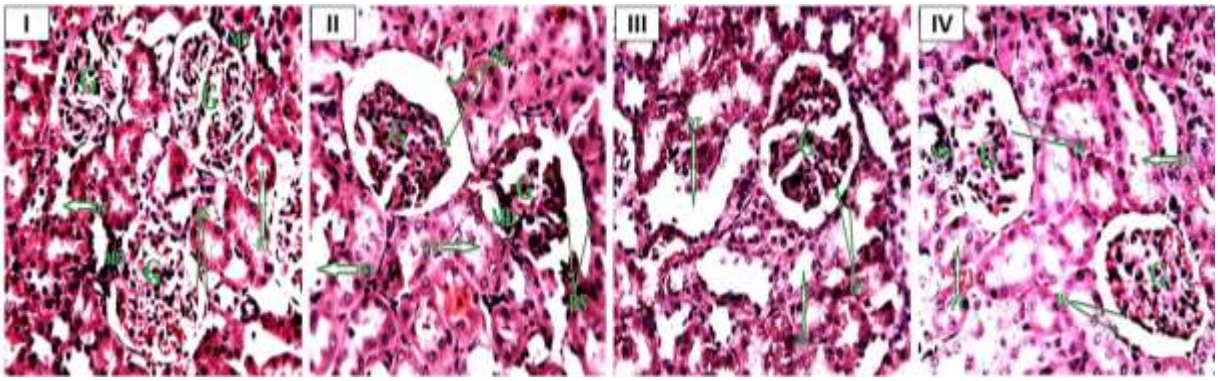

Figure 1: Photomicrograph of the kidney sections of adult male Wistar rats (H and E MagX250). **Group I** (Control) 1 ml/kg water, Group II (5 mg/kg of lead), Group III (Pb + 112.5 mg/kg fish oil); Group IV (Pb + 225 mg//kg fish oil). G - Glomerulus, BS – Bowman's Capsule, DT – Distal Convoluted Tubule, PT – Proximal Convoluted Tubule, MD – Macula Densa Cells

DISCUSSION

Continuous lead exposure in both animals and humans have been associated with various health risk [35]. Despite regulation controlling the use of lead in products in developed countries, lead is still in use in several products in many developing countries [8]. Thus, making it's a exposure a major health challenge. Therefore, this study was aimed at evaluating the ameliorative potential of Fish oil rich in omega 3 fatty acid on nephrotoxicity and oxidative stress induced by lead exposure in the kidney of adult male Wistar rats. Changes in body weight serves as evidence of overall wellbeing status of an animal. In the present study we observed a significant decrease in weight gain of Wistar rats in the group administered lead acetate only when compared to the Control group and groups treated with lead acetate and increasing doses of fish oil. The decrease in weight gain of rats treated with lead acetate alone could be attributed to decrease in the ingestion of food instigated by ingestion of lead acetate. Body weight is regulated in part by the ability of hypothalamic neurons to orchestrate behavioural, endocrine and autonomic responses via afferent and efferent pathways to the brainstem and the periphery [36], therefore it is possible that lead exposure affected the activity of the hypothalamus in weight regulation.

Another possible explanation for decreased body weight gain may be due to oxidative stress induced decrease in muscle mass and cachexia [37]. This is similar to the findings

of Ibrahim *et al.* [38] and Kumar Singh *et al.* [39] who reported significant decrease in weight gain after administration of lead acetate. The marked increase in weight gain in groups' co-administered lead acetate and increasing doses of fish oil rich in omega 3 suggests ameliorative activity of fish oil on reduction in body weight caused by lead exposure and could be attributed to its antioxidant properties. Improved weight gain observed in the group treated with higher dose of fish oil suggest that the oil may be more beneficial at higher doses. This finding is supported by the work of McGlory *et al.* [40] who reported that omega-3 fatty acids ingestion alleviates muscle mass wasting and play an important role in human growth, development and disease prevention.

Organ weight analysis is an important endpoint for the identification of potentially harmful effect of test compound in toxicological studies. There was a significant decrease in mean kidney weight of Wistar rats in the group treated with lead acetate only when compared with control and the groups' treated with lead acetate and increasing doses of fish oil. This decrease in kidney weight may be due to necrosis and lead-induced apoptosis as Wani *et al.* [41] also reported decrease in the weight of the cerebellum explained by the increase in Bax/Bcl-2 ratio and caspase 3 expression in the fetal rat cerebella in lead-exposed group indicating up-regulation of mitochondrial apoptosis pathway. This result is in agreement with the work of Abdou and Hassan [27] who also observed a decrease in kidney weight after administration of lead acetate. Comparable weight observed in control and group's co-administered lead acetate and fish oil suggest the ability of fish oil to protect against degenerative changes incited by lead on the kidney. Decrease in the kidney body weight ratio observed in lead acetate only treated group when compared to the control proposes that there was more rapid decrease in kidney weight of Wistar rats when compared to their body weight, highlighting the deleterious effect of lead exposure on the kidney. However, this was mollified in groups co-administered with fish oil and lead as significant increase in kidney body weight ratio was observed. Similar to the findings of Abdou and Hassan [27] who reported ameliorative effect of fish oil on kidney body weight ratio by reversing the metabolic toxic effect of lead acetate on the kidney organ.

Oxidative stress which occurs when generation of pro-oxidants exceed the capacity of antioxidant defence mechanisms is believed to be one of the major pathways of lead induces its toxicity [42]. A significant decrease in the concentration of CAT, GSH and SOD in kidney of Wistar rats in groups treated with lead acetate was observed when compared to the Control group and groups co-administered lead acetate and fish oil. Reduction in this enzyme could be due to the ability of lead to inactivate the antioxidant enzymes by displacing zinc ions which serve as vital co-factors for these enzymes. This could also due to the affinity of lead acetate for the sulfhydryl groups present in antioxidants [43]. This is similar to the work of Ezejiofor and Orisakwe [44] who reported significant reduction in the levels of total glutathione, superoxide dismutase and gluthatione peroxidase in lead acetate-treated rats. Higher concentration in CAT, GSH and SOD observed in groups' co-administered lead acetate and increasing doses of fish oil may be as a result of the antioxidant properties of omega 3 fatty acid present in the fish oil and suggests positive effect of fish oil in ameliorating lead induced toxicity as similar findings have been reported by Attia *et al.* [45] and Kumar Singh *et al.* [39]. The result also revealed significant increase in MDA levels, in the kidney of Wistar rats after exposure to lead acetate when compared to the Control group and groups' co-administered lead acetate and increasing doses of fish oil. This result coincides with decrease in CAT, SOD and GSH observed in groups treated with lead acetate only, since malondialdehyde which

is the end product of lipid peroxidation of cellular membrane lipid is triggered as a result of offset in antioxidant/ pro oxidant balance as stated by [46]. Decrease in MDA levels observed in groups co-administered lead acetate and increasing doses of fish oil depicts decrease in lipid peroxidation and cell disruption and may be attributed to the antioxidant property as well as reactive oxygen scavenging property of fish oil against lead induced toxicity. This is similar with work of Pauwels and Kostkiewicz [47], who reported reduction in lipid peroxidation after administration of fish oil. Combined together, the level of CAT, SOD, GSH and MDA co-administered lead acetate and higher doses of fish oil was comparable to control. This may suggest that the antioxidant activity of fish oil rich in omega 3 fatty acid is dose dependent with higher doses eliciting better antioxidant and ameliorative activity in lead induced neurotoxicity.

Results from light microscopy examination of the group treated with lead acetate only revealed various degrees of histological changes such as distortion of the cytoarchitecture of the kidney, focal necrosis, spontaneous focal lipid vacuolation of the glomerulus, obliteration of glomerulus, enlargement of the urinary space (Bowman's space) and stenosis of the renal tubules. The tubular and glomerular distortion in the lead acetate treated group may be due to increase in oxidative stress on these cells due to antioxidant enzyme depletion and membrane lipid hydrolysis as reported by Missoun *et al.* [48]. Mohamed and Saleh [49] also reported similar changes when they exposed animals to lead acetate. The cerebral cortices of Wistar rats in groups co-administered lead and increasing doses of fish oil revealed relatively normal cytoarchitecture with mild distortion when compared to control. This shows that administration of fish oil was able to ameliorate distortions observed in the group treated with lead acetate alone. The ameliorative activity of fish oil could be due to its eicosapentaenoic constituent which by itself is a potent antioxidant and its derivatives such as eicosanoids acts as potent activator of nuclear factor 2- related factor 2 (Nrf2) which enhances expression of various antioxidant that attenuate lipolysis which in turn protects the integrity of the membranes and preserving the structure and function of the cell Wang *et al.* [50]. Similar architecture as control observed in kidney of Wistar rats administered lead acetate and higher doses of the lead suggest that fish oil is more potent at higher doses.

CONCLUSION

This study established that Fish oil was able to ameliorate and reverse nephrotoxicity and oxidative stress induced by lead exposure in adult male Wistar rats by scavenging for free radicals, maintaining body weight via alleviation of muscle mass wasting and protecting against distortion of the cytoarchitecture of the kidney via its antioxidant properties.

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