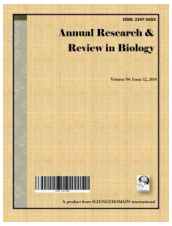

Annual Research & Review in Biology

13(6): 1-14, 2017; Article no.ARRB.33167
ISSN: 2347-565X, NLM ID: 101632869

Lead-induced Oxidative Stress and Chemoprotective Role of Dietary Supplements on Wistar Albino Rats

C. O. Ujowundu[1,2*], G. N. Okwu[2], J. J. Achilike[1], L. A. Nwaogu[1], A. C. Ene[2] and C. I. Iheme[1]

[1]Environmental Biochemistry and Toxicology Unit, Department of Biochemistry, Federal University of Technology, Owerri, Nigeria.
[2]Nutritional and Medical Biochemistry Unit, Department of Biochemistry, Federal University of Technology, Owerri, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors COU, GNO and JJA conceptualized and designed the work, wrote the protocol and interpreted the data. Authors COU, GNO, JJA, LAN, ACE and CII anchored the laboratory work, gathered the initial data and performed preliminary data analysis. Authors JJA, COU and GNO handled the literature searches and produced the initial draft. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2017/33167
Editor(s):
(1) Richard A. Manderville, Departments of Chemistry and Toxicology University of Guelph, Canada.
(2) George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.
Reviewers:
(1) Bechan Sharma, University of Allahabad, India.
(2) Martin Potgieter, University of Limpopo, South Africa.
Complete Peer review History: http://www.sciencedomain.org/review-history/19717

Original Research Article

Received 1st April 2017
Accepted 16th June 2017
Published 27th June 2017

ABSTRACT

The heavy metal lead (Pb) is a common environmental pollutant with widespread distribution, and oxidative stress has been implicated in the pathogenesis of its toxicity. The ameliorative effect of nutritional contents of palm oil and cod liver oil (dietary supplements) following exposure to sublethal concentration of Pb on adult Wistar albino rats was studied. Toxicity was induced by administering intraperitoneally, 30 mg/kg body weight of lead acetate at alternate days for 21 days. Groups treated with supplements received daily oral dose of 2.5 ml palm oil or cod liver oil or 1.25 ml palm oil and 1.25 ml cod liver oil (synergy). Increased activities of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, decreased activities of superoxide dismutase, catalase,

**Corresponding author: E-mail: ujowundu@yahoo.com;*

glutathione peroxidase and increased concentrations of total bilirubin and lipid peroxidation product were observed in Pb exposed rats without nutritional supplement treatment. However, these negative oxidative states were ameliorated in rats by the concomitant administration of nutritional supplements, singly and in combination. Furthermore, non-significant changes were observed in the haematological parameters determined. These observations indicate potential therapeutic benefits in the use of palm oil and cod liver oil in the management of lead-induced toxicity.

Keywords: Heavy metals; lead (Pb); xenobiotics; oxidative stress; antioxidants; Nigeria.

1. INTRODUCTION

Lead (Pb) is a heavy metal and an oxidant that have great potential to accumulate in body tissues. Excess accumulation of lead in living tissues may induce production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), resulting to oxidative stress [1,2] which can disrupt cellular functions by inactivation or precipitation [3]. Oxidative stress induced by free radicals (ROS and RNS) have been implicated in DNA damage, oxidation of thiol group(s) of proteins, and lipid peroxidation [4]. Lead is implicated in various forms of cancer, damage to transport cells, nephron, central nervous and cardiovascular system in animals [5,6].

Lead is regarded as a potent occupational toxin, and its toxicity is closely related to age, sex, and route of exposure, frequency of intake, solubility, metal oxidation state, retention percentage, and duration of exposure [7]. Human exposure to lead occurs through the use of leaded gasoline, lead containing pipes or lead-based solder in water supply systems and industrial activities such as battery recycling, grid and bearings manufacture [8]. Lead toxicity is a particularly insidious hazard with the potential of causing irreversible health effects. It interferes with the central nervous, haematopoietic, hepatic, renal and gastrointestinal systems indicating serious disorders [9,10]. A report by the Centres for Disease Control and Prevention (CDC)-USA, indicate that a blood lead level of 10 µg/dl or above is a cause for concern. Acute toxicity of lead is uncommon (and related to occupational exposure), whereas chronic toxicity is common and occurs at blood lead level of 40-60 µg/dl, characterized by persistent vomiting encephalopathy, lethargy, delirium, convulsions, and coma [11-14].

Some areas in Nigeria such as Zamfara and Niger States are presently experiencing lead poisoning. The outbreak is caused by semi-industrial scale gold ore mining and processing in the affected areas. Since 2010, acute lead poisoning in Zamfara and recently in Niger States has killed hundreds of children. Adult living in these areas experience high rates of infertility and miscarriages in females [15]. It is reported that land surfaces, ponds and rivers within gold mining areas are contaminated with lead from ore-processing [16]. Children and adults bathe, wash their clothes and water their flock of sheep and herds of cattle in the ponds and rivers, and this exposes such individuals and animals to lead poisoning. Furthermore, a large percentage of Nigerians are exposed to mild and chronic levels of lead from vehicular pollution, leaded gasoline use, drinking water obtained from wells, unregulated lead paints, cosmetics, lead solder, crude oil refining etc. [17,18].

However, some bioactive components of plants and animals exhibit immense potentials to either protect or enhance recovery of humans and animals from metal-induced toxicity. Two commonly used plant and animal products are palm oil and cod liver oil. Palm oil is extracted from the fruits of oil palm (*Elaeis guineensis*), which mesocarp contains numerous phytochemicals (mostly phenols) such as catechins, hesperidin, coumaric, ferulic, gallic, chlorogenic, protocatechuic, caffeic acids, 4-hydroxybenzoate etc. [19]. Phenolics are antioxidants, free radical scavengers and chelators of divalent cations [20]. Palm oil is a rich source of vitamin E (a known antioxidant), mainly of tocotrienols and few tocopherols. It contains carotenoids (β- and α-carotene) and chlorophylls, which are potent antioxidants. The simplest carotene is lycopene [21,22]. Cod liver oil on the other hand is a nutritional supplement derived from liver of cod fish (*Gadidae*) [23]. Cod liver oil contains vitamin D, vitamin A (retinol), omega-3 polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These compounds can protect cells from the destructive effect of increased lipid and lipid peroxidation products [24]. The aim of this study was to determine the effect of lead-induced oxidative stress and

hepatoprotective role of dietary supplements on Wistar albino rats.

2. MATERIALS AND METHODS

2.1 Experimental Animals and Samples

Male Wistar albino rats were obtained from a small animal holding unit of the Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, University of Nigeria Nsukka, Enugu State, Nigeria. Palm oil, Cod liver oil and rat feed (growers vital feed) were bought from a local market (Eke-Onuwa) in Owerri Municipal, Imo State. The animals were housed in cages meshed with wire gauze at the animal facility of the Department of Biochemistry, Federal University of Technology Owerri, Imo State, Nigeria. They were maintained under standard laboratory condition with 12hour light and dark cycle and room temperature ranging from 21 – 25℃. This study was approved by the ethics committee (FUTO/BCH/EC/2015/23) of the Department of Biochemistry, Federal University of Technology Owerri. The animals were handled in accordance with the guidelines on care and well being of research animals [25].

2.2 Exposure of Animals to Heavy Metal and Dietary Supplements

Thirty male Wistar albino rats of average weight, 150±30 g were divided into 5 groups of 6 rats each. Animals were allowed to acclimatize for 14 days and allowed free access to standard feed and water only. After this, animals were treated for 21 days as follows:

Group I (Normal control) received standard rat feed and water only *ad-libitum* throughout the treatment period.

Group II (Positive control) received standard rat feed and water *ad-libitum* and intraperitoneal administration of 30 mg/kg body weight (bwt) of lead acetate at alternate days throughout the treatment period.

Group III (Palm oil group) received standard rat feed and water *ad-libitum*, daily oral administration of 2.5 ml palm oil and intraperitoneal administration of 30 mg/kg bwt lead acetate at alternate days throughout the treatment period.

Group IV (Cod liver oil group) received standard rat feed and water *ad-libitum*, daily oral administration of 2.5 ml cod liver oil and intraperitoneal administration of 30 mg/kg bwt lead acetate at alternate days throughout the treatment period.

Group V (Palm oil+Cod liver oil (Synergy) group) received standard rat feed and water *ad-libitum*, daily oral administration of 1.25 ml palm oil and 1.25 ml cod liver oil and intraperitoneal administration of 30 mg/kg bwt lead acetate at alternate days throughout the treatment period.

2.3 Blood and Organ Collection for Biochemical Analysis

At the end of the treatment period, animals were fasted for 12 hours, anaesthetized with moderate ether, and blood collected via cardiac puncture. Blood samples were divided into anticoagulant and plain bottles. Blood samples in plain bottles were allowed to clot, centrifuged (1500 x g) for 10 mins at 4℃ to obtain serum and this was stored in a refrigerator. Afterwards, the rats were sacrificed and liver samples excised, washed in potassium chloride buffer (1.15%), homogenized and centrifuged for 60 mins to obtain supernatant that were used to assay oxidative stress parameters

2.4 Biochemical Assays

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities were determined spectrophotometrically using *in-vitro* methods for quantitative determination as outlined by commercial kits manufacturer of Randox laboratory test kits (Antrin, UK). Serum albumin (ALB), globulin, total protein (TP), and bilirubin were determined spectrophotometrically using manufacturer's instructions in Randox laboratory test kit (Antrin, UK). Activities of superoxide dismutase (SOD) and catalase (CAT) in liver homogenate were assayed as described by Xin et al. [26] and Aebi [27] respectively. Activities of glutathione peroxidase (GPx) were assayed as described by Paglia and Valentine [28]. Concentrations of malondialdehyde (MDA) and glutathione (GSH) were determined as described by Wallin et al. [29] and King and Wootton [30] respectively.

2.5 Determination of Haematological Parameters

Haematological parameters and their differentials were determined by an automated analyzer using the method described by Dacie and Lewis, [31]. Whole blood sample was used to determine Red Blood Cell (RBC) count, White Blood Cell (WBC) count, Platelet (PLT), Hematocrit (HCT), Haemoglobin (HGB), Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH), Mean Cell Haemoglobin Concentration (MCHC), Red Cell Distribution Width (RDW), RDWSD, PDW, PCT, and MPV.

2.6 Statistical Analysis

Statistical analysis was carried out using one way analysis of variance (ANOVA) and significance taken at $p<0.05$. Results obtained were expressed as mean ± standard deviation.

3. RESULTS

3.1 Liver Function Enzymes

Alanine aminotransferase (ALT) activities (Fig. 1) were significantly increased in all lead acetate exposed groups compared to normal control group that was not exposed. Activities of ALT in palm oil, cod liver oil and synergy groups were significantly reduced compared to positive control group. Aspartate aminotransferase (AST) activities (Fig. 2) were significantly elevated in all lead acetate exposed groups compared to the normal control, with the positive control group presenting the highest AST activity. However, significant reduction in activities of AST was observed in the groups treated with dietary supplement as compared to the positive control. In Fig. 3 the activities of alkaline phosphatase (ALP) were significantly elevated in all lead acetate exposed groups compared to the normal control. But the dietary supplement treated groups showed significant reduction in ALP activities when compared to positive control.

3.2 Liver Function Compounds

Total bilirubin concentration (Fig. 4) was significantly elevated in all lead acetate exposed groups except cod liver oil group when compared to the normal group. Total protein concentration was significantly reduced (Fig. 5) in lead acetate (except cod liver oil) exposed groups when compared to normal control. Albumin concentration (Fig. 6) in all lead acetate exposed groups showed no significant difference compared to normal control except, the palm oil treated group that showed significantly reduced albumin concentration. Fig. 7 shows a significant ($p<0.05$) decrease of globulin concentration in the positive control group compared to the normal control and cod liver oil group. However, there was no significant difference amongst the palm oil and synergy group when compared to the positive control group.

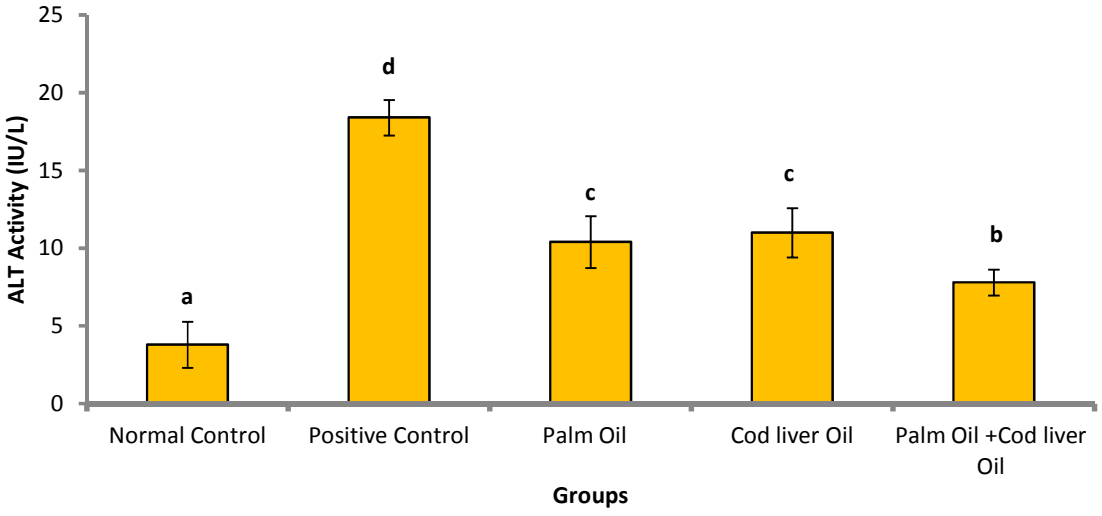

Fig. 1. Alanine aminotransferase (ALT) activities of male Wistar rats exposed to sublethal concentration of lead acetate and treated with dietary supplements

Bars represent mean ± standard deviation of five (n = 5) determinations and bars with different letters are statistically significant ($p<0.05$)

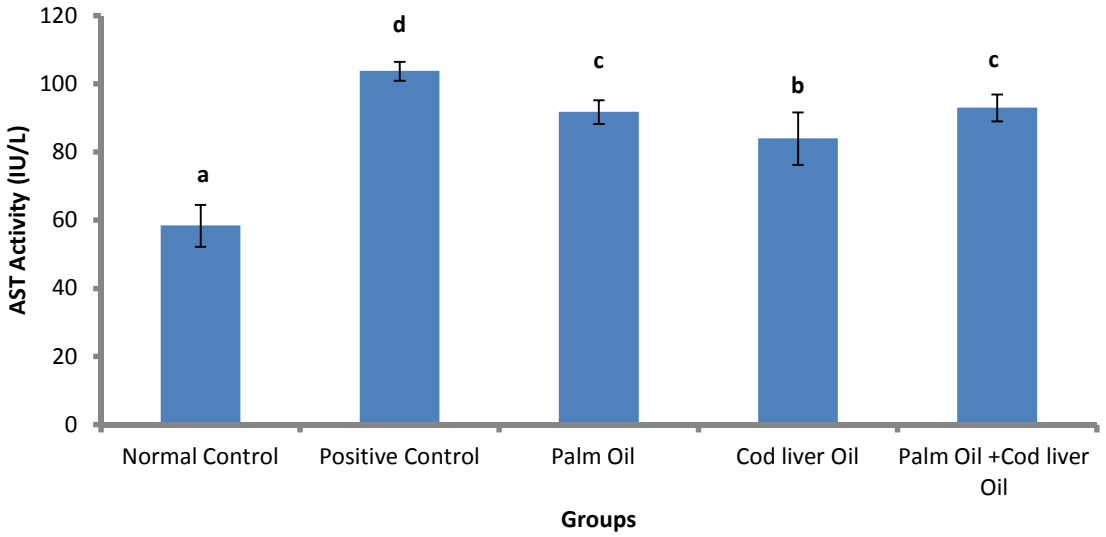

Fig. 2. Aspartate aminotransferase (AST) activities of male Wistar rats exposed to sublethal concentration of lead acetate and treated with dietary supplements

Bars represent mean ± standard deviation of five (n = 5) determinations, bars with different letters are statistically significant (p<0.05)

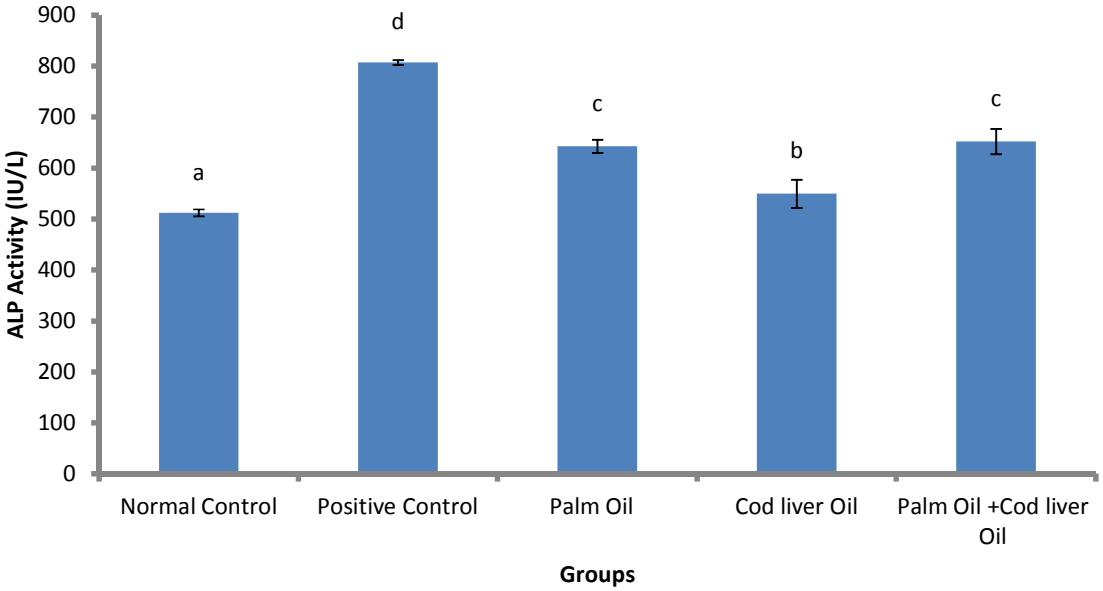

Fig. 3. Aspartate aminotransferase (ALT) activities of male Wistar rats exposed to sublethal concentration of lead acetate and treated with dietary supplements

Bars represent mean ± standard deviation of five (n = 5) determinations and bars with different letters are statistically significant (p<0.05)

3.3 Oxidative Stress Enzymes and Compounds

Superoxide dismutase (SOD) activity (Fig. 8) significantly reduced in positive control when compared to dietary supplement treated groups and normal control group. Also, Fig. 9 shows significant decrease in CAT activity of positive control group when compared to the normal control and dietary supplement treated groups (palm oil, cod liver oil and palm oil+ cod liver oil groups). Rats in cod liver and palm oil groups showed the highest activity of CAT compared to other groups. Furthermore, Fig. 10 shows a

significantly reduced glutathione peroxidase (GPx) activity in positive control rats compared to the normal control and dietary supplement treated groups. Similarly, GSH concentration significantly reduced (Fig. 11) in positive control compared to the normal control and dietary supplement treated groups. However, no significant difference was observed in GSH concentrations of normal control, palm oil, cod liver oil and synergy groups. Finally, malondialdehyde (MDA) concentrations (Fig. 12) were significantly elevated in lead acetate exposed and untreated group (positive control) compared to the normal group and treated groups. Conversely, the dietary supplement treated groups indicated no significant difference in the MDA concentration when compared to the normal control group.

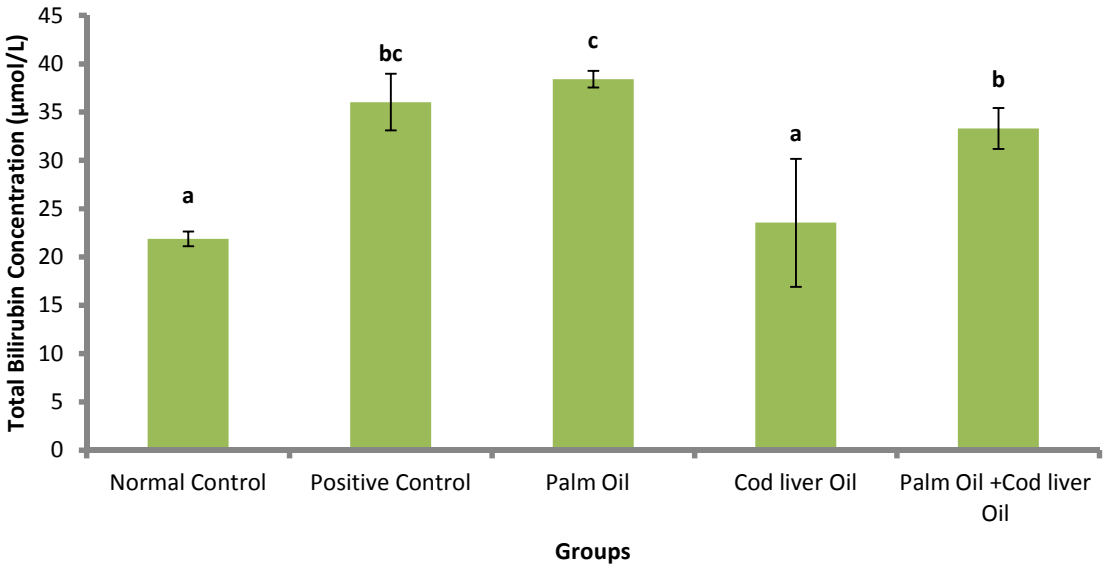

Fig. 4. Aspartate aminotransferase (ALT) activities of male Wistar rats exposed to sublethal concentration of lead acetate and treated with dietary supplements

Bars represent mean ± standard deviation of five (n = 5) determination and bars with different letters are statistically significant (p<0.05)

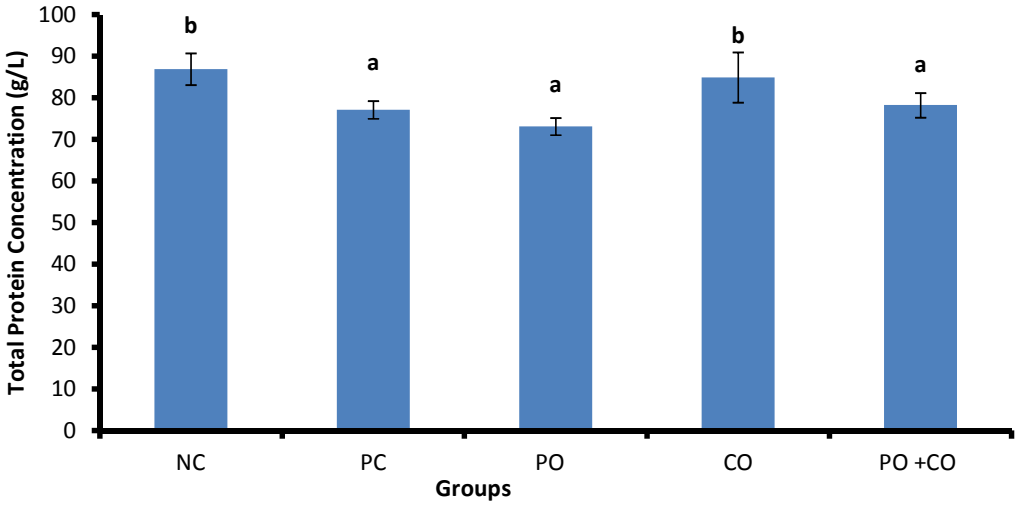

Fig. 5. Total protein concentration of male Wistar rats exposed to sublethal concentration of lead acetate and treated with dietary supplements

Bars represent mean ± standard deviation of five (n = 5) determinations and bars with different letters are statistically significant (p<0.05)

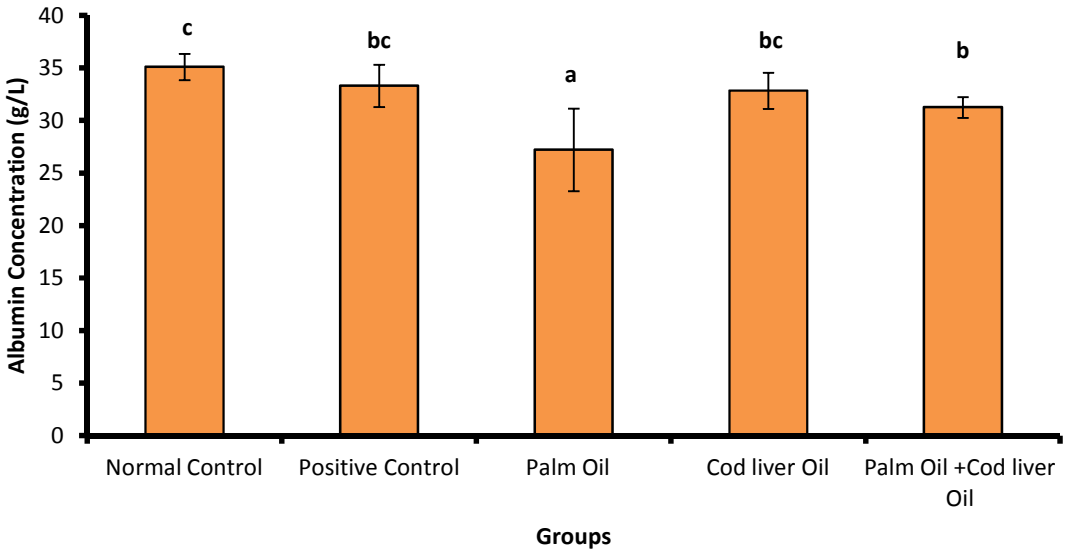

Fig. 6. Albumin concentration of male Wistar rats exposed to sublethal concentration of lead acetate and treated with dietary supplements

Bars represent mean ± standard deviation of five (n = 5) determinations and bars with different letters are statistically significant (p<0.05)

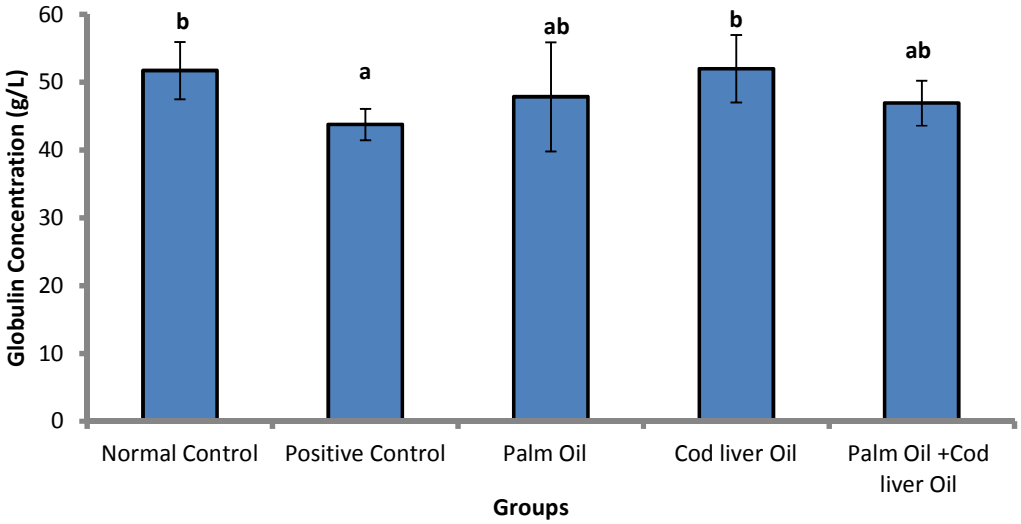

Fig. 7. Globulin concentration of male Wistar rats exposed to sublethal concentration of lead acetate and treated with dietary supplements

Bars represent mean ± standard deviation of five (n = 5) determinations and bars with different letters are statistically significant (p<0.05)

3.4 Haematological Parameters

Table 1 presents the result of haematological studies on rats exposed to lead acetate and treated with dietary supplements. Values shows no significant difference in rats HGB, RBC and HCT of all the groups except palm oil treated rats which showed significant decrease in these parameters. WBC significantly increased in positive control and synergy groups compared to normal control. Palm oil and cod liver oil treated rats showed no significant difference in WBC when compared to normal control. PLT of rats showed no significant difference in all groups. Other blood parameters such as MCV, MCH, MCHC, RDW, RDWSD, MPV, PDW and PCT showed no significant variation amongst the groups.

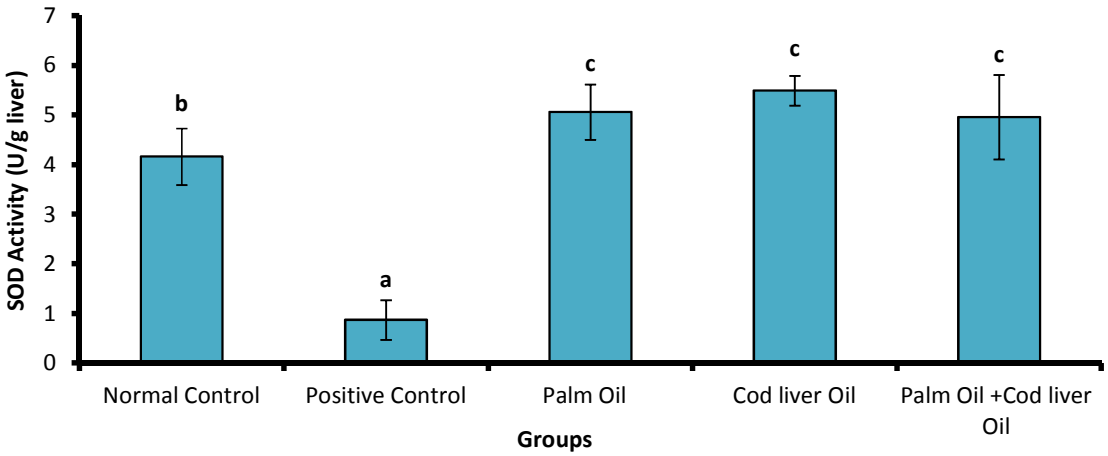

Fig. 8. Superoxide dismutase (SOD) activities of male Wistar rats exposed to sublethal concentration of lead acetate and treated with dietary supplements

Bars represent mean ± standard deviation of five (n = 5) determinations and bars with different letters are statistically significant (p<0.05)

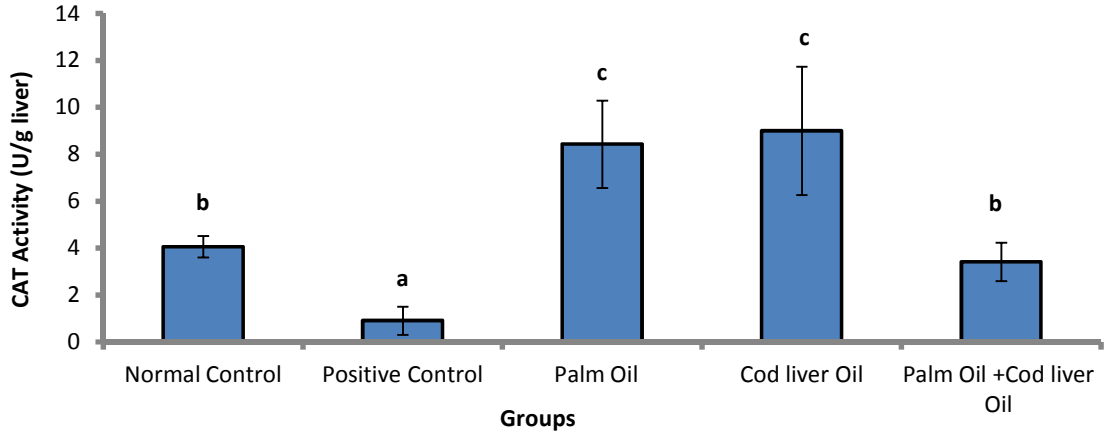

Fig. 9. Catalase (CAT) activities of male Wistar rats exposed to sublethal concentration of lead acetate and treated with dietary supplements

Bars represent mean ± standard deviation of five (n = 5) determinations and bars with different letters are statistically significant (p<0.05)

4. DISCUSSION

Environmental exposure to toxic level of lead (Pb) is a public health problem due to its wide distribution in the environment. Lead poisoning is a common occurrence in Zamfara and Niger States of Nigeria. Here semi-industrial scale gold ore mining and processing is practiced. Lead toxicity induces adverse biochemical, physiological and behavioural dysfunctions and knowledge of its toxicity and health consequences is an objective and effective way of ameliorating the negative health impact.

In this study the observed significant increase in activities of liver function enzymes (ALT, AST and ALP) in animal group exposed to lead acetate without dietary supplementation may indicate impaired liver function. Increased liver enzyme activities may reflect hepatocyte or biliary epithelial necrosis, compromise of hepatocyte membrane integrity, and cholestasis [32-34]. Hepatocyte membrane damage was possibly due to lead acetate exposure. This findings corroborates reports that lead exposure increased activity of liver enzymes [35,36]. Also, cellular membrane is a common target of heavy metal induced oxidative damage [37], with the metal lead showing increased peroxidation of membrane lipid [38]. The elevated activities of serum ALT and AST upregulates liver microsomal membrane fluidity, free radical generation and negative changes in hepatocytes [34].

Similarly, animals treated concomitantly with palm oil and cod liver oil showed significant ameliorative changes of lead induced liver damage as indicated by the significant reduction in the activities of serum ALT, AST, and ALP. The reversal of the elevated activities of serum enzyme in the palm oil and cod liver oil groups after lead administration may be attributed to the stabilizing ability of dietary content (lycopene, retinol, omega-3 PUFAs, EPA, DHA etc.) on cell membrane thus preventing enzyme leakages. Palm oil contains phenols (catechins, hesperidin, coumaric, ferulic, gallic, chlorogenic, protocatechuic, caffeic acids, 4-hydroxybenzoate) that can scavenge free radicals and chelate divalent cations [19,20].

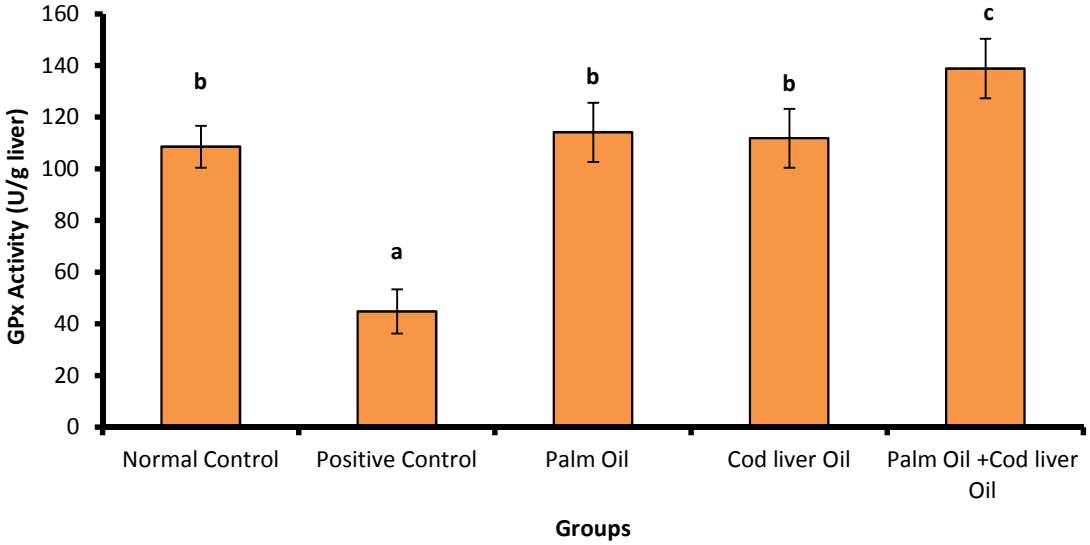

Fig. 10. Glutathione peroxidase (GPx) activities of male Wistar rats exposed to sublethal concentration of lead acetate and treated with dietary supplements

Bars represent mean ± standard deviation of five (n = 5) determinations and bars with different letters are statistically significant (p<0.05)

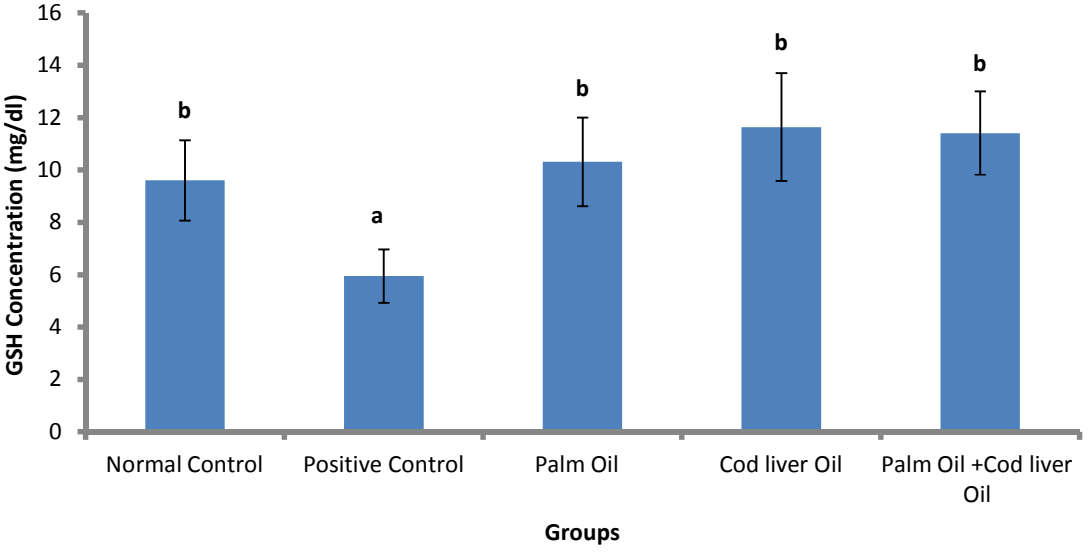

Fig. 11. Glutathione (GSH) concentration of male Wistar rats exposed to sublethal concentration of lead acetate and treated with dietary supplements

Bars represent mean ± standard deviation of five (n = 5) determinations and bars with different letters are statistically significant (p<0.05)

Table 1. Haematological parameters of male Wistar rats exposed to sublethal concentration of lead acetate and treated with dietary supplements

Groups	HGB	WBC	RBC	HCT	PLT	MCV	MCH
Normal control	129.50±5.80^{b}	5.625±1.84^{a}	7.87±0.27^{b}	31.93±1.48^{b}	358.25±78.24^{a}	40.68±2.43^{b}	16.40±0.68^{a}
Positive control	132.00±6.06^{b}	12.27±1.29^{b}	8.12±0.36^{b}	32.05±2.47^{b}	432.50±23.27^{a}	39.50±1.49^{ab}	16.20±0.77^{a}
Palm oil	105.25±4.99^{a}	7.40±3.06^{ab}	6.69±0.33^{a}	25.70±1.92^{a}	471.00±70.03^{a}	38.48±1.09^{ab}	15.70±0.24^{a}
Cod liver oil	114.00±15.12^{ab}	9.80±3.36^{ab}	7.27±1.00^{ab}	27.73±3.39^{ab}	453.00 ±135.90^{a}	38.25±1.00^{ab}	15.70±0.17^{a}
Palm oil+Cod liver oil	132.00±23.27^{b}	12.78±6.18^{b}	8.36±1.27^{b}	31.80±5.43^{b}	395.00±66.91^{a}	38.03±1.52^{a}	15.70±0.48^{a}
Groups	**MCHC**	**RDW**	**RDWSD**	**MPV**	**PDW**	**PCT**	
Normal control	405.25±11.50^{a}	19.75±0.49^{ab}	26.23±2.66^{a}	8.53±0.38^{b}	14.63±0.21^{a}	0.31±0.08^{a}	
Positive control	412.50±22.05^{a}	20.08±0.81^{b}	25.20±1.25^{a}	8.40±0.22^{ab}	14.60±0.14^{a}	0.36±0.02^{a}	
Palm oil	410±16.71^{a}	18.80±0.60^{a}	23.90±0.00^{a}	8.25±0.26^{ab}	14.48±0.15^{a}	0.39±0.05^{a}	
Cod liver oil	410.50±8.06^{a}	19.43±0.87^{ab}	25.23±1.00^{a}	8.00±0.32^{a}	14.25±0.26^{a}	0.36±0.10^{a}	
Palm oil+Cod liver oil	414.50±8.27^{a}	19.33±0.95^{ab}	24.23±0.97^{a}	8.30±0.30^{ab}	14.47±0.38^{a}	0.33±0.05^{a}	

Values are mean ± standard deviation of five (n=5) determinations and columns with different letters are statistically significant (p<0.05)

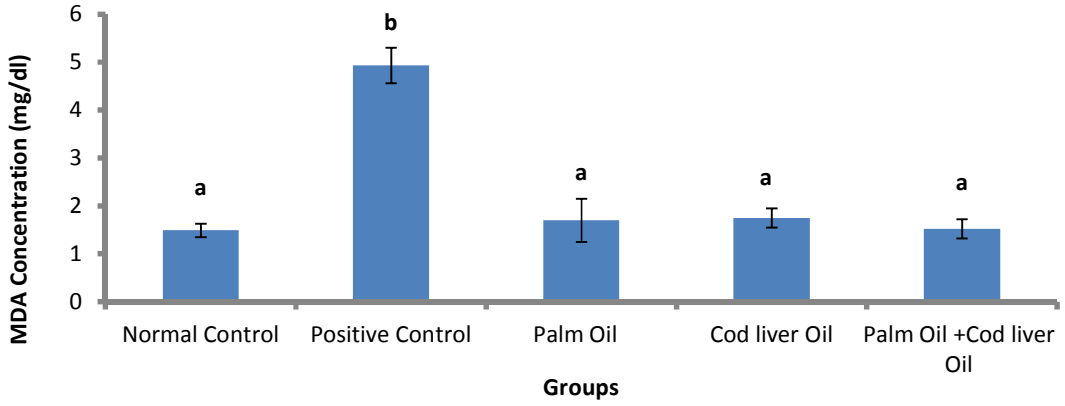

Fig. 12. Malondialdehyde (MDA) concentration of male Wistar rats exposed to sublethal concentration of lead acetate and treated with dietary supplements

Bars represent mean ± standard deviation of five (n = 5) determinations and bars with different letters are statistically significant (p<0.05)

The observed decrease in concentration of serum total protein in positive control when compared to treated groups indicates ameliorative potentials of dietary supplements used in this study. Total protein is decreased in cases of liver dysfunction and malnutrition [39]. Albumin decreases in chronic liver dysfunction such as cirrhosis and in kidney loses, however our result showed no significant decrease in serum albumin concentration. Also, decreased serum globulin of positive control rats may indicate liver dysfunction and nephrosis [40], due to lead toxicity. Similarly, the significantly increased total bilirubin of all Pb exposed groups except cod liver oil group is an oxidative signal for varying metabolic changes such as haemolysis, liver damage, and cholestasis [41].

Furthermore, the significant decrease of catalase activities in positive control agrees with the report of Sharma et al. [42], that reduced activities of catalase in lead nitrate exposure results to peroxidative damage. Cellular systems are sheltered from cell damages caused by reactive oxygen species (ROS) by various defences consisting of antioxidants with diverse functions [43]. When the concentration of ROS present in cellular system increase over the concentration of the defence systems, oxidative stress is induced, and this could result to cellular injuries and ultimately development of diseases. The observed significant decrease in SOD activity of positive control compared to normal control group and groups treated with dietary supplements indicates ameliorative potentials by the dietary supplements. Activities of SOD in tissues and/or blood are markers of oxidative stress [44]. Similarly, Pb exposure may be implicated in the decreased activity of glutathione peroxidase, because under normal conditions cells possesses enzymatic and non enzymatic defences to cope with free radicals [45], however under oxidative stress these conditions are distorted and requires in some cases exogenous antioxidants as supplied by the dietary supplements used in this study.

Oxidative damage, however may occur when antioxidant potential is decreased leading to increase in oxidative stress [46]. These observations give credence to Pb as having high affinity for sulfhydryl (SH) groups. Lead can alter the antioxidant activities of SOD, CAT, GPx, and glucose-6-phosphate dehydrogenase (G6PD) by inhibiting functional SH groups and forming less stable mercaptides complexes [47]. Reduced SOD activity downregulates disposal of superoxide radicals, while a drop in CAT activity impedes the rate at which superoxide radical is scavenged. Apart from targeting the sulfhydryl groups, Pb can also replace the zinc ions that serve as important co-factors for antioxidant enzymes and inactivate them [48]. Increased susceptibility of cells to oxidative stress may arise because Pb toxicity affects uptake of important trace elements (Se, Zn and Cu) required by antioxidant enzymes (GPx, CAT, and SOD) for proper molecular structure and activity.

Similarly, the significant reduction of GSH concentration in positive control implies that administration of dietary supplements enhanced synthesis of GSH, considered as the first line of defence against oxidative damage and free radical generation. Here GSH functions as a scavenger and a co-factor in metabolic detoxification of ROS [49]. Furthermore, the increase in lipid peroxidation (LPO) in positive control confirms the extent of oxidative damage [50] occurring in this group when compared to normal control and groups treated with dietary supplements. Elevated concentrations of MDA are markers for peroxidation of membrane lipids and an indication of possible oxidative injury from oxidative stress [50]. The nature of membrane fatty acids determines susceptibility to peroxidation and because Pb induces elongation membrane arachidonic acids, it is implicated for the enhanced membrane LPO. Cell membrane damage elicited by Pb exposure causes derangement in processes such as activity of membrane enzymes, endo- and exocytosis, transport of solutes across the bilayer, and signal transduction by effecting lateral phase separation [47,51,52]. It could be said that the reduced concentration of MDA in groups treated with dietary supplements indicates ameliorative potentials attributable to *β*-carotene and lycopene content of palm oil which can inhibit LPO, and reduce incidence of oxidative stress-induced conditions and diseases such as cancer, atherosclerosis, age-related macular degeneration, and multiple sclerosis [20,21,22,53]. Also, cod liver oil rich content of retinol, omega-3 polyunsaturated fatty acids, eicosapentaenoic acid and docosahexaenoic acid protect cells from the destructive effect of increased lipids and LPO products [24,54]

The values obtained in haematological studies showed no significant difference in most of haema-parameters of positive control group when compared to normal control, and all dietary supplement treated groups. However, Pb directly

affects the haematopoietic system by inhibiting in a dose dependent manner three key enzymes involved in the synthesis of heme- δ-aminolevulinic acid dehydratase (ALAD), aminolevulinic acid synthetase (ALAS) and ferrochelatase. Lead can reduce the life span of circulating erythrocytes by increasing the fragility of cell membranes. This corroborates the significantly increased total bilirubin, concentration in lead acetate exposed groups because bilirubin is a metabolic product of haemolysis or breakdown of red blood cells [55].The combined effects of these two processes may lead to haemolytic anaemia [56,57]. The no significant difference observed in most of the blood parameters determined may as well be associated to the activity of δ-aminolevulinic acid dehydratase (ALAD). Report by Ahamed et al. [58] indicate that heme biosynthesis does not decrease until the activity of ALAD is inhibited by 80–90%, and this is observed at blood lead concentration of about 55 µg/dl.

5. CONCLUSION

The effects of dietary supplements on lead induced toxicity in Wistar albino rats showed that the dietary supplements cod liver and palm oil decreased the elevated activities of the liver enzymes, and ameliorated the negative effect of oxidative stress. Thus, it may be recommended that adequate amount of these dietary supplements are contained in diets.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Flora SJS, Chouhan S, Kannan GM, Mittal M, Swarnkar H. Combined administration of taurine and monoisoamyl DMSA protects arsenic induced oxidative injury in rats. Oxidative Med Cellular Longevity. 2008;1(1):39–45.
2. Sharma B, Singh S, Siddiqi NJ. Biomedical implications of heavy metals induced imbalances in redox systems. Bio Med Res Inter. 2014;26.
(Article ID 640754)
3. Gupta VK, Pal R, Siddiqi NJ, Sharma B. Acetylcholinesterase from human erythrocytes as a surrogate biomarker of lead induced neurotoxicity. Enzyme Res. 2015;7.
(Article ID 370705)
Available:http://dx.doi.org/10.1155/2015/370705
4. Valko M, Morris H, Cronin MTD. Metals, toxicity and oxidative stress. Curr Medicinal Chem. 2005;12(10):1161–1208.
5. Pitot C, Dragan PY. Chemical carcinogenesis. Mc Graw Hill; New York: Casarett and Doull's Toxicology. 1996; 201–260.
6. Aldahmash BA, El-Nagar DM. Antioxidant effects of captopril against lead acetate-induced hepatic and splenic tissue toxicity in Swiss albino mice Saudi. J Biol Sci. 2016;23(6):667–673.
DOI: 10.1016/j.sjbs.2016.05.005
7. Duruibe J, Ogwuegbu M, Egwurugwu J. Heavy metal pollution and human biotoxic effects. Int. J Phys Sci. 2007;2(5):112-118.
8. Agency for Toxic Substances and Disease Registry (ATSDR) Atlanta, GA: U.S. department of health and human services, public health service. Toxicological profile for lead. (Draft for Public Comment) 2005;43–59.
9. Correia M, Neves NF, Verissimo P. From consensus to atomic broadcast: Time-free Byzantine-resistant protocols without signatures, Computer J. 2006;41(1):82–96.
10. Kalia K, Flora S. Strategies for safe and effective therapeutic measures for chronic arsenic and lead poisoning. J Occup Health. 2005;47:1–21.
11. Flora S, Flora G, Saxena G. Environmental occurrence, health effects and management of lead poisoning. Elsevier Science. B.V. 2006;158-228.
12. Pearce J. Burton's line in lead poisoning. European neurology. 2007; 57:118–9.
13. Liu C, Huo X, Lin P, Zhang Y, Li W, Xu X. Association between blood erythrocyte lead concentrations and hemoglobin levels in preschool children. Environmental Sci Pollution Res. 2015;22(12):9233–9240.
14. Gupta VK, Singh S, Agrawa, A. Siddiqi NJ, Sharma B. Phytochemicals mediated remediation of neurotoxicity induced by heavy metals. Biochemistry Research International. 2015;9.
(Article ID 534769)
Available:http://dx.doi.org/10.1155/2015/534769
15. Biya O, Gidado S, Haladu S, Geoffrey T, Nguku P, Durant J. Notes from the Field:

Outbreak of acute lead poisoning among children aged <5 years, Zamfara, Nigeria. Morbidity Mortality Weekly Report (MMWR). 2010;59(27):846.
16. Tirima S, Bartrem C, von Lindern I, von Braun M, Lind D, Anka SM, Abdullahi A. Environmental remediation to address childhood lead poisoning epidemic due to artisanal gold mining in Zamfara, Nigeria. Environ Health Perspect. 2016;124 (9):1471-1478.
DOI: 10.1289/ehp.1510145
17. Ana GREE, Mynepalli KCS, Elijah AB. Environmental risk factors and health outcomes in selected communities of the Niger Delta Area, Nigeria. Perspect Public Health. 2009;129(4):183-191.
18. Ogbonna CU, Ujowundu CO, Okwu GN, Emejulu AA, Alisi CS. Igwe KO. Biochemical and histological evaluation of benzo[a]pyrene induced nephrotoxicity and therapeutic potentials of *Combretum zenkeri* leaf extract. Afr J Pharm Pharmacol. 2016;10(41):873-882.
19. Tan YA, Sundram K, Sambanthamurthi R, Water-soluble phenolics from the palm oil industry. In: Pfannhauser W, Fenwick GR, Khokhar S, editorss. Biologically-active phytochemicals in food – analysis, metabolism, bioavailability and function. Cambridge: Roy. Soc. Chem. 2001;548-551.
20. Nagendran B, Tan YA, Ravigadevi S, Kalyana S, Samir S. Antioxidant properties of palm fruit extracts. Asia Pac J Clin Nutr. 2005;4(4):319-324.
21. Ravigadevi S, Kalyana S, Tan YA. Chemistry and biochemistry of palm oil. Progress Lipid Res. 2000;39:507-558.
22. Ujowundu CO, Okoye HN, Nwaoguikpe RN, Belonwu DC, Igwe KO, Ujowundu FN. Hepatoprotective effects of crude extracts of tomato and onion in rats exposed to locally processed beef. Int J Biochem Res. Rev. 2014;4(2):193-203.
23. Report of the twenty forth session of the CODEX committee on fats and oils. Joint FAO/WHO Food Standards Programme Codex Alimentarius Commission; 2015.
24. Hünkar T, Aktan F, Ceylan A, Karasu C. Effects of cod liver oil on tissue antioxidant pathways in normal and streptozotocin-diabetic rats. Cell Biochem Funct. 2002;20(4):297-302.
25. National Institute of Health (NIH). Guide for the care and use of laboratory animals. DHEW Publication, Office of Science and Health Reports. Bethesda, USA; 2011.
26. Xin Z, Waterman DF, Henken RM, Harmon RJ. Effects of copper status on neutrophil function, superoxide dismutase and copper distribution in steers. Journal Diary Science. 1991;74:3078.
27. Aebi HE. Catalase. Methods of enzymatics analysis. 3rd Edition. 1983;273-285.
28. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med. 1967;70:158-169.
29. Wallin B, Rosengren B, Shertzer HG, Camejo G. Lipoprotein oxidation and measurement of TBARS formation in a single microlitre plate, its use for evaluation of antioxidants. Analytical Biochemistry. 1993;208:10-15.
30. King EJ, Wootton IDP. Microanalysis in Medical Biochemistry, 3rd ed. London: J. and A. Churchill Ltd; 1956.
31. Dacie JV, Lewis SM. Practical Haematology. Pub.: Churchhill L Livingstone. London. 1991;1-73.
32. Adaramonye O, Osaimeje D, Akinsanya A, Nneji C, Fafunso M, Ademowo O. Changes in antioxidant status and biochemical indices after acute administration of artemether, artemether-lumefantrine and halofantrine in rats. Basic Clin Pharmcol Toxicol. 2008;102:412-418.
33. Ujowundu CO, Nwokedinobi N, Kalu FN, Nwaoguikpe RN, Okechukwu RI. Chemoprotective potentials of *Ocimum gratissimum* in diesel petroleum induced hepatotoxicity in albino wistar Rats. J Applied Pharma Sci. 2011;01(10):56-61.
34. Ibrahim NM, Eweis EA, El-Beltagi HS, Abdel-Mobdy YE. Effect of lead acetate toxicity on experimental male albino rat. Asian Pac J Trop Biomed. 2012;2(1):41–46.
DOI: 10.1016/S2221-1691(11)60187-1
35. Sharma A, Sharma V, Kansal L. Therapeutic effects of *Allium sativum* on lead induced bio-chemical changes in soft tissues of Swiss Albino mice. Pharmacognosy Magazine. 2009;5:364-371.
36. Akilavali N, Radhika J, Brindha P. Hepatoprotective activity of *Ocimum sanctum* linn. against lead induced toxicity in albino rats. Asian J Pharma Clin Res. 2011;4:84-87.

37. Adonaylo VN, Oteiza PI. Pb promotes lipid oxidation and alterations in membrane physical properties. Toxicology. 1999; 132(1):19–32.
38. Yin S, Lin T. Lead-catalyzed peroxidation of essential unsaturated fatty Acid. Biol Trace Element Res. 1995;50:167-172.
39. Merleb EM, Hoehn K, Human anatomy and Physiology. Amazon. Com. Books. 7th Edition; 2007.
40. Jeremy EK. Serum protein. Board Certified Internal Med J. 2011;34-38.
41. Annabi BA, Nehde A. Antioxidant enzymes activity and bilirubin level in adult rat treated with lead. Clinical Research Biology. 2007;330(8):581-588.
42. Sharma V, Sharma A, Kansal L. The effect of oral administration of *Allium sativum* extracts on lead nitrate induced toxicity in male mice. Fd Chem Toxicol. 2010; 48:928–936.
43. Ercal N, Gurer- Orhan H, Aykin-Burns N. Toxic metals and oxidative stress part 1. Mechanisms involved in Metal- Induced Oxidation Damage. Curr Topics Med Chem. 2001;1:529-539.
44. Bariondeau DP, Kass M, Mann CJ, Bruns CK, Taine JA, Getzoff OD. Superoxide dismutase structure and mechanism. Biochemistry. 2004;43(25):8038-8047.
45. Shalana M, Mostafab M, Hassounab M. Amelioration of lead toxicity on rat liver with vitamin and silymarin supplements. Toxicol. 2005;206:1-15.
46. Patra RCD, Swarup SK, Dwivedi AS. Trace minerals in blood of young calves during exposure to lead. Indian J Anim Sci. 2001;71:507-510.
47. Patra RC, Rautray AK, Swarup D. Oxidative stress in lead and cadmium toxicity and its amelioration. Vet Med Int. 2011;457327.
DOI: 10.4061/2011/457327
48. Flora SJ, Flora G, Saxena G, Mishra M. Arsenic and lead induced free radical generation and their reversibility following chelation. Cell Mol Biol. (Noisy-le-grand). 2007;53:26–47.
49. Tandon K, Baldwin E, Scott J, Shewfelt R. Linking sensory descriptors to volatile and non-volatile components of fresh tomato flavor. J Food Sci. 2003;68:2366–2371.
50. Del RD, Stewart AJ, Pellegrine N. A review of recent studies on malonyldialdehyde as toxic molecules and biological marker of oxidative stress. Nutr Met Cardiovascular Dis. 2005;15(4):316-328.
51. Lawton LJ, Donaldson WE. Lead-induced tissue fatty acid alterations and lipid peroxidation. Bio Trace Element Res. 1991;28(2):83–97.
52. Adanaylo V, Oteiza P. Lead intoxication: antioxidant defenses and oxidative damage in rat brain. Toxicol. 1999;135:77-85.
53. Giovannucci E, Clinton SK. Tomatoes, lycopene, and prostate cancer. Proceedings of the Society for Experimental Biology and Medicine. 1998; 218(2):129–139.
54. Salama MF, Abbas A, Darweish MA, El-Hawwary AA, Al-Gayyar MMH. Hepatoprotective effects of cod liver oil against sodium nitrite toxicity in rats. Pharma Bio. 2013;51(11):1435-1443.
55. Annabi BA, Nehde A. Antioxidant enzymes activity and bilirubin level in adult rat treated with lead. Clin Res Bio. 2007;330(8):581-588.
56. Guidotti T, McNamara J, Moses M. The interpretation of trace element analysis in body fluids. Indian J Med Res. 2008;128: 524–532.
57. Vij AG, Hemopoietic, hemostatic and mutagenic effects of lead and possible prevention by zinc and vitamin C. Al Ameen J Med Sci. 2009;2:27–36.
58. Ahamed M, Verma S, Kumar A, Siddiqui MK. Environmental exposure to lead and its correlation with biochemical indices in children. Sci Total Environ. 2005;346:48–55.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/19717