Antioxidants-rich Nutraceutical Ameliorates Lead-induced Oxidative Stress in Albino Rats

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Abstract

Treatment of lead toxicity is usually through aggressive approach such as chelation and bowel irrigation and these therapies are used just to promote body metal excretion and are considered less effective due multiple limitations and side effects. Therefore, the present study was conducted to evaluate the protective and curative potentials of formulated antioxidant-rich nutraceutcals against lead-induced oxidative stress in albino rats. A total of Fifty (50) albino rats of both sexes weighing 200–220 g were equally and randomly divided into two (2) groups (A and B models) of twenty five rats each. The group A was designed to study the protective effect of formulated nutraceutical against lead-induce oxidative stress. While group B was to evaluate the curative effect of same formulated nutraceutical in rats after lead intoxication. The rats in each of the models were randomly divided into five groups. Thirty (20) rats of lead treated groups were administered with different concentration of antioxidant-rich nutraceutical in both models. While 10 rats were treated with standard drug. Our findings reveals that oral administration of antioxidant- rich supplement in lead-intoxicated rats (in both models) significantly reduced blood lead levels, increased the activity of antioxidants enzymes and glutathione levels. Similarly, the levels of antioxidant minerals (Fe, Zn, Cu and Se) and vitamins (A, C and E) were boosted significantly. Concurrently, significant decreased levels of malondialdehyde were observed after administration of the antioxidant-rich supplement, hence, lowering lead-induced oxidative stress. Therefore, oral administration of antioxidant-rich supplement may invariably use as protective and as well as curative regimen to curtail lead-induced oxidative stress.

Key words: *Lead, Toxicity, Antioxidants, Nutraceuticals, Oxidative stress*

Introduction

Lead has been confirmed and affirmed as notorious environmental pollutant affecting all forms of biosphere ((Jangid *et al.*, 2016)). Environmental and occupational exposures to this toxic metal are now on the increase due to high anthropogenic activities, automobile emissions and intensive agricultural practice (Kim *et al.*, 2015; Oboma *et al.*, 2018). Humans are exposed to lead through different routes such as water, air, foodstuff, household products, cosmetics and pharmaceutical products (Jangid *et al.*, 2016). The amount of lead present in the environment for potentials consumption via these routes surpassed the sum of preceding eras (Lamidi and Akefe, 2017; Oboma *et al.*, 2018). Therefore, lead pollution is seen as great environmental health problem facing the globe, especially underdeveloped and developing countries.

Environmental lead access and get absorbed by the body through inhalation of contaminated dust, ingestion of contaminated food and water into gastrointestinal tract or dermal (skin) contact. Inhalation and dermal contact are more of typical occupational exposure while ingestion is mostly for the general population through contaminated foods, cosmetics and herbal preparations (Lamidi and Akefe, 2017). Once lead reaches the bloodstream, it is readily distributed into three compartments: blood, soft tissues such as kidneys, liver, brain, bone marrow and mineralizing tissues such as bone and teeth. It produces several devastating effects on virtually all the body organs causing diseases of the nervous, renal, hepatic and reproductive systems (Dkhil *et al.*, 2016). High blood lead levels following exposure could result into acute or more rampant chronic toxicity. If not properly managed, it becomes severe; characterized by persistent nausea, encephalopathy, fatigue and coma (Sirivarasai *et al.*, 2015).

Mechanisms have been proposed for lead induced toxicity which believed to involve biochemical processes such as lead's ability to inhibit or mimic the action of calcium (which affects Ca^{2+} dependent or related processes), interact with proteins (including those with sulfhydryl, amine, phosphate and carboxyl groups) and more recently, oxidative stress was considered to be the major mechanism of lead-related pathologies (Sadhana *et al.*, 2011).Lead induces oxidative stress via generation of reactive oxygen species (superoxide radicals and hydroxyl radicals), depletes available endogenous antioxidant reserves (glutathione, glutathione peroxidase, superoxide dismutase and catalase) involved in scavenging the free radicals generated, interfering with essential metals and vitamins and increasing susceptibility of cells to oxidative attack by altering membrane integrity and fatty acids composition (Chen *et al.*, 2015).

Over the years, treatment of lead toxicity by physicians is only through aggressive approach such as chelation and bowel irrigation and these therapies are used just to promote body metal excretion. The widely used lead chelators are: dimercaprol (BAL), dimercaptosuccinic acid (DMSA), CaNa2EDTA, unithiol (DMPS) and D-penicillamine (DPA) (Kim *et al.*, 2015). The chelators however, have lots of limitations and associated with many side effects; lack of specificity, some essential minerals are also excreted and can only reduce the blood lead burden, but, it cannot reverse the damage of lead toxicity to the affected organs (Chen *et al.*, 2015). Treatment with CaNa2EDTA has been reported to cause renal toxicity especially when used in high dose. DMSA is characterized with consequent loss of appetite, nausea and diarrhea.

Consequent upon that, chelation therapy is considered less effective due its multiple limitations and side effects. They can also cause the depletion of essential minerals and the metabolic

disorder of antioxidant. This has led to presumption that supplementation of diet- rich antioxidant could be an alternative regimen for chelation therapy. The use of essential metals, vitamins, edible plants and dietary phytochemicals, probiotics and other dietary supplements in the treatment of lead toxicity are now appearing in the literature (Chen *et al.*, 2015; Dkhil *et al.*, 2016; Kordas, 2017; Oboma *et al.*, 2018).

A nutraceutical product is a substance, which has physiological benefit or provides protection against chronic disease. Formulation of antioxidants-rich nutraceutical to serve as supplement in the treatment and management of chronic disease is currently receiving attention from different researchers. Saidu *et al.* (2012) reported that antioxidant-rich supplement reduced blood pressure in hypertensive rats. Wali *et al.* (2013) showed that supplementation with nutraceutical rich in antioxidants may reduce the risk of oxidative stress and dyslipidemia in diabetic mellitus. It is therefore, believed that supplementation of antioxidant-rich nutraceutical may invariably boost body's antioxidant defense system thereby preventing or reversing the devastating effects of lead toxicity.

MATERIALS AND METHODS

Chemicals

Lead acetate $Pb(C_2H_3O_2)_2$ was obtained from Sigma-Aldarich Corporation (St. Louis, Missouri, USA), plastic wares including test tubes, blood collection containers and pipettes were autoclaved and made metal free, following a standard protocol. All other chemicals used were of analytical grade. Antioxidant enzymes were assayed using commercial kits (Cayman Chemical Company, USA) with the following item numbers: superoxide dismutase (706002), catalase (707002), glutathione peroxidase (703102) and MDA (10009055).

Antioxidant-rich supplement

The supplement was in form of fine granules mixture of tomatoes, onions, garlic, ginger, lemon, melon seed and palm oil, in a ratio of 4:4:4:4:2:1:1 (Saidu *et al.*, 2012). The nutraceutical was prepared in form of oral suspension characterized with attractive taste. The suspension was fed to the lead intoxicated rats with an oral gavage. The tomato, onion, garlic, ginger and melon seeds were obtained and dried in an oven at a temperature of 40°C. The dried materials were then crushed into fine powder using electric blender. Palm oil was added to the powder and mixed thoroughly; lastly lemon juice was added, mixed thoroughly and spread on a clean flat tray to dry at room temperature.

Experimental animals

The study was undertaken in the Animal House of Biological Sciences, Faculty of Science, Usmanu Danfodiyo University Sokoto Nigeria from August to September 2019. A total of Fifty (50) albino rats of both sexes (Wistar strain) weighing 200–220 g were housed in groups of 5, in plastic cages, in an air-conditioned room maintained at a temperature of 24 ± 2 °C and with a 12 h light/dark illumination cycle. The animals were fed a standard commercial laboratory pelleted diet and tap water *ad libitum*. The study was conducted in accordance with ethical guidelines for care and use of laboratory animals and granted approval by the Animals Research Committee of Usmanu Danfodiyo University Sokoto Nigeria.

Experimental Design

After about two weeks of acclimatization, the animals were equally and randomly divided into two (2) groups (A and B models) of twenty five rats each. The group A was designed to study the protective effect of formulated nutraceutical against lead-induce oxidative stress. While group B was to evaluate the curative effect of same formulated nutraceutical in rats after lead intoxication. Therefore, rats in the group A (preventive model) were randomly distributed into five (5) groups of follows:

Group 1: Control (distilled water only) and termed as NCG
Group 2: Lead-induced (60 mg/kg Lead acetate only) and termed as LAO
Group 3: 60 mg/kg Lead acetate + 250 mg/kg formulated nutraceutical and termed as LAF_{250}
Group 4: 60 mg/kg Lead acetate + 500 mg/kg formulated nutraceutical and termed as LAF_{500}
Group 5: 60 mg/kg Lead acetate + 18.86 mg/kg (standard drug) and termed as LAS

The treatment was given orally accordingly to all the groups an hour after lead acetate was given to the rats except the control group on daily basis for 28 days. The 18.86 mg/kg of the standard drug was based on the oral dose for human adult 1g/70kg body weight per day.
The second group (B) (curative model) was also randomly subdivided into five groups below. All the groups except the control were intoxicated with 60 mg/kg Lead acetate for period of 14 days and thereafter, treatment with respective doses of nutraceutical and standard drug were given orally to group 2,3 and 4 accordingly for another 14 days.

Group 1: Control (distilled water only) and termed as $_{C}$NCG
Group 2: Lead-induced (60 mg/kg Lead acetate only) and termed as $_{C}$LAO
Group 3: 60 mg/kg Lead acetate + 250 mg/kg formulated nutraceutical and termed as $_{C}LAF_{250}$
Group 4: 60 mg/kg Lead acetate + 500 mg/kg formulated nutraceutical and termed as $_{C}LAF_{500}$
Group 5: 60 mg/kg Lead acetate + 18.86 mg/kg (standard drug) and termed as $_{C}$LAS

Sample collection and preparation

After 28 days of the experiment, animals were allowed to fast overnight and anesthetized with chloroform. This was done by placing each in a jar which contains a cotton wool soaked in chloroform. About 5 ml blood sample was collected through cardiac puncture and serum was, thereafter, obtained by centrifuging the blood sample at 3,000rpm for 10 minutes. The serum was aspirated and transferred to another sets of clean test tubes and stored at -4°C before use.

Metal Analysis
Serum concentrations of Pb, Zn, Cu, Fe and Se determination was carried out using Atomic Absorption Spectrophotometer (AAS Perkin Elmer, 6300 model USA). Wet digestion was carried out on the serum samples as described by Madiha *et al.* (2018). One milliliters of blood sample was taken into test tube and 2 ml of nitric acid was added and heated for about 45 minutes and allowed to reach 160^{0}C to reduce the volume. After completion of digestion the sample was allowed to cool and hydrogen per oxide was added to make it up to 5ml and filtered. The calibration curve was prepared for each element by running different concentration of standard solutions. The machine was set to zero by running reagent blank. Three replicate results were taken for each sample and average values were obtained.

Determination of Enzymatic activity of the Antioxidant Enzymes

The activity of serum superoxide dismutase (SOD) was measured according to the method of Sun *et al.* (1988). The method utilizes a tetrazolium salt for the detection of superoxide radicals generated by xanthine oxidase and hypoxanthine in the presence of a range concentration of superoxide dismutase. The absorbance was read at 450nmwavelength. The activity of serum glutathione peroxidase (GPx) was measured according to the method of Paglia and Valentine (1967). The assay measures glutathione peroxidase activity indirectly through a coupled reaction with glutathione reductase. The oxidized glutathione, produced upon reduction of hydroperoxide by glutathione peroxidase, is recycled to its reduced state by glutathione reductase using NADPH. The oxidation of NADPH to $NADP^+$ is accompanied by a decrease in absorbance at 340 nm.

The activity of serum catalase (CAT) was measured according to the method of Johansson and Borg (1998). The method was based on the reaction of the enzyme with methanol in the presence of an optimal concentration of hydrogen peroxide. The formaldehyde produced was measured spectrophotometrically with 4-amino-3-hydrazino-5-mercapto-1, 2, 4-triazole (Purpald) as a chromogen at 450nm wavelength.

Measurement of Glutathione

Glutathione concentration was determined by the method of Baker *et al. (*1990**).** The complex formed between GSH and 5, 5'dithiodis-2-nitrobenzoic acid (DTNB) produces thionitrobenzoic acid (TNB) was quantified by taking the absorbance at 412 nm.

Estimation of Lipid Peroxidation Levels

Serum malondialdehyde (MDA) levels were measured according to the method of Buege and Aust (1978). The coloured complex of MDA-(TBA) 2 produced by the reaction of MDA and TBA was measured by using Shimadzu UV-1201V spectrophotometry at 532 nm.

Measurement of Antioxidant Vitamins

The vitamin A, C and E levels were assayed by the method of Rutkowski and Grzegorczyk (2007).

STATISTICAL ANALYSIS

Statistical analysis was conducted using Graph Pad Prism version 5.0. Parameters were analyzed statistically by one way analysis of variance (ANOVA) and Tukey Kramer multiple comparison tests was used to establish the significance of the observed difference among various groups. Results were expressed as the Mean ± SD. Differences were considered significant when $P<0.05$.

RESULTS

Increased in body weight were observed in all groups of both models (Table 1and 2). Rats supplemented with 500 mg nutraceutical accumulated more weight, though not significant ($p>0.05$) than those supplemented with 250mg nutraceutical and standard supplement at the end of 28 days.

As shown in Figure 1, Blood lead concentration reduced significantly ($P<0.05$) in all supplemented groups compared to Pb-only (LA) group. Oral supplementation of nutraceutical reduced blood lead concentrations of lead-intoxicated rats in the curative model (cLA) than in the intervention model (LA) and the reduction was dose defendant. However, non-significant ($P>0.05$) change was recorded between groups supplemented with antioxidant-nutraceutical and standard supplement.

Activities of antioxidant enzymes and levels of GSH and MDA

Lead toxicity distorts activity of antioxidant enzymes, in this study, activity of SOD, CAT and GPx decreased significantly ($P<0.05$) in lead-intoxicated rats of both preventive and curative models compared to supplemented and normal groups. (Figure 1and 2). In preventive model, group supplemented with 500mg antioxidant-rich nutraceutical, activity of SOD, CAT and GPx enzymes improved significantly ($P<0.05$) to near that of normal control group. The administration of 500mg antioxidant-rich nutraceutical had boosted the activity of these enzymes significantly ($P<0.05$) compared to other supplemented groups in the curative model (Figure 3). Similarly, supplementation of 250mg antioxidant-rich nutraceutical had improved the activity of these antioxidant enzymes ($P<0.05$) and the improvement was in dose-defendant fashion. Lead exposure was also known to cause alteration in the level of GSH and induce increase MDA concentration. There was significant ($P<0.05$) decrease level of GSH in all lead-intoxicated groups compared to normal group. Oral supplementation of antioxidant-rich nutraceutical and standard supplement showed significant ($P<0.05$) recovery of GSH levels compared to no supplemented (cLA) group in the curative model (Figure 3). The improvement was more in antioxidant-rich nutraceutical than standard supplement. Non-significant ($P>0.05$) change was observed in the preventive model (Figure 2). MDA concentrations had increased significantly ($P<0.05$) in all lead-intoxicated groups compared to normal group. Supplementation of antioxidant-rich nutraceutical and standard supplement reversed the changes significantly ($P<0.05$) compared to lead-only group. However, MDA concentration was not reversed to that of normal control group (Figure 1 and 2).

Level of antioxidant minerals

The level of Fe and Zn decreased significantly ($P<0.05$) in all lead-intoxicated groups except that supplemented with standard supplement compared to control both in preventive and curative models. Significantly ($P<0.05$) high levels of Fe and Zn were recorded in group administered with standard supplement compared to those supplemented with antioxidant-rich nutraceutical (Figure 3 and 4). There was significant ($P<0.05$) increased levels of Cu in lead-intoxicated group compared to control in preventive model, but the level remain unchanged in curative model. The Se levels increased significantly in all lead-intoxicated groups of both models compared to control (Figure 4 and 5). Group administered with standard supplement showed significantly ($P<0.05$) high level of Se compared to those supplemented with antioxidant-rich nutraceutical.

Level of antioxidant Vitamins

Figure 5 and 6 illustrate the levels of antioxidant vitamins. Level of vitamin A and E decreased significantly ($P<0.05$) in all lead-intoxicated groups of both preventive and curative models compared to control, but the vitamin A level improved significantly ($P<0.05$) in group supplemented with 500mg antioxidant-rich nutraceutical of curative model. Vitamin C levels decreased significantly ($P<0.05$) only in lead untreated group of curative model (Figure 6).

Table 1: Weight of Experimental Groups and Control in Preventive Model

GROUP	**LA**	**LA250**	**LA500**	**LAS**	**NCG**
Initial wgt (g)	265.60±5.32	214.20±5.93	244.80±11.47	249.60±11.52	213.80±9.55
Final wgt(g)	270.00±5.15	220.60±5.94	254.40±11.48	255.20±11.86	220.20±9.15
% Difference	0.8%	1.3%	1.9%	1.1%	1.5%

n = 5, NCG: Normal Control Group, LA: Lead Acetate, LA250: Lead Acetate with 250mg antioxidant–rich nutraceutical, LA500: Lead Acetate with 500mg antioxidant–rich nutraceutical and LAS: Lead Acetate with Standard Supplement (centrum)

Table 2: Weight of Experimental Groups and Control in Curative Model

GROUP	**cLA**	**cLA250**	**cLA500**	**cLAS**	**cNCG**
Initial wgt(g)	270.60±2.61	208.60±14.22	287.00±16.59	245.20±3.63	204.20±4.60
Final wgt(g)	280.60±7.16	219.60±9.34	304.80±16.05	252.20±3.68	211.20±4.92
% Difference	1.8%	2.4%	3.0%	1.4%	1.7%

n = 5, NCG: Normal Control Group, LA: Lead Acetate, LA250: Lead Acetate with 250mg antioxidant–rich nutraceutical, LA500: Lead Acetate with 500mg antioxidant–rich nutraceutical and LAS: Lead Acetate with Standard Supplement (centrum)

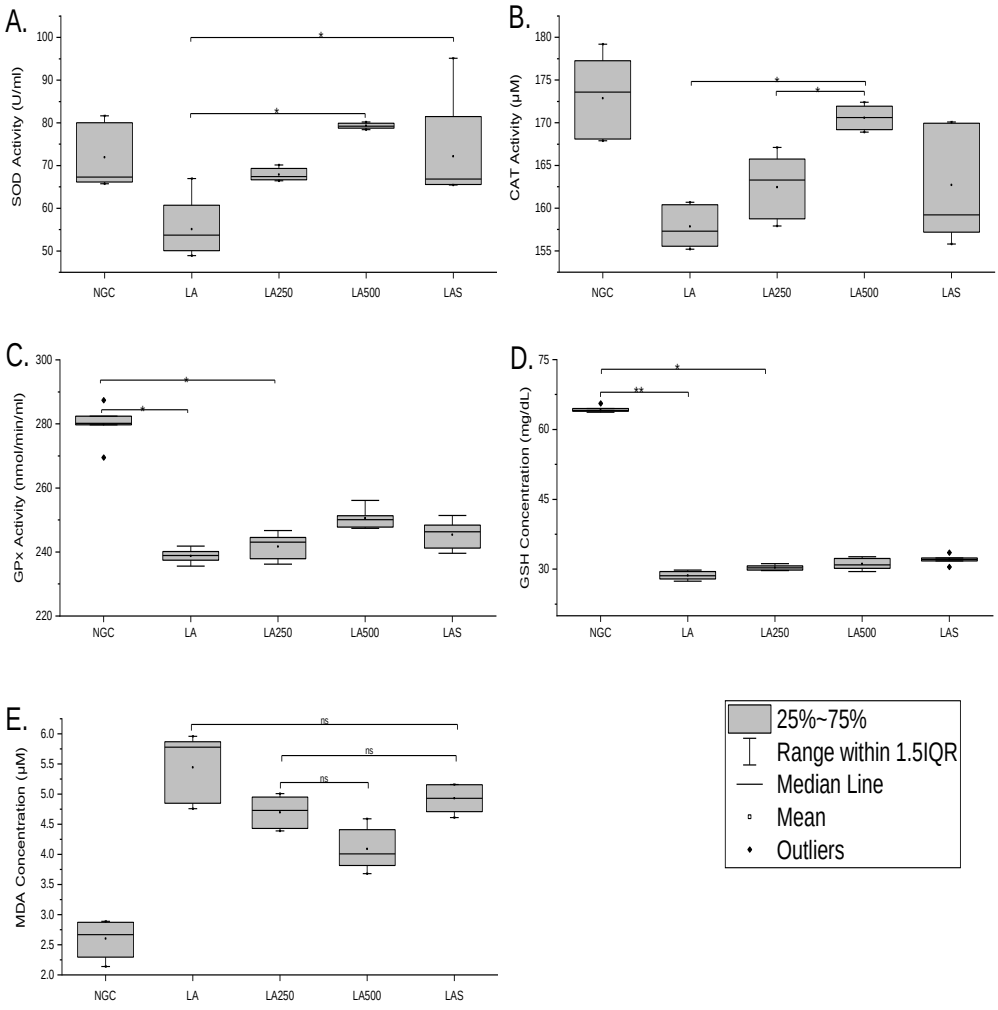

Figure 1: Effect of Antioxidant-Rich Supplement on the Activities of SOD, CAT, GPx and the Concentration of GSH and MDA in Lead-Intoxicated rats (Preventive Model).

Values are mean ± SD A: SOD activity, B: CAT activity, C: GPx activity, D: GSH concentration and E: MDA concentration, NGC: Normal Control Group, LA: Lead Acetate, LA250: Lead Acetate with 250mg antioxidant–rich nutraceutical, LA500: Lead Acetate with 500mg antioxidant–rich nutraceutical and LAS: Lead Acetate with Standard Supplement (centrum)

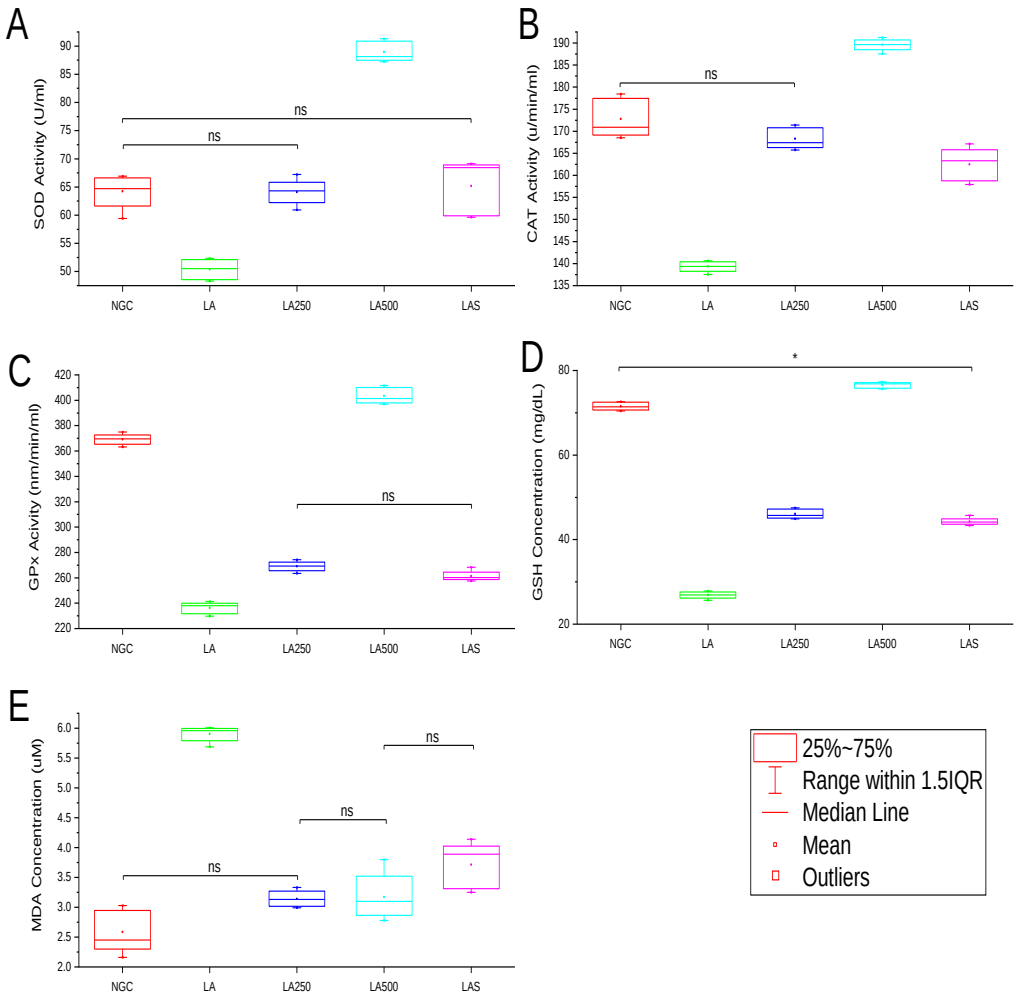

Figure 2: Effect of Antioxidant-Rich Supplement on the Activities of SOD, CAT, GPx and the Concentration of GSH and MDA in Lead-Intoxicated Rats (Curative Model).

Value are mean ± SD A: SOD activity, B: CAT activity, C: GPx activity, D: GSH concentration and E: MDA concentration, NGC: Normal Control Group, LA: Lead Acetate, LA250: Lead Acetate with 250mg antioxidant–rich nutraceutical, LA500: Lead Acetate with 500mg antioxidant–rich nutraceutical and LAS: Lead Acetate with Standard Supplement (centrum)

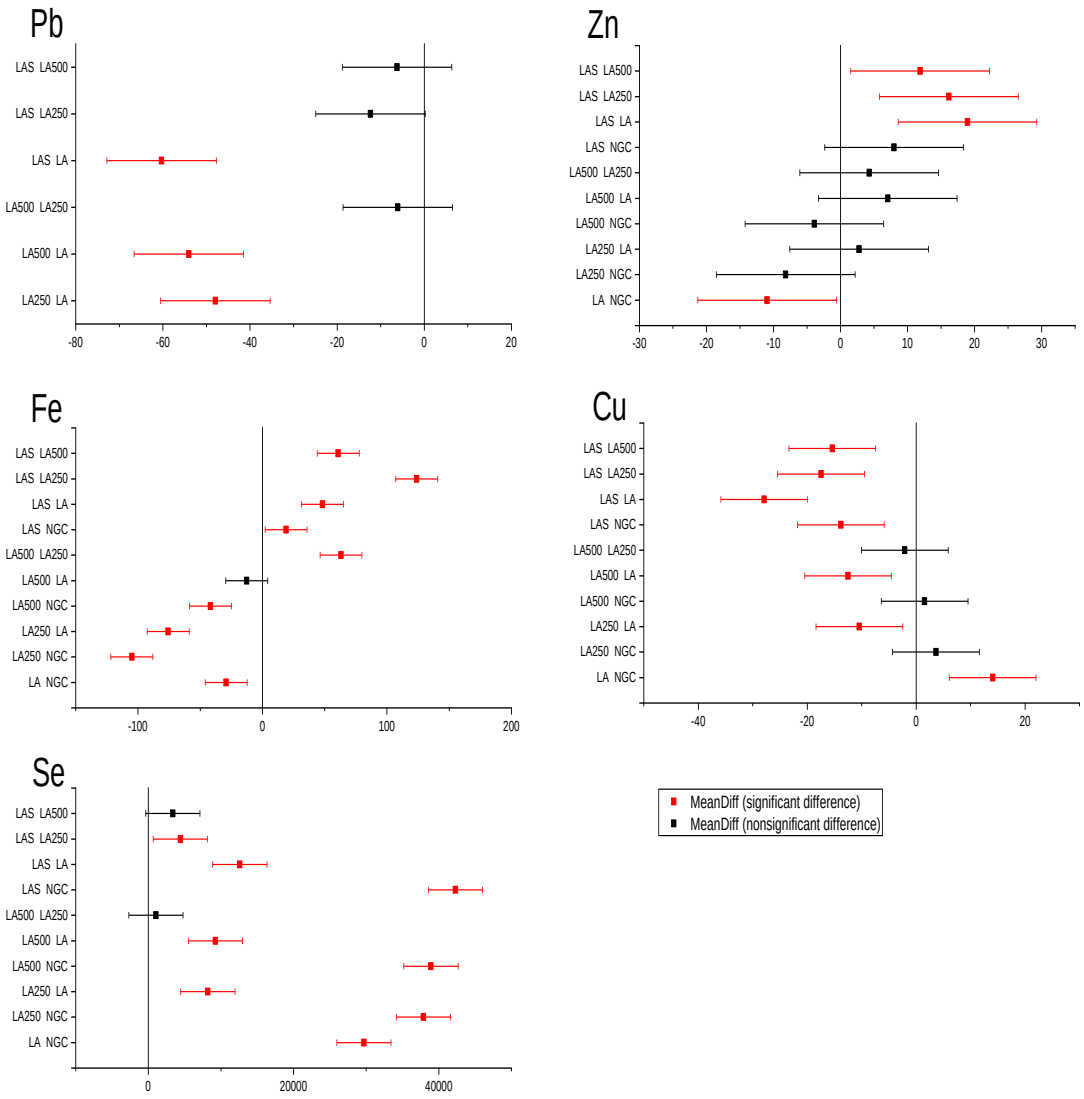

Figure 3: Effect of Antioxidant-Rich Supplement on the Serum Concentrations of Pb, Zn, Fe, Cu and Se in Lead-Intoxicated Rats (Preventive Model). NGC:

Normal Control Group, LA: Lead Acetate, LA250: Lead Acetate with 250mg antioxidant–rich nutraceutical, LA500: Lead Acetate with 500mg antioxidant–rich nutraceutical and LAS: Lead Acetate with Standard Supplement (centrum)

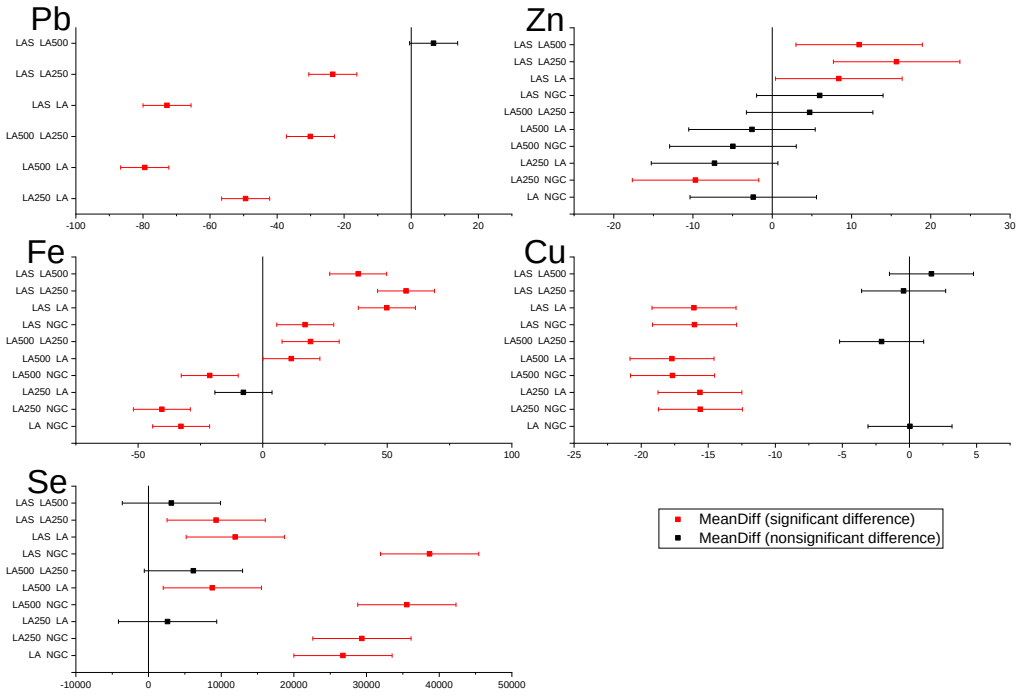

Figure 4: Effect of Antioxidant-rich supplement on the serum concentrations of Pb, Zn, Fe, Cu and Se in lead-intoxicated rats (Curative model).

NGC: Normal Control Group, LA: Lead Acetate, LA250: Lead Acetate with 250mg antioxidant–rich nutraceutical, LA500: Lead Acetate with 500mg antioxidant–rich nutraceutical and LAS: Lead Acetate with Standard Supplement (centrum)

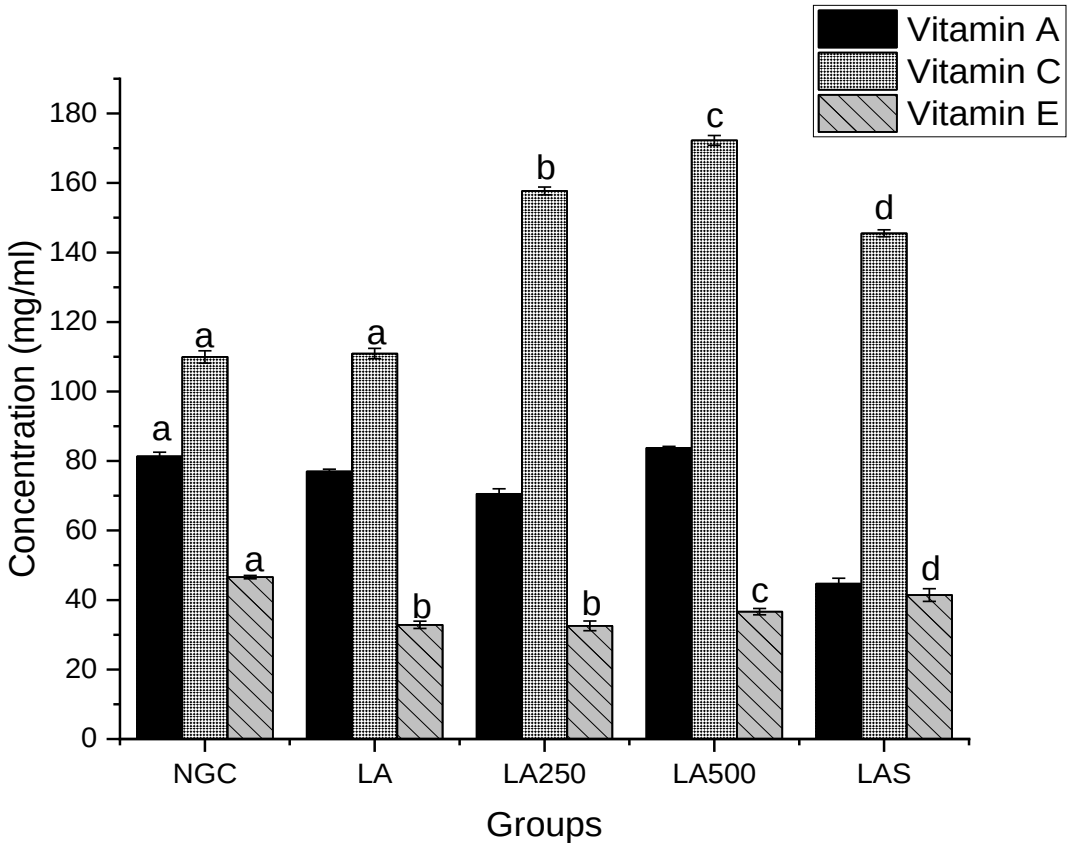

Figure 5: Effect of Antioxidant-Rich Supplement on the Serum Concentrations of Vitamin A C and E in Lead-Intoxicated Rats (Preventive Model).

Values are mean ± SD NGC: Normal Control Group, LA: Lead Acetate, LA250: Lead Acetate with 250mg antioxidant–rich nutraceutical, LA500: Lead Acetate with 500mg antioxidant–rich nutraceutical and LAS: Lead Acetate with Standard Supplement (centrum)

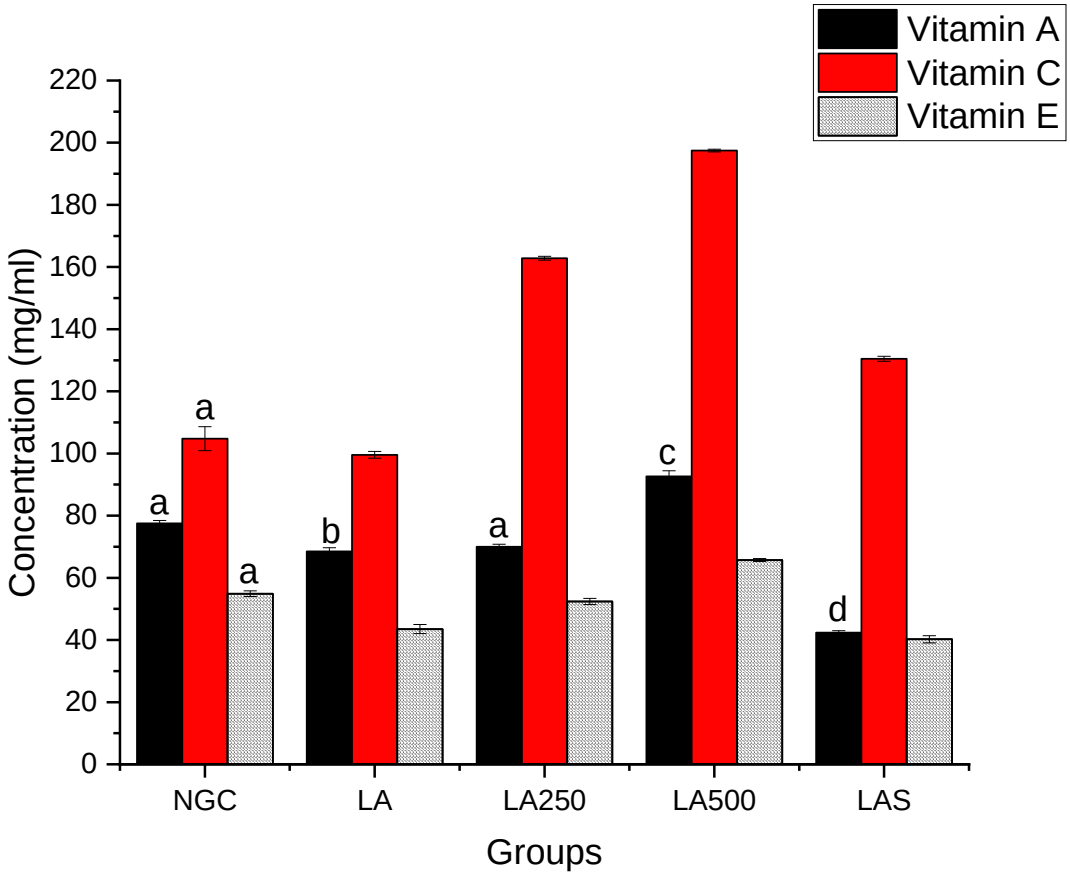

Figure 6: Effect of Antioxidant-Rich Supplement on the Serum Concentrations of vitamin A C and E in Lead-Intoxicated Rats (Curative Model).

Values are mean ± SD, NGC: Normal Control Group, LA: Lead Acetate, LA250: Lead Acetate with 250mg antioxidant–rich nutraceutical, LA500: Lead Acetate with 500mg antioxidant–rich nutraceutical and LAS: Lead Acetate with Standard Supplement (centrum)

DISCUSSION

Increased industrial activities, modern agricultural practices and human lifestyle impacted largely on the body burden of Pb in the general population. In the last decade, consumption of Pb has continued to increase and serious Pb pollution inflicts many devastating health consequences in developing countries (Zhai *et al.*, 2015).

Lead induces oxidative stress via generation of reactive oxygen species, depletion of available endogenous antioxidant reserves, interfering with essential metals and vitamins there by increasing susceptibility of cells to oxidative attack by altering membrane integrity and fatty acids composition (*Chen et al.*, 2015).

In our previous investigation on individuals environmentally exposed to lead showed that Pb induced oxidative stress there by causing decreased activities of antioxidant enzymes, GSH levels and increased lipid peroxidation, as well as alteration of antioxidant minerals and

vitamins. The results obtained in these animal models show that lead-intoxicated rats had decreased activities of SOD, CAT and GPx and GSH levels in both preventive and curative models (Figure 1 and 2) accompanied by increased lipid peroxidation.

The use of chelation therapy in treatment of lead poisoning is considered less effective due its multiple limitations and side effects. It causes depletion of essential minerals, produce toxic potentials themselves and metabolic disorder of antioxidants. This has led to presumption that supplementation of diet- rich antioxidant could be an alternative regimen for chelation therapy. The use of essential metals, vitamins, edible plants and dietary phytochemicals, probiotics and other dietary supplements in the treatment of lead toxicity are now appearing in the literature (Chen *et al.,* 2015; Dkhil *et al.,* 2016; Kordas, 2017; Oboma *et al.,* 2018).

In the present study, tomatoes, onions, garlic, ginger, lemon, melon seed and palm oil were selected and incorporated into formulation of antioxidant-rich nutraceutical supplement. The food ingredients used for the formulation are good sources of vitamins and essential minerals. Therefore, administration of this supplement can boost the level of vitamins and minerals in the body, in turns, may decrease the risk of Pb toxicity. Supplementation of antioxidant-rich nutraceutical may significantly reduce blood lead levels in rats (Figure 2 and 4). The protection observed in the groups supplemented with 250mg/kg and 500mg/kg antioxidant-rich nutraceutical could be due to Pb-chelating properties of some constituents present in the supplement. Studies have shown that garlic has protective property against lead toxicity by reducing lead intestinal absorption. The protection may be attributed to its sulfur-containing amino acids such as S-allyl cysteine and S-allyl mercaptocystein (Sharma *et al.,* 2010). Oral administration of probiotics and grape seed extract can protect intestinal barrier and hence, inhibit lead absorption in the gut (Goodrich *et al*., 2012; Zhai *et al*., 2016). Dietary supplement containing probiotics, micronutrients and plant extracts has also been reported to significantly reduced Pb levels in the tissue and blood of mice (Zhai *et al*. 2018).

Lead binds to antioxidant enzymes and other molecules that have functional sulhydryl groups thereby making them inactive and consequence increase ROS production. Decreased activity of antioxidant enzymes are often implicated in oxidative stress. The present study indicated decreased activity of SOD, CAT and CAT of lead-intoxicated rats in preventive and curative models (Figure 1 and 2). The decrease activity of these enzymes may be due to increase ROS production and accumulation. Haleagrahara *et al* (2010) reported marked decreased in SOD, CAT and GPx level in lead acetate–intoxicated rats. Similarly, decreased activity of these enzymes was reported in Wister rats after exposure to lead (Anjum et al., 2017).

Supplementation of antioxidant-rich nutraceutical significantly increases the activity SOD, CAT and GPx in dose defendant fashion. The supplementation of antioxidant-rich nutraceutical show greater impact in retrieving their defensive activity especially in the curative model. Oral administration of 500mg/kg of antioxidant-rich nutraceutical might have better effect of improving antioxidant status; reducing oxidative stress caused lead exposure and providing certain treatment to the body. Jawhar *et al.* (2017) investigated the effect of *Berveris vulgaris* on oxidative stress and liver injury in lead-intoxicated mice. Berberis vulgaris extract was shown to effectively normalize the activity of antioxidant enzymes (SOD, CAT and GPx) and GSH level.

In the present study, decreased GSH level and concurrent increased MDA concentration were observed in lead-intoxicated rats. The reduction of GSH level may consequently facilitate increase lipid peroxidation. Moreover, Pb inhibits GSH synthesis from cysteine via γ – glutamyl cycle, which further depresses the GSH content (Flora *et al.*, 2012).

The supplementation of 500mg/kg antioxidant-rich nutraceutical in the curative model brought significant increased GSH level (Figure 2). The increase in the GSH level should definitely improve the antioxidant defense system and integrity of the cell. The antioxidant-rich supplement may facilitate either in maintaining the steady state of GSH level or its rate of biosynthesis thus reduces oxidative stress.

Elevation of MDA concentration has been considered the major effect of ROS which may affect physicochemical properties, fluidity and integrity of the cell membrane and with consequent alteration of calcium and sulfhydryl homeostasis and distortion of antioxidant defense system. Supplementation with antioxidant-rich nutraceutical effectively decreased oxidative stress induced by lead in preventive and curative models which resulted in significant decrease of MDA concentration. Decreased MDA level indicates a remarkable protection against oxidative onslaught induced by lead exposure. Ahmed *et al*. (2000) reported that extract of ginger effectively reduced ROS formation in rats.

Significant ($P<0.05$) decreased of Fe and Zn concentrations were observed in lead-intoxicated rats (Figure 3 and 4). In contrast, the study noted increased concentration of Cu and Se in the lead-intoxicated rats. Supplementation of antioxidant-rich supplement had improved the concentration of Fe and Zn in dose dependent manner. It is well documented that Pb competes with essential metals like Fe, Zn, Cu, Ca, Se and Mg. Perhaps, some play important role in antioxidant enzymes functionality. Interaction of these essential minerals with lead may distorts several important biochemical processes including antioxidant defense system. Essential metals have been reported to facilitate lead's absorption, distribution, deposition, post exposure and excretion processes (Reddy *et al*., 2014). Calcium and iron deficient diet results increased susceptibility to Pb toxicity via its increased intestinal absorption. However, supplementation of Zn and Fe has been reported to reduce intestinal absorption of Pb (Reddy *et al*., 2011). Tariq *et al*. (2018) reported synergistic effect of calcium and zinc supplementation against oxidative stress in rats. The restoration of Fe, Zn, Cu and Se levels observed by supplementation of antioxidant-rich nutraceutical in this study may be attributed to the food ingredients used in the formulation of the supplement as they are good sources of minerals.

Significant alterations of antioxidant vitamins concentration in serum of rats intoxicated with lead were observed (Figure 5 and 6).Supplementation of antioxidant-rich nutraceutical had remarkably improved the concentration of vitamin C in preventive and curative models. This may be connected with the fact that the antioxidant-rich supplement is a good source of vitamin C. Evidence has shown that diet containing vitamins A, B, C, E and antioxidant were capable of protecting sperm DNA from attack of ROS (Jedlinska-krakowska *et al*., 2006). Vitamin C as a potent scavenger of ROS has also been reported to reduce lipid peroxidation and facilitated in the regeneration of α- tocopherol in liver and brain tissue of lead exposed animals (Patra *et al*., 2001; Khamroubi *et al*., 2008).

CONCLUSION

The present study concluded that Pb induced oxidative stress by progressive generation of ROS that leads to increased lipid peroxidation with consequent reduction in activity of antioxidant enzymes, concentration of GSH content, antioxidant minerals and vitamins. Supplementation of natural and synthetic compounds capable of offering protection and /or alleviation of lead-induced oxidative damage has been advocated in growing literature. Antioxidant-rich

supplement indicated protective as well as curative roles against devastative effects of lead exposure.

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