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EFFECT OF ANTIOXIDANTS (VIT C & E) ON THE WEIGHT AND IMMUNOGLOBULINS OF MALE WISTAR RATS EXPOSED TO LEAD ACETATE

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ABSTRACT
The aim of the present study was to investigate the impact of the selective administration of Vitamin C and Vitamin E on lead immune-toxicity. Male albino rats were subdivided into six groups: the first is the control, the second group received lead acetate in bolus as 10 mg/kg diet daily, the third group receive vitamin C only (200 mg/kg), fourth group receive vitamin E only (1000iu/kg), fifty group received the same lead acetate dose and supplemented with Vitamin C, and the sixth group received the same lead acetate dose and supplemented with Vitamin E orally daily. Blood samples were taken after four weeks of treatment. Significant lead-induced immune-toxicity disruptions in serum Immunoglobulins (IgG, IgM, and IgA) and whole white blood cells, differential white blood cells, Platelets and total leucocyte count activities were observed after end of treatment. However, serum immunoglobulins were initially increased by the Lead assault but upon treatment with the anti-oxidants, decreased towards normal. But there was decreased by the Lead assault but upon treatment with the anti-oxidants, increased towards normal on the differential white blood cells and platelets over time. Lead acetate decreased the cellular integrity and homeostasis of the Liver, Spleen and Thymus tissues. After four weeks of lead administration dilation and congestion of terminal hepatic veins and portal vein branches were observed. Lead also induced hepatocyte proliferation without any localized distribution among zones 1-3. Portal inflammatory infiltrate with disruption of the limiting plates (interface hepatitis), splenotoxicity and thymus fibrosis were detected especially by fourth week of lead administration. Combined treatment of lead-exposed animals with Vitamin C and E showed marked improvement of the biochemical, molecular and histopathological findings. These experimental results strongly indicate the protective effect of Vitamin C and E against toxic effects of lead on liver, spleen and thymus tissues.

KEYWORDS: However, serum immunoglobulins were initially increased by the Lead assault but upon treatment with the anti-oxidants, decreased towards normal.

INTRODUCTION
Lead is a toxic metal pollutant persisting in nature as oxides or salts (Moneim, 2016). Lead poses as an environmental and occupational hazard, that adversely affecting multiple systems such as the hematopoietic, hepatic, nervous, and renal systems in both humans and animals. Lead toxicity occurs through oxidative damage, and chelating agents conventionally used for treatment exert detrimental effects and are incapable of alleviating some toxic outcomes of lead (Shatha et al., 2016). The environmental lead, accesses the body through inhalation of airborne contaminated dust or ingestion of food and water into the digestive tract. Once absorbed, the lead diffuses swiftly through the bloodstream to diverse systems and organs including the liver, kidneys, brain and to well calcify tissues that includes bones and teeth (Alya et al., 2015). The lead induced oxidative damage has been proposed as one of the important mechanisms of lead related pathologies (Flora et al., 2012). Additionally, chelating agents exert detrimental effects and are incapable of alleviating some toxic effects of lead (Ajayi et al., 2009). Antioxidants are believed to be playing very important role in the body defence system against ROS (Boxin et al., 2002), (Vivek and Surendra, 2006). The pollutants or toxins that successfully enter an organism encounter the cells and mechanisms of the innate immune system. The innate response is usually triggered when the microbes are identified by pattern recognition receptors, which recognize components that are conserved among broad groups of microorganisms (Medzhitov, 2007).

Recent researches using antioxidants in the prevention and treatment of lead poisoning have yielded promising results in many body systems (Xhyrel et al., 2016). But in the area of immune system, the reports on the use of antioxidants have been very scanty.

Hence, the reason for the present effort to validate the use of antioxidants (Vitamin C and E) in the prevention and treatment of lead induced immune-toxicity in experimental animal model.

MATERIAL AND METHODS

Forty eight male Wistar rats with weight between 180 and 200 g were obtained from the Experimental Animal Farm at the University of Port Harcourt, Nigeria. The Wistar rats were housed in animal wooden cages in a well-ventilated experimental room. The rats were allowed to acclimatize for a period of two weeks before the commencement of treatments. The rats had free access to standard rat chow and clean water ad libitum. Handling of animals was in accordance with relevant institutional and ethical guidelines as approved for scientific study.

The rats were randomly sorted into six groups and appropriate treatments were commenced subsequently.

Experimental design

Grouping	Dosage / Administration
Group 1 Control	Saline + normal meal
Group 2 Lead only	10mg/kg body weight of lead acetate/rat.
Group 3 Vit C only	200mg/kg body weight of vitamin C/rat.
Group 4 Vit E only	1000iu/kg body weight of vitamin E/rat.
Group 5 Lead + Vit C	10mg/kg body weight of lead acetate + 200mg/kg body weight of vitamin C/rat.
Group 6 Lead + Vit E	10mg/kg body weight of lead acetate + 1000iu/kg body weight of vitamin E/rat.

Administration

Lead acetate

The lead acetate was obtained from sigma Chemical Company, Egypt. A total 48 (2-3 month old) male albino rats of body weight raging from (180-200g) (Albino Wistar rat strain) were obtained from the animal house University of Port Harcourt. These animals were housed in the laboratory Animal centre of faculty of basic medical sciences. The animals were divided into six groups (eight rats per group) and kept under the normal healthy laboratory conditions and acclimatized for two weeks. The first group represent the health control animals, while the second, third, fourth, fifth and sixth groups were ingested orally with sub lethal doses of lead acetate orally. Food and distilled water were supplied ad libitum for all groups during the period of the experiment.

Experimental induction of lead

10mg/kg body weight of lead acetate per rat was administered orally daily for four weeks.

Vitamin c administration

Lead acetate at dose level of 10mg/kg followed by 200mg/kg body weight of vitamin C was administered orally daily for four weeks. (Sheweita et al., 2001).

Vitamin e administration

Vitamin E was produced by Yasoo Health Inc., TN, USA. Lead acetate at dose level of 10mg/kg[1] followed 1000iu/kg body weight of water soluble vitamin E was administered orally daily for four weeks (Sheweita et al., 2001).

Haematological analysis

Whole blood was obtained from a puncture of the retroorbital sinus by the conventional method (Van Herck et al., 1992). Blood samples collected in ethylene diamine tetra-acetic acid (EDTA) anticoagulant tubes (8.5%) was quickly returned by mixing with anticoagulant in the tube. All blood samples were labeled and immediately conveyed to the laboratory for analysis. Hematological parameters were analyzed: white blood cell count (WBC), platelet count (PLT) and the number of lymphocytes (LYM). All hematological parameters were analyzed using the automated method with the automatic analyzer "Haematology auto analyzer Sysmex KX-21N".

Immunoglobulin analysis

Biochemical methods : at the end of experiment, rat were sacrificed, blood sample was collected and centrifugated at 3000 rpm for 10 minutes in room temperature; the serum was separated and kept in clean stopper glass vial at -20 °C unit assay. Serum was subjected to the following parameters; IgG, IgA and IgM (Narayanan, 1982).

Histological techniques (Liver, Spleen & Thymus)

The standard tissue processing techniques which include dehydration, clearing, impregnation, embedding, sectioning and staining were employed. The formalin-fixed tissues were stored at 4 ◦C until examination. Tissues were processed using standard histology laboratory techniques. They were first fixed in formalin, then embedded in paraffin wax and cut into 3–4 µm sections for hematoxylin and eosin (H&E) staining.

Statistical analysis

The version 23.0 of the statistical package for social sciences (SPSS) and Microsoft Excel 2013 for the statistical analysis were employed for analyzing the various sets of data except otherwise.

The Mean, standard errors of mean and ranges were determined for the different parameters for the respective groups of rat. The analysis of variance (ANOVA) was used to determine the variations amongst the animals. The P-value of less than 0.05 was regarded as significant.

RESULTS

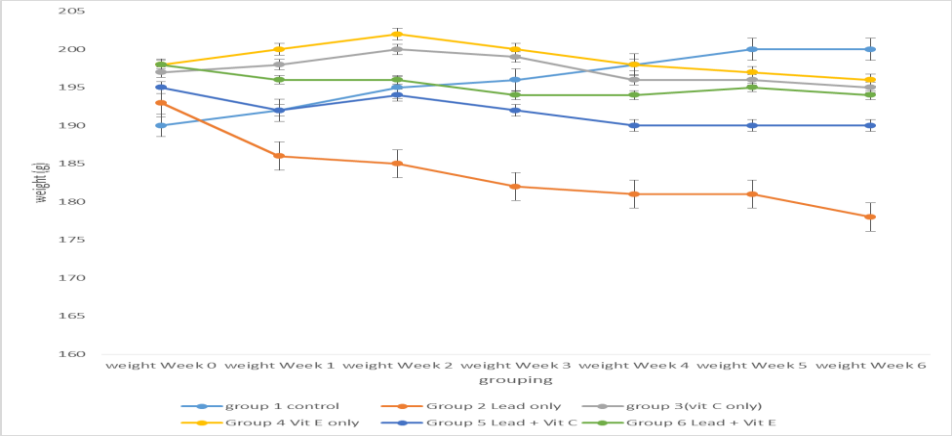


Figure 1: Variations in the weights of rats in various groups for a period of 6 weeks.

Table 1: Immunoglobulins (IgG, IgM and IgA) parameters in various groups.

Grouping	Immunoglobulin G	Immunoglobulin M	Immunoglobulin A
Group 1 (Control)	247.0 ± 1.23	98.4 ± 1.47	121.2 ± 2.94
Group 2 (Lead only)	768.8 ± 57.8 [a]	154.0 ± 24.4 [a]	205.6 ± 32.8 [a]
Group 3 (Vit C Only)	277.7 ± 25.3 [a]	96.7± 7.68	126.0± 5.89[a]
Group 4 (Vit E Only)	261.3± 31.43 [a]	102.3± 27.21[a]	123.7± 6.46
Group 5 (Lead + Vit C)	719.2 ± 75.9 [b]	97.2 ± 13.3 [b]	315.4 ± 108.2 [b]
Group 6 (Lead + Vit E)	691.6 ± 72.9 [b]	172.4 ± 59.9 [b]	228.6 ± 74.2 [b]

Values are presented as mean ± sem. n=5, [a, b] are Mean significant differences relative to the control and lead groups respectively, at $p < 0.05$.

Table 2: Total white blood cell count, Platelets count and Total Lymphocyte Count Parameters in various Groups.

Grouping	White Blood Cell Count (X 10^8µ/L ± sem)	Platelets Count (µg/L ± sem)	Total Leucocyte Count (µg/L ± sem)
Group 1 (Control)	6.58 ± 0.29	257.6 ± 54.87	431.4 ± 2.69
Group 2 (Lead only)	4.66 ± 0.91 [a]	352.0 ± 72.41 [a]	312.5 ± 61.25 [a]
Group 3 (Vit C Only)	9.33 ± 0.34 [a]	264.0 ± 34.21[a]	695.6 ± 50.18 [a]
Group 4 Vit E Only	11.2 ± 5.13 [a]	266.67 ± 23.39[a]	865.87 ± 65.67 [a]
Group 5 (Lead + Vit C)	5.60 ± 0.52	324.8 ± 91.3 [b]	354.8 ± 20.5 [b]
Group 6 (Lead + Vit E)	5.22 ± 0.35	206.4 ± 16.6 [b]	369.2 ± 47.4 [b]

Values are presented as mean ± sem. n=5, [a, b] are Mean significant differences relative to the control and lead groups respectively, at $p < 0.05$.

Table 3: Differential White blood cell count parameters in various groups

Grouping	Neutrophil Count (% ± sem)	Lymphocytes Count (% ± sem)	Monocyte Count (% ± sem)	Eosinophil Count (% ± sem)
Group 1 (Control)	27.6 ± 0.98	64.0 ± 2.45	8.0 ± 0.0	2.0 ± 0.0
Group 2 (Lead only)	24.2 ± 1.53[a]	67.4 ± 2.06[a]	5.4 ± 1.17[a]	4.0 ± 0.84 [a]
Group 3 (Vit C Only)	32.33 ± 3.24 [a]	74.0 ± 5.34 [a]	9.33 ± 2.16	1.33 ± 1.21
Group 4 Vit E Only	30.67 ± 4.38 [a]	79.2 ± 4.39 [a]	10.67 ± 1.13	4.67 ± 1.67
Group 5 (Lead + Vit C)	26.6 ± 2.18	70.6 ± 3.54 [b]	6.1 ± 1.12	3.2 ± 0.74
Group 6 (Lead + Vit E)	25.6 ± 3.47	70.0 ± 4.93 [b]	6.8 ± 0.86	3.8 ± 1.63

Values are presented as mean ± sem. n=5, [a, b] are Mean significant differences relative to the control and lead groups respectively, at $p < 0.05$.

For Photomicrographs of the Spleen, Liver and Thymus of the Test and Control Groups in Response to Lead Toxicity and Vitamin E and C Treatment (see plate sheets).

DISCUSSION

The aim of the present study was to investigate the effects of antioxidants (Vitamins C and E) on lead induced immune-toxicity. Toxicity by heavy metals could have delirious and detrimental effects on both human and animals. In this study, there was significant

mean body weight reduction in the lead acetate group when compared with the mean value of the control group. This harmful effect of lead on the body weight gradually increases with the increase in lead acetate doses. This findings agreed with many studies such as Haouas et al. (2014), who observed significant reduction of body weight as effects of Lead ingestion in rats, Pentenusci, (1988); Hamilton, (1994) and Djebli et al. (2004), who reported that the reduced growth was due to reduced food consumption through the inhibition of the appetite centre.

There was skewed in the level of immunoglobulins (IgG, IgM and IgA) parameters of lead treated group (group 2) when compared with the control as showed in table; 1. This might be because the antibodies, recognize the lead as antigens, thereby enhancing its activities to fight against the lead toxicity by covering the toxic antigenic sites so it can neutralize the toxic effects on the rats. These results suggest that oxidative stress contributes to serum immunoglobulins levels during lead intoxication in vivo. This findings agreed with Patrick, (2006) who stated that lead induced oxidative damage is one important mechanisms of lead related pathologies (Patrick, 2006).

There was significant decrease in the white blood cell (WBC) count and total leucocyte count (TLC) in lead treated group (group 2) when compared with the control as showed in table 2; WBC play important role in body defense system, The breakdown in white blood cell counts and total leucocyte count might be due to oxidative stress. According to studies by (Stohs and Bagchi, 1995; Mateo et al., 2003), who stated that lead has a potential to induce oxidative stress and acts as a catalyst in the oxidative reactions of biological macromolecules. Also observed in the study was a significant decrease in the Neutrophil and Monocytes counts in group 2 when compared with control. The direct effect of lead acetate on them might have decrease their count.

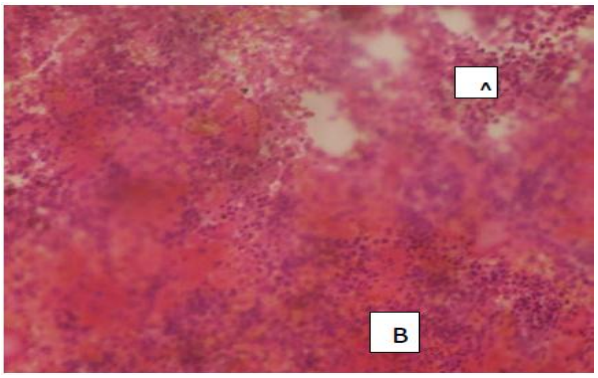
This finding validates previous studies of lead-induced immunosuppression which demonstrated significant decreased in natural killer (NK) cytotoxicity (Neilan et al., 1983 and Talcott et al., 1985) and macrophage motility and migration (Blakley and Archer 1981 and Kiremidjian-Schumacher et al., 1981). In vitro studies also suggest that lead causes oxidative stress in red blood cells (Ribarov et al., 1981; Ribarov and Bochev, 1982 and Quinlan et al., 1988). This finding is supported by studies that showed the oxidative damaged in red blood cells (RBCs) from lead-exposed workers and Fisher 344 rats (Ito et al., 1985; Monterio et al., 1985; Sugawara et al., 1991 and Gurer et al., 1998). Photo micrographic assessment of spleen histology under study revealed normal structures in the control group but there was alteration in group treated with lead only. The alteration include Hypo- cellular white pulp, enlargement of venous sinusoids, clustering of heterochromatin in nucleus, vacuolation in the cytoplasm, swelling of mitochondria, and the distortion of rough endoplasmic reticulum cisterns were observed upon microscopic examination of the spleen tissue cells. These cytopathology alterations indicate that lead acetate has some drastic toxicological effects on immune organ.

There was significant weight lost due to inhibition of appetites caused by lead acetate toxicity on the male Wistar rats, but this was ameliorated through the introduction of antioxidants and there was weight gain in groups treated with lead and vitamins C and E. This might be because the antioxidants (Vitamins C and E) were able to activate the appetite centre, thereby enhancing the feeding rate of the rats which may have contributed to their abilities to gaining weight.

In this study lead induced immune toxicity might have caused significant increase in immunoglobulins (IgG, IgM and IgA) level but the antioxidants (Vitamins C and E) were able to ameliorate their effects on group threated with lead and Vitamins C and E. This showed antioxidants potential in detoxifying and reducing drug cravings. In the present study, it was observed that the three immune proteins were favored though at different level of response by the administration of the anti-oxidants over a period of four weeks. These results suggest that oxidative stress contributes to serum IgG levels during lead intoxication in vivo, and that intervention with either antioxidants such as (Vitamins C and E) might have alleviate this lead-induced damaged. This finding validated the report of Mark Perciva (1998), who reported that antioxidants are our first line of defence against free radical damage, and are critical for maintaining optimum health and wellbeing (Mark Perciva 1998).

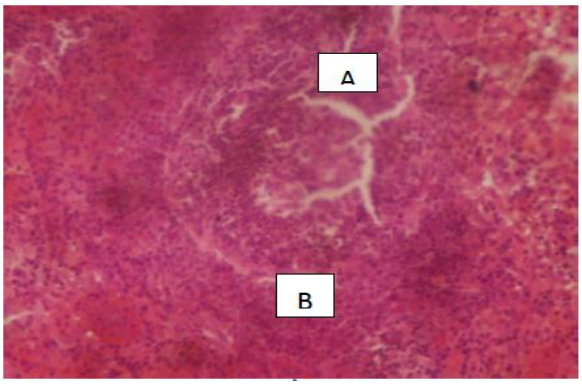
There was decrease in white blood cell count, total leucocyte count, % neutrophil and % monocyte count, this pathological condition was caused by lead induced immune toxicity. But this was ameliorated by the introduction of antioxidants in groups 5 and 6 and proved the boosting potentials of antioxidants (Vitamins C and E). Also this study results showed increased in platelet count, % lymphocyte count and % eosinophil count these physiological and pathological conditions were due to lead immune toxicity poisoning, but this was also ameliorated through the introduction of antioxidants (Vitamins C and E). This showed the potent of antioxidants in converting the radicals during the oxidative stress to less reactive species. Selected tissues such as the spleen, Liver and thymus were histologically examined to ascertain the potential tissue damage that could result on exposure to Lead acetate. It was observed that the liver sinusoids were encroached which could set a pace for hepatocyte breakdown on the long run. The portal apparatus of the liver was equally affected by the toxic effects as seen in the photomicrographs but this scenario seemed to be arrested by the introduction of Vitamins C and E though the potency of Vitamin C was

more significant on the liver tissue when compared with the status of vitamin E. Hepatic response to metal toxicity in this case was more pronounced due to continuous feeding with the Lead treatment and it was just normal that hepatic damage would result. The restoration of new and emerging hepatocytes proved that these antioxidants could arrest liver damage by stimulating new cells to replace the dead ones as observed in the study. Liver cells are saddled with the responsibility of producing these immune proteins and their levels can be affected adversely in toxic scenarios. The spleen and thymus was also distorted being an immune tissue and heavy metals have history of halting their functionality as observed in the photomicrograph but they were ameliorated by the introduction of antioxidants.



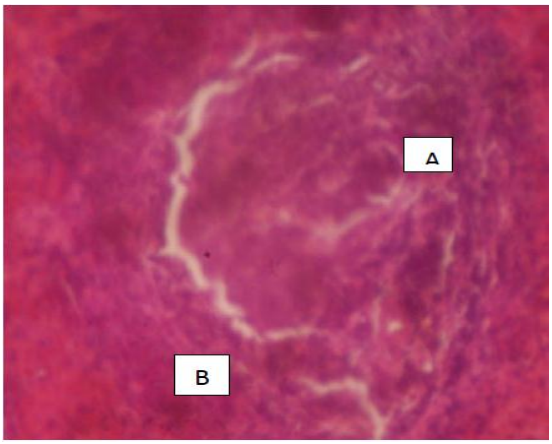
A= Lymphocytes, both in the white and red pulp of the spleen, exhibited normal structures
B=Normal nucleus, organelles, and mitochondria with typical inner membrane

Plate 1: Histology of Spleen in control group 1 (X 400).



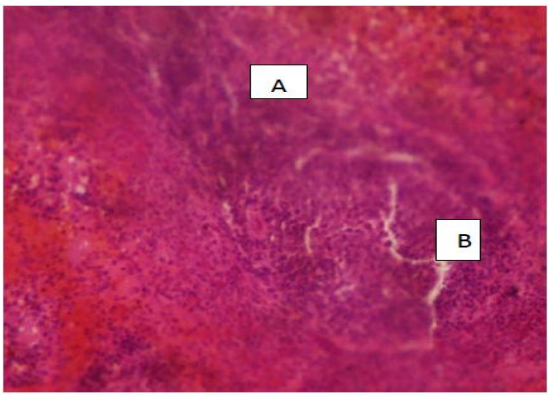
A=Multiple microvacuolations
B=Macrophage and lymphocyte infiltration

Plate 2: Histology of Spleen in test group 2 (Lead only, X400).



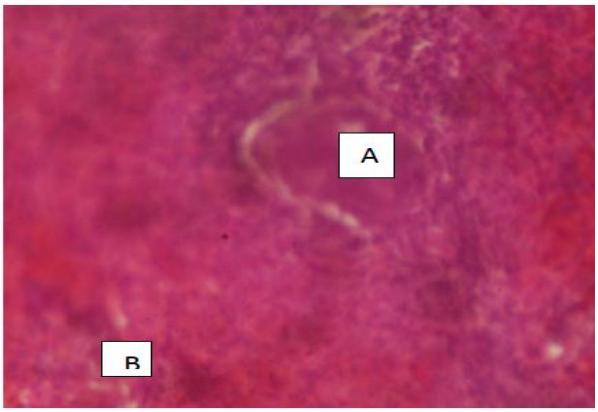
A=Rich, condensed cellular matrix devoid of debris
B=Clear hepatotic kupfer cells at low active state

Plate 3: Histology of Spleen in group 3 (Vit C only, X400).



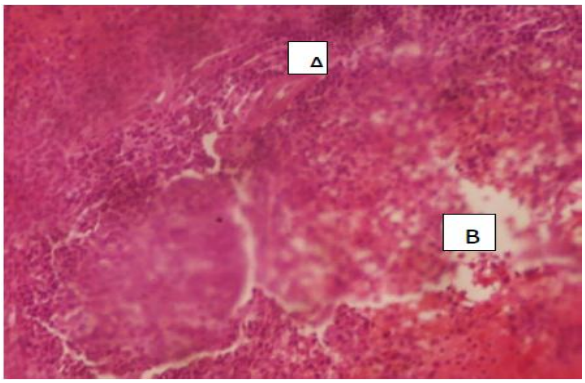
A=Well preserved cellular matrix
B=Portal triad showing no congestion or debris

Plate 4: Histology of Spleen in group 4 (Vit E only, X400).

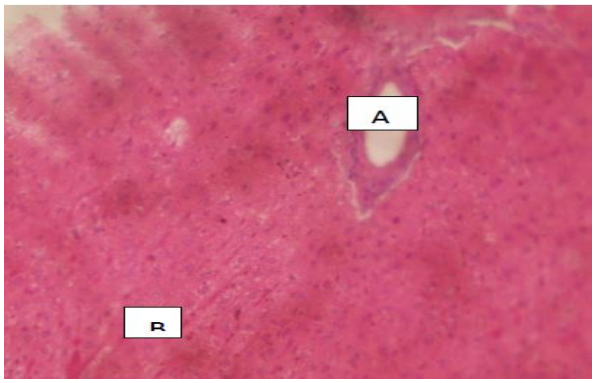


A=Macrovasculation and encroached space undergoing restoration
B=Emerging healthy sinusoids in the matrix

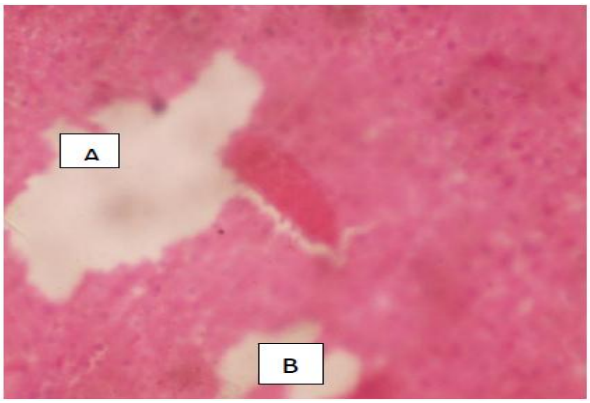
Plate 5: Histology of Spleen in group 5 (Lead + Vit C X400).



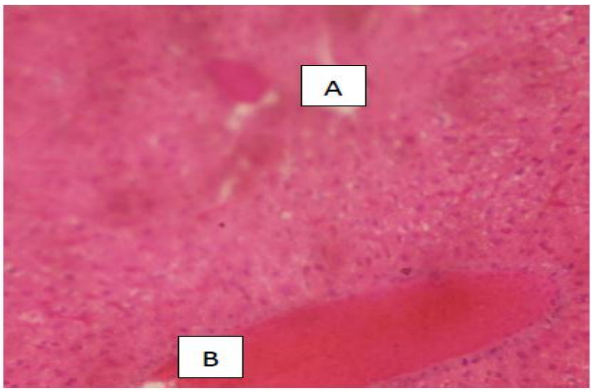
A=Healthy matrix upon cellular assault
B=Hypocellular & Necrotic patches and re-alignment
Plate 6: Histology of Spleen in group 6 (Lead + Vit E, X400).



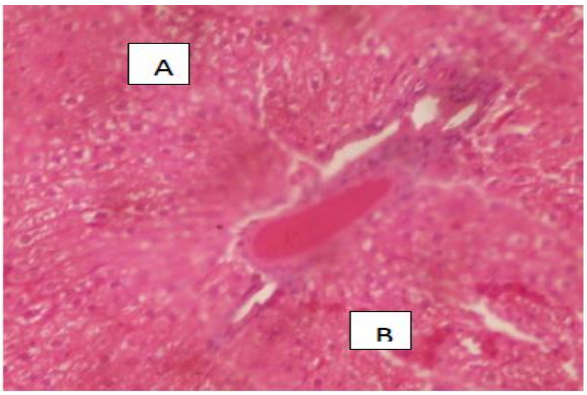
A=Patches of encroached interlobular segment
B=Clear matrix with healthy sinusoidal cells
Plate 7: Histology of Liver in control group 1 (X400)



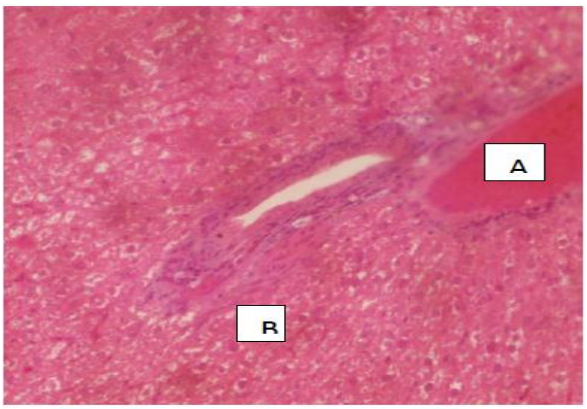
A=Portal inflammatory infiltrate
B=Dilation and congestion of terminal hepatic veins
Plate 8: Histology of Liver in group 2 (Lead only, X400).



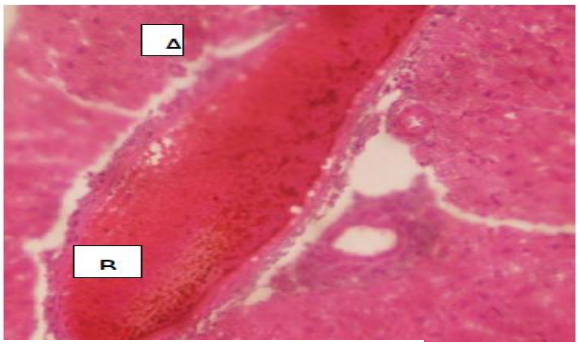
A=Interlobular area with healthy hepatocyte
B=No derangement of Nissil particles
Plate 9: Histology of the Liver in group 3 (Vit C) (X400).



A=Healthy hepatic matrix
B=Well scattered kupfer cells with no visible assault
Plate 10: Histology of the Liver in group 4 (Vit E only, X400).

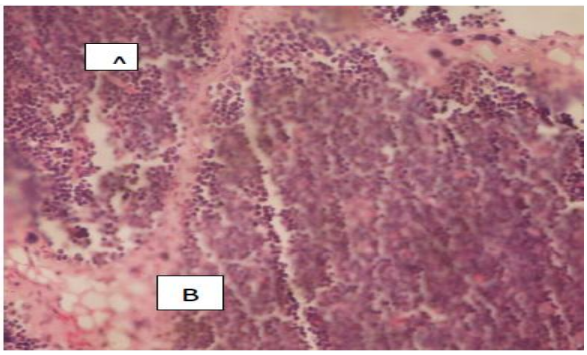


A=Inflamed portions of the lobular patches: evidence of toxicity
B=Aggregates of recovered cells in the matrix
Plate 11: Histology of Liver in group 5 (Lead + Vit C, X400).



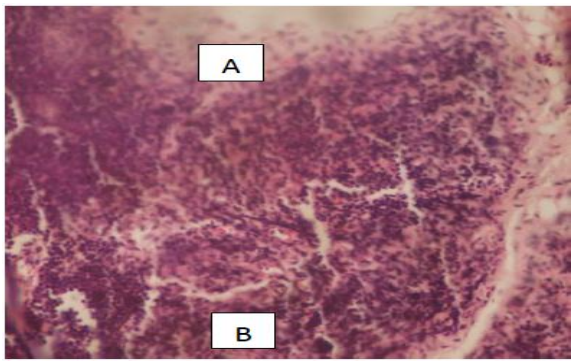
A=Normal portal hepatic tissue emerging
B=Evidence of chronic portal triaditis due to lead toxicity

Plate 12: Histology of Liver in group 6 (Lead + Vit E, X40.



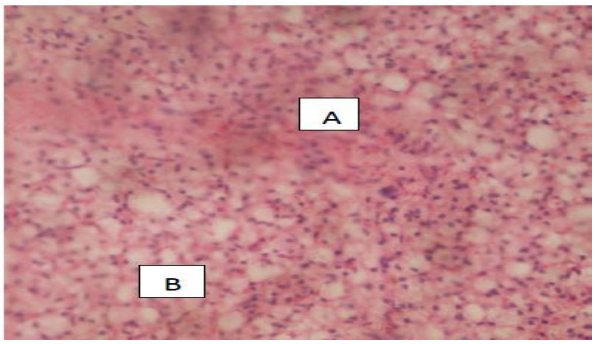
A=Well aligned sinusoids
B=Healthy cellular matrix devoid of debris encroachment

Plate 13: Histology of Thymus in control group 1 (X400).



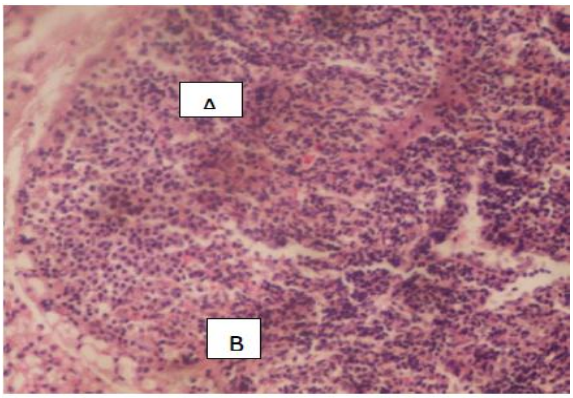
A=Hypocellular white pulp: evidence of Lead
B=Enlargement of venous sinusoids

Plate 14: Histology of Thymus in test group 2 (Lead only, X400).



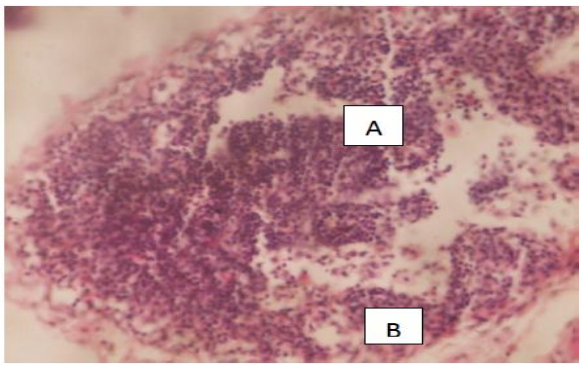
A=No visible evidence of hypocellular white pulp
B=No visible evidence of enlarged venous sinusoids

Plate 15: Histology of Thymus in group 3 (Vit C only, X400).



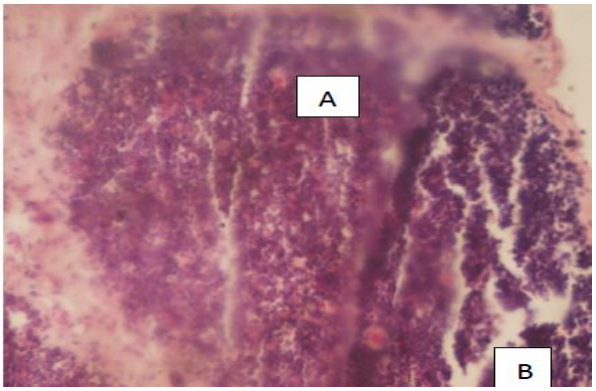
A=Well aligned cellular sinusoids
B=Low level clustering of heterochromatin substances nucleus

Plate 16: Histology of Thymus in group 4 (Vit E only, X400).



A=Vacuolation in the cytoplasm & swelling of mitochondria.
B=Clustering of heterochromatin in nucleus & Hypocellular white pulp.

Plate 17: Histology of Thymus in group 5 (Lead + Vit C, X400).



A=Distortion of rough endoplasmic reticulum cisterns reduced upon treatment
B=Enlargement of venous sinusoids

Plate 18: Histology of Thymus in group 6 (Lead + Vit E, X400).

CONCLUSION

The results of the study show that exposure to lead acetate have negative effects on weights, immunoglobulin parameters (IgM, IgG and IgA), the white blood cell counts and differentials, platelet count, total leucocyte count, immune organs (liver, Spleen and thymus) of male Wistar rats significantly. But the appropriate intervention by the treatment with antioxidants (Vitamins C and E) have significant ameliorative effect on the lead induced damage to immune system.

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