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METHANOLIC LEAVES EXTRACT OF TERMINALIA CATAPPA MITIGATES THE HEPATOTOXIC EFFECTS OF LEAD ACETATE IN ADULT WISTAR RATS

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ABSTRACT

Aim: This study assessed the effect of methanolic extract of *Terminalia catappa* leaves on the liver of adult wistar rats following lead induced oxidative stress. **Methods:** Thirty five (35) adult wistar rats of both sexes weighing between 150g and 200g were grouped into five groups A, B, C,D and E of seven (7) per group. They were fed on grower's marsh and given water *ad-libitum.* Groups A served as control. Group B received 25mg/kg of *Terminalia catappa* only. Group C received 8.75mg/kg of lead acetate with 50mg/kg of *Terminalia catappa.* Group D received 10mg/kg of lead acetate with 100mg/kg of *Terminalia catappa*. Group E received 13mg/kg of lead acetate only. **Results:** Results indicated that lead acetate caused a significant decrease ($P<0.05$) in body weights which was more pronounced in group E compared to that of the group C and D. it also showed a significant increase ($P<0.05$) in the level of the hepatic enzymes ALT, AST and ALP which was an indication that lead acetate caused a severe pathological changes in the liver parenchyma. This was supported by histological analysis that showed massive degenerative changes and dispersed hepatocyte cells, necrosis with depletion in the glycoprotein granules inside the hepatocyte cells on histological analysis. **Conclusion:** exposure to lead acetate causes severely distorted histoarchitecture of the liver resulting in significant increase in liver enzymes with decrease in body weights. However, methanolic leaf extract of *Terminalia catappa* exhibited a protective effect against the lead acetate induced-toxicity in the liver of wistar rats.

KEYWORDS: Lead Acetate, *Terminalia catappa.* Hepatocytes, liver enzymes, hepatotoxicity.

INTRODUCTION

Lead, a naturally occurring bluish-gray metal found in small amounts in the Earth's crust can be found in all parts of our environment. It is an environmental contaminant as a result of its significant role in modern industry.[1] Occupantional and environmental exposures remain a serious problem in many developing and industrializing countries.[2,3] Lead (Pb) is ubiquitous and one of the earliest metals discovered by the human race.[4,5] It is a toxic heavy metal and harmful even in small amounts.[4] Previous studies reported some of its effects as an enzymatic toxicant, neurotoxic, hemato and cardiovascular toxic, nephrotoxic, immunotoxic, carcinogenic, teratogenic and mutagenic.[6] The manifestations of lead poisoning in humans are nonspecific.[7,8] They may include weight loss, anemia,[9-11] nephropathy, infertility, liver, testis and heart damages.[11,12]

Lead is known to produce oxidative damage in the liver tissues by enhancing peroxidation of membrane lipids,[13-16] a deleterious process solely carried out by free radicals. Lead-induced oxidative stress in blood and other soft tissues has been postulated to be one of the possible mechanisms of lead- induced toxic effects.[2,17-20] Disruption of pro-oxidant/antioxidant balance might lead to tissue injury.[2,21,22] It was reported that lead increased the level of lipid peroxides and altered the antioxidant defense system in the hepatic tissues.[23,24]

MATERIALS AND METHODS

List of materials

Thirty five[35] Wistar Rats, Lead (Pb), Tropical almond *(Terminalia catappa*) Leaves, Methanol, Animal Feed(grower), water, dissecting set, hand gloves, Plastics Experimental cages, oral cannulas, distil water, weighing balance, permanent marker, methylated spirit, dissecting set.

Animal model

Thirty five[35] adult wistar rats of both sexes weighing between 150g and 200g were obtained using a top

loading digital scale (NV2101 model, OHAUS Corporation USA).

The animals were acclimatizes for two (2) weeks and throughtout the period of acclimatization they were seen healthy. They were housed in plastics experimental cages under standard laboratory condition in the Animal House in Faculty of the Basic Medical Sciences, Ladoke Akintola University of Technology Ogbomoso. They fed on grower's marsh obtained from BOVAJAY FEEDS, Orita Naira, Ogbomoso, Oyo State and were given water *ad-libitum.* Animals were cared for in accordance with 'Guide for the care and use of Laboratory Animal' prepared and compiled by the National Academy of Science and published by the National Institute of Health.[25]

Experimental Design and Grouping

The animals were picked based on weight and grouped into five groups A, B, C,D and E of seven (7) in each group. Group A rats were the controls, Group B, C, D and E were experimental treated groups.

- Groups A served as control and received distil water.
- Group B received 25mg/kg of *Terminalia catappa* only.
- Group C received 8.75mg/kg of lead acetate with 50mg/kg of *Terminalia catappa*
- Group D received 10mg/kg of lead acetate with 100mg/kg of *Terminalia catappa*
- Group E received 13mg/kg of lead acetate only

They were fed with grower's marsh obtained from BOVAJAY FEEDS, Orita Naira, Ogbomoso, Oyo state and given water substantially. The animals were acclimatized for two (2) weeks before the experiment began and after the acclimatization, it was recorded an increase in the weight of the animals which ranges from 150g to 275g. The Lead acetate was obtained from an open market in Ogbomoso, Oyo State.

Lead Acetate and Terminalia catappa administation

Table 1: The full detail of the administration of the experimental groups.

Groups	Terminalia catappa (g)	Lead acetate (g)
Groupa (control)	0	0
Group b	25mg/kg	0
Group c	50mg/kg	8.75mg/kg
Group d	100mg/kg	10mg/kg
Group e	0	13mg/kg

The LD50 for lead acetate used was 50mg/kg. All groups took equal amounts of feed and water not excluding the control group. Lead acetate were administered through intra-peritoneal for the first seven (7) days and *Terminalia catappa* were administered orally through the oral cannula for seven days. Administration was done for (14) days after which they were all sacrificed on the 15th day of experiment.

Sacrifice of the animals

The rats were sacrificed on the 15th day through cervical dislocation to make them be in a near living state while still breathing. The whole liver was harvested after which it was fixed in 10% normal saline and stored in sample for tissue preparation, the bottle was labeled according to the group in which the animals belong and then marked for identification. Blood samples collected via cardiac puncture for liver function test [mainly Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline Phosphatase (ASP)]. The blood sample was processed and these enzymes activities determined as previously described.[12]

Preparation of the liver tissue for histological analysis

The steps involved in tissue processing included fixation, dehydration, clearing, infiltration, embedding, blocking, sectioning, and staining. Liver tissues harvested were fixed in 10% normal saline (fixative) to prevent autolysis and putrefaction. The tissues were dehydrated in ascending grade alchohol (70%, 80%, 90%, 95%, absolute alcohol for 1h each but twice in the absolute alcohol), and cleared using two changes of xylene for an hour each. Once cleared, the tissues were infiltrated in molten paraffin wax in the oven at 58°C. Two changes of molten paraffin wax at one-hour intervals were made, after which the tissues were embedded in wax and made into blocks of wax. After cooling the cassette blocks were fixed in position on the microtome. The microtomes were set at 3-5 microtome and the tissues were sectioned serially. The ribbons formed were picked and placed gently on the surface of warm water in a water bath (at a temperature approximately 10 c lower than the melting point of wax) to allow spreading out of the tissue with the surrounding wax. After words, a clean albumen smeared slide was dipped obliquely into the water to attach the section. The slide was removed from the water and blotted dry after which diamond pencil was used to label the slide. The sections were fixed on clean slides and later stained with hematoxylin and eosin. Finally **t**issues were mounted using dextrose plasticizer and xylene mountant covered with cover slip avoiding air bubbles and viewed under light microscope and photomicrographs taken at magnifications x10, x20 and x40 respectively.

Statistical analysis

Data were analyzed using Graph Pad Prism 5 to determine student *t*- test and one way ANOVA when necessary. Results were expressed as mean ± SEM and $p < 0.05$ was accepted as significant.

RESULTS

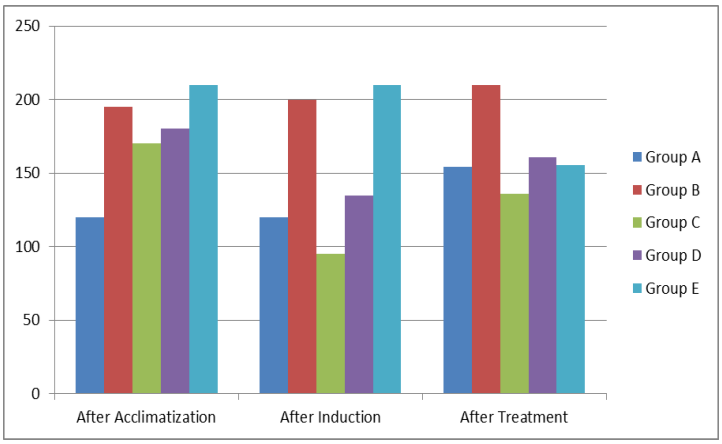


Figure 1: Graphical representation of the body weights of wistar rats before and after treatment. Values were expressed as mean ± SD and $p < 0.05$ was accepted as significant.

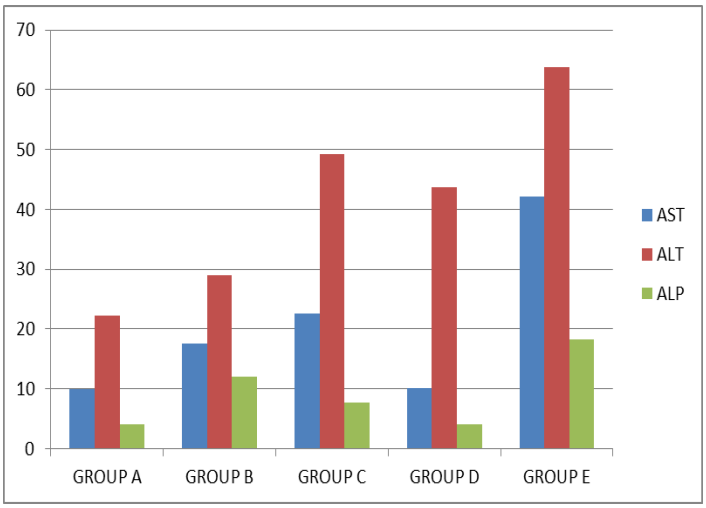
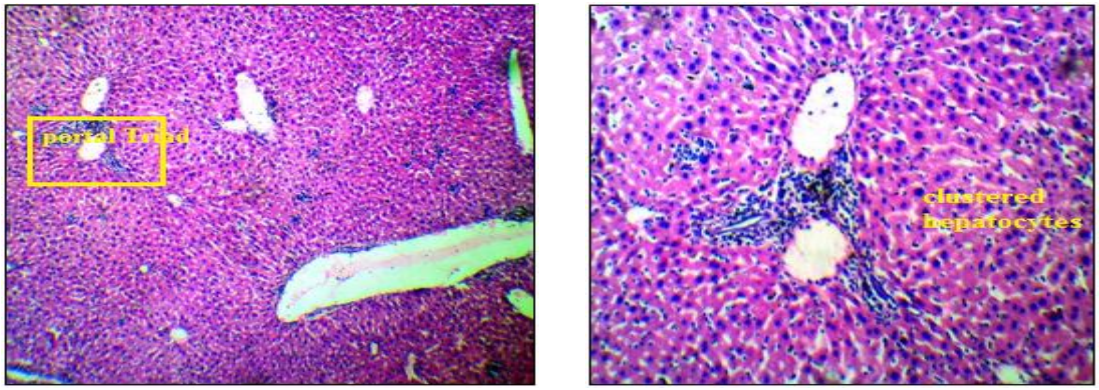


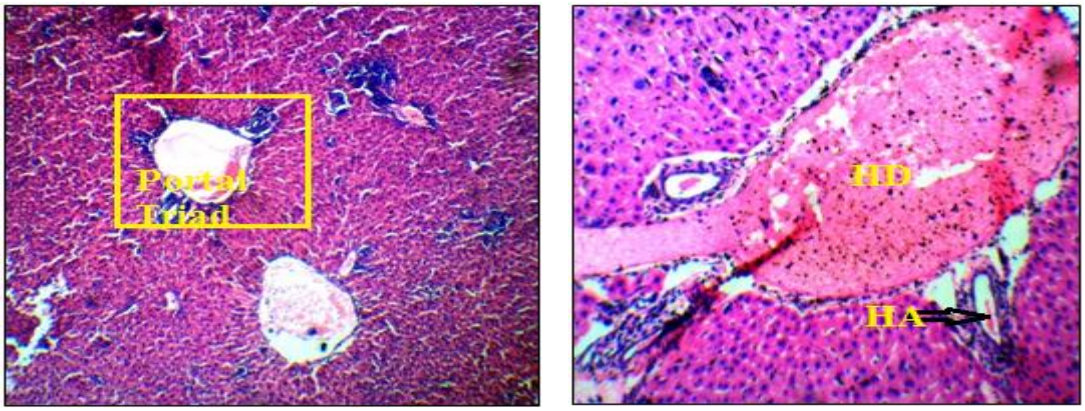
Figure 2: Graphical representation of AST, ALT and ALP. Values were expressed as mean ± SEM and $p < 0.05$ was accepted as significant.

Liver (Normal control)



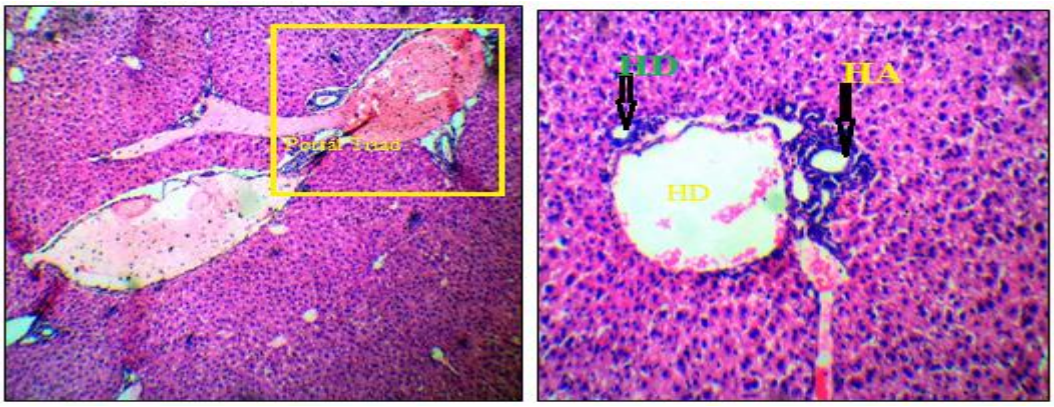
X100 **x400**

Plate 1: Photomicrograph of a liver section of group A showing normal liver architecture. No altered panoramic morphological presentation of the hepatic cytology was observed on group A.



X100 x400

Plate 2: Photomicrograph of a liver section of group B administered *Terminalia catappa* only. No altered panoramic morphological presentation of the hepatic cytology was observed on group B. Hepatic vein (HV), Hepatic artery (HA), Hepatic duct (HD). H&E x 100.



x100 x400

Plate 3: Photomicrograph of a liver section of group C administered lead acetate and lower dosage of *terminalia catappa*. There is some mild hemorrhage but general outline present are similar to the control groups A. Hepatic artery (HA), Hepatic vein (HV), Hepatic duct (HD). H&E x 100.

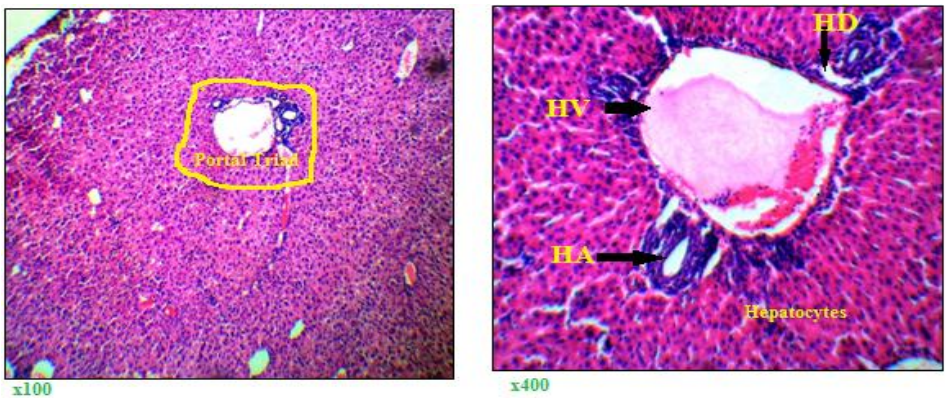
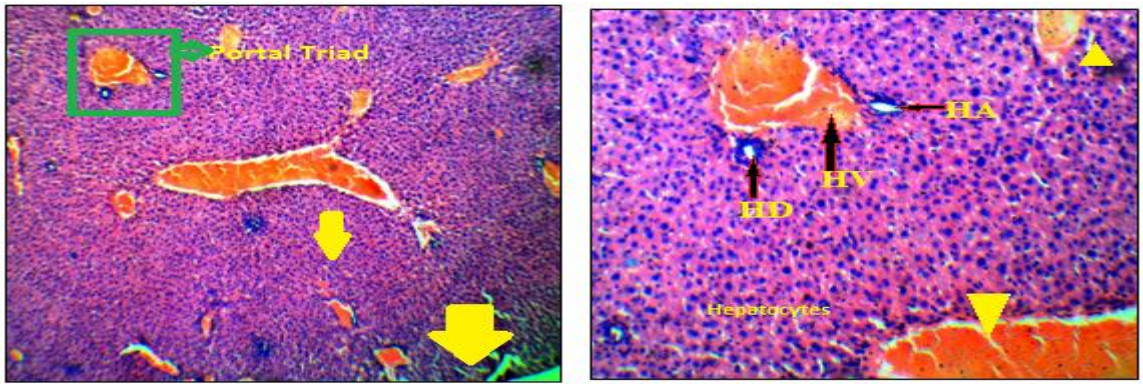


Plate 4: Photomicrograph of a liver section of group D administered lead acetate and higher dosage of *terminalia catappa*. There was mild hemorrhage but general outline present are similar to the control groups A. Hepatic artery (HA), Hepatic vein (HV), Hepatic duct (HD). H&E x 100 and x400.



X 100 x400

Plate 5: Photomicrograph of liver section of animals in group E administered lead acetate only. Showing severe pathological changes (yellow arrows). Enlarge hepatic vein and necrosis. Hepatic duct (HD), Hepatic vein (HV), Hepatic artery (HA) H&E x 100.

DISCUSSION

This report shows some effect of the administration of lead acetate on the liver of adult wistar rats. The effect of lead acetate on body weight of adult wistar rat was progressively decreased during the experimental period in different group (group C, D and E). The final body weight of intoxicated rats with lead (group E) was significantly reduced compared to that of the group A and B. These results clearly indicated that lead caused a significant decrease in body weight. This was in agreement with the findings of Ibrahim *et al.*[12] Banu *et al.*[26] and Khaki,[10] who found that lead cause decrease in body weight and growth rate in rat when fed lead.

The results of the present investigation have shown the effectiveness of methanolic extract of *Terminalia catappa* leaf as antioxidant on the body weight of group C and D that received both lead acetate and *Terminalia catappa* extract. There was an increase in body weight after receiving the extract compared to group E that received lead acetate only.

This present study showed an increase in the level of the liver enzymes ALT, AST and ALP is an indication of pathological changes within the liver. This was in agreement with the report of Ibrahim *et al.*[12] who demonstrated that ingestion of lead induced significant increase in ALT and AST. The present results showed that effect of lead acetate on the transaminases activity is dose independent. The high plasma ALT and AST activities are accompanied by high liver microsomal membrane fluidity and alteration in the liver tissue histogram. These observations are in agreement with previous study by Abdou *et al.*[27] who reported that lead has hepatotoxic effect.

Histopathological examination of the control and different experimental groups shows control group demonstrated normal architectural appearance of hepatocyte cells with sinusoidal dilation and glycoprotein accumulation inside the cells. In contrast, in the lead acetate group (group E), the liver tissue showed massive degeneration changes and dispersed hepatocyte cells necrosis with depletion in the glycoprotein granules inside the hepatocyte cells.

CONCLUSION

There is considerable evidence to suggest that exposure to lead acetate is a serious public health problem. The present study shows that the administration of lead acetate causes severe damages to the histology of the liver and decrease in body weight when lead acetate is applied in any amount of dosage. Also the serum AST, ALT and ALP shows functional activity of liver.

Recommendation

There must be a preventing aid to stop the exposure of lead by removing lead containing items from home, improved ventilation at workplace and nationwide policies such as laws that ban lead in products such as paint and gasoline, reduce allowable levels in water or soil and provide for cleanup of contaminated soil.

Ethical approval

All authors hereby declare that the principles of laboratory animal care [NIH publication No. 85-23 revised 1985] were followed as well as specific national laws where applicable.[28] All experiments have been examined and approved by the relevant ethics committee.

Conflict of interest

We declare that no conflict of interest.

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Authors' contributions

All authors worked together and contributed to the concepts and experimental design, literature review,

administration of the aqueous extract, animal sacrifice, data and statistical analysis.

List of Abbreviation
ALT – Alanine amino transferase
AST – Aspartate amino transferase
ALP – Alkaline phosphatase
SD – standard deviation
ANOVA – Analysis of variance
NIH – National Institutes of Health

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