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PROTECTIVE EFFECT OF ANACARDIUM OCCIDENTALE AQUEOUS LEAF EXTRACTS ON LEAD-INDUCED HEPATOTOXICITY IN MALE WISTAR RATS: HISTOLOGICAL AND BIOCHEMICAL EVALUATION

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ABSTRACT

This study investigated the protective effect of *Anacardium occidentale* (A.O) aqueous leaf extract on lead acetate-induced hepatotoxicity in male Wistar rats. Thirty male rats were divided randomly into six equal groups. Group 1 (normal control), received 0.5ml normal saline; group 2 received 50mg/kg body weight (b.wt) lead acetate (Pb) for 28 days; groups 3 & 4 received A.O (150 and 300 mg/kg b.wt respectively) for first 14 days and Pb (50mg/kg b.wt) for the next 14 days; group 5 received concurrently (150 mg/kg b.wt A.O and 50 mg/kg b.wt Pb) for 28 days; group 6 received concurrently (300 mg/kg b.wt A.O and 50 mg/kg b.wt Pb) for a period 28 days. Animals were sacrificed and blood sample collected for assay of activity of serum liver enzymes; Alkaline phosphatase (ALP), Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST). Result on histological analysis in lead only treated rats revealed vacuolar degeneration, necrosis of the hepatocytes and biochemical evaluation showed significant increases ($p<0.05$) in the activities of serum liver enzymes compared to normal control group indicative of hepatotoxicity. Pre and co-administrations of A.O at high and low doses to lead exposed rats, showed significant ($p<0.05$) reduction in serum levels of liver enzymes and cytoarchitectural preservation of liver tissue against lead intoxication. In conclusion, result obtained from this study, suggests that A.O possesses protective potentials against lead acetate-induced hepatotoxicity.

KEYWORDS: *Anacardium occidentale*; Hepatotoxicity; Lead Acetate; Liver Enzymes.

INTRODUCTION

Hepatotoxins are chemical, dietary or medicinal substances that have the potential to damage the liver cells.[1] The liver is a primary target to both natural and artificial chemical substances and plays a major role in drug metabolism and detoxification.[2] Liver toxicity is a global health burden that affects millions of people all over the world.[3] Lead is a soft, grey-blue poisonous and toxic heavy metal that is widely distributed in the environment.[4] It can occur both as an organic compound, in form of Tetraethyl lead and inorganic compound in form of lead acetate or lead chloride.[5] Because of its unparalleled attributes like softness, high malleability, ductility, low melting point and resistance to corrosion, lead has been very useful in manufacture of automobile, paint, ceramics and plastics.[6] This wide utilization has led to a rise in the distribution of free lead in human biological systems and the external surroundings. Lead is an occupational toxin with potent toxicology and the mechanism of lead toxicity has been reported[6] to occur via induction of oxidative stress by a rise in reactive oxygen species like hydrogen peroxides and radicals of superoxide.

The medicinal value of plants has assumed an important dimension owing mainly to the discovery that extracts from plants contain a diverse array of secondary metabolites with antioxidant potential.[7] These antioxidants help prevent damages to cell membranes due to cellular oxidative processes that may result in diseases.[8] Various studies by[9,10,11,12] have shown the protective effect of some medicinal plants against lead-induced liver damage.

The cashew is a tree in the family of the flowering plant Anacardiaceae. It has its roots all over the world. Several parts of the tree are used either individually or collectively to treat several diseases. Research studies on extracts from roots, leaves, stems, and fruits of A. Occidentale, have been documented to exhibit hypoglycemic effect.[13] Investigation on the effect of hydroethanolic leaf extract of Anacardium occidentale in ulceration showed that the extract inhibited gastric

lesions induced by HCl/ethanol in female rats.[14] Studies have also revealed that *Anacardium occidentale* leaf extracts have efficient antimicrobial activity against P. intermedia.[15] This study, therefore, investigated the protective potential of *Anacardium occidentale* (A.O) aqueous leaf extract against hepatotoxicity induced by lead acetate.

MATERIALS AND METHODS

Experimental animals

Thirty Wistar rats, weighing 200-240g were purchased from the animal house of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka. They were housed in cages in the animal house of the Department of Anatomy, University of Nigeria, Enugu Campus, under controlled conditions of 12-hour light/ 12- hour dark cycles. The rats were acclimatized to the environment for 2 weeks before experimental use and allowed free access to clean water and standard livestock pellets (Guinea Feed Nigeria Limited) ad libitum.

Ethical consideration

Ethical clearance was obtained from the Research Ethics Committee of the College of Medicine, University Nigeria, Enugu Campus (the institution of the principal author).

Chemicals and reagents

Lead acetate (C4H6O4Pb_H2O) was purchased from M&B chemicals, England. All other biochemical reagents and chemicals were of analytical grade.

Plant material

The fresh leaves of *Anacardium occidentale* were collected from the cashew plantation area in Opi Nsukka, Enugu state, Nigeria. Its botanical identity was authenticated by a botanist in the University of Nigeria Nsukka Herbarium with voucher specimen number (240a).

Preparation of leaf extract

The collected leaves were washed in tap water, dried at room temperature for 2 weeks and ground into powder. 500g of plant powder sample was extracted in 2L of distilled water by the maceration method for 24 hours. The mixture was stirred after every 8 hours using a sterile glass rod. The macerated sample was filtered using muslin cloth at room temperature. The filtrate was concentrated using a rotary evaporator and finally dried on the water bath in an evaporating dish until it became completely dry. The dried extract was weighed to be 49.1g yielding 9.8%. The extract was stored at 4°C in a refrigerator.

Experimental design

Thirty Wistar rats used for this study were randomly divided into six equal groups. Group 1 served as normal control and received 0.5ml normal saline/day; Group 2 was administered 50mg/kg b.wt lead acetate (Pb) only; Group 3 received A.O (150mg/kg b.wt) for first 14 days and 50mg/kg b.wt Pb for next 14 days; Group 4 received A.O (300mg/kg b.wt) for first 14 days and 50mg/kg b.wt Pb for next 14 days; Group 5 received (150 mg/kg b.wt A.O and 50 mg/kg b.wt Pb) concurrently for 28 days. Group 6 received (300 mg/kg b.wt A.O and 50 mg/kg b.wt Pb) concurrently for 28 days. All administration was by oral gavage.

Experimental protocol

After the completion of treatments, all animals were sacrificed by cervical dislocation. Through a midline incision on the anterior abdominal wall, the heart was accessed and by cardiac puncture, blood sample was collected in a 5 ml plain bottle for biochemical evaluation. The liver was quickly excised and processed for subsequent histological analysis.

Histological studies

All the groups were subjected to histological studies at the end of 28 days, following standard procedures.[16] Tissues were fixed in 10% formalin solution in a phosphate buffer and processed for paraffin wax embedding and sectioned on a rotary microtome at 5µm thickness.

Estimation of activity of serum liver enzymes

Using a bench centrifuge, collected blood samples were centrifuged at 5000 rpm for 15 mins to obtain serum. Standard biochemical diagnostic kits were spectrophotometrically used to estimate the activity of serum liver enzymes (AST, ALT, and ALP). Detailed procedures for the above estimations were carried out, following the kit manufacturer's instructions.

Data analysis

The data obtained were analyzed statistically with one-way analysis of variance (ANOVA) followed by student's t-test with the aid of SPSS (V20; USA). Data were presented as mean±SEM (standard error of mean). P value ($p<0.05$) was considered statistically significant.

RESULTS

Biochemical analysis

At the end of the experiment, animals exposed to lead-acetate only (group 2) showed significantly ($P<0.05$) increased serum levels of the liver enzymes (AST, ALT, and ALP) compared to normal control. In group 3 and 4, administration of A.O extract to Wistar rats at a dose of 150mg/kg bwt and 300mg/kg bwt before exposure to lead-acetate, showed significantly ($p<0.05$) reduced serum levels of ALT, ALP, and AST when compared to the lead-acetate only treated group. A significant decrease ($p<0.05$) in serum levels of AST, ALP, ALT when compared to the lead only treated group was also observed in groups 5 and 6, administered A.O extracts at a dose of 150mg/kg and 300mg/kg, concurrently with lead-acetate (50mg/kg) (Table 1).

Table 1: Effects of aqueous leaf extract of *A.O* on AST, ALT and ALP in serum of lead induced toxicity in rats (Mean ± SEM).

NUMBER	GROUP	AST (U/L)	ALT (U/L)	ALP (U/L)
1	(Normal control)	25.6 ± 0.80	25.68 ± 0.44	19.1 ± 1.15
2	Lead acetate only	57.01 ± 1.27[a]	59.65 ±1.32[a]	59.1 ± 1.30[a]
3	Protective (low dose)	34.8 ± 0.04[b]	33.52 ±2.33[b]	36.6 ± 2.80[b]
4	Protective (high dose)	38.85 ± 0.35[b]	30.88 ±0.22[b]	38.3 ± 0.27[b]
5	Ameliorative (low dose)	33.90±1.30[b]	37.91 ±0.84[b]	30.2 ± 0.10[b]
6	Ameliorative (high dose)	32.0 ± 2.05[b]	38.76 ±0.04[b]	39.2 ± 0.80[b]

KEY: [a] significantly different (p<0.05) from normal control group (A); [b] significantly different (p<0.05) from lead only group (B).

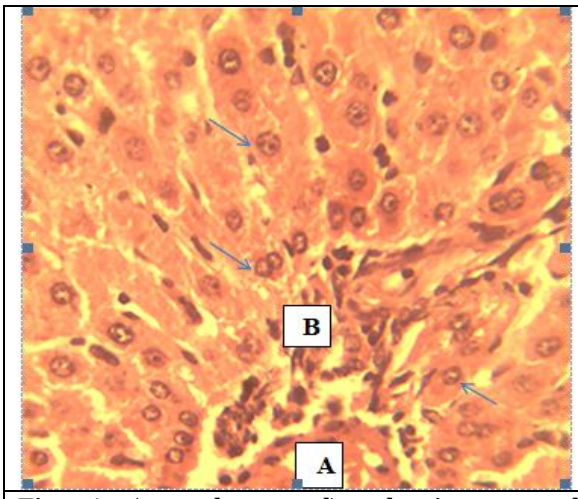

Fig. 1 (normal control): showing normal histological structure of hepatocytes (arrow), hepatic artery (A), and bile duct (B) at the periphery of the lobules H&E X400.

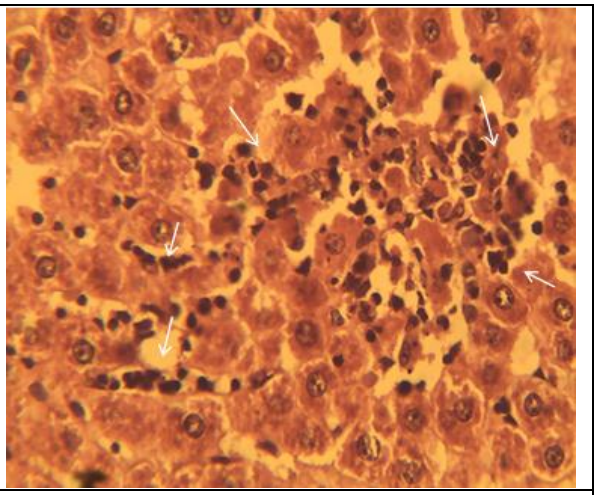

Fig. 2 (lead only): showing vacuolar degeneration and necrosis of the hepatocytes (white arrow). The affected hepatocytes show multiple clear vacuoles in their cytoplasm. H&Ex400.

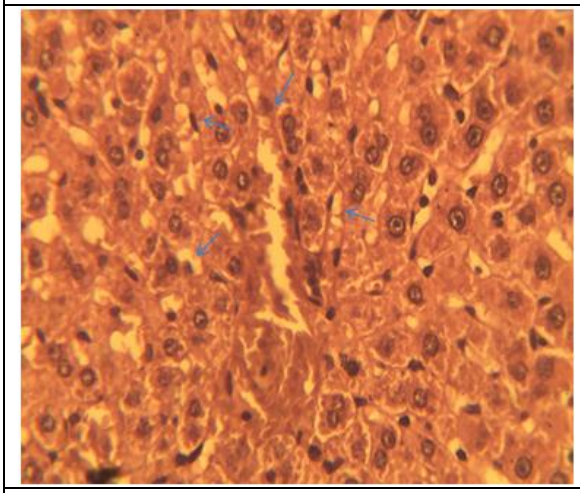

Fig. 3 (pre-treatment low dose); Liver tissue section shows mild degeneration of the hepatocytes (arrow). H&Ex400

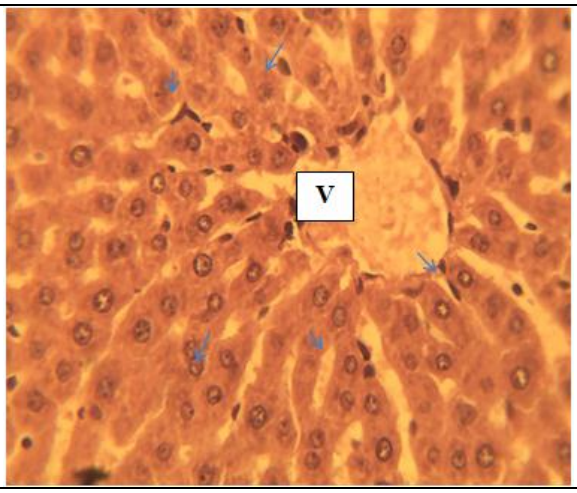

Fig. 4 (pre-treatment high dose): showing of normal hepatocytes arranged in interconnecting chords around the central vein (V). H&Ex400

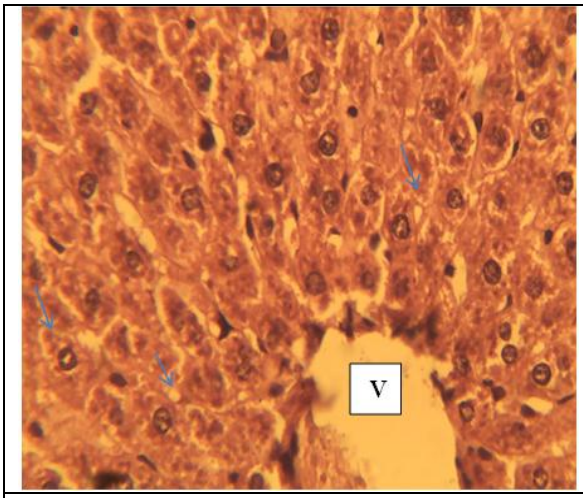

Fig. 5 (Co-treatment low dose); showing few degenerating hepatocytes (arrow) arranged in a radiating manner around the central vein (V). H&E x400.

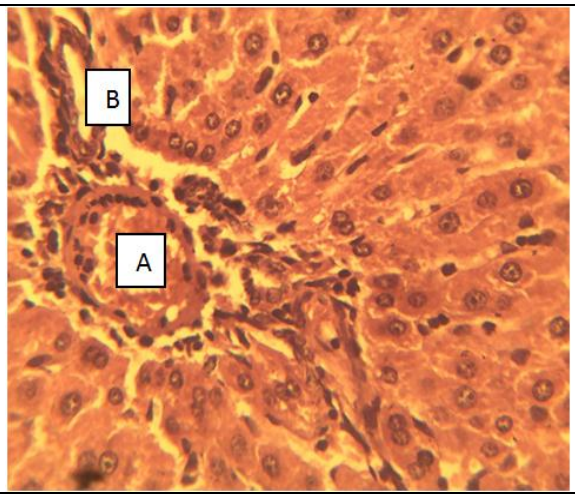

Fig. 6 (Co-treatment high dose): showing normal hepatic architecture with some components of the portal triad- hepatic artery (A) and Bile duct (B). H&Ex400.

Histological findings

These results have been summarized in figures 1 to 6. The Group 2 rats induced with Lead acetate without any treatment showed vacuolar degeneration and necrosis of the hepatocytes, indicative of hepatotoxicity. The histological structure of the animals pre-treated before induction with Lead acetate (3 &4) and also those co-treated with the toxic agent (5 & 6) were better preserved and showed less signs of hepatotoxicity when compared with Group 2. The higher doses of A.O extracts showed more therapeutic potentials in both pre-treatment and co-treatment groups.

DISCUSSION

Inorganic lead is an environmental and occupational toxin. Man can be exposed to lead by swallowing or breathing it in.[17] After it has been absorbed, lead conjugates in the liver and passes on to the kidney, from where a little amount is removed in urine while the remnant builds up in other organs of the body.[18] As a result, biological activities at various levels (cellular, intercellular and molecular) are affected; leading to changes in morphology that will last over a long period of time even after blood and soft tissue lead levels has decreased.[19] Report on research studies[17,20] has revealed that in lead exposed animals, 33% of lead accumulates in the liver tissue, making it the soft tissue with the highest depository for lead.

Estimation of serum aminotransferases (ALT, AST) and alkaline phosphatase (ALP) levels are broadly used as markers in accessing the extent of hepatic injury.[21] Increased activity of these liver enzymes may indicate disruption of hepatocyte membrane integrity and hepatocyte epithelial necrosis.[22]

Report from previous studies[2,6] has shown that exposure to Lead can damage the membrane of hepatocytes, resulting in a marked rise in the serum levels of the liver enzymes (AST, ALT and ALP).

A rise in AST levels reflects an acute damage to hepatic tissue, although the enzyme is not specific to liver tissue and can be used as a marker for muscle injury or cardiac infarction.[23] However, ALT is a more specific marker for detecting hepatic damage. Increase in serum ALT levels may reflect loss of functional integrity of hepatic cell membrane, resulting in cellular leakage of the enzyme into the blood.[24,25,26] Serum alkaline phosphatase (ALP) level also determines hepatic cell function. Increase in synthesis of ALP, increases the activity and level of this enzyme in the blood.[27]

The present study demonstrated that, Pb treated rats (group 2) showed significant increase ($p<0.05$) in serum AST, ALT and ALP levels when compared with normal control, an indication of tissue injury. The increase in the aminotransferases (ALT and AST) and alkaline phosphatase (ALP) levels may be as a result of loss of functional integrity of the liver membrane and leakage of these enzymes into the blood. This finding is supportive of previous studies by[28,29,30] who reported that exposure of animals to Lead resulted in elevated levels of serum ALT, AST, and ALP.

Structurally, the liver cytoarchitecture of the Lead treated rats in this study was severely altered when compared with the liver sections of the control group and the morphological changes observed include; necrosis of hepatocytes, multifocal vacuolar degeneration and nuclear pyknosis, and cellular infiltration of the liver, indicative of lead related hepatotoxicity. These changes observed may be because the liver is one of the major organs implicated in depository and elimination of lead. This result was in accordance with the findings of[31] where histopathological examination of liver tissue in

lead exposed rats revealed focal areas of massive hepatic degeneration.

A report by[32] suggested that medicine derived from plants may serve as a potent therapeutic and are less expensive than synthetic alternatives. Several phytomedicine have been reported to possess antioxidant properties which acts to protect against damages to cell membrane resulting from cellular oxidative processes due to exposure to toxic chemical substances.[33,34]

From the present study, the significant ($p<0.05$) decrease in the activity of serum aminotransferases (AST, ALT) and alkaline phosphatase (ALP) across the A.O treatment groups (3, 4, 5 and 6), suggests a reduced severity of toxic damages caused by lead exposure. The effect of A.O at low and high doses on AST, ALT and ALP levels, points out the potent protective effect of this plant for the liver, which may be due to the presence of antioxidant properties in A.O. The findings and pattern of activity of liver enzymes in this study are in consonance with those described by other researchers[35],[36] on the hepatoprotective potential of various medicinal plants on toxicity induced by free-radical releasing substances.

Histopathological analysis of liver tissue sections from rats in these groups (3, 4, 5 and 6), showed preservation of the liver cytoarchitecture with very mild necrosis of hepatocytes when compared to the lead only treated group (2). This is an indication that A.O at low and high doses mitigates the toxic effect of lead in the liver tissue by stabilizing the membrane integrity and preventing leakage of intracellular enzymes. This finding is supportive of medicinal plant related studies that have reported hepatoprotective activity of plant extracts decrease the severity of histopathological changes induced by lead.[37]

The hepatoprotective efficacy of A.O may be attributed to the presence of phytochemicals which act as antioxidants. A. occidentale extracts has been reported to possess some important phytochemicals such as flavonoids, phenols, tannins, terpenoids, alkaloids, steroids, glycosides and volatile oils.[38,39] Flavanoids, saponins and phenolic plant phytochemicals, are well known[40] for their antioxidant activities and have the ability to protect against peroxidative damage by scavenging reactive oxygen species released by environmental toxicants.

CONCLUSION

This study suggests that aqueous leaf extracts of *Anacardium occidentale* particularly at a higher dose (300mg/kg) is of considerable benefit in alleviating the damaging effects of lead acetate on the functional health of the liver owing mainly to the fact that it contains potent antioxidant properties.

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