Niger. J. Physiol. Sci. 37 (December 2022): 219 – 224
www.njps.physiologicalsociety.com

Niger. J. Physiol. Sci.

Full length Research Article

Antioxidant and Chemopreventive Effects of *Azadirachta indica* on Lead Acetate-induced Hepatotoxicity in Male Wistar Rats

Asenuga E.R.A, Ajibade T.O.B.,*, Adejumobi O.A.C, Alaba B.A.C, Akinboye O.A.C, Badejo J.A.D, Nabofa E.W.E, Oyagbemi A.A.B, Omobowale T.O.C,

[a]*Department of Veterinary Physiology & Biochemistry, University of Benin, Nigeria*
[b]*Department of Veterinary Physiology and Biochemistry, University of Ibadan, Nigeria*
[c]*Department of Veterinary Medicine, University of Ibadan, Nigeria*
[d]*Department of Pharmacology and Therapeutics, University of Ibadan, Nigeria*
[e]*Physiology Department, Babcock University, Ilishan-Remo, Nigeriae*

Summary: Toxic metals such as lead cause severe liver damage in humans and animals, with oxidative stress prominently implicated in the pathogenesis of lead acetate-induced liver injury. *Azadirachta indica* is hepatoprotective due to its antioxidative effect. This study investigated the antioxidative role of *A. indica* (AI) and its chemopreventive effect on lead acetate (LA)-induced hepatocellular dysfunction with seventy adult male rats classified into group A- Control (distilled water), group B 0.1% LA only, group C and D- 0.1% LA + 100 mg/kg and 0.1% LA + 200 mg/kg AI respectively, group E- 0.2% LA, group F and G- 0.2% LA + 100 mg/kg and 0.2% LA + 200 mg/kg AI. Oxidative stress markers (MDA and H_2O_2), antioxidant parameters (GSH, SOD, CAT, GPx, GST), inflammatory markers (MPO and NO), alanine aminotransferase (ALT) and histopathological studies of the liver were evaluated. The results showed that LA administration caused a decrease in GSH, GPx, and GST while AI co-administration increased the activities of the antioxidants. Moreover, LA administration increased MPO, NO, MDA, and H_2O_2 levels whereas AI significantly reduced ($P<0.05$) these parameters. Histopathological examination revealed necrosis and mild infiltration by inflammatory cells in LA administered rats, whereas these lesions were absent in AI administered rats. In conclusion, *A. indica* demonstrates a protective role in lead acetate-induced hepatotoxicity, mainly via oxidative stress inhibition.

Keywords*: Azadirachta indica, antioxidant, hepato-protection, oxidative stress, lead-acetate*

**Authors for correspondence: toajibade@gmail.com; +2349071842142*

Manuscript received- July 2022; Accepted- November 2022

DOI: https://doi.org/10.54548/njps.v37i2.9

©Physiological Society of Nigeria

INTRODUCTION

The tree, *Azadirachta indica* (AI) popularly called neem is widely grown in Africa primarily due to its numerous valuable properties for medicinal and agricultural uses as pesticide and insecticide among rural dwellers (Islas *et al*., 2020). Various phytochemical constituents of *A. indica* including nimbin, nimbandiol, nimbiol, nimbolide and 6-desacetylnimbinene are used medicinally for their chemopreventive and chemotherapeutic properties (Singaravelu *et al*., 2019). The aqueous leaf extract of *A. indica* reportedly is hepatoprotective against paracetamol-induced liver necrosis in rats (Aneja *et al*., 2013). Furthermore, Arivazahagan *et al*. (2000) suggested that neem leaf extract exerts potential chemopreventive effects via the downregulation of lipid peroxidation, concurrently increasing the level of reduced glutathione (GSH) and GSH-dependent enzymes. The antioxidant effect of neem relied primarily on the total phenolic compounds, such as the flavonoids. Increased serum ALT and AST levels indicate liver damage, and these have been shown to reduce significantly after administration of aqueous leaf extract of neem (Nwobodo *et al*., 2018).

Lead (Pb) is a persistent toxic pollutant of the environment with documented deleterious effects on the liver and most other parts of the digestive system (Andjelkovic *et al*., 2019). Following ingestion, Pb is absorbed through intestinal membranes into the blood stream where it binds to the erythrocyte proteins, subjected to first pass metabolism in the liver, and subsequently undergoes systemic distribution (García-Niño and Pedraza-Chaverri, 2014), where lead induces numerous macromolecular damages and eventually cell death (Abdelhamid *et al*., 2020). Oxidative mechanisms are actively involved in Pb-induced toxicity, since exposure to lead causes an imbalance between the antioxidant and prooxidant systems, leading to protein or DNA damage, mitochondrial impairment, and apoptosis (Dobrakowski et al., 2017). Thus, this study was designed with the aim of investigating antioxidant and chemopreventive modulatory roles of *A. indica* on oxidative stress mediated hepatotoxic changes associated with lead exposure in male Wistar rats.

MATERIALS AND METHODS

Treatment groups: The first group (group A) which served as the control was administered water, which was replaced with corn oil after 8 weeks. Group B, C and D were dosed with 0.1% lead acetate (LA) orally and was withdrawn after

8 weeks. On withdrawal, group B received water, and groups C and D received 100 and 200 mg/kg of *Azadirachta indica* (AI) methanolic extract, respectively. Group E, F, and G were given 0.2% LA orally and was withdrawn after 8 weeks. On withdrawal, group E received water while groups F and G were given 100 and 200 mg/kg of *Azadirachta indica* methanol extract.

Preparations of tissues and sera for biochemical assay: The liver tissue was excised using scissors and forceps, rinsed in potassium chloride solution, blotted with filter paper and weighed. Thereafter, the tissues were homogenized in ice cold buffer before centrifugation to obtain the post mitochondrial fraction that was used for biochemical analysis. In order to obtain the sera, blood samples collected into plain haematological sample bottles were left in a standing position to allow clotting and separation of the serum, which was subsequently decanted into clean bottles and preserved.

Biochemical assay: In this study, enzymes such as alanine aminotransferase (Reitman and Frankel, 1957), superoxide dismutase (Misra and Fridovich, 1972), Catalase (Sinha, 1971), glutathione peroxidase (Beutler *et al*., 1963), and glutathione S-transferase were analysed using standard biochemical assay methods. Furthermore, the nonenzymatic antioxidant - reduced glutathione- was assayed as described by Beutler, *et al*. (1963), whereas protein concentration was analysed in accordance with the procedure reported by Gornal *et al*., (1949). Also in this study, markers of oxidative stress including malondialdehyde (Varshney and Kale, 1970), and hydrogen peroxide (wolff, 1994), were analysed. Moreover, the nitrite levels in the liver were evaluated as described by Olaleye *et al*. (2017), with optical density measured at 550 nm spectrophotometrically.

Determination of Hydrogen Peroxide (H_2O_2): The level of hydrogen peroxide was determined according to the method by Wolff (1994). The concentrations of the standard were plotted against absorbance.

Histopathological Studies: After treatment, the animals were euthanised and carefully opened up through the ventral abdomen to collect hepatic tissues of the control as well as the exposed animals. Before the assessment, the organ was stored in formalin solution, washed with ethanol (70%), dehydrated via a graded series of ethanol, and later sectioned serially in a rotary microtome. The fixed sections were stained with Hematoxylin and Eosin (H&E) and later viewed under a light microscope (Drury, 1976).

Statistical analysis

The data obtained from this study were expressed as Mean ± Standard Deviation, and student's t-test used to estimate the significance between two groups using GraphPad Prism software. Furthermore, the variances among more than three groups were analysed using ANOVA and Turkey's comparison. Confidence limit was set at 95%.

RESULTS

Results in Table 1 show that the GSH level in group exposed to 0.1% LA + 200mg/kg of *A. indica* increased significantly relative to control. Also, a significant ($p<0.05$) increase in GSH level was observed in group exposed to 0.1% LA + 100mg/kg of *A. indica* (4%) and in group exposed to 0.1% LA + 200mg/kg of *A. indica* (7%) when compared to group that received 0.1% LA only (Table 2). GPx activity decreased in group exposed to 0.1% LA (4%) and 0.2% LA (4%) only, while a significant ($p<0.05$) increase was seen in groups exposed to 0.1% LA + 200mg/kg of *A. indica* (5%) and 0.2% LA + 100mg/kg *A. indica* (5%) when compared to the control group. However, a significant ($p<0.05$) increase was observed in group that received 0.1% LA + 100mg/kg *A. indica* (9%) and 0.1% LA + 200mg/kg *A. indica* (10%) when compared to group that received 0.1% LA only. Also, a significant ($p<0.05$) increase in GPx activity was observed in group that received 0.2% LA + 100mg/kg *A. indica* (9%) and 0.2% LA + 200mg/kg *A. indica* (8%) when compared to group that received 0.2% LA only (Table 3).

Table 1:
The effects of *Azadirachta indica* on reduced glutathione (GSH) (µmole/g/tissue)

GROUP	MEAN ± SD
A (Control)	42.08±0.29
B (0.1% LA)	40.75±1.5
C (0.1% LA + 100mg/kg neem)	42.25±0.5 [b]
D (0.1% LA + 200mg/kg neem)	43.44±1.38[a,b]
E (0.2% LA)	41.83±1.18
F (0.2% LA + 100mg/kg neem)	42.5±1.34
G (0.2% LA + 200mg/kg neem)	42.5±1.25

LA- Lead acetate
Values are presented as mean ± standard deviation. [a] indicates significant difference at p<0.05 with respect to group A, [b] indicates significant difference at p<0.05 in comparison with group B while [c] indicates significance difference at p<0.05 in comparison with group E.

Table 2:
The effects of *Azadirachta indica* on glutathione peroxidase (GPx) activity (units/mg protein)

GROUP	MEAN ± SD
A (Control)	105.7±3.47
B (0.1% LA)	101.2±2.39 [a]
C (0.1% LA + 100mg/kg neem)	110.5±4.79 [b]
D (0.1% LA + 200mg/kg neem)	111.4±1.94 [a, b]
E (0.2% LA)	101.9±2.15 [a]
F (0.2% LA + 100mg/kg neem)	110.9±1.04 [a, c]
G (0.2% LA + 200mg/kg neem)	110.2±5.67 [c]

LA- Lead acetate
Values are presented as mean ± standard deviation. [a] indicates significant difference at p<0.05 with respect to group A, [b] indicates significant difference at p<0.05 in comparison with group B while [c] indicates significance difference at p<0.05 in comparison with group E.

Table 3:
The effects of *Azadirachta indica* on superoxide dismutase (SOD) (units/mg protein)

GROUP	MEAN ± SD
A (Control)	13.04±0.7
B (0.1% LA)	15.76±1.49 [a]
C (0.1% LA + 100mg/kg neem)	15.35±0.6 [a]
D (0.1% LA + 200mg/kg neem)	14.46±0.9 [a]
E (0.2% LA)	15.77±0.6 [a]
F (0.2% LA + 100mg/kg neem)	14.77±0.35 [a, c]
G (0.2% LA + 200mg/kg neem)	14.86±1.25 [a]

LA- Lead acetate
Values are presented as mean ± standard deviation. [a] indicates significant difference at p<0.05 with respect to group A, [b] indicates significant difference at p<0.05 in comparison with group B while [c] indicates significance difference at p<0.05 in comparison with group E.

Table 4:
The effects of *Azadirachta indica* on glutathione-S-transferase (GST) (Glutathione S-transferase; mmole1-chloro-2, 4-dinitrobenzene-GSH complex formed/min/mg protein)

GROUP	MEAN ± SD
A (CONTROL)	12.51±0.61
B (0.1% LA)	11.58±1.21
C (0.1% LA + 100mg/kg neem)	14.81±1.04 [a, b]
D (0.1% LA + 200mg/kg neem)	15.01±0.76 [a, b]
E (0.2% LA)	8.73±0.19 [a, b]
F (0.2% LA + 100mg/kg neem)	14.67±0.61 [a, c]
G (0.2% LA + 200mg/kg neem)	14.93±0.9 [a, c]

LA- Lead acetate
Values are presented as mean ± standard deviation. [a] indicates significant difference at p<0.05 with respect to group A, [b] indicates significant difference at p<0.05 in comparison with group B while [c] indicates significance difference at p<0.05 in comparison with group E.

Table 5:
The effects of *Azadirachta indica* on Catalase (mmoleH_2O_2 consumed/min/mg protein)

GROUP	MEAN ± SD
A (CONTROL)	38.1±1.94
B (0.1% LA)	50.2±5.33 [a]
C (0.1% LA + 100mg/kg neem)	45.1±1.74 [a, b]
D (0.1% LA + 200mg/kg neem)	41.7±1.47 [a, b]
E (0.2% LA)	47.2±1.20 [a]
F (0.2% LA + 100mg/kg neem)	43.6±1.32 [a, c]
G (0.2% LA + 200mg/kg neem)	46.9±1.45 [a, c]

LA- Lead acetate
Values are presented as mean ± standard deviation. [a] indicates significant difference at p<0.05 with respect to group A, [b] indicates significant difference at p<0.05 in comparison with group B while [c] indicates significance difference at p<0.05 in comparison with group E.

Increase in the activities of SOD in groups exposed to 0.1% LA only (21%), 0.1% LA + 100mg/kg (18%), 0.1% LA + 200mg/kg (11%), 0.2% LA only (21%), 0.2% LA + 100mg/kg (13%) and 0.2% LA + 100mg/kg (14%) when compared to the control group, was observed. Meanwhile, a significant (p<0.05) decrease was observed in group exposed to 0.2% LA + 100mg/kg (6%) when compared to group exposed to 0.2% LA only.

In table 4, there was a significant (p<0.05) increase in GST activities in groups exposed to 0.1% LA + 100mg/kg (18%), 0.1% LA + 200mg/kg (20%), 0.2% LA + 100mg/kg (17%) and 0.2% LA + 200mg/kg (19%), while increase in GST activities in groups to which 0.1% LA + 100mg/kg (28%), and 0.1% LA + 200mg/kg (30%) was given when compared to group that receives 0.1% LA only. GST activity increased significantly (p<0.05) in groups to which 0.2% LA + 100mg/kg (68%) and 0.2% LA + 200mg/kg (71%) was given when compared to group that receives 0.2% LA only.

Elevated activities were recorded for catalase in groups that receives 0.1% LA only (32%), 0.1% LA + 100mg/kg (18%), 0.1% LA + 200mg/kg (9%), 0.2% LA only (24%), 0.2% LA + 100mg/kg (14%) and 0.2% LA + 200mg/kg (23%) when compared to the control group. Meanwhile, there was a significant (p<0.05) decrease in the catalase activity in groups that receives 0.1% LA + 100mg/kg (10%) and 0.1% LA + 200mg/kg (17%) when compared to group that receives 0.1% LA only. Also, there was a significant (p<0.05) decrease in groups that receives 0.2% LA + 100mg/kg (8%) and 0.2% LA + 200mg/kg (1%) when compared to group that receives 0.2% LA only (Table 5).
In figure 1, there was a significant (p<0.05) increase in the ALT activity in groups exposed to 0.1% LA only (788%), 0.1% LA + 100mg/kg *A. indica* (767%), 0.1% LA + 200mg/kg *A. indica* (797%), 0.2% LA only (897%), 0.2% LA + 100mg/kg *A. indica* (738%) and 0.2% LA + 200mg/kg *A. indica* (798%) when compared to the control group. Meanwhile, there was a significant (p<0.05) decrease ALT activity in group exposed to 0.1% LA + 100mg/kg *A. indica* (2%) when compared to group that received 0.1% LA only. Also, a significant (p<0.05) decrease in ALT activities was observed in groups that was exposed to 0.2% LA + 100mg/kg *A. indica* (16%) and 0.2% LA + 200mg/kg *A. indica* (10%) when compared to group that received 0.2% LA only.

In figure 2, there was a significant (p<0.05) increase in MDA level in groups that receives 0.1% LA (21%) and 0.2% LA only (34%), while a significant (p<0.05) decrease was observed in groups exposed to 0.2% LA + 100mg/kg *A. indica* (8%) and 0.2% LA + 200mg/kg *A. indica* (1%) when compared to the control group. Also, a significant decrease was observed in groups exposed to 0.1% LA + 100mg/kg *A. indica* (16%) and 0.1% LA + 200mg/kg *A. indica* (26%) compared to the group exposed to 0.1% LA only.

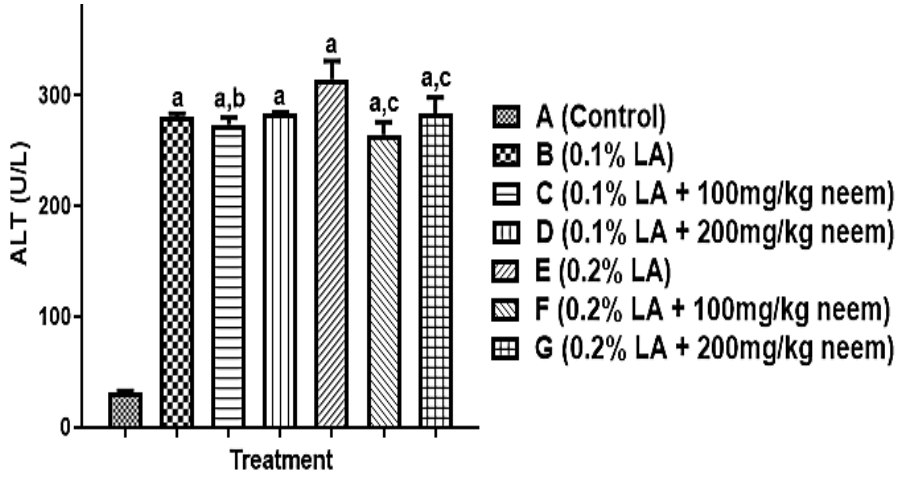

Figure 1:
The effects of *Azadirachta indica* (neem) on alanine aminotransferase (ALT) activity
LA- Lead acetate.
Values are presented as mean ± standard deviation. [a] indicates significant difference at p<0.05 with respect to group A, [b] indicates significant difference at p<0.05 in comparison with group B while [c] indicates significance difference at p<0.05 in comparison with group E.

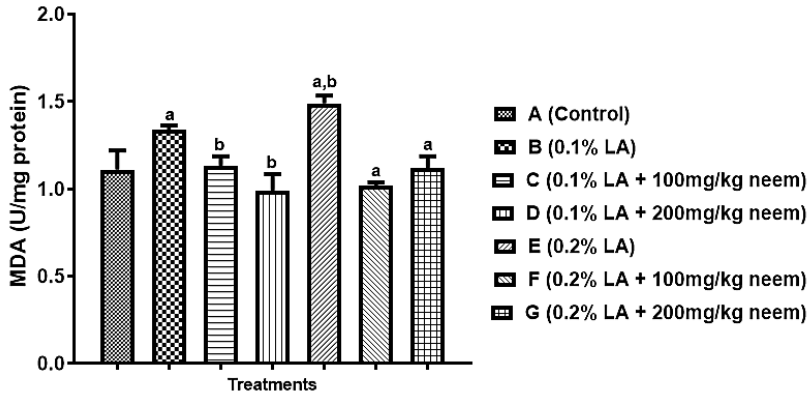

Figure 2:
The effects of *Azadirachta indica* (neem) on malondialdehyde (MDA)
LA- Lead acetate
Values are presented as mean ± standard deviation. [a] indicates significant difference at p<0.05 with respect to group A, [b] indicates significant difference at p<0.05 in comparison with group B while [c] indicates significance difference at p<0.05 in comparison with group E

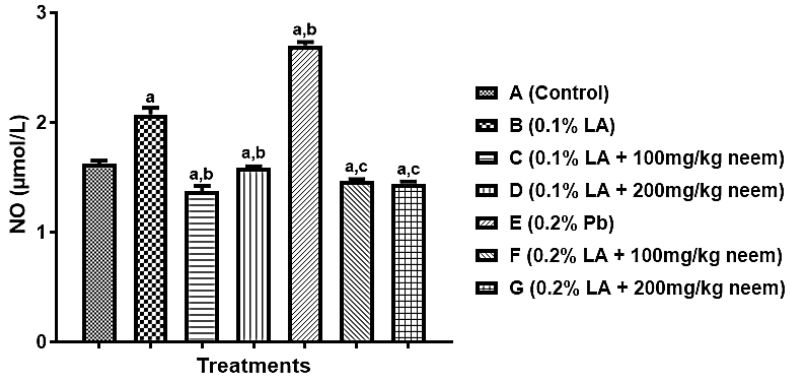

Figure 3: The effects of *Azadirachta indica* (neem) on nitric oxide (NO) content
LA- Lead acetate
Values are presented as mean ± standard deviation. [a] indicates significant difference at p<0.05 with respect to group A, [b] indicates significant difference at p<0.05 in comparison with group B while [c] indicates significance difference at p<0.05 in comparison with group E.

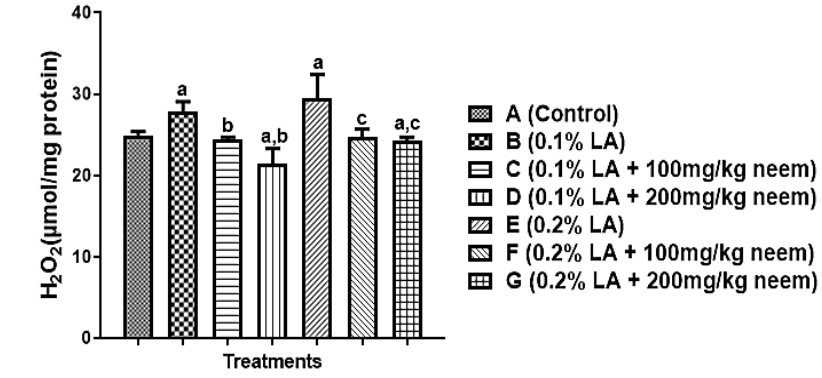

Figure 4: The effects of *Azadirachta indica* (neem) on hydrogen peroxide (H_2O_2) generation (µmol/mg protein)
LA- Lead acetate
Values are presented as mean ± standard deviation. [a] indicates significant difference at p<0.05 with respect to group A, [b] indicates significant difference at p<0.05 in comparison with group B while [c] indicates significance difference at p<0.05 in comparison with group E.

Nitric oxide increased in groups that received0.1% LA (28%) and 0.2% LA only (67%). In comparison, a significant (p<0.05) decrease was observed in groups that receives 0.1% LA + 100mg/kg *A. indica* (15%), 0.1% LA + 200mg/kg *A. indica* (2%), 0.2% LA + 100mg/kg *A. indica* (9%) and 0.2% LA + 200mg/kg *A. indica* (11%) when compared to the control group. A significant (p<0.05) decrease in the NO level was observed also in groups that receives 0.1% LA + 100mg/kg *A. indica* (34%) and 0.1% LA + 200mg/kg *A. indica* (34%) when compared to group that receives 0.1% LA only. Compared to group that receives 0.2% LA only, NO level decreased significantly in groups that receives 0.2% LA + 100mg/kg *A. indica* and 0.2% LA + 200mg/kg *A. indica* (47%) (figure3).

In figure 4, there was a significant (p<0.05) increase in the hydrogen peroxide (H_2O_2) level in groups that was exposed to 0.1% LA only (12%) and 0.2% LA only (19%), while a significant (p<0.05) decrease in groups exposed to 0.1% LA + 200mg/kg *A. indica* (14%) and 0.2% LA + 200mg/kg *A. indica* (3%) when compared to the control group. A significant (p<0.05) reduction was seen in the level of hydrogen peroxide in group exposed to 0.1% LA + 100mg/kg *A. indica* and 0.1% LA + 200mg/kg *A. indica* (12%) when compared to the group that receives 0.1% LA only. Also, a significant (p<0.05) decrease was observed in groups exposed to 0.2% LA + 100mg/kg *A. indica* (1%) and 0.2% LA + 200mg/kg *A. indica* (18%) when compared to group that receives 0.2% LA only.

In Plate 1, following histopathological analysis, group (A) showed moderate periportal infiltration by inflammatory cells (black arrow), Group B showed focal areas of necrosis as well as moderate periportal infiltration by inflammatory cells (Black arrows). Groups C and D show mild congestion of the vessel (blue arrow), mild infiltration by inflammatory cells (Black arrows) and moderate microvesicular steatosis (orange arrow). Group E shows mild infiltration by inflammatory cells (Black arrows) and moderate disseminated steatosis (orange arrow). Finally, groups F and G showed very mild periportal infiltration by inflammatory cells (black arrows).

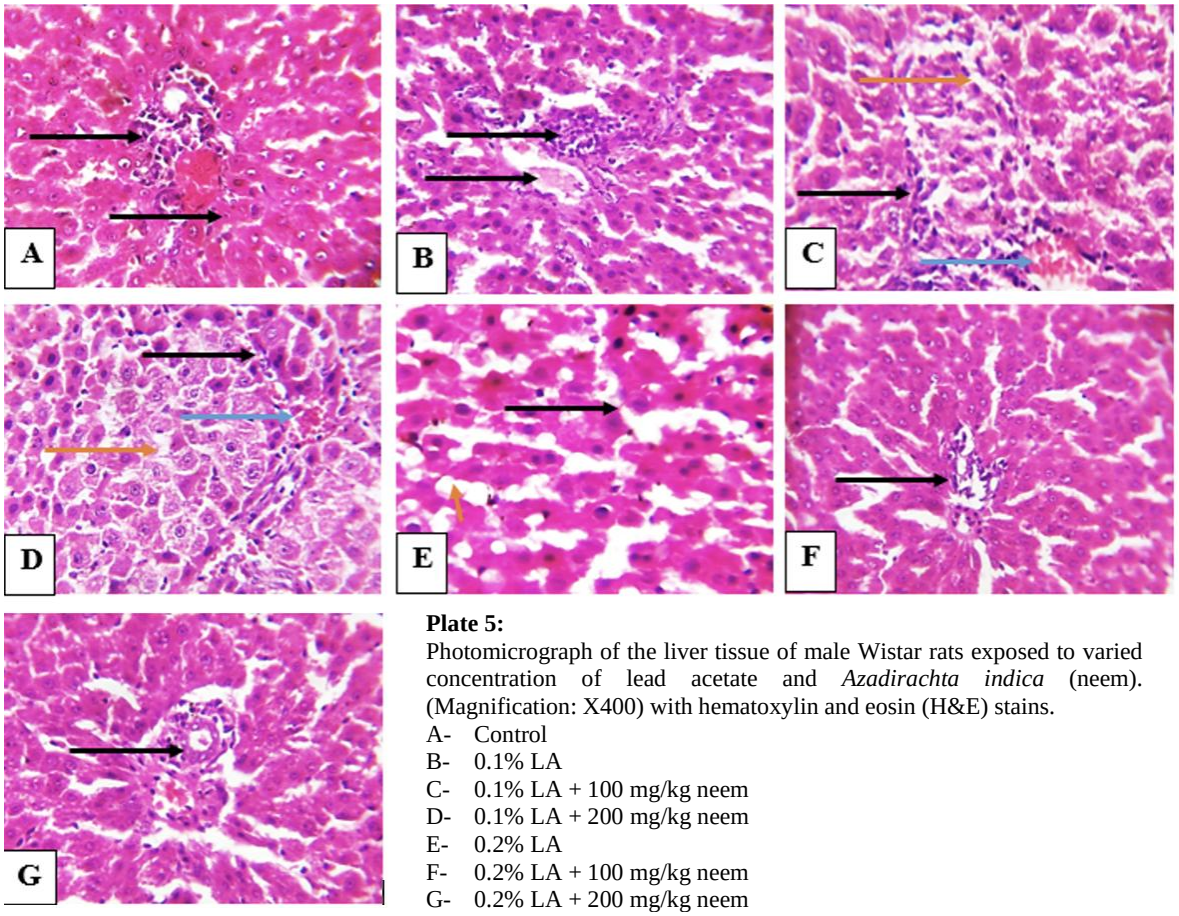

Plate 5:
Photomicrograph of the liver tissue of male Wistar rats exposed to varied concentration of lead acetate and *Azadirachta indica* (neem). (Magnification: X400) with hematoxylin and eosin (H&E) stains.
A- Control
B- 0.1% LA
C- 0.1% LA + 100 mg/kg neem
D- 0.1% LA + 200 mg/kg neem
E- 0.2% LA
F- 0.2% LA + 100 mg/kg neem
G- 0.2% LA + 200 mg/kg neem

DISCUSSION

Lead-acetate (LA) exposure, in this study, resulted in increased ALT activity, which is indicative of liver toxicity, with a corresponding high degree of hepatic histological alterations. The results of this study corroborate an earlier report of Abdelhamid et al. (2020), who reported the ameliorative effect of the antioxidant curcumin against lead acetate–induced hemato-biochemical alterations and hepatotoxicity in rats. Lead acetate reportedly depletes the antioxidant system and aggravates free radical generation (Laamech *et al*., 2017). The increased MDA level, observed in this study, indicates a compromised or damaged hepatic cellular membrane integrity. Results of this study is similar to an earlier report of Daggett et al. (1998), who attributed a 2.5-fold increase MDA levels to acute lead acetate exposure in experimental rats. Increased MDA suggests heightened lipid peroxidation which modifies cellular membrane integrity and consequently leads to hepatocellular damage (Samarghandian *et al*., 2017). However, administration of *A. indica* significantly reduced the MDA level in a dose-dependent manner. Also, the elevated level of nitric oxide, an inflammatory marker and hydrogen peroxide in the animal group administered with LA- only relative to those administered *A. indica* extract which has decreased NO levels at 100 and 200 mg/kg, confirms the antioxidant property of the plant extract.

The mechanism for lead hepatotoxicity is associated with decreased radical scavenging capacity of antioxidants in the liver. Observation of increased SOD and CAT levels, as seen in this study, may suggest an adaptive response to increased production of prooxidants, including superoxide, as well as hydroxyl radicals. Furthermore, from this study, LA-administration at different concentrations brought down the systemic levels of GPx, CAT and GST. However, administration of neem extract ameliorates this effect by increasing activities/ levels of the antioxidant enzymes and molecules. A slight difference observed in GSH level, after administration of *A. indica* was similar to the report of Chattopadhyay and Bandyopadhyay (2005) on the hepatoprotective activity of *A. indica*, where supplementation with *A. indica* increased its level. This could be adduced to the anti-lipid peroxidative effect of the extract (Nwobodo, 2017), thereby maintaining the cell membrane integrity. Therefore, the decrease in antioxidant enzyme activity with increased MDA levels suggest decreased antioxidant potential. Previous studies also revealed that LA induced toxicities may involve the modulation of delta-aminolevulinic acid (ALA) amassed in tissue as a response to ALAD (delta-aminolevulinic acid dehydratase) activity inhibition, as earlier reported by Kumar *et al*. (2017). In this study, LA exposure partially causes hepatic structural alteration, similar to the study of Abdelhamid *et al*. (2020) following histopathological research associated with increased liver biomarkers levels, which were moderately ameliorated by neem extract.

From the observations in this study, it can be concluded that *A. indica* could increase enzymatic/ non-enzymatic antioxidants and thus has hepatoprotective effects on lead-acetate exposed Wistar rats.

REFERENCES

Abdelhamid, M. F., Mahgoub, A. H., & Ateya, I. A. (2020). Ameliorative effect of curcumin against lead acetate–induced

hemato-biochemical alterations, hepatotoxicity, and testicular oxidative damage in rats. Environmental Science and Pollution Research, 27(10), 10950-10965.

Andjelkovic, M., Buha, D. A., Antonijevic, E., Antonijevic, B., Stanic, M., Kotur-Stevuljevic, J., Spasojevic-Kalimanovska, V., Jovanovic, M., Boricic, N., Wallace, D., & Bulat, Z. (2019). Toxic effect of acute cadmium and lead exposure in rat blood, liver, and kidney. International Journal of Environmental Research and Public Health, 16, 274.

Aneja, S., Vats, M., Aggarwal, S., & Sardana, S. (2013). Phytochemistry and hepatoprotective activity of aqueous extract of Amaranthus tricolor Linn. roots. Journal of Ayurveda and integrative medicine, 4(4), 211–215.

Arivazhagan, S., Balasenthil, S., & Nagini, S. (2000). Modulatory effects of garlic and neem leaf extracts on N-methyl-N0-nitro Nnitrosoguanidine (MNNG)-induced oxidative stress in Wistar rats. Cell Biochemistry & Function, 18,17–21

Beutler, E., & Kelly, B. M. The effect of sodium nitrite on red cell GSH. Cellular and Molecular Life Sciences, 19, 96 – 97

Chattopadhyay, R.R., & Bandyopadhyay M. (2005). Effect of Azadirachta indica leaf extract on serum lipid profile changes in normal and streptozotocin induced diabetic rats. African Journal of Biomedical Research, 8,101 – 104.

Dobrakowski, M., Pawlas, N., Kasperczyk, A., Kozłowska, A., Olewińska, E., Machoń-Grecka, A., Kasperczyk, S. Oxidative DNA damage and oxidative stress in lead-exposed workers. Human & Experimental Toxicology. 36, 744-754.

Drury, RA.B., Wallington, E.A, & Cancerson, R. (1976). Carleton's Histopathological Techniques, 4th ed., Oxford University Press, Oxford/London/New York, 1976.

García-Niño, W.R., & Pedraza-Chaverri, J. (2014). Protective effect of curcumin against heavy metals-induced liver damage. Food and Chemical Toxicology 69:182–201.

Gornall, A.G., Bardawill, C.J., David, M.M. (1949). Determination of serum proteins by means of the biuret reaction. Journal of Biological Chemistry, 177, 751–766.

Islas, J. F., Acosta, E., Buentello, Z., Delgado-Gallegos, J.L., Moreno-Treviño, M. G., Escalante, B., & Moreno-Cuevas, J. E. (2020). An overview of Neem (Azadirachta indica) and its potential impact on health. Journal of Functional Foods, 74, 104171.

Laamech, J., El-Hilaly, J., Fetoui, H., Chtourou, Y., Gouitaa, H., Tahraoui, A., & Lyoussi, B. (2017) Berberis vulgaris L. effects on oxidative stress and liver injury in lead-intoxicated mice. Journal of Complementary and Integrative Medicine, 14(1).

Misra, H.P., & Fridovich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biological Chemistry. 247 3170–3175.

Nwobodo, D.C, Nwosu, S.O., Ugwuene, F. O., Ihim, N. O., Ani, JK, & Ani, O. (2018). Effects of Azadirachta indica leaf aqeous extract on the antioxidant enzymes in paracetamol-induced hepatotoxicity in Wistar rats. International Journal of Biological and Chemical Sciences. 12(1), 1-10.

Nwobodo, E.I. (2017). Evaluation of antilipid peroxidation and hypolipidemic potentials of Azadirachta Indica leaf aqueous extract in paracetamol-induced hepatotoxicity in wistar rats. International Journal of Information Research and Review, 4 (2), 3615-3619.

Olaleye, S.B., Adaramoye, O.A., Erigbali, P.P., & Adeniyi, O.S. (2007). Lead exposure increases oxidative stress in the gastric mucosa of HCl/ethanol-exposed rats. World Journal of Gastroenterology. 13, 5121–5126.

Reitman, S., & Frankel, S.A. (1957). Colorimetric method for the determination of serum glutamate oxaloaceteate and pyruvate transaminases. American Journal of Clinical Pathology, 28, 56-63.

Sinha, K. A. (1972). Colorimetric assay of catalase. Analytical Biochemistry, 47, 389 94.

Singaravelu, S., Sankarapillai, J., Chandrakumari, A.S., & Sinha, P. (2019). Effect of Azadirachta indica crude bark extracts concentrations against gram-positive and gram-negative bacterial pathogens. Journal of Pharmacy and Bioallied Sciences, 2019, 11, 33-37.

Varshney, R., & Kale, R. K. (1970). Effects of calmodulin antagonists on radiation induced lipid peroxidation in microsomes. International Journal of Radiation Biology, 58, 733– 743.

Wolff, S.P. (1994). Ferrous ion oxidation in the presence of ferric ion indicator xylenol orange for measurement of hydroperoxides. Methods in Enzymology, 233, 182–189.