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RESEACH ARTICLE

PROTECTIVE EFFECT OF AQUEOUS MORINGA OLEIFERA LEAF EXTRACT AGAINST MERCURIC CHLORIDE-INDUCED HEPATOTOXICITY IN WISTAR RATS

M. A. Abubakar, M. S. Saleh*

Department of Anatomy, Faculty of Basic Medical Sciences, college of Health Sciences, Bayero University, Kano, Nigeria.

Abstract

Background: Mercury is a well-known environmental pollutant that induces severe hepatic toxicity. This study evaluated the protective effects of aqueous Moringa oleifera leaf extract on mercuric chloride-induced structural and biochemical alterations in the liver of Wistar rats**. Methods:** Twenty-five rats (90–170 g) were randomly assigned into five groups (n = 5): Group I (control), Group II (0.5 mg/kg mercury chloride), Group III (500 mg/kg Moringa extract), Group IV (500 mg/kg Moringa + 0.5 mg/kg mercury chloride), and Group V (750 mg/kg Moringa + 0.5 mg/kg mercury chloride). Treatments were administered orally for 21 days. Blood and liver tissues were collected for biochemical and histopathological analysis at the end of the study. **Results:** Mercuric chloride exposure significantly elevated serum liver enzyme levels (AST, ALT, ALP) and caused histopathological damage, including sinusoidal dilatation, fibrosis, and lymphocyte infiltration. Co-administration with Moringa oleifera extract mitigated these effects in a dose-dependent manner, as evidenced by reduced enzyme activity and preservation of liver architecture. The differences observed were statistically significant ($p < 0.001$)**. Conclusion:** These findings suggest that aqueous Moringa oleifera leaf extract exhibits hepatoprotective properties against mercury-induced toxicity, potentially through antioxidant mechanisms.

Keywords: Moringa oleifera, Mercury chloride, Liver enzymes, Hepatopathological, Wister rats

INTRODUCTION

Industrial pollution of the environment with metal compounds is becoming a significant problem. In recent years, there has been an increasing ecological and global public health concern associated with environmental contamination by these metals. Also, human exposure has risen dramatically as a result of an exponential increase of their use in several industrial, agricultural, domestic and technological applications (Bradl, 2002). Reported sources of heavy metals in the environment include geogenic, industrial, agricultural, pharmaceutical, domestic effluents, and atmospheric sources (He *et al*., 2005). Environmental pollution is very prominent in point source areas such as mining, foundries and smelters, and other metal-based industrial operations (Singh *et al*., 2022).

Moringa oleifera belongs to the Moringaceae family of perennial angiosperm plants. It is a fast growing tree that can attain a height of about 10 – 12 m with a diameter of about 45 cm. Although native to the Sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan, it is now cultivated throughout the tropical and sub-tropical regions of the world because of its numerous benefits (Mahmood *et al*., 2023). In Nigeria, Moringa oleifera is planted in all parts of the country but more in the northern part and is identified by a variety of local names including, 'Zogale' (Hausa); 'ewe igbale' (Yoruba) and 'ikwa oyibo' (Ibo) (Thilza *et al*., 2010).The leaves, fruit, flowers and immature pods of Moringa oleifera are highly nutritious and have also been utilized in ethno medicine for the treatment of various human ailments (Kumar *et al*., 2016). Specifically, the leaves are reported to be rich in proteins, mineral elements, vitamins A, C, E, beta-carotene, various

* Corresponding Author: Author: M. S. Saleh
Tel: +2348023098344
Email: sagirsaleh007@gmail.com

polyphenolic compounds and natural antioxidants (Xiao *et al*., 2023). Moreover, a variety of pharmacological activities have been attributed to the leaf extract of Moringa including anticancer, anti-inflamatory, bactericidal, hypocholesterolemic, antiatherosclerotic, antioxidant, neuro and hepatoprotective (Xiao *et al*., 2023).

Despite all the aforementioned benefits of moringa oleifera, there were limited attempts at establishing the possible protective effects of Moringa oleifera on hepatoxic effect of Mercuric chloride on the liver in wistar rats. Therefore, this study investigates the potential of Moringa oleifera to mitigate mercury-induced liver damage in Wistar rats

MATERIALS AND METHODS

A total 25 Wistar rats, grower feed (chikum), distilled water, weighing balance, Moringa Oleifera leaves, Mercury chloride, cage, syringes, needles, killing chamber, 10% formal saline, dissection kit.

Experimental design

Twenty-five adult Wistar rats weighing between 90-170g were purchased from the Animal house of the Department of Pharmacy, Bayero University, Kano. The animals were housed in the animal house of Anatomy department and were allowed to acclimatize for two weeks, and fed with Chikum grower feed and distilled water. The animals were then randomly selected and assigned into five groups (n = 5), Group I were the control, Group II were given 0.5 mg/kg mercury chloride, Group III were given 500 mg/kg Moringa extract, Group IV were given 500 mg/kg Moringa extract and 0.5 mg/kg mercury chloride concurrently, and Group V were as well given 750 mg/kg Moringa and 0.5 mg/kg mercury chloride concurrently. Treatments were administered orally for 21 days.

Ethical Consideration

The study was carried out under the guidance of the revised International Council for Laboratory Animal Science and Council for International Organizations of Medical Sciences Principles for Biomedical Research Involving Animals (2012). The guideline was followed during the care, handling, treatment, sacrifice and disposal of the chemicals and the sacrificed animals during the study.

Extract preparation

The Moringa oleifera leaves were plucked from a farm along Madobi road, Kano state. Placed in a polythene bag and transported to the Department of Plant Biology at Bayero University Kano for identification, and was assigned a reference number (BUKHAN 0011). The leaves were air-dried under a shade and were allowed to dry for five days. The dried leaves were collected and grinded to powder using mortar and pestle. The powder was weighed and was recorded to weigh 1.8kg. The powder was soaked in a brown reagent bottle with 4 liters of distilled water for 48 hours and was allowed to suspend completely. A clean cloth was used to filter the solution and separate the filtrate from the particles. The filtrate was further filtered using grade one filter paper. Then the filtrate was heated at 33 degrees Celsius in a water bath and was allowed to evaporate for 72hrs, The final residue after the water fully evaporated, wasthe extract, which was then weighed and was recorded to be 56g; with % yield of 3.11 The extract was then kept in a clean plastic container and stored in the refrigerator at 12.8 degree Celsius until usage.

Procurement of Mercury chloride

Mercury chloride was obtained from the Department of Biochemistry Bayero University, Kano. 0.1g of mercury chloride was dissolved in 100ml of distilled water.

Animal treatment

For three weeks the body weight changes were monitored daily throughout the experimental period. All drugs were administered orally and the study was conducted from December 2023 to January 2024.The oral median lethal dosage (LD50) of Moringa oleifera Lam, and mercury chloride of rats were discovered to be above 3000mg/kg (milligram per kilogram) of the body weight, and 1mg/kg (milligram per kilogram) respectively.

Animal sacrifice

The rats were sacrificed on the 19th of January 2024. The rats were then sacrificed after 24 hours from the last administration. Chloroform was used to anaesthetize the rats. Cardiac puncture was used to collect blood sample for liver enzymes assay, then the liver was harvested and fixed into a 10% formal saline. Standard method for dissection, harvesting, and processing of tissue was employed during the whole process.

Tissue processing

Following the fixation of the liver in 10% formal saline, the tissue processing continued using the following procedure:

Tissues were dehydrated in ascending grades of alcohol, from 50%, 70%, and 90% to absolute alcohol 1 and finally to absolute alcohol 2 for two hours each.Cleared in xylene 1 and xylene 2 for two hours each.Infiltrated in molten paraffin wax 1 for one hour and molten paraffin 2 for one hour.Embedded with embedding mould in molten paraffin wax.Fixed in the refrigerator for five minutes until it solidifies.The tissue block obtained was sectioned using a rotatory microtome (thickness of 5 microns). Good tissue sections which came out as ribbons s were collected on a glass slide, and placed in a water bath to float at the temperature of 30 degrees Celsius. The floating tissue was collected with another glass slide containing a tissue adhesive

(albumen) already spread on in xylene to de-wax (remove excess paraffin wax) for two minutes.

The tissues were rehydrated by passing through descending alcohols of absolute 1, absolute 2, 90%, 70%, and 50% for two minutes each.The tissues were then stained with haematoxylin for 30 minutes and rinsed in water for 10 seconds. Differentiated in 1% acid alcohol for two minutes, and rinsed with water for 10 seconds. Stained in eosin solution for 15 seconds, and rinsed with water.Then dehydrated in ascending grades of alcohol from 50%, 70%, 80%, 90%, absolute 1, and absolute 2 respectively.Cleared in xylene for 1 minute.The slides and allowed to dry for 24 hours.

Staining process

One type of staining method was employed; The Harris (1990) H & E routine laboratory tissue staining technique.

Haematoxylin and Eosin Staining

The glass slides containing the tissue for the H&E staining were placed in xylene to de-wax (remove excess paraffin wax) for two minutes. The tissues were rehydrated by passing through descending alcohols of absolute 1, absolute 2, 90%, 70%, and 50% for two minutes each.The tissues were then stained with haematoxylin for 30 minutes and rinsed in water for 10 seconds. Differentiated in 1% acid alcohol for two minutes, and rinsed with water for 10 seconds. Stained in eosin solution for 15 seconds, and rinsed with water.Then dehydrated in ascending grades of alcohol from 50%, 70%, 80%, 90%, absolute 1, and absolute 2 respectively.Cleared in xylene for 1 minute.Finally, the tissue slides were dropped with a mountant (DPX) and covered with a cover slip, ready to view with a microscope.

Biochemical Analysis

Blood samples were collected through cardiac puncture into plain sample bottles. The blood samples were centrifuged at 1000revs/min for 10 minutes to obtain the serum. The serum was then subjected to the biochemical analysis using Randox kit to check for the liver enzymes Aspartate transaminase (AST), Alanine Transaminase (ALT) and Alkaline Phosphatase (ALP) activity.

Statistical Analyses

The result of the liver enzyme assayed were presented as mean and standard deviations, comparison between groups in the measured liver enzymes was done using one-way analysis of variance (ANOVA), Turkeys' post-hoc testing was used and assessed further difference between groups. All data were analyzed with statistical package for social science (SPSS) version 21 for windows, data with p-value <0.001 was considered significant.

RESULTS

Plate I shows the H&E micrograph of the cross section of the liver of wistar rats in the control (Group I). There was no evidence of any change in the histomorphology of the hepatocytes (H), sinusoids (S) can also be seen with no any congestion, and the connective tissue (CS) component of the liver tissue is also preserved. Plate II shows the H&E micrograph of the cross section of the liver of wistar rats in the group II that were given 0.5mg\kg mercury chloride and these changes were observed in the micrograph; Fibrosis (F), Steatosis (S), Dilatation of sinusoids (DS), and Aggregation of lymphocytes (AOG). Plate III shows the H&E micrograph of the cross section of the liver of wistar rats in group three that were given 500mg/kg moringa extract only, and it showed Aggregation of lymphocytes (AOL) within the

hepatocytes. While Plate IV shows the H&E micrograph of the cross section of the liver of wistar rats in group IV that were given 0.5mg\kg of mercury chloride solution and 500mg/kg extract of Moringa oleifera, and showed only infiltration of Central vein with inflammatory cells. Plate V shows the H&E micrograph of the cross section of the liver of wistar rats in group V that were given 0.5mg\kg of mercury chloride solution and 750mg/kg extract of Moringa showed as well Infiltration of Central vein by inflammatory cells.

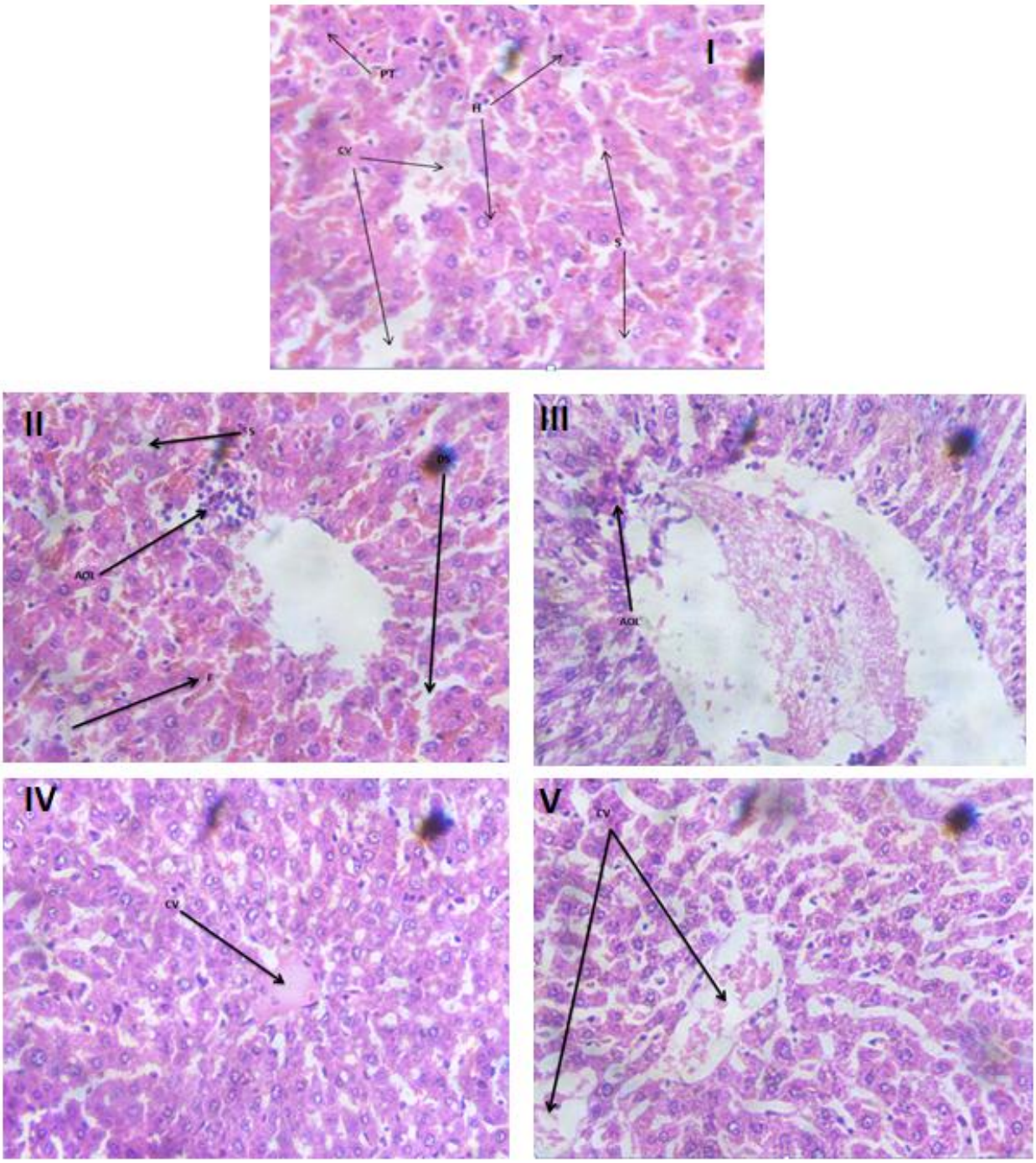

Plate I: Photomicrographs of Liver Cross Sections H&E ×100 in all the Groups; Plate I (control) showed Hepatocytes (H), Sinusoids (S), and preserved connective tissue (CS); Plate II (Group II) showed Fibrosis (F), Steatosis (S), Dilatation of sinusoids (DS), and Aggregation of lymphocytes (AOG); Plate III (group III) showed Aggregation of lymphocytes (AOL) around the hepatocytes; Plate IV (group IV) and Plate V (Group V) showed infiltration of Central vein with inflammatory cells.

Analysis of serum biochemical parameters showed an increased in liver enzymes Aspartate Transaminase (AST), Alanine transaminase (ALT) and alkaline phosphatase (ALP) activities in the group treated with mercuric chloride when compared with control group. Significant increase in (ALP) was observe in groups II ($p<0.001$) and Group V when compared to the control and a slight increase in Group III and Group IV (Table 1) (ALT) level was also observed to have significantly increased in Groups II and IV when compared to control groups ($p<0.001$).

Table1 shows the liver enzymes assessed among the different groups of rats treated with different doses of moringa extract and /or mercury over 21 days period.

Variables	**Mean ± SD**					**F-value**	**p-value**
	Group-I	Group-II	Group-III	Group-IV	Group-V		
AST	6.05±0.1	9.93±0.1	4.01 0.7	12.3±0.5	7.02±0.1	935.5	<0.001
ALT	8.02±0.06	12.00±0.06	5.95±0.05	11.03±0.05	3.97±0.07	805.65	<0.001
ALP	100.15±0.4	199.73±0.5	91.95±0.24	101.47±0.4	93.16±0.8	42566	<0.001

AST: Aspartate Transaminase; ALT: Alanine Transaminase; ALP: Alkaline Phosphatase; SD: Standard Deviation

DISCUSSION

In this study, there was no alteration in the histomorphology and enzymatic features of the liver of the control group while the distortion in both structure and enzymes of the liver were observed in rats treated with mercuric chloride. Mild aggregation of lymphocytes, enlarged or dilated sinusoids, with diffused necrotic hepatocytes were some of the characteristic changes seen on the liver section after treatment with mercury and this finding has been reported earlier in the literature (Olatuji *et al*., 2023). The specific mechanism underlying these changes may involve a role for the antioxidant defense system (Valle-Martinez *et al*., 2008). Exposure to mercury had been documented to increase the activity of liver enzymes and also causes vascular degeneration and necrotic changes in the liver of experimental animals (Jadhav *et al*., 2011).

Treatment of Wistar rats with aqueous Moringa olifera leaves extract at 500mg/kg and 750mg/kg showed preserved histoarchitecture of the liver with an intact central vein membrane and little perivascular aggregation of lymphocytes around central vein. Thus, suggesting some level of protection provided by the extract at 500mg/kg and 750mg/kg. Earlier findings suggested that the aqueous leaves extract of Moringa olifera have some adverse drug effect at high dose (Jadhav *et al*., 2011). Thus, the morphological and biochemical distortion seen following treatment with extracts and mercury could partly be contributed by both the mercury and the higher dose of moringa (Jadhav *et al*., 2011). Nevertheless, low dose of moringa has been well known to play a very important anti-oxidant effect against mercury toxicity with associated normalisation in mean value of the liver biochemical parameters (Saalu *et al*., 2008). Evaluation of liver function can be made by assay of serum enzymes like AST, ALT, ALP and total Bilirubin, which are present in higher concentration in cytoplasm (Kumar *et al*., 2016). When there is hepatopathy, these enzymes leak into the blood stream in conformity with the extent of liver damage (Ahamad & Ahmad, 2024; Yousefi *et al*., 2023), Normally AST and ALP are present in high concentration in liver, due to hepatocyte necrosis or abnormal membrane permeability; these enzymes are released from the cells and their levels in the blood increase (Ahmad & Ahmad, 2024). The present study shows a significant increase in AST, ALP and ALT levels in animals administered mercuric chloride only.

Conclusion

The present study showed that exposure to mercury caused histopathological damage and also alters the level of liver enzymes in Wistar rats. Administration of aqueous extract of Moringa oleifera leaves showed a dose dependent protective effect on the histology and liver enzyme activity in Wistar rats.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Authors' Contribution

MAA; methodology, treatment, and handling of animals, tissue processing and MSS Literature review, review and reporting of tissue micrographs and biochemical analysis.

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