Mitigating Mercury Chloride-Induced Hepatotoxicity in Wistar Rats: The Protective Potential of *Mangifera indica*

[1]Imafidon*, E.O.; [1]Aig-Unuigbe, J.E.

[1]Department of Anatomy, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Nigeria.

[*]Corresponding author
Email: endurance.imafidon@uniben.edu

Abstract

Background: Mercuric chloride, a potent toxicant containing mercury, poses a serious environmental threat, accumulating in air, water, soil, and food sources. Its impact on human health and ecosystems, particularly its tendency to target the liver, underscores the urgent need for protective strategies. *Magnifera indica*, known for its delicious taste and rich in polyphenols, vitamins, antioxidants, and phytochemicals like mangiferin and quercetin, shows promise in defending against oxidative stress, inflammation, and bolstering cellular defenses. This study is aimed at investigating protective effects of *Mangifera indica* leaves on mercuric chloride-induced liver damage in adult Wistar rats. **Materials and Methods**: Thirty (30) adult Wistar rats were randomly divided into six groups of five (5) rats each. Group A served as control. Group B was given 250 mg/kg BW of *Mangifera indica* only. Group C was given 500 mg/kg BW of *Mangifera indica* only. Group D was given 250 mg/kg BW of *Mangifera indica* and 5 mg/Kg BW of Mercuric chloride. Group E was given 500 mg/kg BW of *Mangifera indica* and 5 mg/Kg BW of Mercuric chloride. Group F was given 5 mg/Kg BW of Mercuric chloride. The administration lasted for twenty-eight (28) days. At the end of the administration, the animals were sacrificed under chloroform anaesthesia. Blood sample was collected for liver function assessment. The liver tissue was also collected for histological assessments. **Results:** Mercury exposure led to liver damage evidenced by a significant increase in alkaline phosphatase, aspartate aminotransferase, and alanine transaminase. Also, mercuric chloride resulted in significant histological alterations to the liver architecture, evidenced by periportal infiltrates of inflammatory cells and portal congestion. However, *Mangifera indica* mitigated these effects, evidenced in the significant decrease of alkaline phosphatase, aspartate aminotransferase, and alanine transaminase activities, and significant improvements in the liver histo-architecture. **Conclusion**: *Mangifera indica* possesses protective properties against mercury chloride-induced Hepatotoxicity.

Keywords: *Mercuric chloride, Magnifera indica, hepatotoxicity, Wistar rats,*

Introduction

Mercury chloride, a highly toxic compound, has been utilized for various purposes, including its historical use as a disinfectant and antiseptic (1). However, its toxicity has led to a decline in its use. Despite this, exposure to mercury chloride remains a concern due to its presence in industrial settings, contaminated water sources, and certain traditional medicines (1,2). Exposure to mercury, including mercury chloride, poses significant risks to human health. One of the most concerning aspects of mercury chloride exposure is its potential to cause liver damage. The liver, being the primary organ responsible for detoxification and metabolism, is particularly susceptible to the toxic effects of mercury chloride (1,3). This toxicity arises from mercury's ability to induce oxidative stress, disrupt cellular function, and promote inflammation within the liver (1,2,3). The consequences of

mercury-induced hepatotoxicity can range from mild liver dysfunction to severe liver damage, including cirrhosis and liver failure (3,4). In the search for effective strategies to combat mercury-induced hepatotoxicity, natural compounds with hepatoprotective properties have garnered considerable attention. Among these, *Mangifera indica*, commonly known as mango, has emerged as a promising candidate. This tropical fruit, widely consumed and utilized in traditional medicine, possesses a rich repertoire of bioactive compounds, including polyphenols, flavonoids, and vitamins (5,6,7). *Mangifera indica* exhibits potent antioxidant activity, effectively scavenging free radicals and mitigating oxidative stress (6,7), a key mechanism underlying mercury-induced liver damage. Moreover, studies have demonstrated its ability to modulate liver enzyme levels, reduce inflammation, and protect against liver cell death (8,9). Thus, this study was aimed at investigating the effects of *Mangifera indica* leaves on mercury chloride-induced liver damage in adult Wistar rats.

Materials and methods

Plant extract*: Magnifera indica* leaves were collected from a farm in Ovia North East Local Government Area of Edo state, Nigeria. The collected *Magnifera indica* leaves were thoroughly washed and air dried for some days under a shed at room temperature. The leaves were cut into smaller pieces and distilled water was then added. The mixture was boiled after which it was left to simmer in the water for about 15-20 minutes. After simmering, it was then left to cool slightly and then the liquid was strained through a fine mesh strainer into a sterile glass container. The aqueous extract was then allowed to cool to room temperature before sealing the container. The extract was then stored in the refrigerator.

Experimental animals: Thirty (30) adult Wistar rats weighing between 160g and 180g were purchased and bred at the animal house, Department of Anatomy, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Nigeria. The rats were then acclimatized for two weeks before the commencement of the study. During this period, the animals were allowed access to standard animal feed (Top feed growers mash) and clean water *ad libitum*.

Experimental design: The animals were randomly assigned into six (6) of five (5) rats each. Group A served as control; Group B was given 250 mg/kg body weight of *Magnifera indica*; Group C was given 500 mg/kg body weight of *Magnifera indica*; Group D was given 5 mg/kg body weight of mercury Chloride and 250 mg/kg body weight of *Magnifera indica*; Group E was given 5 mg/kg body weight of Mercury Chloride and 500 mg/kg body weight of *Magnifera indica*; Group F was given 5 mg/kg body weight of Mercury Chloride. All administrations lasted for 28 days and were done via oral route.

Sample collection: After 28 days, the rats were weighed. Subsequently, the rats were sacrificed under chloroform anesthesia. The rats' livers were excised, blotted clean of blood. Blood samples were collected using 5 ml syringes and then put into heparin bottles for biochemical assessments.

Liver function assessment: The blood sample collected was centrifuged at 3000 rev/min using a centrifuge for 10 min. Serum alanine aminotransaminase, alkaline phosphatase, aspartate aminotransferase, and total bilirubin were assayed for spectrometrical analysis using Randoxdiagnostic kits by calorimetric method (10).

Histological assessment: Briefly, the excised liver tissues were fixed in 10% buffered formal saline. They were then processed and routinely stained using hematoxylin and eosin, according to the method previously reported by Drury and Wallington (11).

Statistical Analysis: Data were analyzed using IBM Statistical Package for Social Sciences. Results were presented as mean ± standard error of mean (Mean ± SEM). The

parameters for all the groups were compared using Analysis of Variance (ANOVA). *Post hoc* analysis was done using Least Square Differences (LSD). Differences in means were considered significant at 95% confidence level ($p<0.05$).

Results and discussion

Mercury chloride exposure in humans initiates its metabolic conversion within the body, typically to mercuric ions. These mercuric ions bind to various proteins and biomolecules, including those crucial for cellular function, such as enzymes and structural proteins (12). As a result, they are transported via the bloodstream to different organs, prominently affecting the liver due to its role as a primary site of metabolism and detoxification. Mercury chloride is a well-known hepatotoxic agent implicated in the development and progression of liver damage, including hepatocellular injury and oxidative stress (13).

Liver enzymes are essential for maintaining overall health as they participate in numerous metabolic processes crucial for proper functioning. The levels of these enzymes serve as key indicators of liver health and function (14). Hepatocellular damage results in the release of these enzymes into the bloodstream. Elevated serum AST levels indicate liver damage similar to viral hepatitis, infarction, and muscle deterioration. ALT, which is specific to the liver and mediates the conversion of alanine to pyruvate and glutamate, is a valuable biomarker of hepatic injury. Increased levels of these enzymes signify cellular invasion and disruption of liver cell membrane integrity (14,15). Additionally, ALP binds to membranes, and its alteration can affect membrane permeability and disrupt metabolite transport (15). This study showed that mercury chloride significantly increased the activity levels of key liver enzymes, including AST, ALT and ALP in rats exposed to mercury chloride only when compared to control. Mercury chloride has been reported to induce hepatotoxicity by disrupting liver function and affecting key biomarkers (15,16). However, *M. indica* significantly improved liver enzyme activity; as evidenced by the significant reduction in AST, ALT and ALP in mercury chloride-exposed rats treated with *M. indica* when compared to the mercury chloride-only group. *M. indica* has been reported to be a rich source of antioxidants which is implicated in the induction of detoxifying enzymes (17). These antioxidants help stabilize liver cell membranes, preventing the leakage of liver enzymes into the bloodstream (17). Also, *M. indica* has been reported to possess regenerative potential which promote the healing and repair of damaged liver tissues, leading to a reduction in elevated liver enzymes (18).

Mercury chloride is well-documented for its profound hepatotoxic effects, eliciting significant histological alterations in liver tissue that underscore its harmful impact on hepatic architecture and function (19). Findings from this study showed that upon exposure to mercury chloride, liver tissue exhibited a spectrum of pathological changes indicative of severe toxicity. Notably, there was a marked inflammatory response characterized by periportal infiltrates of inflammatory cells, including lymphocytes and macrophages. These infiltrates signify an active immune reaction against mercury-induced cellular injury within the liver. In addition to the inflammatory response, histological examination revealed prominent vascular disturbances, most notably portal congestion. This condition manifested as dilatation and engorgement of portal veins, disrupting normal blood flow and exacerbating liver dysfunction. Concurrently, hepatocytes, the principal functional cells of the liver, demonstrated varying degrees of damage ranging from cellular swelling and vacuolation to necrosis. This aligns with previous reports indicating that mercury chloride disrupts liver function and initiates a cascade of cellular events that result in histological alterations. These alterations include inflammation, formation of vacuoles, and

necrosis (18,20). However, *M. indica* mitigated these mercury chloride-induced histological alterations. Rats treated with *M. indica* extract showed varying degrees of improvement in liver histology as evidenced by relatively normal histo-architecture.

Conclusion

Findings from this study suggest that exhibits a protective effect against mercury chloride-induced hepatotoxicity in Wistar rats. This effect is attributed to the plant's potent antioxidant, anti-inflammatory, and hepatoprotective properties.

Table 1: showing body weight change, liver weight and hepatosomatic indices across the experimental groups.

	Control	***M. indica* (250mg/kg)**	***M. indica* (500mg/kg)**	**$HgCl_2$ (5mg/Kg) + *M. indica* (250mg/kg)**	**$HgCl_2$ (5mg/Kg) + *M. indica* (500mg/kg)**	**$HgCl_2$ (5mg/Kg)**	**P Value**
Body weight change (g)	55.525 ±5.775	35.540±10.044	37.080 ±3.278	36.900 ±9.763	28.950 ±15.050	47.675 ±6.266	0.335
Liver weight (g)	7.925 ±0.496	7.160 ±0.778	7.420 ±0.312	7.275 ±0.202	8.500 ±0.500	8.700 ±0.609	0.312
Hepatosomatic index	4.318 ±0.181	4.406 ±0.359	4.510 ±0.185	4.538 ±0.085	5.285 ±0.745	5.123 ±0.247*	0.190

Values are given as mean ±SEM.

Table 2: showing the liver function indices across the experimental groups.

	Control	***M. indica* (250mg/kg)**	***M. indica* (500mg/kg)**	**$HgCl_2$ (5mg/Kg) + *M. indica* (250mg/kg)**	**$HgCl_2$ (5mg/Kg) + *M. indica* (500mg/kg)**	**$HgCl_2$ (5mg/Kg)**	**P Value**
ALP (u/l)	336.50±48.92	345.60±38.79	311.25±41.41	385.25±40.88$^{\#}$	396.25±30.47$^{\#}$	545.20±40.67*	0.033
AST (u/l)	2.60±0.71	2.74±0.84	2.70±0.74	3.00±0.75$^{\#}$	3.30±0.29$^{\#}$	4.50±0.29*	0.028
ALT (u/l)	1.00±0.48	1.25±0.37	2.00±0.51	1.50±0.29$^{\#}$	2.80±0.01$^{\#}$	3.25±0.48*	0.031
Total bilirubin (mg/dl)	0.93±0.11	1.08±0.24	0.84±0.12	1.03±0.08	0.75±0.03	0.95±0.23	0.201

*Values are given as mean ±SEM. * $p<0.05$ (significantly different) compared with the control group; # $p<0.05$ (significantly different) compared to the mercury chloride-only group.*

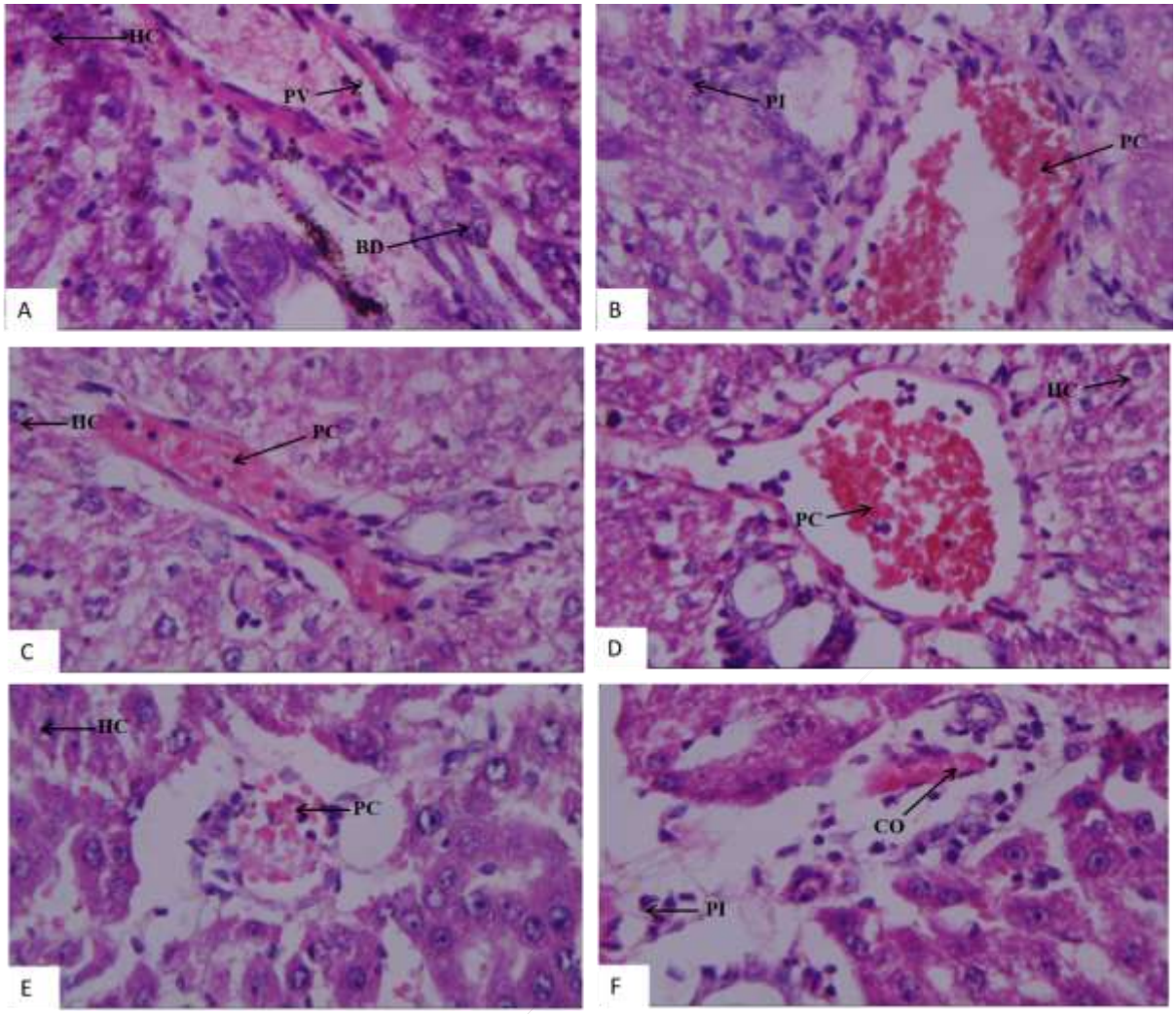

Figure 1: *Representative histology of the liver in* ***(A)*** *Control showing normal architecture: hepatocytes (HC), portal vein (PV), bile duct (BD);* ***(B)*** *Rat given $HgCl_2$ showing: periportal infiltrates of inflammatory cells (PI), portal congestion (PC);* ***(C)*** *Rat given 250mg/kg Magnifera indica showing normal architecture: hepatocytes (HC), active portal congestion (PC);* ***(D)*** *Rat given 500mg/kg Magnifera indica showing normal architecture: hepatocytes (HC), active portal congestion (PC);* ***(E)*** *given 250mg/kg Magnifera indica + $HgCl_2$ showing normal architecture: hepatocytes (HC), active portal congestion (PC);* ***(F)*** *Rat given 500mg/kg Magnifera indica + $HgCl_2$ showing: portal congestion (CO), periportal infiltrates of inflammatory cells (PI). [H&E; 400x].*

References

1. Zhao, M., Li, Y., and Wang, Z. (2022). Mercury and mercury-containing preparations: history of use, clinical applications, pharmacology, toxicology, and pharmacokinetics in traditional Chinese medicine. *Frontiers in Pharmacology*, *13*, 807807.
2. Broussard, L. A., Hammett-Stabler, C. A., Winecker, R. E., and Ropero-Miller, J. D. (2002). The toxicology of mercury. *Laboratory Medicine*, *33*(8), 614-625.
3. Balali-Mood, M., Naseri, K., Tahergorabi, Z., Khazdair, M. R., and Sadeghi, M. (2021). Toxic mechanisms of five heavy metals: mercury, lead, chromium, cadmium, and arsenic. *Frontiers in Pharmacology*, *12*, 643972.

4. Bjørklund, G., Dadar, M., Mutter, J., and Aaseth, J. (2017). The toxicology of mercury: Current research and emerging trends. *Environmental Research*, *159*, 545-554.
5. Shah, K. A., Patel, M. B., Patel, R. J., and Parmar, P. K. (2010). Mangifera indica (mango). *Pharmacognosy reviews*, *4*(7), 42.
6. Kim, H., Moon, J.Y., Kim, H., Lee, D.S., Cho, M., Choi, H.K., Kim, Y.S., Mosaddik, A. and Cho, S.K. (2010). Antioxidant and antiproliferative activities of mango (Mangifera indica L.) flesh and peel. *Food Chemistry*, *121*(2), 429-436.
7. Jahurul, M.H.A., Zaidul, I.S.M., Ghafoor, K., Al-Juhaimi, F.Y., Nyam, K.L., Norulaini, N.A.N., Sahena, F. and Omar, A.M. (2015). Mango (Mangifera indica L.) by-products and their valuable components: A review. *Food Chemistry*, *183*, 173-180.
8. Das, J., Ghosh, J., Roy, A., and Sil, P. C. (2012). Mangiferin exerts hepatoprotective activity against D-galactosamine induced acute toxicity and oxidative/nitrosative stress via Nrf2–NFκB pathways. *Toxicology and applied pharmacology*, *260*(1), 35-47.
9. Yoopum, S., Wongmanee, N., Rojanaverawong, W., Rattanapunya, S., Sumsakul, W., & Hanchang, W. (2023). Mango (Mangifera indica L.) seed kernel extract suppresses hyperglycemia by modulating pancreatic β cell apoptosis and dysfunction and hepatic glucose metabolism in diabetic rats. *Environmental Science and Pollution Research*, *30*(59), 123286-123308.
10. Reitman, S., & Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology*, *28*(1), 56-63.
11. Drury, R. A. B., & Wallington, E. A. (1980). General Staining Procedures. In R. A. B. Drury & E. A. Wallington (Eds.), Carleton's Histological Techniques (5th ed., pp. 125-150). Oxford University Press.
12. Carocci, A., Rovito, N., Sinicropi, M. S., & Genchi, G. (2014). Mercury toxicity and neurodegenerative effects. *Reviews of environmental contamination and toxicology*, 1-18.
13. Goudarzi, M., Kalantar, M., & Kalantar, H. (2017). The hepatoprotective effect of gallic acid on mercuric chloride-induced liver damage in rats. *Jundishapur Journal of Natural Pharmaceutical Products*, *12*(4).
14. Ueno, T., & Komatsu, M. (2017). Autophagy in the liver: functions in health and disease. *Nature reviews Gastroenterology & hepatology*, *14*(3), 170-184.
15. Elewa, M. A., Al-Gayyar, M. M., Schaalan, M. F., Abd El Galil, K. H., Ebrahim, M. A., & El-Shishtawy, M. M. (2015). Hepatoprotective and anti-tumor effects of targeting MMP-9 in hepatocellular carcinoma and its relation to vascular invasion markers. *Clinical & experimental metastasis*, *32*, 479-493.
16. Caglayan, C., Kandemir, F. M., Darendelioğlu, E., Yıldırım, S., Kucukler, S., & Dortbudak, M. B. (2019). Rutin ameliorates mercuric chloride-induced hepatotoxicity in rats via interfering with oxidative stress, inflammation and apoptosis. *Journal of Trace Elements in Medicine and Biology*, *56*, 60-68.
17. Venkatachalam, P., Jayalakshmi, N., Geetha, N., Sahi, S.V., Sharma, N.C., Rene, E.R., Sarkar, S.K. and Favas, P.J., (2017). Accumulation efficiency, genotoxicity and antioxidant defense mechanisms in medicinal plant Acalypha indica L. under lead stress. *Chemosphere*, *171*, 544-553.
18. Lam, P., Cheung, F., Tan, H. Y., Wang, N., Yuen, M. F., & Feng, Y. (2016). Hepatoprotective effects of Chinese medicinal herbs: a focus on anti-inflammatory and anti-oxidative activities. *International Journal of Molecular Sciences*, *17*(4), 465.
19. El-Shenawy, S. M., & Hassan, N. S. (2008). Comparative evaluation of the protective effect of selenium and garlic against liver and kidney damage induced by mercury chloride in the rats. *Pharmacological reports*, *60*(2), 199.

20. Trivedi, S. P., Singh, S., Trivedi, A., & Kumar, M. (2022). Mercuric chloride-induced oxidative stress, genotoxicity, haematological changes and histopathological alterations in fish Channa punctatus (B loch, 1793). *Journal of fish biology*, *100*(4), 868-883

How to Cite this article: **Imafidon, E. O. & Aig-Unuigbe, J. E. (2024).** Mitigating Mercury Chloride-Induced Hepatotoxicity in Wistar Rats: The Protective Potential of *Mangifera indica. Journal of Basic and Applied Medical Sciences*, 4(1&2), 28-34.