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EFFECTS OF ETHANOL EXTRACT OF *NEWBOULDIA LAEVIS* LEAVES ON LIVER MORPHOLOGICAL CHANGES IN MERCURY CHLORIDE EXPOSED WISTAR RATS

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ABSTRACT

Mercury chloride ($HgCl_2$), an extremely poisonous inorganic chemical, is widely used in industries and laboratories, posing substantial environmental and health concerns. This study investigates the preventive effects of Newbouldia laevis, a plant known for its therapeutic characteristics, on mercury chloride-induced hepatotoxicity in Wistar rats. Eighteen adult Wistar rats weighing between 160g and 200g were separated into six groups (n=6) and given simultaneous doses of mercury chloride and ethanol leaf extract of Newbouldia laevis (ELEN) for twenty-eight days. Mercury chloride exposure caused considerable changes in body and liver weights, with histological examination demonstrating severe liver damage characterized by vasodilation, vascular congestion, and periportal inflammatory infiltrates. However, co-administration of Newbouldia laevis had a protective effect, minimizing these negative alterations. Histological study of liver tissues from rats treated with Newbouldia laevis revealed low to mild damage, with doses of 250mg/kg revealing near-normal liver architecture and doses of 500mg/kg resulting in significant recovery, while some vascular ulcers remained. This study demonstrates that Newbouldia laevis ethanolic extract offers significant hepatoprotection against mercury chloride-induced toxicity in Wistar rats. The plant's anti-inflammatory and antioxidant properties, likely due to its flavonoids, alkaloids, and glycosides, mitigate oxidative stress and cellular damage caused by mercury exposure. Rats treated with the extract showed improved liver architecture and overall health, including significant weight gain compared to those exposed to mercury alone. These findings suggest that Newbouldia laevis could be a promising natural therapeutic agent for managing mercury-induced hepatotoxicity.

Keywords: Liver protection, Hepatotoxicity, *Newbouldia laevis*, Mercury chloride, Wistar rats.

INTRODUCTION

In recent years, health research has moved its focus away from simply treating diseases with potent pharmaceuticals and improved diagnostics and toward improving health outcomes. While synthetic medications are effective, their considerable side effects have sparked a renewed interest in herbal therapy as a safer option. *Newboudialaevis*, is a medium-sized angiosperm in the Bignoniaceae family, also known as the border tree, chieftaincy tree, or the tree of life (Rashed, 2021). It is

commonly known as Ogirisi (Igbo), Akoko (Yoruba), and Aduruku (Hausa) and has considerable cultural and therapeutic value in West Africa (Hutchison and Dalziel, 1963). The plant is known for its luxuriant leaves, tubular pink blossoms, and capacity to resist frigid temperatures (Barwick, 2004). It thrives in moist, well-drained soil ranging from guinea savannah to deep forest areas (Barwick, 2004). It contains medicinal characteristics such as antibacterial (Chukwuma and Nwachukwu, 2016), anti-inflammatory, antimalarial, and antioxidant (Ukwubile*et al.*, 2023). Various portions have been used traditionally to cure coughs, diarrhea, skin disorders, and discomfort (Burkill, 2004). Alkaloids, flavonoids, and glycosides have been identified by phytochemical investigations (Anaduaka*et al.*, 2013).

Although environmental and occupational mercury exposures have decreased, mercury continues to pose health concerns through air, water, and food (Budnik and Casteleyn, 2019). Mercury has an effect on all tissues after it has been absorbed, with the kidneys and liver being particularly vulnerable (Bridges and Zalups, 2017). Mercury chloride ($HgCl_2$), is a poisonous metallic element (Fraise, Lambert and Maillard, 2008). It is a major contaminant that causes tissue damage and peripheral neuropathy yet it was previously employed by Arab medics to cure wounds until it was considered dangerous (Norn *et al.*, 2008; Pérez, Shah and Butler, 2020). Mercury's detrimental effects vary depending on its chemical form, which influences absorption and tissue distribution. It has been linked to hepatotoxicity (Zhang *et al.*, 2022; Ozoani*et al.*, 2023) and genotoxicity (Sánchez-Alarcón*et al.*, 2021), as well as a deleterious impact on the male reproductive system (Massányi*et al.*, 2020; Kandemir*et al.*, 2020). Mercury chloride causes oxidative stress, resulting in kidney and liver damage (Yadav *et al.*, 2019; Liao *et al.*, 2022; Francis *et al.*, 2023). The liver metabolizes carbohydrates, proteins, and fats, stores glucose as glycogen, lipids, vitamins, and iron, detoxifies chemicals, creates bile, and regulates immunological function and blood flow (Kalra and Tuma, 2023). *Newbouldia laevis'* ability to prevent or reduce liver damage caused by mercury chloride has not been previously investigated despite its known therapeutic properties (Osigwe, Akah and Nworu, 2017; Oyewopo *et al.*, 2019), with the absence of prior research on the specific effects of the ethanol extract of *Newbouldia laevis* on mercury chloride-induced hepatotoxicity, this study aims to fill that gap through histopathological analyses and organ-body weight evaluation to assess the plant's efficacy and related morphological changes.

MATERIAL AND METHODS

1. Procurement and Preparation of Newbouldia laevis Leaf

Fresh leaves of *Newbouldia laevis* were sourced from Uselu Market in Egor Local Government Area, Edo State, Nigeria. The leaves were identified and authenticated with herbarium voucher number in the Department of Plant Biology and Biotechnology, University of Benin. After collection, the leaves were air-dried and ground into a fine powder using a British milling machine (Viking Exclusive Joncod, Type YL112M-2), yielding 130g of powder.

2. Preparation of Ethanol Extract of Newbouldia laevis

The powdered Newbouldia laevis leaves (130g) were soaked in absolute ethanol for 48 hours. After soaking, the mixture was filtered, and the ethanol was evaporated using a water bath at 40ºC, producing 37.2g of concentrated extract (28.6% yield). The extract was then stored in a refrigerator in the Department of Anatomy, University of Benin. The yield was calculated as follows:

$$\textit{Yield (\%)} = \frac{\textit{Final weight of ELEN (g)}}{\textit{Weight of powdered Newbouldia laevis (g)}} x\ 100$$

3. Stock Preparation and Administration of Mercury Chloride

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Mercury chloride crystals (10g) from ANOSANTEC LABORATORY® (catalog number 2040, batch number D7p6) were dissolved in 100ml of distilled water to prepare a mercury chloride solution for experimental administration.

Experimental Design

Eighteen (18) experimental adult male Wistar rats ware assigned into six (6) groups; Groups A-F comprising of three (3) rats per group and administered as follows:

Group A: Control group received 1ml of distilled water

Group B: Negative control received 4mg/kg body weight of $HgCl_2$ only

Group C: 250mg/kg body weight of ELEN only

Group D: 500mg/kg body weight of ELEN only

Group E: 250mg/kg body weight of ELEN + 4mg/kg body weight of $HgCl_2$

Group F: 500mg/kg body weight of ELEN + 4mg/kg body weight of $HgCl_2$

Group A served as the control group, whereas Groups B, C, D, E, and F served as experimental groups. All groups were allowed to acclimatize for a period of two weeks prior to administration. They were provided with unrestricted access to feed (growers mesh) and water, and daily administrations were carried out orally using an orogastric tube for a duration of twenty-eight days after acclimatization.

Histological Assessment

On the 28th day of administration, rats were euthanized with chloroform, and the heart was extracted and fixed in 10% buffered formalin for 72 hours before being processed using the hematoxylin and eosin staining technique described by Drury and Wallington (1980). Processed tissue slides were evaluated using a Leica DM750 research microscope equipped with a digital camera (LeicaICC50). Tissue sections were digitally photographed at 400x magnification.

Ethical Clearance

In the course of this research work, ethical clearance was applied for and approved by ethics committee of the College of Medical sciences, University of Benin, Benin city, Nigeria; Resignation number: CMS/REC/ 2023/340

Statistical Analysis

Data were subjected to statistical analysis using the IBM SPSS statistic software (statistical package for social science) (version 25) and relevant statistical values were obtained. One-way analysis of variance (ANOVA) was carried out and presented as mean SEM. LSD post-hoc test was used. Value of $p< 0.05$ were converted into graphical representations in form of bar charts.

RESULTS

Figure 1 - Total Body Weight:

The body weight changes in Wistar rats exposed to mercury chloride ($HgCl_2$) and treated with extracts were observed across groups. Significant weight decreases were seen in groups treated with 500 mg/kg extract alone and 250 mg/kg extract + $HgCl_2$, as compared to the $HgCl_2$-only group ($P<0.05$). No significant weight changes were noted in groups treated with only $HgCl_2$ or in those given extracts compared to the control.

Figure 2 - Liver Weight:

Across all groups, there were no significant differences in liver weight compared to the control meaning neither $HgCl_2$ exposure alone nor extract treatments caused notable changes in liver weight.

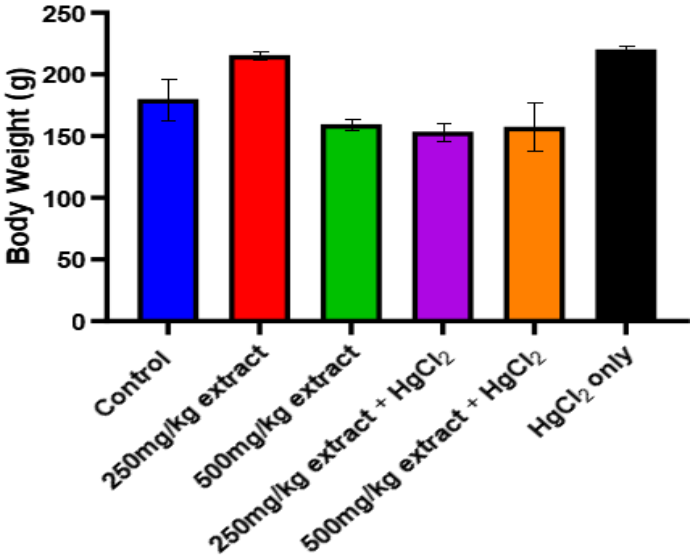

Fig 1: Total body weight of Mercury chloride toxicity induced Wistar rat treated with extract.

Figure 1: Shows the body weight change across the experimental groups. There was significant decrease in 500mg/kg extract only and 250mg/kg + $HgCl_2$ compared with $HgCl_2$ only($P<0.05$) in the body weight change of rats across the treated group when compared to the control.

There were no significant changes in groups with only $HgCl_2$ and groups treated with extract compared with control respectively. Though there were significant decrease in 500mg/kg extract only and 250mg/kg + $HgCl_2$ compared with $HgCl_2$ only.

$^{\times}P<0.05$ indicates significant difference in other groups compared with control.

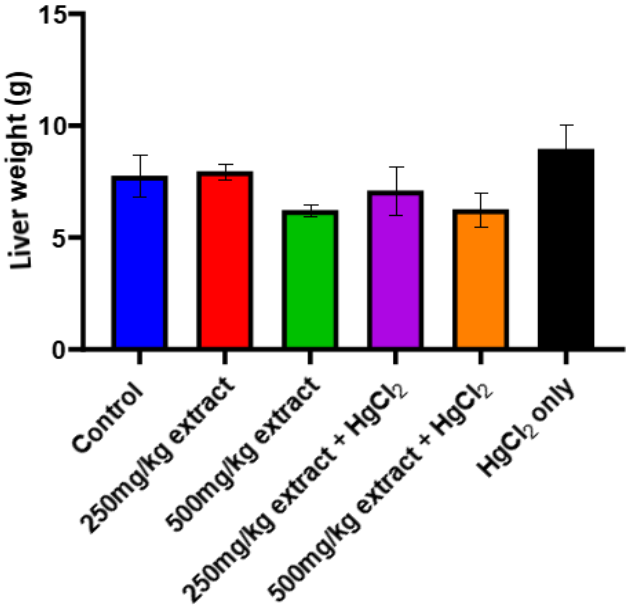

Fig 2: Liver weight of Mercury chloride toxicity induced Wistar rats treated with extract.

Figure 2: shows the liver weight change across the experimental groups. There was no significant difference ($P<0.05$) in the liver weight of rats across the treated group compared to the control. There were no significant changes in groups with HgCl2 only and groups treated with extract compared with control respectively.

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ˣP<0.05 indicates significant difference in other groups compared with control.

αP< 0.05 indicate significant difference when treated groups are compared with only HgCl.

Histopathology (Plates A-F):

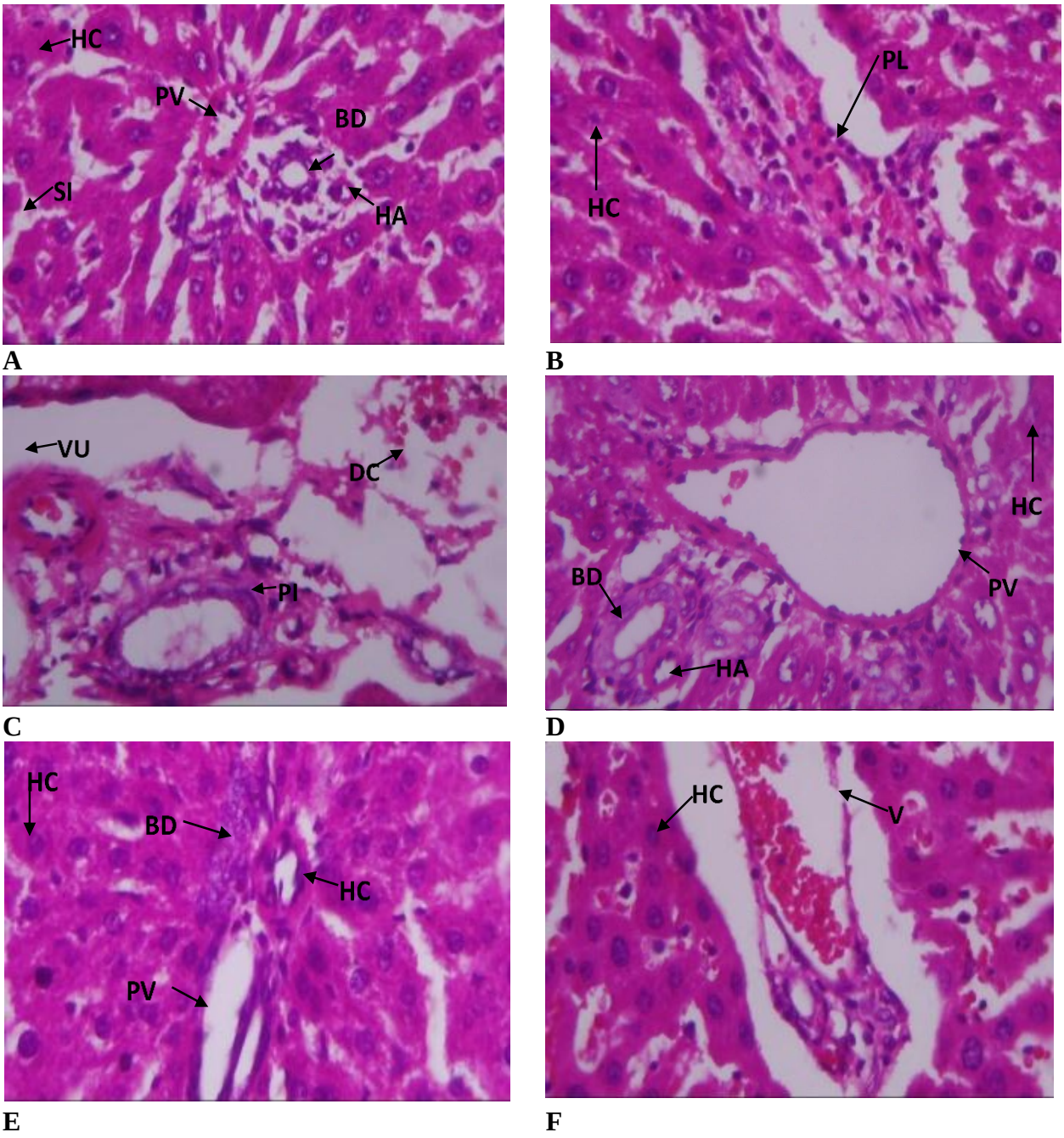

Plate A: Rat liver. Group A Composed of normal architecture: hepatocytes (HC), sinusoids (SI), portal vein (PV), bile duct (BD), hepatic artery (HA): H&E 400x, **Plate B:** Rat liver administered $HgCl_2$ only showing: severe vascular dilatation and congestion (DC), ulceration (VU), Periportal infiltrates of inflammatory cells (PI): H&E 400x, **Plate C**: Rat liver administered 250mg ELEN only showing: hepatocytes (HC), bile ducts (BD), hepatic artery (HA), portal vein (PV): H&E 400x, **Plate D:** Rat liver administered 500mg ELEN only showing: normal hepatocytes (HC), periportal mobilization of lymphocytes (PL): H&E 400x, **Plate E:** Rat liver administered $HgCl_2$ + 250mg ELEN showing normal architecture: hepatocytes (HC), portal vein (PV), bile duct (BD), hepatic artery (HA): H&E 400x and **Plate F:** Rat liver administered $HgCl_2$ + 500mg ELEN showing: normal hepatocytes (HC), vascular ulceration (VU): H&E 400x.

- Plate 1 (Control Group): Liver showed normal architecture with healthy hepatocytes, sinusoids, portal vein, bile duct, and hepatic artery.

- Plate 2 ($HgCl_2$ Only): Liver showed severe vascular dilatation, congestion, ulceration, and periportal inflammatory cell infiltration, indicating toxicity.

- Plate 3 (250 mg Extract Only): Normal hepatocytes, bile ducts, hepatic artery, and portal vein were observed, with no signs of damage.

- Plate 4 (500 mg Extract Only): Normal hepatocytes with periportal lymphocyte mobilization, indicating mild immune activity without toxicity.

- Plate 5 ($HgCl_2$ + 250 mg Extract): Liver maintained normal architecture similar to the control, suggesting protective effects.

- Plate 6 ($HgCl_2$ + 500 mg Extract): Presence of normal hepatocytes but with vascular ulceration, suggesting partial protective effects.

DISCUSSION

Weight changes serve as a sensitive indication of the general health status. This means that significant fluctuations in weight can often signal underlying health (Porwal, Khan and Maheshwari, 2017). Mercury toxicity induces weight loss through metabolic disruption and digestive impairment. It disrupts enzyme functions, affecting nutrient metabolism and absorption, leading to reduced energy availability. Mercury also triggers inflammation, increasing energy demands and further depleting the body's resources. Additionally, it disrupts hormonal balance, including thyroid and insulin regulation, which may affect appetite and metabolism, contributing to weight loss (Kawakami *et al.*, 2012). Findings from this study showed that mercury chloride intoxication resulted in decreased body weight. This weight loss may possibly be due to reduced food intake or impaired nutrient absorption. This agrees with previous studies who reported the weight loss effect of mercury chloride (Kawakami *et al.*, 2012; Rice *et al.*, 2014; Zhao *et al.*, 2020). However, on treatment with *Newbouldia laevis*, there was a significant weight gain when compared to mercury chloride only group. The *Newbouldia laevis* treatment, particularly at the higher dose (500mg), appeared to reverse the mercury chloride induced weight loss, indicating a potential improvement in overall health. This agrees with studies by Mbagwu*et al.*and Igbokwe*et al* who reported that *Newbouldia laevis*was implicated in weight gain in their respective studies.

Histological findings in this study showed that mercury caused severe damage, evident by vascular dilatation and congestion, ulceration, and periportal infiltration of inflammatory cells. These findings are consistent with known effects of mercury, which disrupts vascular function, causes cell death, and triggers inflammatory responses (Joshi *et al.*, 2014; Goudarzi, Kalantar and Kalantar, 2017; Caglayan*et al.*, 2019). However, the livers of rats pretreated with *Newbouldia laevis* showed minimal to mild damage. At the 250mg/Kg dose the liver architecture appeared almost entirely normal. Even at the 500mg/Kg dose, while some vascular ulceration persisted, the overall liver structure was significantly improved compared to the mercury-only group. Also, *Newbouldia laevis* contains antioxidants that neutralize free radicals generated by mercury chloride, reducing oxidative stress and protecting liver cells.

CONCLUSION

This study shows that *Newbouldia laevis* extract has the capacity to protect Wistar rats against mercury chloride-induced hepatotoxicity. This proves that it may be useful as an alternative treatment for mercury chloride-induced hepatotoxicity.

Recommendation

Further research is recommended to understand the mechanisms that underpin *Newbouldia laevis'* hepatoprotective

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properties against mercury chloride-induced liver damage. Furthermore, studying the ideal dosage and long-term effects of *Newbouldia laevis* treatment would yield useful information for clinical applications.

Conflict of Interest

None declared.

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