IBOM MEDICAL JOURNAL
Vol.18 No.1 | January - April, 2025 | Pages 173 - 180
www.ibommedicaljournal.org

Mitigating Mercury Chloride-Induced Spleen Toxicity in Wistar Rats: The Efficacy of *Newbouldia laevis* Ethanol Extract

Silvanus Olu INNIH[1], *Deborah CALMDAY-OMBO*[1,2]

[1]*Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin City, Nigeria.*
[2]*Department of Anatomy, Faculty of Basic Medical Sciences, Federal University Otuoke, Nigeria.*

Abstract

Background: Mercury chloride toxicity poses significant health risks, particularly affecting organs such as the spleen. *Newbouldia laevis* is reputed for its medicinal properties, yet its potential to counteract mercury chloride-induced splenic toxicity remains largely unexplored. This study investigated the effects of ethanol extract of *N. laevis* on mercury chloride-induced spleen toxicity in Wistar rats.
Method: Eighteen adult Wistar rats, weighing between 160g and 200g, were randomly divided into six groups of three rats each. Group A served as the control, while Group B and C received 250mg/kg and 500mg/kg of *N. laevis* ethanolic extract, respectively. Group D and E were treated with 250mg/kg and 500mg/kg of the extract combined with 4mg/kg of mercury chloride, respectively, and Group F received 4mg/kg of mercury chloride only. After 28 days of treatment, the rats were sacrificed under chloroform anesthesia. Blood samples were collected for hematological analysis, and the spleens were harvested for histological assessment.
Results: The results indicated a significant decrease in white blood cell count in the group treated with 500mg/kg of the extract alone, while other hematological parameters remained unchanged. There were no significant changes in body and organ weights, except for a decrease in body weight in the 500mg/kg extract-only and 250mg/kg + mercury chloride groups. Histologically, mercury chloride induced splenic necrosis and follicular hypertrophy, whereas the extract showed no effect on the follicle but caused red cell sequestration.
Conclusion: Combined treatments displayed varying effects, with a low extract dose and mercury chloride showing a normal follicle but increased red cell sequestration. These findings suggest that *Newbouldia laevis* offer protective properties against mercury chloride-induced spleen damage.

Keywords: *Newbouldia laevis*, mercury chloride toxicity, spleen protection, Wistar rats, ethanol extract

Introduction

The use of herbs and plants in traditional medicine has been an integral part of indigenous healing practices worldwide for centuries[1]. Across diverse cultures, herbal remedies have played a crucial role in treating various health ailments and promoting overall well-being. Among these medicinal plants, *Newbouldia laevis*, commonly known as the African border tree or "Ogirisi" in Nigeria[2], stands out in traditional African folk medicine[3]. Native to West Africa, *N. laevis* is highly valued for its medicinal properties and versatile applications in treating numerous health conditions. Different parts of this plant, including its leaves, stem bark, and roots, have been traditionally used for their therapeutic benefits, reflecting the rich botanical heritage of the region. Phytochemical analyses of *N. laevis* have

Corresponding Author:

Deborah CALMDAY-OMBO
Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin City, Nigeria
ORCID number: 0009-0002-6987-6521

deborah.calmday-ombo@bmedsci.uniben.edu

DOI: 10.61386/imj.v18i1.605

identified the presence of bioactive compounds such as alkaloids, flavonoids, tannins, and terpenoids[2]. These compounds contribute to the plant's pharmacological activities, including antioxidant[4], anti-inflammatory[5], analgesic[5], antimicrobial[6], and hepatoprotective effects[7]. This traditional knowledge highlights *N. laevis* as a valuable resource for promoting health and well-being within local communities.

The spleen in Wistar rats located in the upper left abdomen features red pulp for filtering damaged red blood cells and white pulp for immune functions, including lymphocyte activation[8]. During embryonic development, it forms from mesodermal tissue, differentiating into red and white pulp regions[9]. The spleen's arterial supply is via the splenic artery from the celiac trunk, while venous drainage occurs through the splenic vein to the portal vein[10]. Lymphatic drainage from the spleen supports immune function, and nerve supply from the sympathetic nervous system regulates blood flow and immune responses[9].

Mercury chloride ($HgCl_2$) is a toxic and corrosive substance used in various industrial and medical applications[11]. Mercury poisoning remains a significant global health concern, particularly in regions with high levels of environmental contamination from industrial activities, mining, and improper waste disposal. The World Health Organization (WHO) estimates that tens of millions of people are exposed to unsafe levels of mercury, leading to widespread toxicity affecting various organs[11]. Its toxicity is linked to mechanisms like oxidative stress, inflammation, and disruption of cellular processes[12]. The spleen, an essential organ for immune function and detoxification, is particularly susceptible to mercury chloride-induced damage[13]. Splenotoxicity, or damage to the spleen, poses significant health risks due to its critical role in filtering blood, storing red blood cells, and producing immune cells. Inflammation of the spleen, known as splenitis, can lead to symptoms and complications such as splenomegaly (enlarged spleen) and impaired immune function[14]. The severity of splenotoxicity and splenitis depends on various factors, including the cause, duration of inflammation, and individual health conditions. Acute splenitis may result from infections and present with symptoms like abdominal pain, fever, and fatigue[15]. Chronic inflammatory conditions, such as autoimmune diseases, can lead to persistent splenitis, resulting in long-term complications and organ damage[13]. Mercury chloride exposure can cause splenic damage, characterized by histopathological changes, immune suppression, and altered cytokine production[16,17]. Additionally, mercury chloride toxicity is associated with an increased risk of autoimmune diseases and cancer[18].

Recent interest has grown in exploring natural remedies for managing splenotoxicity and inflammatory disorders affecting the spleen. *N. laevis*, with its long history of use in African folk medicine, has emerged as a potential candidate[19]. Studies have shown that *N. laevis* possesses anti-inflammatory properties due to its bioactive compounds like flavonoids, alkaloids, and terpenoids, which inhibit pro-inflammatory cytokines, reduce oxidative stress, and modulate immune responses[2,5]. Furthermore, the plant has demonstrated hepatoprotective, antioxidant, and immunomodulatory effects, suggesting its potential efficacy in alleviating splenitis and mitigating splenotoxicity[4,7,20]. This study aims to investigate *N. laevis'* ability to alleviate splenotoxicity and reduce spleen inflammation. By assessing changes in body and organ weight, hematological indices, and histopathological alterations in splenic tissues, the study seeks to determine the extent of *Newbouldia laevis's* protective effects against splenitis and splenotoxicity.

Materials and method

The leaves of *Newbouldia laevis* used in this research were collected from the Uselu area in Benin City, Edo State. These trees, known locally as boundary trees, are commonly used as fences or boundary markers. The leaves were air-dried for several days and then pulverized into a fine powder. About 500g of the powdered leaves were exhaustively extracted through maceration for 72 hours with 2.8 liters of 70% ethanol. Filtration was carried out using a funnel and filter paper. The extract was then concentrated in an evaporating dish over a hot water bath, yielding 38.28%.

Experiment animals

Eighteen (18) adult Wistar rats weighing 165-200g were used for this study. The rats were bred in the

animal holding of Anatomy Department, University of Benin, Benin City. The animals had free access to water and Growers' mash (manufactured by Premier Feed Mills Co. Ltd, a subsidiary of Flour Mills of Nigeria Plc.) ad libitum. About 4mg/kg body weight of $HgCl_2$ was administered orally to induce splenotoxicity. Daily administration of extract and $HgCl_2$ lasted for twenty-eight days.

Histological assesment

At the end of the 28-day treatment period, the rats were euthanized using chloroform. Their Spleens were then removed and preserved in 10% buffered formalin for 72 hours. The spleen tissue was processed using the hematoxylin and eosin staining method as described by Drury and Wallington (1980). The prepared tissue slides were examined under a Leica DM750 research microscope equipped with a digital camera (Leica ICC50). Digital photos of the tissue sections were taken at 100 and 400x magnification.

Exerimental design

Eighteen (18) experimental adult Wistar rats of either sexes ware randomly assigned into six (6) groups; Groups A-F comprising of three (3) rats per group.

GROUP A: Rats served as control, they were fed with standard animal feed and clean water ad libitum.

GROUP B: rats were treated daily with 4mg/kg body weight $HgCl_2$ only.

GROUP C: rats were treated with oral administration of 250mg/kg body weight of Newbouldia laevis extract.

GROUP D: Rats were treated with oral administration of 500mg/kg body weight of *Newbouldia laevis* extract.

GROUP E: Rats were treated with oral administration of 250mg/kg body weight of extract and 4mg/kg body weight $HgCl_2$.

GROUP F: Rats were treated with oral administration of 500mg/kg body weight of extract and 4mg/kg body weight $HgCl_2$.

Statistical analysis

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

Results

The results revealed several key findings:

Body Weight: Rats treated with 500 mg/kg of the extract alone and those receiving 250 mg/kg of the extract combined with mercury chloride exhibited significant decreases in body weight (fig 1). There were no significant changes observed in other treatment groups compared to the control.

Organ Weight: There were no significant differences in spleen weight across the different treatment groups when compared to the control group (fig 2).

Hematological Results: The administration of 500 mg/kg of the extract alone led to a significant reduction in white blood cell (WBC) count and MID percentages. In contrast, the combined treatment of 250 mg/kg of the extract and mercury chloride resulted in a notable increase in WBC count. Hemoglobin levels, hematocrit, and red blood cell (RBC) counts were lower in the group treated with

Table 1: showing the mean values of hematological indices of Mercury chloride ($HgCl_2$) induced toxicity Wistar rats treated with doses of extract.

Parameters	Control	250mg/kg *extract* only	500mg/kg *extract* only	250mg/kg *extract* + $HgCl_2$	500mg/kg *extract* + $HgCl_2$	$HgCl_2$ only	p-value
WBC ($x10^9$/L)	8.133 ± 2.193	7.033 ± 1.68	4.700±0.665	11.40±3.26	7.100±0.70	8.133±1.03	0.000128
Lymphocytes (%)	94.80 ± 1.266	92.57 ± 0.74	94.80±0.56	94.33±0.80	92.73±0.87	94.03±1.56	0.000257
MID (%)	3.733 ± 1.20	5.467 ± 0.77	2.367±0.39	4.300±0.82	5.200±0.56	4.400±1.30	0.000644
Granulocytes (%)	1.467 ± 0.067	1.967 ± 0.03	2.167±0.441	1.367±0.067	2.067±0.318	1.567±0.33	0.000407
RBC ($x10^{12}$/L)	7.033 ± 0.376	7.590 ± 0.096	7.080±0.288	6.720±0.456	7.073±0.386	6.593±0.590	0.000566
Haemoglobin (mg/dl)	13.73 ± 0.617	14.57 ± 0.176	13.70±0.346	12.47±0.93	13.50±1.16	12.47±0.97	0.000118
Haematocrit (%)	39.33 ± 1.49	41.90 ± 0.50	38.10±0.929	35.47±2.73	38.73±2.76	35.43±1.91	0.000102
MCV (fl)	56.10 ± 1.50	55.30 ± 1.33	53.97±1.56	52.80±1.23	54.80±2.13	54.13±1.91	0.000102
MCH (pg)	19.50 ± 0.173	19.13 ± 0.03	19.30±0.38	18.50±0.36	19.03±1.19	18.90±0.35	0.000605
MCHC (g/dl)	34.87 ± 0.71	34.73 ± 0.84	35.97±0.98	35.13±0.186	34.77±0.87	35.07±0.817	0.000133
Platelet count ($x10^9$/L)	9.100 ± 0.173	8.133 ± 0.13*	8.300±0.27	8.033±0.26	8.933±0.41	8.500±0.10	0.000734

*P<0.05 indicates significant difference with control

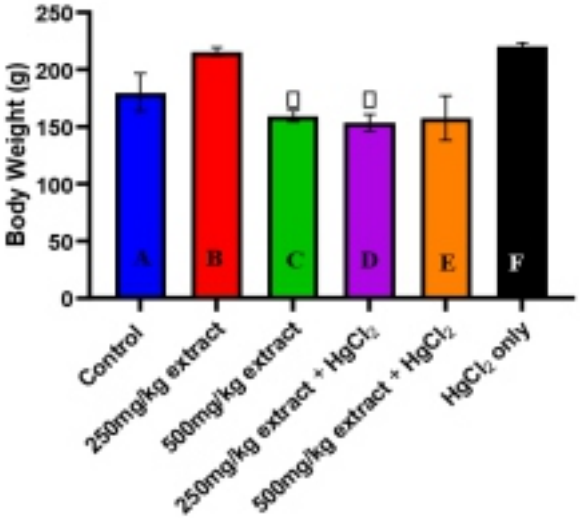

Fig 1: Total body weight of Mercury chloride toxicity induced Wistar rat treated with extract.

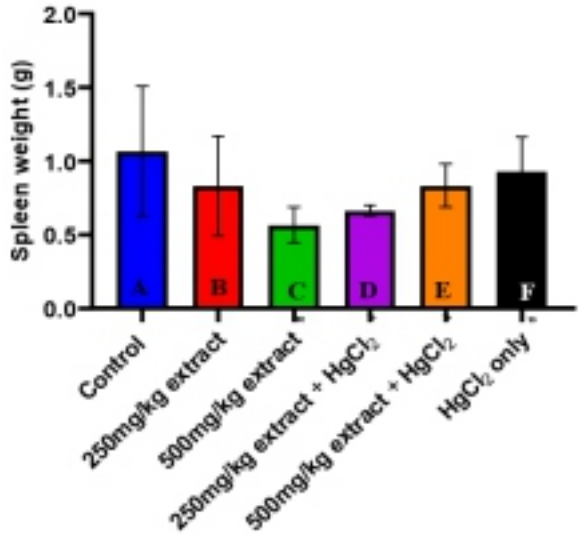

Fig 2: Spleen weight of Mercury chloride toxicity induced Wistar rats treated with extract.

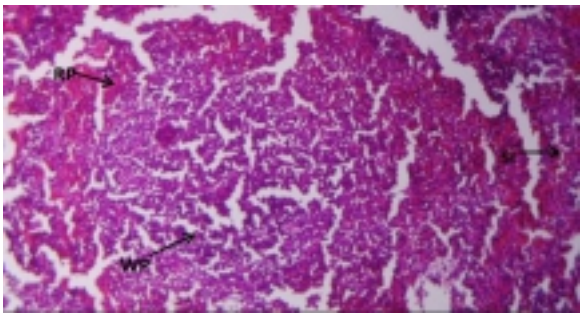

Plate 1a: Rat spleen. Control. Composed of: normal architecture: red pulp (RP), white pulp (WP), sinuses (SI): H&E 100x.

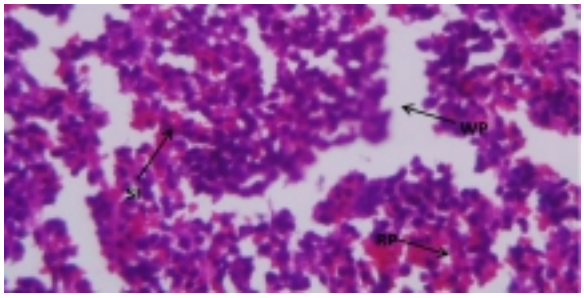

Plate 1b: Rat spleen. Control. Composed of: normal architecture: red pulp (RP), white pulp (WP), sinuses (SD): H&E 400x.

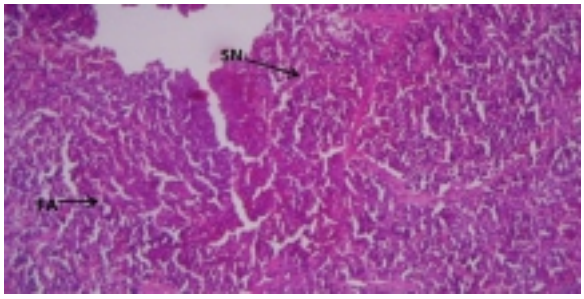

Plate 2a: Rat spleen given HgCl2 only showing: splenic necrosis (SN), follicular hypotrophy (FA): H&E 100x.

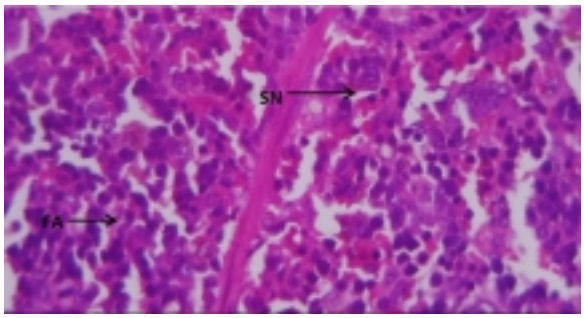

Plate 2b: Rat spleen given HgCl2 only showing: splenic necrosis (SN), follicular hypotrophy (FA): H&E 400x.

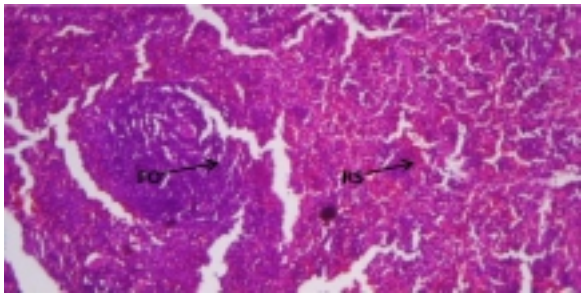

Plate 3a: Rat spleen given 250mg Extract only showing normal follicle (FO), increased red cell sequestration (RS): H&E 100x.

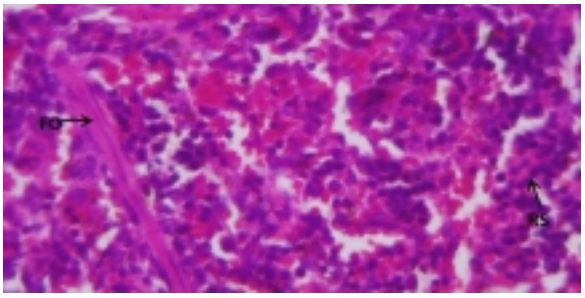

Plate 3b: Rat spleen given 250mg Extract only showing normal follicle (FO), increased red cell sequestration (RS): H&E 400x.

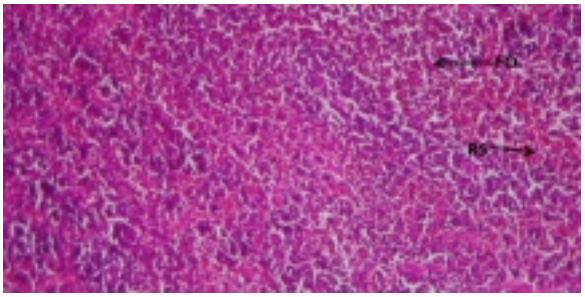

Plate 4a: Rat spleen given 500mg Extract only showing: normal follicle (FO), increased red cell sequestration (RS): H&E 100x.

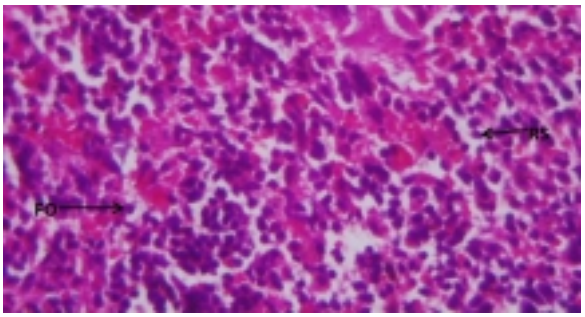

Plate 4b: Rat spleen given 500mg Extract only showing: normal follicle (FO), increased red cell sequestration (RS): H&E 400x.

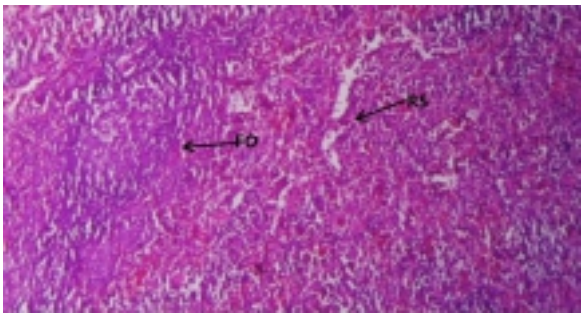

Plate 5a: Rat spleen given HgCl2 + 250mg Extract showing: normal follicle (FO) and increased red cell sequestration (RS): H&E 100x.

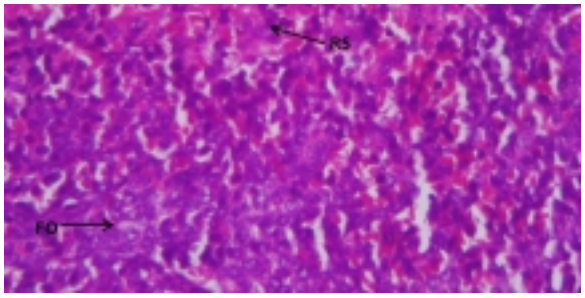

Plate 5b: Rat spleen given HgCl2 + 250mg Extract showing: normal follicle (FO) and increased red cell sequestration (RS): H&E 400x.

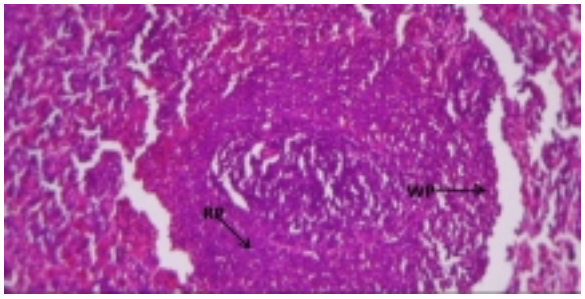

Plate 6a: Rat spleen given HgCl2 + 500mg Extract only showing: normal architecture: white pulp (WP), red pulp (RP): H&E 100x.

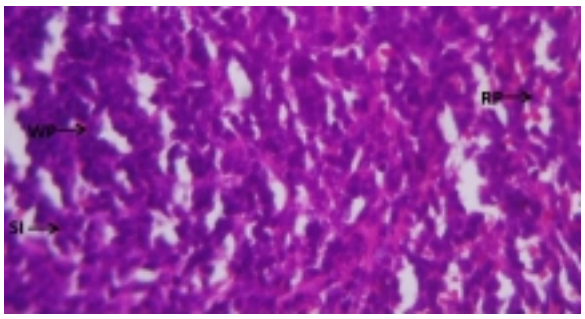

Plate 6b: Rat spleen given HgCl2 + 500mg Extract only showing: normal architecture: white pulp (WP), red pulp (RP): H&E 400x.

250 mg/kg of the extract combined with mercury chloride. Other hematological parameters, including lymphocytes, granulocytes, MCV, MCH, MCHC, and platelet counts, showed minimal changes, with some groups displaying variations but not significant deviations (table 1).

Histological Results: Histological examination revealed that mercury chloride exposure led to splenic necrosis and follicular hypotrophy (plates 2a and 2b). In contrast, treatment with 250 mg/kg or

500 mg/kg of the extract alone maintained normal follicular architecture, though increased red cell sequestration was observed (plates 3a, 3b, 4a and 4b). The combination of 250 mg/kg of the extract with mercury chloride also preserved normal follicular structure with increased red cell sequestration (plates 5a and 5b). The 500 mg/kg extract combined with mercury chloride showed normal spleen architecture (plates 6a and 6b), with no significant histopathological alterations.

Discussion

Mercury chloride ($HgCl_2$) is a highly toxic substance known to cause oxidative stress and damage to various biological systems[11]. Its toxicity involves complex interactions with cellular components, leading to the disruption of antioxidant defenses. Mercury chloride can enter cells through ingestion, inhalation, or skin contact, where it interacts with sulfhydryl (-SH) groups in proteins and enzymes, disrupting their structure and function[12].

The body weight of rats is an important indicator of their overall health and any toxic effects caused by experimental treatments. In this study, no significant changes were observed in the body weights of groups treated with $HgCl_2$ alone or in combination with *Newbouldia laevis* extract compared to the control. However, there were significant decreases in the body weight of rats treated with 500mg/kg extract only and those treated with 250mg/kg extract combined with $HgCl_2$ compared to the $HgCl_2$-only group. This suggests that *N. laevis* extract might have a modulating effect on body weight when combined with $HgCl_2$. This finding is consistent with Kolawole *et al.* (2013)[21], who reported significant reductions in body weight gains with *N. laevis* leaf extract. Interestingly, this contradicts Ibegbu et al. (2018)[22], who found that mice exposed to mercury chloride had a dose-dependent decrease in body weight compared to controls.

Similarly, spleen weights showed no significant changes in groups treated with $HgCl_2$ alone or in combination with the extract compared to the control. The absence of significant changes in organ weights indicates that *N. laevis* extract, at the given doses, does not cause any adverse effects on the spleen's mass.

Histological analysis showed that exposure to mercury chloride caused splenic necrosis and follicular hypotrophy, indicating significant pathological changes in the spleen. Splenic necrosis disrupts the normal structure and function of the spleen, leading to tissue damage and cell death, and can result from toxic exposures such as mercury chloride[23]. Necrosis can impair the spleen's microcirculation, leading to reduced blood flow (ischemia) and oxygen deprivation (hypoxia) in affected areas, compromising the viability of splenic follicles and surrounding tissues[24]. Groups treated only with *N. laevis* extract showed normal follicle structure and increased red blood cell sequestration. Groups treated with *N. laevis* extract after mercury chloride-induced damage exhibited restorative properties. This finding aligns with previous literature establishing the anti-inflammatory, antioxidant, and immunomodulatory effects of *N. laevis*[4,5,25]. The splenoprotective properties of *N. laevis* are likely due to these properties.

Hematological parameters are crucial for assessing the overall health and physiological status of experimental animals. In this study, there were no significant changes in most hematological indices among the different groups, except for a notable decrease in white blood cell (WBC) count in the group treated with 500mg/kg of *N. laevis* extract alone. This decrease in WBC count might indicate an immunomodulatory effect of the extract at higher doses. This in the agreement with the findings from the immunosuppressive activity of *N. laevis* by Ujam *et al.* (2021)[25]. However, the lack of significant changes in other hematological parameters suggests that *N. laevis* extract does not adversely affect blood health in Wistar rats. Many studies have reported decreases in WBC counts following exposure to mercury compounds, including $HgCl_2$. For instance, Hounkpatin et al. (2013)[26] demonstrated a significant reduction in WBC counts in mice exposed to mercury chloride compared to control animals. The administration of *N. laevis* extract, particularly at 500mg/kg dose, mitigated the detrimental effects of mercury chloride on the spleen, as evidenced by the preservation of normal follicle structure and the reduction of splenic necrosis and follicular hypotrophy. Additionally, the extract's

administration did not induce adverse changes in body weight, spleen weight, or most hematological parameters, suggesting its safety and efficacy at the tested doses.

Conclusion

In conclusion, the ethanol extract of *Newbouldia laevis* demonstrated protective effects against mercury chloride-induced splenic toxicity in Wistar rats, preserving spleen structure and reducing necrosis. However, at a higher dose (500 mg/kg), the extract caused a significant decrease in white blood cell count, indicating a potential immunosuppressive effect. To mitigate this negative outcome, future studies should focus on optimizing dosage to balance its protective properties with minimal immunosuppressive effects, potentially through dose adjustments or combining it with other treatments that support immune function. Further research is needed to refine its safe therapeutic application.

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