Annals of Pharmacy Practice and Pharmacotherapy
ISSN: 3062-4436
2025, Volume 5, Page No: 43-52

Available online at: www.galaxypub.co/page/journals

Galaxy Publication

Galaxy Publication

Evaluation of Long-Term Toxicity of Bischofia javanica Leaf Nanoparticles: Considerations for Therapeutic Applications

Sarah Taylor[1], Ethan Wilson[1]*, Grace Lee[1]

[1]Department of Pharmacy Administration, College of Pharmacy, University of Minnesota, Minneapolis, USA.

***E-mail** ✉ ethan.wilson@gmail.com

Received: 19 December 2024; **Revised:** 09 March 2025; **Accepted:** 11 March 2025

ABSTRACT

This research examines the long-term toxicity of nano-formulated Bischofia javanica leaves, a medicinal plant widely used in Indonesia, following OECD guideline 452. Mice were administered four different doses (2, 4, 6, and 8 g/kg body weight) of the nano-formulated extract for 60 days, while the control group received only water. On the 61st day, animals were euthanized for the collection of blood and tissue samples for biochemical, hematological, and histopathological evaluation. Data analysis was performed using one-way ANOVA with Tukey's post hoc test. Low to moderate doses (2–4 g/kg BW) enhanced liver structure and function, whereas higher doses (6–8 g/kg BW) induced liver damage characterized by fat accumulation in hepatocytes, degeneration of central blood vessels, and sinusoidal disruption. Lung tissues in high-dose groups displayed alveolar inflammation, epithelial cell shedding, cellular debris, and infiltration of inflammatory cells. Heart tissue was largely normal at lower doses, but higher doses resulted in hemorrhages and deposition of amorphous material. Apart from elevated liver injury markers at high doses, other hematological and biochemical parameters, including blood glucose, remained largely unaffected, and no treatment-related deaths were observed. Overall, moderate dosing of nano-formulated Bischofia javanica leaves appears to support organ function, but high doses can compromise vital organs, underscoring the need for cautious therapeutic use.

Keywords: Proper dosage, Bischofia javanica, Biochemical, Toxicity, Microanatomy, Hematological

How to Cite This Article: Taylor S, Wilson E, Lee G. Evaluation of Long-Term Toxicity of Bischofia javanica Leaf Nanoparticles: Considerations for Therapeutic Applications. Ann Pharm Pract Pharmacother. 2025;5:43-52. https://doi.org/10.51847/Xny0XyNCyi

Introduction

Traditional medicine continues to be widely utilized across the globe, particularly in low- and middle-income countries, due to its perceived safety when properly administered. The effective use of herbal therapies requires careful consideration of factors such as dosage, timing, route of administration, selection of ingredients, thorough evaluation of relevant information, and appropriate clinical indications. Many traditional remedies contain multiple bioactive compounds, which can produce complementary or synergistic effects to achieve therapeutic outcomes. Additionally, traditional medicine offers flexibility in its mode of administration, including decoctions, infusions, and incorporation into food, highlighting its potential as a complementary or alternative healthcare approach.

In recent years, there has been growing scientific interest in herbal medicines, driven by their potential benefits and safety profile. A fundamental step in developing these remedies is the evaluation of their toxicity. Animal-based toxicity studies provide essential evidence supporting the safety of these herbal preparations, and the choice of testing method is guided by the intended use and potential human exposure risks. One medicinal plant that has attracted considerable research attention is Bischofia javanica. Several studies have investigated its pharmacological activities. For example, locomotor effects were assessed using the Open Field (OP) test [1] and the Hole Cross (HC) test [2], while sedative effects were evaluated through thiopental sodium-induced sleep assays [3]. Its anxiolytic properties were examined by Lister (1987) and Sonavane *et al*. (2002), and its anti-diabetic potential was investigated through alpha-amylase inhibition assays [4–6]. Furthermore, the

phytochemical components of Bischofia javanica have been extensively characterized for various biological activities, including thrombolytic-preventing effects [7], anticancer activity [8], antioxidant effects [9], anti-inflammatory activity [10], anti-allergic effects [11], anti-diabetic activity [12], and anti-H. pylori activity [13]. Most of these studies administered the plant extracts orally to experimental animals.

Previous toxicity studies on Bischofia javanica have predominantly focused on acute toxicity (LD50) and cytotoxicity (LC50) evaluations [14, 15], while chronic toxicity remains largely unexplored. This study aims to address this gap by examining the long-term effects of repeated administration of nano-formulated Bischofia javanica leaves over a three-month period. The investigation also evaluates the impact of prolonged exposure on physiological parameters in growing mice, including complete blood counts, liver and kidney function markers, and histological assessments of vital organs such as the liver, kidneys, heart, and lungs, with detailed documentation of the experimental methods employed.

Materials and Methods

Preparation of nano-formulated bischofia javanica leaves

Nano-formulated Bischofia javanica leaves were produced using High Energy Milling (HEM). Initially, 2 kg of fresh leaves were thoroughly washed and air-dried in a shaded environment for one week. The dried leaves were then coarsely ground using a mechanical grinder. The resulting coarse powder was placed into a grinding container with alumina grinding balls in a 1:20 powder-to-ball mass ratio. The grinding process was performed at 350 rpm following a structured schedule: an initial 3-hour grinding period, a 1-hour break, a 6-hour grinding session, another 1-hour pause, and a final 9-hour grinding session. Particle size analysis confirmed that the leaves were successfully reduced to the nano-scale, ensuring their suitability for pharmaceutical applications.

Animal handling and treatment

Forty healthy adult male Mus musculus (20–40 g) were used in the study. Each mouse was individually labeled for identification and randomly assigned to five groups (n=6 per group). Following a ten-day acclimatization period under controlled temperature, humidity, and a 12-hour light/dark cycle, the animals were provided with standard pellet feed and water ad libitum. Four groups received daily graded doses of nano-formulated Bischofia javanica leaves, while the control group was given only water and feed. Mice were weighed weekly before administration. On day 60, the animals were humanely euthanized under mild chloroform anesthesia.

Nano-formulated leaf doses were designated as T1 (2 g/kg BW), T2 (4 g/kg BW), T3 (6 g/kg BW), and T4 (8 g/kg BW), based on previous LD50 studies that determined the LD50 at 12.6 g/kg BW. Oral administration of the leaves and sterile water followed OECD guideline 452 for chronic toxicity studies. The experimental design adhered to the 3R principles (reduce, refine, replace) to minimize animal use, and complied with international ethical standards, including the ARRIVE guidelines and the 2013 revision of the Helsinki Declaration, to ensure both scientific validity and animal welfare.

General observations

Throughout the study, animals were monitored daily to identify any potential adverse effects arising from the administration of nano-formulated Bischofia javanica leaves. Observations included feeding behavior, changes in fur color, signs of social withdrawal, indications of discomfort or pain, and any occurrences of mortality.

Hematological assessment

Blood samples were collected from the tail vein, treated with anticoagulants, and analyzed using an automated hematology system (Mindray BC-2800 Auto Hematology Analyzer). Parameters measured using the Automatic Hematology Analyzer BF-6800 included White Blood Cell (WBC) count, Red Blood Cell (RBC) count, hemoglobin (HGB), hematocrit (HCT), mixed cell count (MXD), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count, Red Cell Distribution Width-Standard Deviation (RDW-SD), lymphocyte count, neutrophil count, Red Cell Distribution Width-Coefficient Variation (RDW-CV), Platelet Distribution Width (PDW), mean platelet volume (MPV), and Platelet-Large Cell Ratio (P-LCR).

Biochemical evaluation

Twelve hours prior to euthanasia, mice were weighed, and blood was collected via cardiac puncture. Approximately 5 mL of blood was drawn into gel separator tubes, allowed to clot, and centrifuged at 3000 rpm for 15 minutes. Serum was separated and stored at -20 °C until analysis. Biochemical parameters were measured using the Automatic Biochemistry Analyzer NEUES480 (MedGroup) and included Albumin, Globulin, Total Protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), direct and indirect bilirubin, total bilirubin, Low-Density Lipoprotein (LDL), High-Density Lipoprotein (HDL), Very Low-Density Lipoprotein (VLDL), total cholesterol, triglycerides, creatinine, uric acid, blood glucose, Gamma-Glutamyl Transferase (GGT), and Blood Urea Nitrogen (BUN).

Histological analysis

Vital organs, including the liver, heart, kidneys, and lungs, were carefully excised, weighed, and fixed in 10% neutral buffered formalin. The tissues were processed for histological examination by slicing sections, dehydrating in graded alcohols, and embedding in paraffin. Sections of 4–10 µm thickness were mounted in neutral DPX medium, stained with hematoxylin and eosin, and observed under a light microscope at 40×, 100×, and 400× magnifications.

Data analysis

Data were analyzed using one-way analysis of variance (ANOVA) with a 95% confidence level, followed by Tukey's post hoc test using SPSS version 21. Results were presented in tables and graphs, with additional graphical analysis performed using GraphPad Prism version 8.0.

Results and Discussion

Macroscopic observations

Routine monitoring of food and water intake, physical appearance, and activity levels is essential in toxicity studies. In this investigation, macroscopic examination of the mice revealed no significant alterations in feeding, drinking, or exploratory behavior throughout the treatment period. Fur color, incisor height, and general appearance were also observed to ensure normal health parameters were maintained.

Body weight

Exposure to xenobiotic compounds can affect appetite, digestion, and metabolic processes, often leading to weight loss or growth retardation. This study assessed the effect of graded doses of nano-formulated Bischofia javanica leaves on body weight over an eight-week period. All experimental groups showed a progressive increase in body weight **(Figure 1)**, with no statistically significant differences compared to the control group.

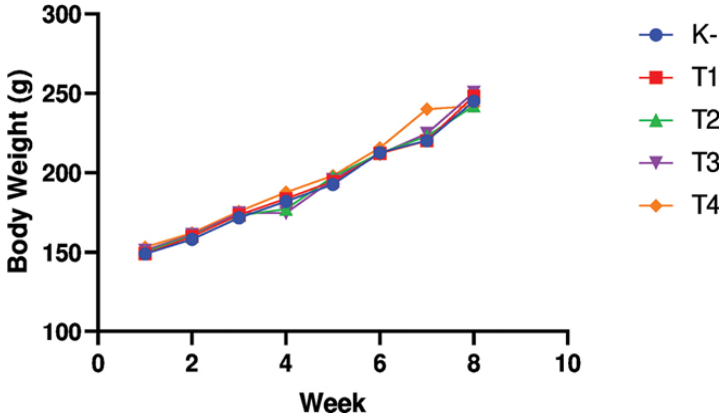


Figure 1. Weekly Body Weight Changes. Data are expressed as mean ± SEM. Groups: K- (Control), T1 (2 g/kg BW), T2 (4 g/kg BW), T3 (6 g/kg BW), T4 (8 g/kg BW).

Hematological assessment

Bioactive compounds, including herbal extracts, can influence immune function, enzymatic activity, and the formation of blood cells, sometimes leading to hematological imbalances [16]. Xenobiotic exposure may trigger

an increase in white blood cell (WBC) production as a defense mechanism [17, 18]. To evaluate the impact of Bischofia javanica leaves on blood health, key hematological parameters were measured.

In this study, WBC counts were elevated in the T3 group (6 g/kg BW), reaching 12.10 ± 0.31 compared to 8.94 ± 0.56 in the control group **(Figure 2)**. The mixed cell fraction (MXD), which includes monocytes, eosinophils, and basophils, showed significant increases at low and high doses: 19.29 ± 2.24 in T1 (2 g/kg BW) and 16.62 ± 0.84 in T4 (8 g/kg BW), versus 14.43 ± 0.93 in controls. Among all measured parameters, neutrophil levels exhibited the most pronounced dose-dependent changes. The proportion of neutrophils rose sharply in T1 (20.42 ± 1.20), T3 (15.57 ± 1.84), and T4 (14.22 ± 3.13), while T2 (4 g/kg BW) displayed a slight decrease to 7.30 ± 0.29, which did not differ significantly from the control. Similarly, neutrophil counts (×10³) were significantly higher in T1, T3, and T4 relative to controls, whereas T2 remained comparable to baseline values.

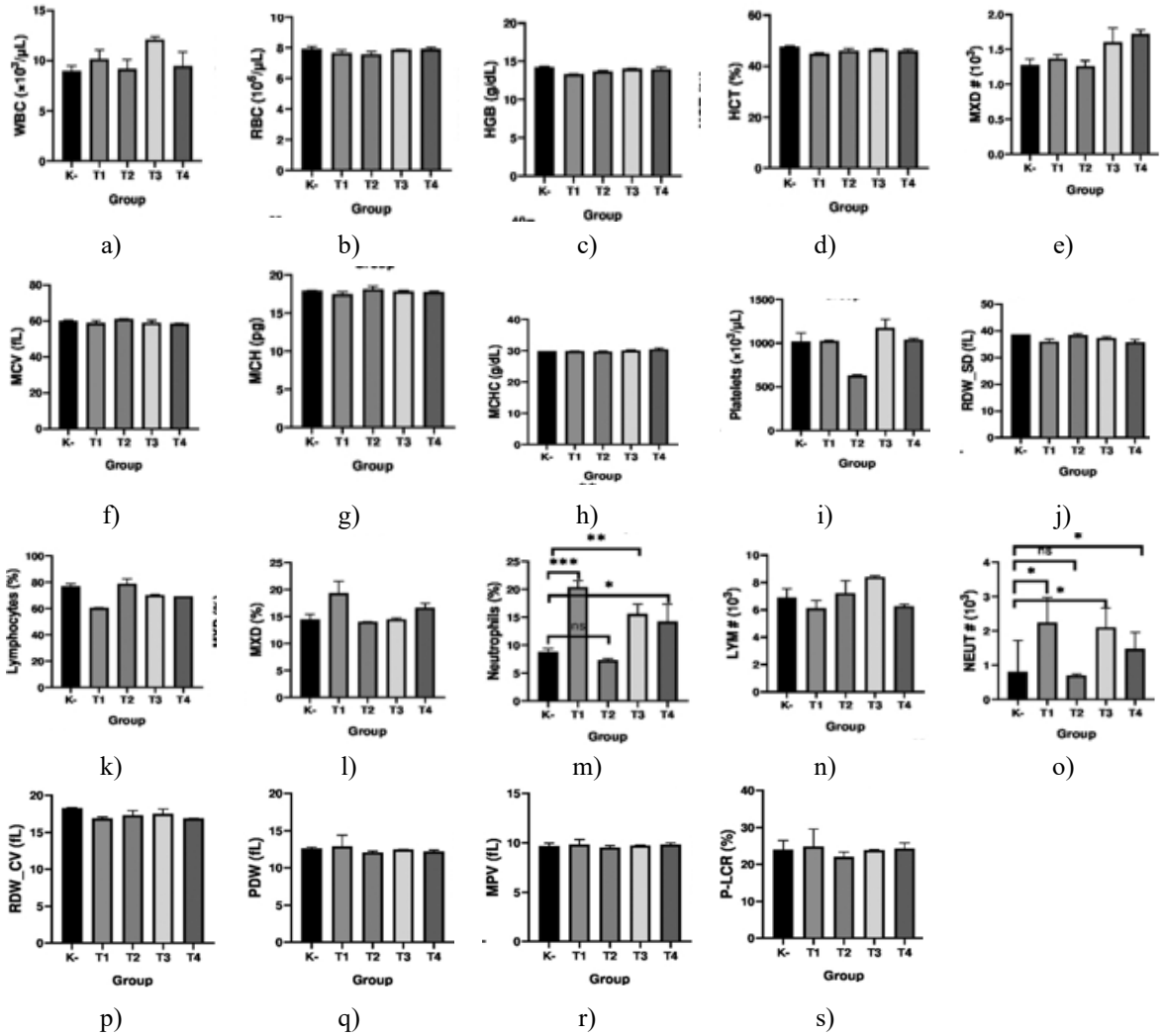


Figure 2. Influence of nano-formulated Bischofia javanica leaf extract on hematological parameters, presented as mean ± SEM. K- (Control), T1 (2 g/kg BW), T2 (4 g/kg BW), T3 (6 g/kg BW), and T4 (8 g/kg BW).

Biochemical function parameters

AST, an enzyme found in the cytoplasm and mitochondria of cardiac, hepatic, and skeletal tissues [19], and ALT, primarily present in hepatocyte cytosol, are released into the blood during liver damage, necrosis, or changes in cellular permeability, making them reliable markers for liver injury [20]. Bilirubin metabolism begins with indirect bilirubin produced from hemoglobin and red blood cell breakdown, which is converted in the liver to direct bilirubin for bile excretion. Monitoring bilirubin is critical in toxicity studies to assess red blood cell destruction and liver catabolic function. The kidneys facilitate the elimination of urea, bilirubin, and other

metabolic wastes, while the liver synthesizes triglycerides and proteins and produces roughly 80% of the body's cholesterol, reflecting hepatic synthetic capacity after herbal treatment.

Renal function was further assessed using serum creatinine and blood urea nitrogen, indicators of the kidney's ability to clear protein metabolism byproducts [21]. In this study, bilirubin concentrations remained stable at T1, T2, and T3 doses but were elevated at T4. Albumin levels were consistent with those of the control group. Both total and direct bilirubin increased in T3 and T4, while AST levels rose in all treated groups. Lipid profile changes included a reduction in HDL across all treatments, with LDL and total cholesterol showing increases; LDL was significantly higher in T2, T3, and T4 compared to controls **(Figure 3)**. However, most lipid measures did not differ significantly from control values. Blood glucose remained within normal limits and was unaffected by treatment across all groups **(Figure 3)**.

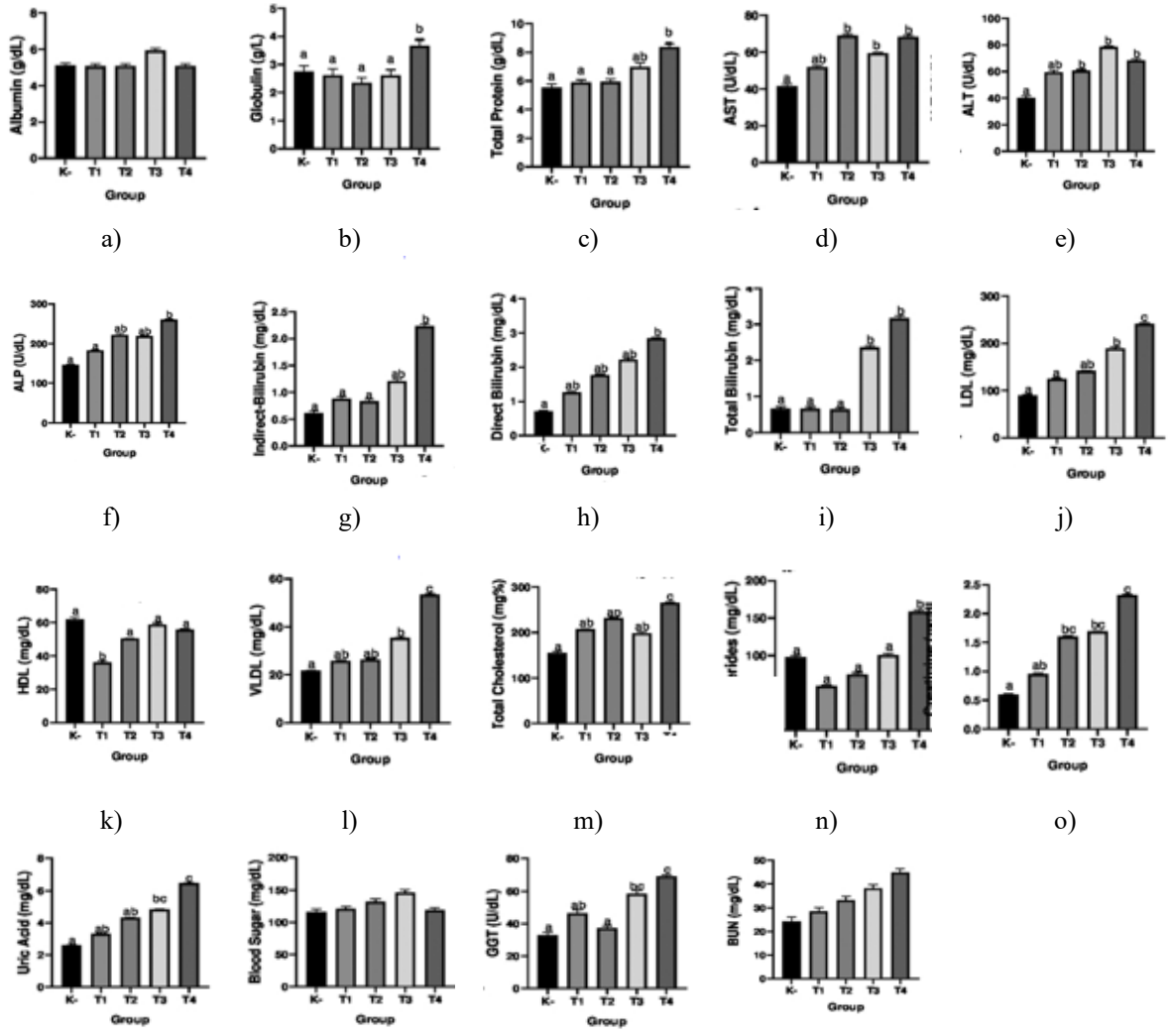


Figure 3. Influence of nano-formulated Bischofia javanica leaf extract on biochemical function parameters, reported as mean ± SEM. K- (Control), T1 (2 g/kg BW), T2 (4 g/kg BW), T3 (6 g/kg BW), and T4 (8 g/kg BW).

Organ weight

Exposure to bioactive compounds can provoke tissue and organ inflammation, which may manifest as changes in both body and organ masses. Monitoring these weight variations between treated and control groups is an important approach for detecting potential toxic effects. According to the Society of Toxicologic Pathology, organ weight analysis is a fundamental tool in general toxicity screening for bioactive substances [22–24]. In this investigation, the weights of the heart, liver, kidneys, spleen, and testes were evaluated. While reductions in organ

mass were observed across most treated groups, the liver, kidneys, and heart demonstrated significant decreases specifically in the negative control group **(Figure 4)**.

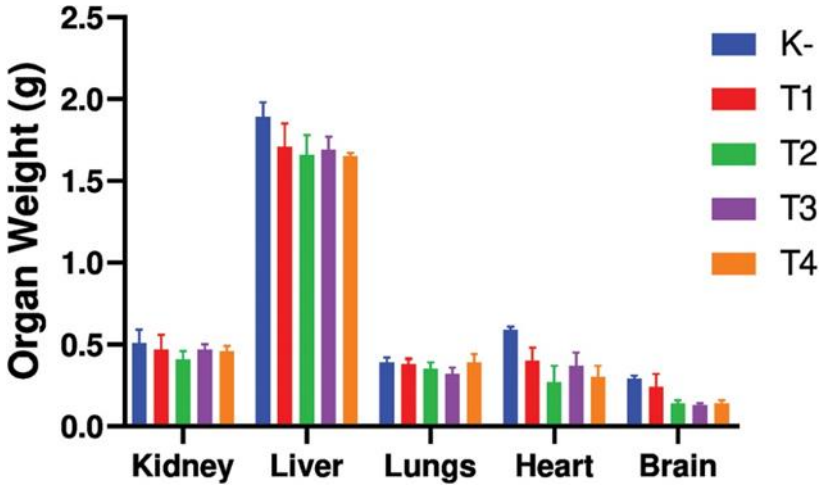


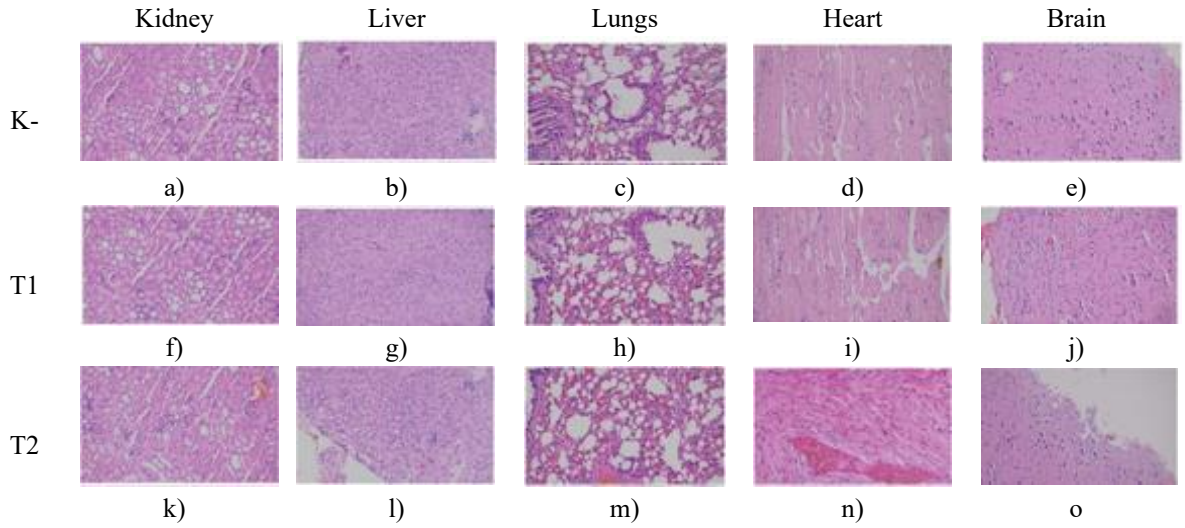
Figure 4. Relative organ weights after administration of nano-formulated Bischofia javanica leaf extract, presented as mean ± SEM. K- (Control), T1 (2 g/kg BW), T2 (4 g/kg BW), T3 (6 g/kg BW), and T4 (8 g/kg BW).

Histological analysis

To uncover subtle or early toxic effects that may not be detected through biochemical tests, microscopic examination of vital organs is indispensable. In this study, the hearts, lungs, livers, kidneys, and brains of animals treated with nano-formulated Bischofia javanica extract were evaluated and compared to those from the control group.

Liver

In untreated control animals, central veins displayed moderate to severe congestion **(Figure 5)**. By contrast, treated groups showed subcapsular hemorrhages and congestion involving central veins, sinusoids, and veins beneath the liver capsule. The lowest dose group (T1) revealed necrotic regions without inflammatory infiltration. At higher doses (T3 and T4), hepatocytes with intracellular lipid droplets were scattered throughout the tissue. Central venous congestion was also noted in livers from T1, T2, and T3 groups. The T2 group exhibited hepatocytes with microvesicular morphology and localized fatty changes. Overall, liver pathology was more pronounced in T2, T3, and T4 groups, with congestion affecting multiple regions, including subcapsular areas, sinusoids, major vessels, and central veins, and T4 livers additionally displayed foamy hepatocytes **(Figure 5)**.



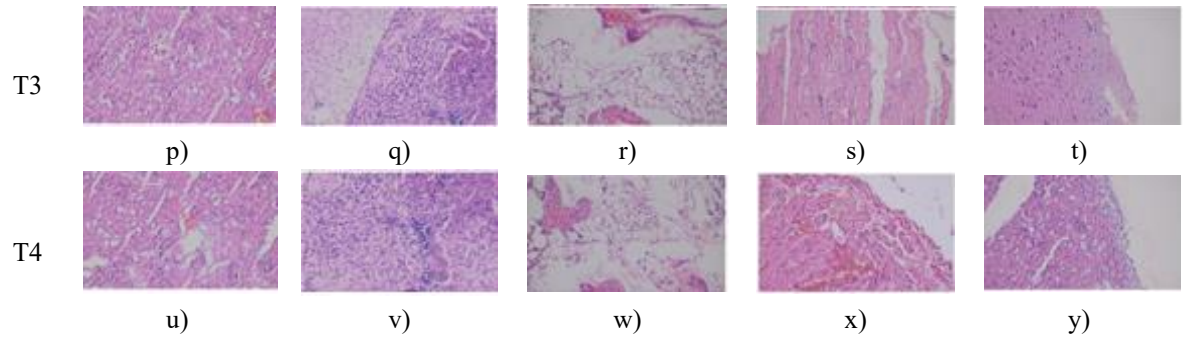


Figure 5. Microscopic examination of key organs in Mus musculus following chronic exposure to nano-formulated Bischofia javanica leaf extract. K- (Control), T1 (2 g/kg BW), T2 (4 g/kg BW), T3 (6 g/kg BW), and T4 (8 g/kg BW).

Kidney

Renal histology in control and T1 mice showed normal architecture, with well-defined glomeruli, tubules, and collecting ducts, and only minimal stromal congestion **(Figure 5)**. In contrast, T2, T3, and T4 groups displayed marked stromal congestion affecting glomeruli, tubules, and collecting ducts. The T4 group, receiving the highest extract dose, exhibited persistent inflammatory changes **(Figure 5)**.

Heart

All treated groups exhibited severely congested cardiac arteries without evidence of inflammation, fibrosis, or infarction **(Figure 5)**. Specifically, the T4 group showed highly eosinophilic myocardial fibers, localized hemorrhage with amorphous exudates, and scattered congested arteries **(Figure 5)**.

Lungs

Control lungs showed mild airway epithelial shedding, whereas chronic inflammatory changes dominated by lymphocytes and macrophages were apparent in T2–T4 groups. The T3 group had alveolar spaces filled with inflammatory cells and epithelial debris, while T4 lungs displayed moderate chronic inflammation with epithelial cell exfoliation. In T2, localized inflammatory clusters were observed, and T1 lungs showed only minor inflammatory changes **(Figure 5)**.

Brain

Brain tissue in control mice was normal, without edema or congestion **(Figure 5)**. Mild edema and focal congestion were observed in T1, while T2 and T3 exhibited more pronounced congestion after 60 days. The T4 group showed extensive focal congestion and severe edema affecting nearly all neurons in the cerebrum, indicating that prolonged high-dose exposure significantly impacts brain histology **(Figure 5)**.

Bischofia javanica, widely used in Indonesia for its therapeutic potential, was assessed here for chronic toxicity in its nano-formulated form due to limited safety data. Hematological analysis revealed no significant adverse effects or treatment-related mortality across all groups. Red and white blood cell counts remained within normal ranges, with minor increases in some groups considered non-toxicologically relevant. Medium and high doses led to slight elevations in WBC counts, but differences were not statistically significant. Macroscopic examination at euthanasia revealed minor small intestine ulceration in T4 mice.

High doses of nano-formulated Bischofia javanica caused elevated AST levels, suggesting possible hepatocyte damage or long-term liver toxicity, consistent with previous studies indicating moderate toxicity [25]. Although prior reports suggest hepatoprotective effects of active components such as ursolic acid and betulinic acid [25–27], histological analysis in this study revealed sinusoidal congestion under the capsule and within large vessels at high doses. T4 livers displayed hepatocytes with fat globules, fatty changes, and microvesicular alterations; T3 livers contained foamy hepatocytes; and T2 livers showed fat globules and reduced albumin levels, reflecting altered synthetic capacity. The decreased albumin/globulin ratio in T2 emphasizes the need for cautious dosing of Bischofia javanica. The high oil content of its seeds, previously considered inedible, further supports avoiding excessive intake [28].

Mice administered a moderate dose of nano-formulated Bischofia javanica (T3; 6 g/kg BW) demonstrated elevated blood glucose compared to controls, indicating potential disturbances in glucose metabolism, pancreatic

stress, or a predisposition toward diabetes mellitus, consistent with previous research on hyperglycemia and diabetes [29]. Chronic administration of doses exceeding 2 g/kg BW (the human-equivalent recommended dose) resulted in a reduction of blood glucose levels within normal physiological ranges, but exposure beyond this threshold may increase the risk of diabetes development [25]. Across all treatment groups, lung histology revealed inflammatory infiltration, congestion, and the presence of chronic inflammatory cells, pointing to moderate pulmonary toxicity. Lung weights were notably higher in the T4 group than in controls, highlighting that the lungs are particularly vulnerable to high doses of nano-formulated Bischofia javanica through absorption and systemic distribution.

Gastrointestinal effects were also observed, especially in the small intestine, alongside alveolar inflammation dominated by lymphocytes and macrophages, with alveolar spaces filled with cellular debris in animals receiving doses above 8 g/kg BW. Given that drugs are absorbed, distributed, and bind to target tissues to exert effects, these changes reflect the systemic impact of high-dose administration. The brain, with its high metabolic demand—receiving roughly 20% of cardiac output (about 750 ml/min)—showed histological alterations in white mice (Mus musculus), including congestion and perivascular edema. While some pathological findings are expected even in control animals, differences in lesion extent and distribution underscore the effects of high-dose exposure. Animals not bred as specific pathogen-free (SPF) may also show unanticipated variations [30]. Extended exposure to high-sulfur compounds present in the nano-formulated leaves may exacerbate cellular toxicity in sensitive organs such as the brain.

Renal toxicity was dose-dependent: low-dose (T1) treatment caused mild kidney changes, 4 g/kg BW (T2) induced moderate effects characterized by capillary obstruction in glomeruli, renal tubules, and collecting ducts, and 6 g/kg BW (T3) resulted in chronic inflammation. Kidney weights in T4 mice were significantly lower than those of controls, likely reflecting inflammation-induced atrophy [21]. These findings suggest that chronic, high-dose administration contributes to sustained inflammatory foci in renal tissue.

Cardiac effects were also observed following repeated exposure. Mice displayed minor structural damage, including dispersed but heavily congested vessels, eosinophilic muscle fibers, hemorrhagic areas with amorphous exudates, and isolated congested vessels. These changes likely result from the high concentration and prolonged exposure to the nano-formulated herb, with toxicity modulated by dose, duration, composition, and environmental factors [31]. Chronic cardiac alterations were primarily associated with vascular congestion and inflammatory changes, consistent with previous observations of herb-induced cardiac toxicity [32].

Conclusion

Prolonged administration of nano-formulated Bischofia javanica leaves at doses exceeding 4 g/kg BW for 60 days or more may adversely affect critical organ systems. Conversely, lower doses (<4 g/kg BW) appear relatively safe and may offer therapeutic benefits, including support of liver, kidney, blood, and heart function, with minimal effects on brain and lung tissue. Careful monitoring and controlled dosing are recommended, and further safety evaluations—particularly regarding teratogenic effects in pregnancy—are warranted. Additionally, ensuring the quality and safety of the plant material through proper agricultural and processing practices is crucial to prevent contamination with pesticides or other harmful substances.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

References

1. Kulkarni SK, Reddy DS. Animal behavioral models for testing antianxiety agents. Methods Find Exp Clin Pharmacol. 1996;18(3):219-30.

2. Takagi K, Watanabe M, Saito H. Studies of the spontaneous movement of animals by the hole cross test; effect of 2-dimethyl-aminoethanol and its acyl esters on the central nervous system. Jpn J Pharmacol. 1971;21(6):797-810. doi:10.1254/jjp.21.797
3. Ferrini R, Miragoli G, Taccardi B. Neuro-pharmacological studies on SB 5833, a new psychotherapeutic agent of the benzodiazepine class. Arzneimittelforschung. 1974;24(12):2029-32.
4. Lister RG. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology (Berl). 1987;92(2):80-185. doi:10.1007/BF00177912
5. Sonavane GS, Sarveiya VP, Kasture VS, Kasture SB. Anxiogenic activity of Myristica fragrans seeds. Pharmacol Biochem Behav. 2002;71(1-2):239-44. doi:10.1016/S0091-3057(01)00660-8
6. Hansawasdi C, Kawabata J, Kasai T. Alpha-amylase inhibitors from roselle (Hibiscus sabdariffa Linn.) tea. Biosci Biotechnol Biochem. 2000;64(5):1041-3. doi:10.1271/bbb.64.1041
7. Prasad S, Kashyap RS, Deopujari JY, Purohit HJ, Taori GM, Daginawala HF. Development of an in vitro model to study clot lysis activity of thrombolytic drugs. Thromb J. 2006;4(1):14. doi:10.1186/1477-9560-4-14
8. Lingadurai S, Roy S, Joseph RV, Nath LK. Antileukemic activity of the leaf extract of Bischofia javanica Blume on human leukemic cell lines. Indian J Pharmacol. 2011;43(2):143-9. doi:10.4103/0253-7613.77348
9. Lingadurai S, Kumar KP, Kant NL, Besra SE, Siromoni JRV. Free radical scavenging activity of leaves of Bischofia javanica Blume and Fraxinus floribunda Wallich. Pharmacologyonline. 2009;1:1324-32.
10. Andersson DC, Ylva N, Gudelia S, Cox PA, Premila P, Lars B. Evaluation of some Samoan and Peruvian medicinal plants by prostaglandin biosynthesis and rat ear edema assay. J Ethnopharmacol. 1997;57(1):35-46. doi:10.1016/S0378-8741(97)00043-3
11. Florin B, Asako H, Naoki H, Ryoichi Y, Toshiki E, Hisayuki M, et al. Potential use of Bischofia javanica as an active ingredient of functional foods and cosmeceutical products possessing hyaluronidase, collagenase, tyrosinase and urease inhibitory effects. Jpn J Complement Altern Med. 2010;7:129-33. doi:10.1625/jcam.7.129
12. Hutahaean S, Ilyas S, Rumahorbo CGP. Insulin expression and insulitis degree of diabetic mice after giving Bischofia javanica leaves. J Pharm Pharmacogn Res. 2021;9(5):598-608. doi:10.56499/jppres20.998_9.5.598
13. Wang YC, Huang TL. Screening of anti-Helicobacter pylori herbs deriving from Taiwanese folk medicinal plants. FEMS Immunol Med Microbiol. 2005;43(2):295-300. doi:10.1016/j.femsim.2004.09.008
14. Chowdhury MR, Chowdhury KH, Hanif NB, Sayeed MA, Mouah J, Mahmud I, et al. An integrated exploration of pharmacological potencies of Bischofia javanica leaves through experimental and computational modeling. Heliyon. 2020;6(9):e04895. doi:10.1016/j.heliyon.2020.e04895
15. Rumahorbo CGP, Ilyas S, Hutahaean S, Zuhra CF, Situmorang PC. Oral acute toxicity study of nano-formulated Bischofia javanica leaves. In: 6th International Conference on Agriculture, Environment, and Food Security. Medan (Indonesia): IOP Publishing; 2022. In press.
16. Lubran MM. Hematologic side effects of drugs. Ann Clin Lab Sci. 1989;19(2):114-20.
17. Wang DH, Wang MY, Shen WH, Yuan JF. Analysis of chemical compounds and toxicological evaluation of Forsythia suspensa leaves tea. Food Sci Biotechnol. 2021;30(2):305-14. doi:10.1007/s10068-020-00855-y
18. Han JS, Lee BS, Han SR, Han HY, Chung MK, Min BS, et al. A subchronic toxicity study of Radix Dipsaci water extract by oral administration in F344 mice. Regul Toxicol Pharmacol. 2016;81:136-45. doi:10.1016/j.yrtph.2016.07.017
19. Upur H, Amat N, Blazeković B, Talip A. Protective effect of Cichorium glandulosum root extract on CCl4- and galactosamine-induced hepatotoxicity in mice. Food Chem Toxicol. 2009;47(8):2022-30. doi:10.1016/j.fct.2009.05.022
20. Wong LL, Lacar L, Roytman M, Orloff SL. Urgent liver transplantation for dietary supplements: an under-recognized problem. Transplant Proc. 2017;49:322-5. doi:10.1016/j.transproceed.2016.11.041
21. Kluwe WM. Renal function tests as indicators of kidney injury in subacute toxicity studies. Toxicol Appl Pharmacol. 1981;57(3):414-24. doi:10.1016/0041-008X(81)90239-8
22. Michael B, Yano B, Sellers RS, Perry R, Morton D, Roome N, et al. Evaluation of organ weights for rodent and non-rodent toxicity studies: a review of regulatory guidelines and current practices. Toxicol Pathol. 2007;35(5):742-50. doi:10.1080/01926230701595292

23. Simanjuntak LJ, Rumahorbo CGP. Acute toxicity test of nanoherbal Phaleria macrocarpa fruit. Pharmacia. 2022;69(4):1063-74. doi:10.3897/pharmacia.69.e94436
24. Rumahorbo CGP, Ilyas S, Hutahaean S, Zuhra CF, Situmorang PC. Oral chronic toxicity test of nano herbal Phaleria macrocarpa. Pharmacia. 2023;70(2):411-8. doi:10.3897/pharmacia.70.e106744
25. Rumahorbo CGP, Ilyas S, Hutahaean S, Zuhra CF, Situmorang PC. Improvement of the physiological effects of nano-formulated Bischofia javanica leaves. Rasayan J Chem. 2023;16(2):766-72. doi:10.31788/RJC.2023.1628245
26. Li JS, Wang WJ, Sun Y, Zhang YH, Zheng L. Ursolic acid inhibits the development of nonalcoholic fatty liver disease by attenuating endoplasmic reticulum stress. Food Funct. 2015;6(5):1643-51. doi:10.1039/C5FO00083A
27. Rumahorbo CGP, Hutahaean S, Ilyas S. The effect of Bischofia javanica leaf extract on blood sugar levels and islet of Langerhans in alloxan-induced diabetic rats. IOP Conf Ser Earth Environ Sci. 2021;713:012046. doi:10.1088/1755-1315/713/1/012046
28. Ameen M, Zafar M, Ramadan MF, Ahmad M, Makhkamov T, Bokhari A, et al. Conversion of non-edible Bischofia javanica seed oil into methyl ester via recyclable zirconia-based phyto-nanocatalyst. Environ Technol Innov. 2023;30:103101. doi:10.1016/j.eti.2023.103101
29. Richter B, Hemmingsen B, Metzendorf MI, Takwoingi Y. Development of type 2 diabetes mellitus in people with intermediate hyperglycaemia. Cochrane Database Syst Rev. 2018;10:CD012661. doi:10.1002/14651858.CD012661.pub2
30. Tsatsakis AM, Vassilopoulou L, Kovatsi L, Tsitsimpikou C, Karamanou M, Leon G, et al. The dose-response principle from philosophy to modern toxicology. Toxicol Rep. 2018;5:1107-13. doi:10.1016/j.toxrep.2018.10.001
31. Sexton K, Hattis D. Assessing cumulative health risks from exposure to environmental mixtures. Environ Health Perspect. 2007;115(5):825-32. doi:10.1289/ehp.9333
32. Wang H, Wei J, Zheng Q, Meng L, Xin Y, Yin X, et al. Radiation-induced heart disease: a review of classification, mechanism and prevention. Int J Biol Sci. 2019;15(10):2128-38. doi:10.7150/ijbs.35460