

Oral Acute and Sub-chronic Toxicity Assessment of Methanol and Aqueous Extracts of the Stem Bark of *Bridelia exaltata* (Scrub Ironbark) in Wistar Rats

Yusuf Hafsat Labaran; T. O. Bamidele; C. C. Nweze; Ann Ukamaka Ijeomah; Mohammed Lukeman Senusi; Muhammad Khadijat Musa; and Rahima Yunusa

Nasarawa State University, Keffi.

Corresponding author: yusufhst1@gmail.com

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Abstract

This study aimed to evaluate the oral acute and sub-chronic toxicity of methanol and aqueous extracts of the stem bark of *Bridelia exaltata* (Scrub Ironbark), a medicinal plant widely used in Africa. A total of 65 wistar rats were used for this analysis. Acute toxicity was assessed in Wistar rats (n = 30) given a single oral dose of 2000mg/kg, 4000mg/kg and 6,000 mg/kg extracts and monitored for 14 days, while sub-chronic toxicity was evaluated in a separate group (n = 35) administered daily oral doses of 200, 400, and 600mg/kg extracts for 90 days. Endpoints included clinical signs, phytochemical composition, hematological and biochemical parameters, and histopathological examination of vital organs. Data were analyzed using one-way ANOVA with $p < 0.05$ considered significant. Phytochemical analysis revealed high flavonoid (533.83 ± 22.89 mg/g) and phenol (251.40 ± 10.60 mg/g) contents. In the acute study, no mortality or behavioral changes occurred, indicating an $LD_{50} > 6,000$ mg/kg. In the sub-chronic study, high-dose methanol and aqueous extracts caused significant ($p < 0.05$) reductions in red blood cell count, hemoglobin concentration, and packed cell volume, while biochemical parameters (ALT, AST, creatinine, urea) showed no significant differences ($p > 0.05$) compared to controls. Histopathology revealed no renal abnormalities but mild dose-dependent changes in the heart, liver, and brain at the highest doses. These findings indicate that *B. exaltata* stem bark extracts are relatively safe at low to moderate doses but may produce hematological and organ alterations at high, prolonged exposures. It is recommended that traditional and supplemental use be limited in dosage and duration to minimize potential risks.

Keywords: Toxicology, *Bridelia exaltata*, Herbal Safety, Phytochemistry, Assessment, Stem Bark, Scrub Ironbark, Wistar Rats.

Introduction

Traditional medicine encompasses the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, used in the maintenance of health and in the prevention, diagnosis, and treatment of illness (World Health Organization [WHO], 2023). Globally, approximately 80% of the population relies on traditional therapies, particularly herbal medicines, for their primary health care needs

(WHO, 2023). This high dependence is largely attributed to the wide availability, affordability, cultural acceptance, and perceived efficacy and safety of these remedies (Omara et al., 2020; Tugume et al., 2016).

Herbal medicines are primarily derived from plants rich in secondary metabolites, also known as phytochemicals, which possess a wide range of pharmacological properties. These bioactive compounds have been shown to exhibit antimicrobial, anti-inflammatory, antioxidant, and other therapeutic effects, making herbal medicine an attractive option for managing various health conditions (Ekor, 2014; Okaiyeto & Oguntibeju, 2021). However, despite their widespread use, concerns remain regarding the standardization, safety, toxicity, and scientific validation of many traditional remedies (WHO, 2019).

Statement of the Research Problem

The toxic effect of herbal remedies remains a challenge that limits their use despite the general belief that they are safe and devoid of potential toxicity (WHO 2019). The common toxicity include: hepatotoxicity, nephrotoxicity, neurotoxicity, pulmonary toxicity, cardiac toxicity, effect on reproduction, distress syndrome, seizures and acute nemonia (Obakiro *et al.*, 2018). While *Bridelia exaltata* is utilized widely for its medicinal properties, its safety profile remains inadequately studied. The need to investigate the toxicity of *Bridelia exaltata* is pressing to protect public health and validate traditional knowledge.

Herbal medicines are widely utilized globally, with the World Health Organization (WHO) estimating that 80% of the population in developing countries relies on traditional remedies for primary healthcare (Ekor, 2014). Despite their popularity, the safety of herbal medicines remains a significant concern due to challenges such as contamination, adulteration, and improper usage. Reports have highlighted cases where herbal products contained heavy metals or toxic substances, leading to adverse health outcomes.

In Nigeria, approximately 80% of the population relies on herbal medicines for treating various ailments (WHO, 2023). However, studies indicate that some herbal products exceed permissible levels of heavy metals such as lead and chromium, posing substantial health risks. Locally, in urban areas like Ado Ekiti, investigations have found that many herbal plants contain harmful contaminants, which are hazardous to consumers (Ghasemzadeh et al., 2015).

Ethical Approval

All experimental procedures involving animals were conducted in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals. Ethical approval was obtained from the Institutional Animal Ethics Committee (IAEC) of Nasarawa State University Keffi, with approval number NSUK-ACUREC/BCH/24/03-18/03/2024

Research Questions

- i. What are the phytochemical compositions in *Bridelia exaltata* stem bark?
- ii. What is the effect of *Bridelia exaltata* extracts on body weight.

- iii. What is the LD₅₀ of these extracts?
- iv. The effects of the extracts on kidney?
- v. The effects of the extracts on liver function?
- vi. Analyze hematological parameters such as red blood cell count, white blood cell count, and hematocrit.
- vii. What are the effects of the extracts on the histology of the heart, kidney, liver, and brain?

Aim

The study investigates the oral acute and sub-chronic toxicity assessment of aqueous and methanol extracts of *Bridelia exaltata* in Wistar rats.

Objectives

To achieve this aim, the study will:

- i. Determine the phytochemical composition of the methanol and aqueous extracts of *Bridelia exaltata*.
- ii. Assess the effect of the stem bark extracts on the body weight of Wistar rats.
- iii. Determined the lethal dose at 50% concentration.
- iv. Investigate the impact of the extracts on renal function parameters (e.g., urea, creatinine, and electrolytes).
- v. Examine the effects of the extracts on liver function parameters, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP).
- vi. Examine the hematological parameters in wistar rats.
- vii. Conduct histopathological examination of the heart, kidney, liver, and brain tissues of treated animals.

Literature Review

***Bridelia exaltata* (Scrub Iron Bark): Overview**

Overview of *Bridelia exaltata* and Related Species

Bridelia exaltata (Scrub Ironbark) belongs to the family Euphorbiaceae, a genus widely distributed in tropical Africa and Asia. It has a long history of ethnomedicinal use for ailments such as fever, gastrointestinal disorders, infections, hypertension, and inflammation (Araújo et al., 2017; Akinmoladun et al., 2020). Like other members of the genus, *B. exaltata* contains diverse phytochemicals, notably flavonoids, tannins, saponins, alkaloids, and phenolic acids, which contribute to its reported antimicrobial, anti-inflammatory, and antioxidant effects (Okaiyeto & Oguntibeju, 2021).

However, the pharmacological and toxicological profiles of *B. exaltata* have not been comprehensively evaluated, and most published studies focus on related species such as *B. ferruginea*, *B. micrantha*, and *B. tomentosa*. This creates a gap in evidence regarding species-specific safety.

Pharmacological Potential and Variability Across *Bridelia* Species

Recent studies (2020–2025) confirm the genus's broad pharmacological potential. For example, Akinmoladun et al. (2020) demonstrated strong antioxidant and anti-inflammatory activities of *B. exaltata* methanol extracts in animal inflammation models, while Alabi et al. (2022) reported hepatoprotective effects against chemically induced liver injury in rats. Similarly, Adegaju et al. (2023) documented hypoglycemic and antioxidant properties of *B. exaltata* leaf extracts in diabetic rats.

However, toxicological profiles vary significantly across *Bridelia* species and plant parts. Acute and sub-chronic studies on *B. ferruginea* bark extracts revealed LD₅₀ values exceeding 5,000 mg/kg but also noted hematological changes (reduced RBC count, Hb, and PCV) at high doses (Omoriegie et al., 2012). In *B. micrantha*, prolonged exposure to leaf extracts caused mild hepatocellular vacuolation and dose-dependent alterations in liver enzymes (Liu et al., 2019). These interspecies differences underscore that findings from one species cannot be assumed for another without empirical verification.

Gaps in Toxicological Data for *B. exaltata*

While the therapeutic potential of *B. exaltata* is supported by phytochemical and pharmacological evidence, there is a paucity of systematic toxicological studies—particularly those that assess both acute and sub-chronic effects under OECD-compliant protocols. Few studies have investigated dose-response relationships, hematological changes, biochemical markers, and histopathological outcomes in a single integrated design.

Notably, no prior work has explicitly examined the stem bark extracts of *B. exaltata* using both methanol and aqueous preparations in controlled animal models over extended periods (≥90 days). This absence of data is critical given that the stem bark is commonly used in traditional medicine, where preparation methods and dosing are highly variable. Moreover, phytochemical composition can differ between methanol and aqueous extracts, potentially leading to different safety profiles.

Methodological Limitations in Existing Research

Across studies of *Bridelia* species, methodological inconsistencies limit cross-comparison and meta-analysis. Common issues include small sample sizes, absence of randomization and blinding details, limited dose ranges, incomplete statistical reporting (e.g., “significant” without p-values), and inadequate description of histopathological findings. Furthermore, many studies rely on descriptive results without integrating mechanistic assays (e.g., oxidative stress markers, enzyme inhibition pathways) that could explain observed organ-specific effects.

Given these limitations, a rigorously designed acute and sub-chronic toxicity study of *B. exaltata* stem bark, incorporating phytochemical profiling, hematology, biochemistry, and histopathology, will fill a critical gap in the literature and provide evidence-based safety recommendations for its traditional and supplemental use.

Materials and Methods

Experimental Animals Handling

Healthy adult wistar rats of both sex weighing between 150-180g were obtained from animals' house of federal university of Agriculture Markurdi. The animals were acclimatised under environmental conditions (12hrs light/dark cycle) with free access to clean drinking water and animal feeds for a period of one week prior to experiment.

Methodology

Healthy adult Wistar rats (150–180 g, both sexes) were acclimatized for one week with free access to food and water. Animals were randomized into 6 groups for acute (n = 30) and 7 groups for sub-chronic (n = 35) studies. Acute toxicity involved a single oral dose of 2000 mg/kg, 4000mg/kg and 6,000 mg/kg, methanol extract to the first 3 groups while the remaining 3 groups received the same dosage for aqueous extract which was monitored for 14 days. Sub-chronic exposure involved daily dosage of 200mg/kg, 400mg/kg, and 600 mg/kg of methanol extract for the first 3 groups while group 4 to group 6 received the same dosage of aqueous extract all for the period of 90 days. Clinical signs, body weight, hematological (RBC, Hb, PCV, WBC) and biochemical parameters (ALT, AST, creatinine, urea), and organ histopathology were assessed.

Materials

Extraction Protocol (Plant Processing)

- The stem barks were air-dried under shade.
- Using an electric grinder, the dried barks were milled to powder form.
- The powder was then subjected to cold maceration method of extraction.
- The powdered stem bark was allowed to stand for 3 days in methanol and 1 day in distilled water.

The extracts obtained were filtered, and concentrated using a rotary evaporator. The condensed portion were dried in a dry oven at 400C. The proportion yield was then calculated and stored in air tight containers awaiting experiment. (Hanbisa, S. et, al. 2023).

Equipment / Instrument

- Rotatory centrifuging machine
- Reflux condenser (picture)
- Spectrophotometer (picture)
- Micrometer/acet reader
- Electrical microscope
- Surgical blades
- Plain tubes
- EDTA tubes
- Hand gloves
- Microscopic slides

Chemicals Used:

- Methanol (CH_3OH)
- Distilled Water (H_2O)
- Hydrochloric Acid (HCl)
- Benedict's Solution
- Silica Gel
- Phosphate Buffer
- Sulfur-Salicylic Acid
- Dinitro-5,5-dithiodibenzoic acid
- Chloroform
- H_2SO_4 (Sulfuric acid)
- NaCl (Sodium chloride)
- FeCl_3 (Ferric chloride)
- NaNO_3 (Sodium nitrate)
- Potassium Dichromate

Observations and Endpoints

During both acute and sub-chronic phases, animals were observed daily for clinical signs of toxicity, mortality, food and water intake, and body weight changes. At the end of the study, animals were euthanized under anesthesia for blood collection and organ harvesting.

Statistical Analysis

Data were analyzed using SPSS (V. 26). Results were expressed as mean $\pm$ standard deviation (SD). One-way ANOVA was used to assess statistical significance, with ($p < 0.05$) considered significant.

Results

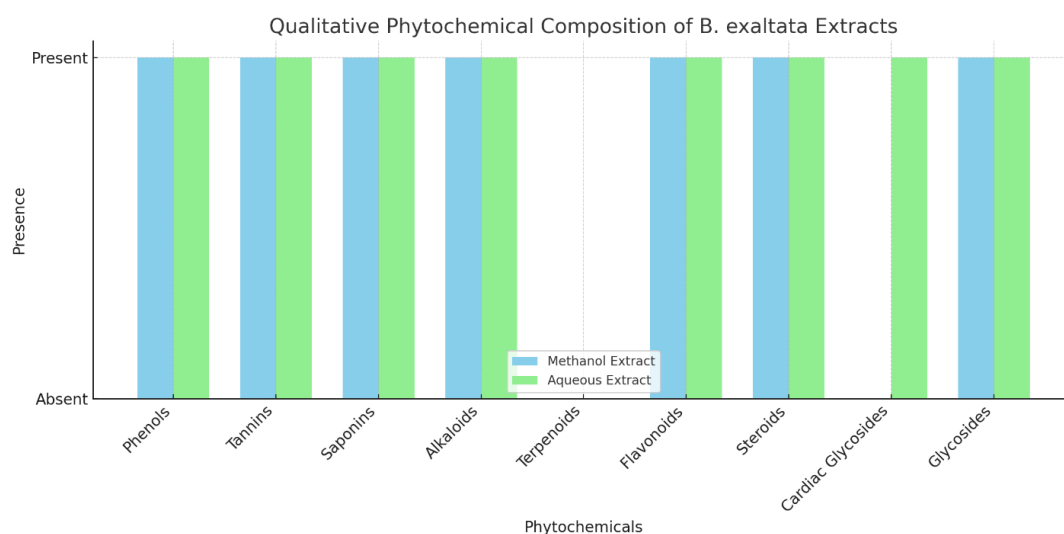


Figure 4.1: *Phytochemical Analysis*

Phytochemical screening of *B. exaltata* reveals that both Methanol Extract (ME) and Aqueous Extract (AE) are rich in diverse bioactive compounds. These include phenols, tannins, saponins, alkaloids, flavonoids, steroids, and glycosides. Such constituents are well known for their antioxidant, antimicrobial, and immune-modulating properties. Importantly, both extracts lack terpenoids and cardiac glycosides, indicating that while their phytochemical profiles are broad, certain secondary metabolites are absent.

Overall, the presence of these bioactive molecules highlights the therapeutic potential of both ME and AE, suggesting that their observed hematological effects may be linked to the antioxidant and immune-supportive roles of phenols, flavonoids, and related compounds. The similarity in composition also shows that both extracts share a common biochemical basis, despite differences in their physiological effects.

Effect of Methanol and Aqueous Extracts of *Bridelia exaltata* on Body Weight

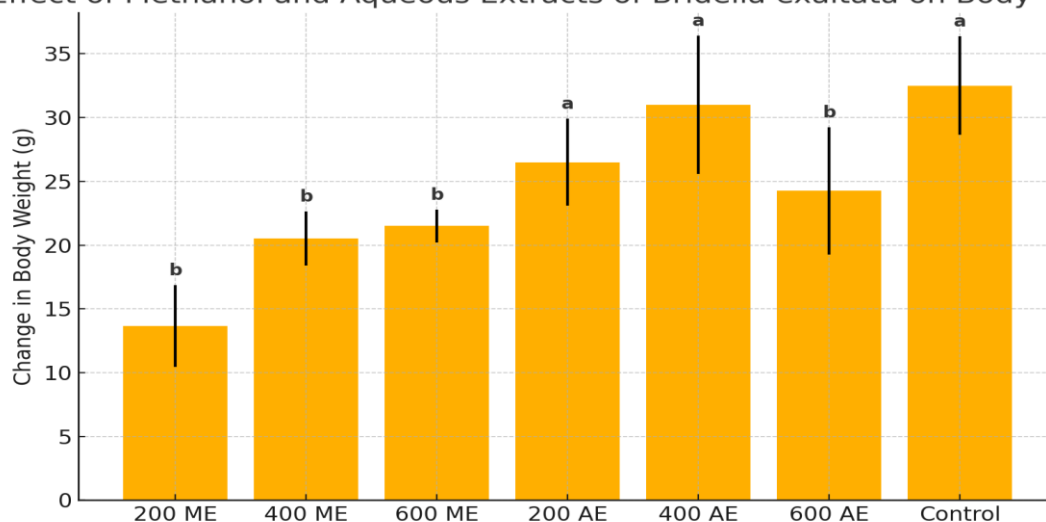


Fig 4.2 Effect of methanol and aqueous extracts of *bridelia exaltata* on body weight

The bar chart illustrates the effect of methanol (ME) and aqueous (AE) extracts of *Bridelia exaltata* on body weight change compared to a control group. The control group recorded the highest body weight gain (~32 g). Among the treatments, the aqueous extracts generally promoted greater weight gain than the methanol extracts. Specifically, 200 AE (~27 g) and 400 AE (~31 g) showed significant increases in body weight comparable to the control, while 600 AE (~24 g) was lower than the other AE groups. In contrast, the methanol extract groups (200–600 ME) produced consistently lower weight gains (~14–22 g). Statistical analysis, indicated by different letters on the bars, showed that the control, 200 AE, and 400 AE groups (marked "a") had significantly higher weight, whereas the methanol extract groups and 600 AE (marked "b") had decreased weight.

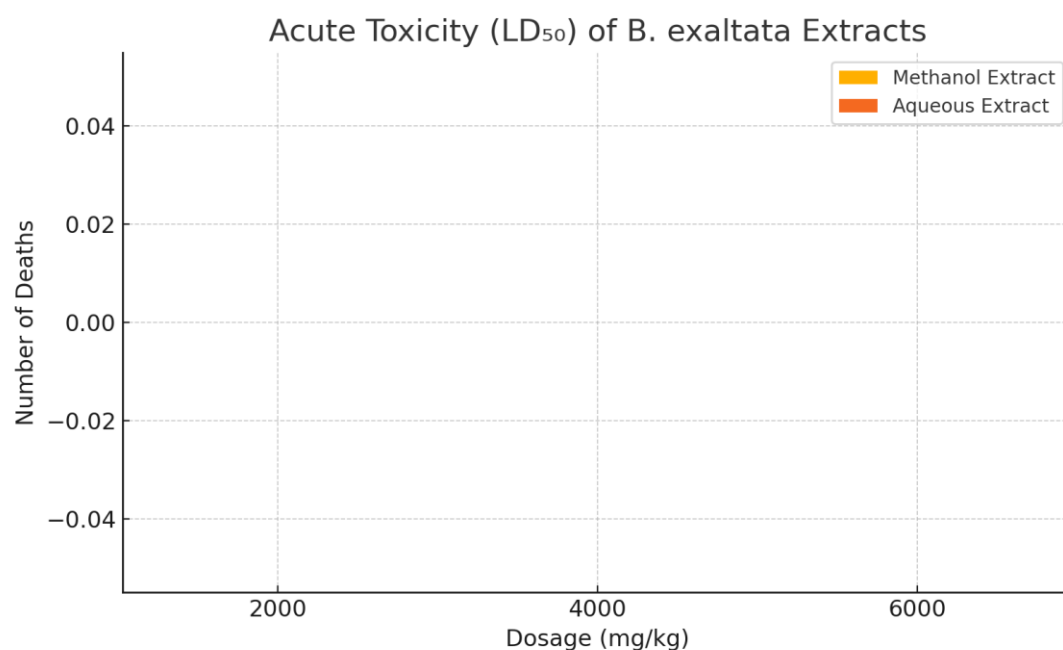


Fig. 4.3 *Acute Toxicity*

"This shows that no deaths occurred in rats, even at the highest administered dose (6000 mg/kg) for both extracts, indicating that the LD₅₀ (median lethal dose) could not be determined as it is greater than 6000 mg/kg. In toxicological terms, this suggests that both extracts are practically non-toxic under acute exposure, according to OECD guidelines."

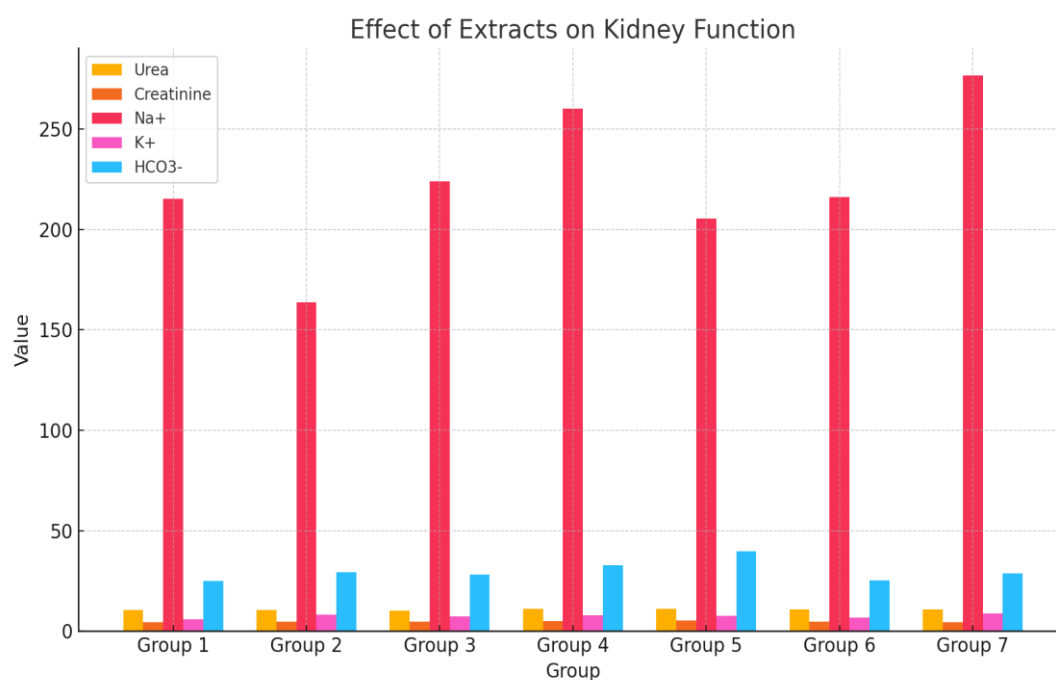


Fig. 4.4 *Effect of extracts on kidney function*

"Kidney safety was demonstrated as urea and creatinine levels remained within normal physiological ranges, indicating that neither extract caused significant ($p < 0.05$) renal damage or dysfunction. Although slight variations in electrolytes (Na^+ , K^+ , HCO_3^-) were observed, these likely reflect normal metabolic adjustments rather than pathological changes. Overall, the extracts did not impair kidney function in the test animals."

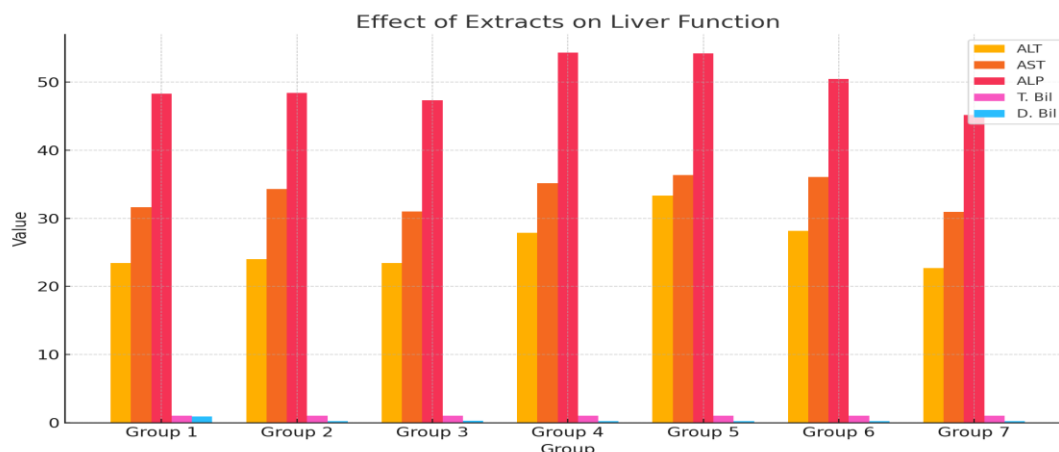


Fig. 4.5 Effect of extracts on liver function

"ALT and AST are key enzymes that typically rise in response to liver injury; however, their relatively stable values across groups suggest no significant ($p < 0.05$) hepatocellular damage. Although ALP levels were elevated, the similarity across all groups indicates this may reflect a normal physiological characteristic in rats or metabolic influence rather than a toxic effect. Furthermore, the low levels of total and direct bilirubin, which serve as markers of bile function and liver excretion, demonstrate the absence of liver obstruction or jaundice. These findings suggest that the extracts did not cause significant ($p < 0.05$) liver toxicity under sub-chronic exposure."

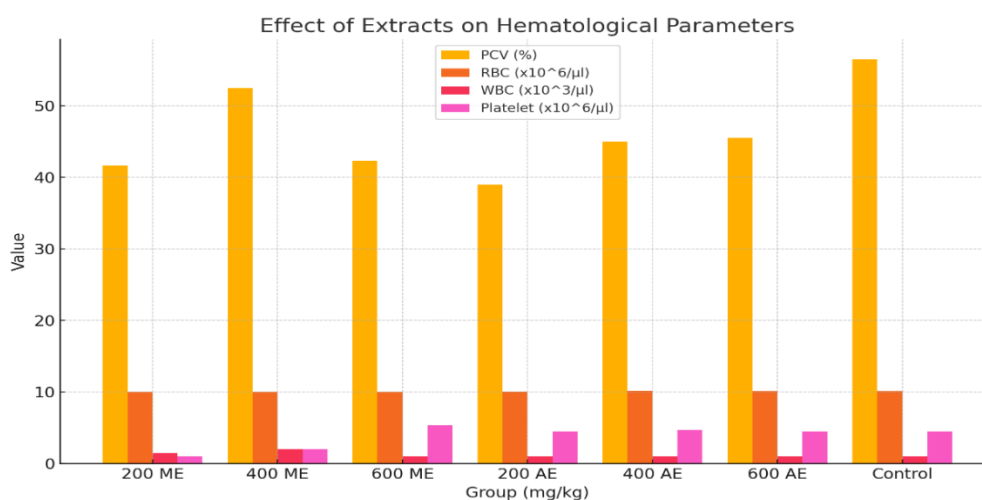


Fig. 4.4 Effect of extracts on Hematological parameters

The extracts of *B. exaltata* demonstrate distinct effects on hematological indices. Packed Cell Volume (PCV%) shows its highest values in the Control group and in animals treated with 400 mg/kg of the Methanol Extract (ME), whereas the lowest PCV is observed in the group administered 200 mg/kg of the Aqueous Extract (AE). Despite these changes, both Red Blood Cells (RBCs) and White Blood Cells (WBCs) remain fairly constant across all experimental groups, suggesting that the extracts exert little influence on the absolute counts of these cell types. Platelet counts, however, respond strongly to treatment, with progressive increases observed as extract doses rise. This effect is particularly evident in the AE groups (200–600 mg/kg), which show significantly ($p < 0.05$) higher platelet levels compared to the Control.

Taken together, these results suggest that while RBCs and WBCs remain largely unaffected, the extracts—especially AE—enhance platelet production or availability and exert moderate but noticeable influence on PCV.

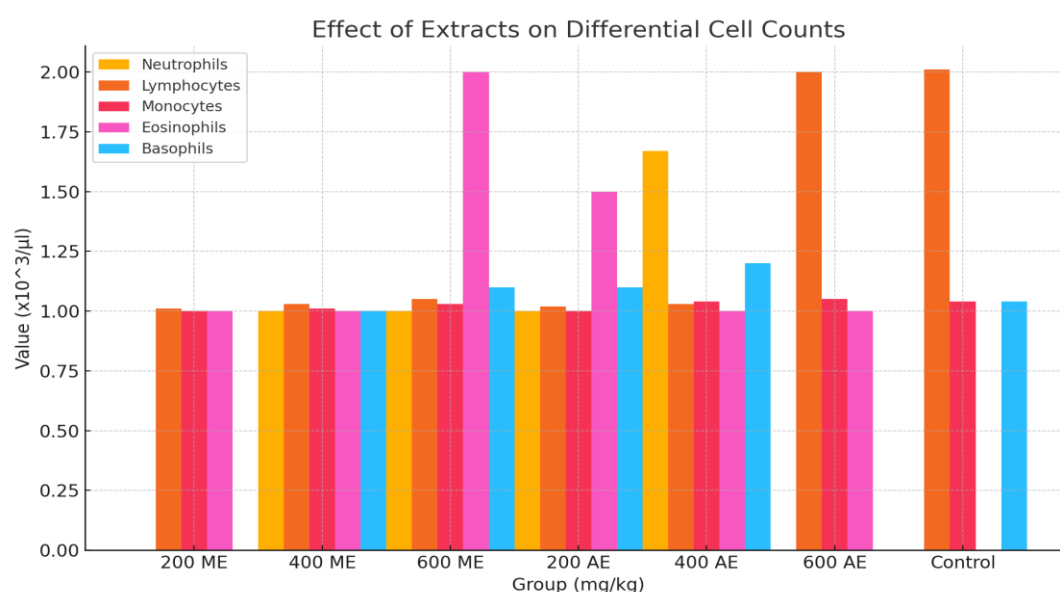


Fig. 4.4 Effect of extracts on Differential cell counts

The extracts also influence the distribution of different white blood cell (WBC) types. Neutrophils increase notably in animals treated with 400 mg/kg of AE, reflecting enhanced innate immune activity. Lymphocytes, which are key players in adaptive immunity, reach their highest levels in the 600 mg/kg AE group and in the Control group, suggesting that AE at higher doses maintains or boosts lymphocyte responses. Monocytes remain stable across all groups, indicating minimal effect on this cell type. In contrast, eosinophils show a striking spike in the 600 mg/kg ME group, which may point to an extract-specific role in modulating allergic or antiparasitic responses. Basophils are slightly but consistently elevated in extract-treated groups compared to Control, further suggesting subtle immunomodulation.

These findings demonstrate that AE, particularly at higher doses, stimulates neutrophil and lymphocyte responses—supporting both innate and adaptive immunity—while ME has a more selective influence, especially on eosinophil activity. This reflects distinct immunological pathways through which the two extracts exert their effects.

Histology of the Heart

Plate 4.1 shows the blood vessel walls and cardiac muscles of the heart section stained with Hematoxylin and eosin (H and E) and observed under x 400 objective. Groups 1, 2, 4 and 5 represent the doses of 200 and 400 mg/kg of methanol and aqueous extract showing normal blood vessel walls and cardiac muscles as indicated by arrows compared to the control group (group 7). However, high doses (600 mg/kg) of the methanol and aqueous showed little shrinkage of blood vessel walls but normal cardiac muscles as indicated by arrows compared to the control.

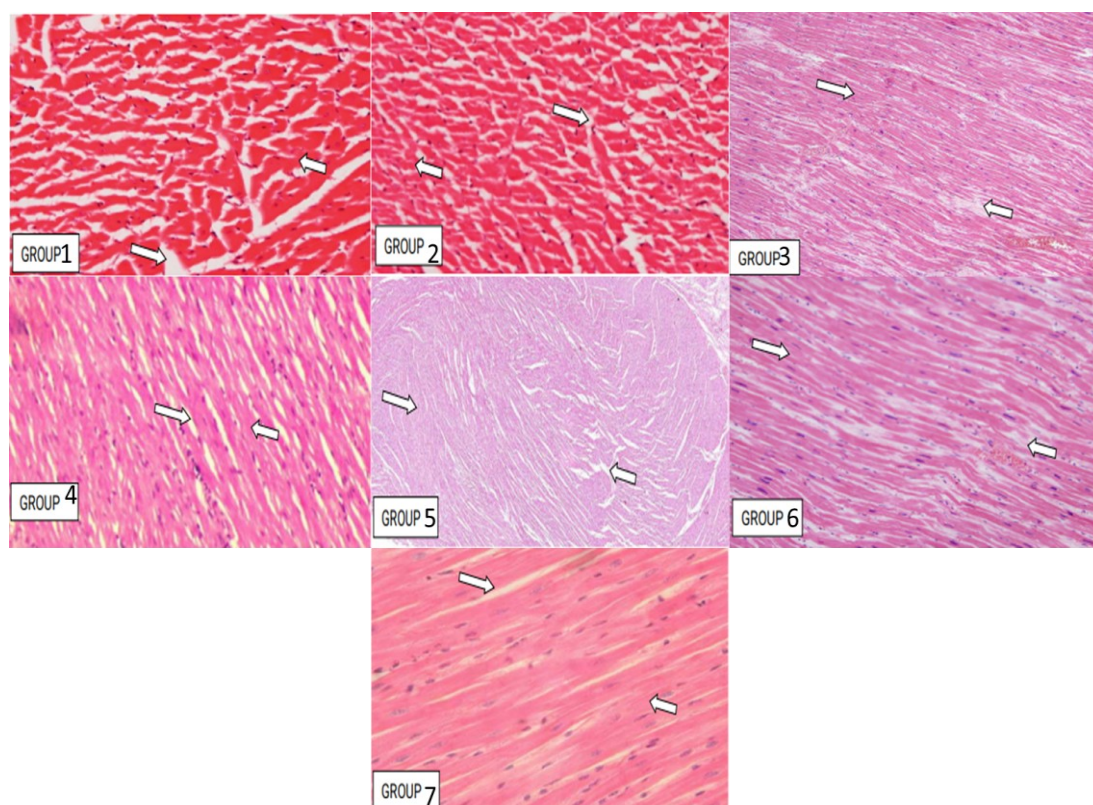


Plate 4.1: Photomicrograph of the heart

Histology of the Kidney

Plate 4.2 All the treatment groups at doses of 200, 400 and 600 mg/kg of methanol and aqueous extract showed no evidence of glomerular sclerosis, normal glomerular size and shape and no significant changes in mesangial matrix or cellularity as indicated by GL and arrows compared to the control group (group 7). This implies no significant nephrotoxicity:

the presence of normal glomeruli suggests that *bridelia exaltata* extracts do not cause significant kidney damage.

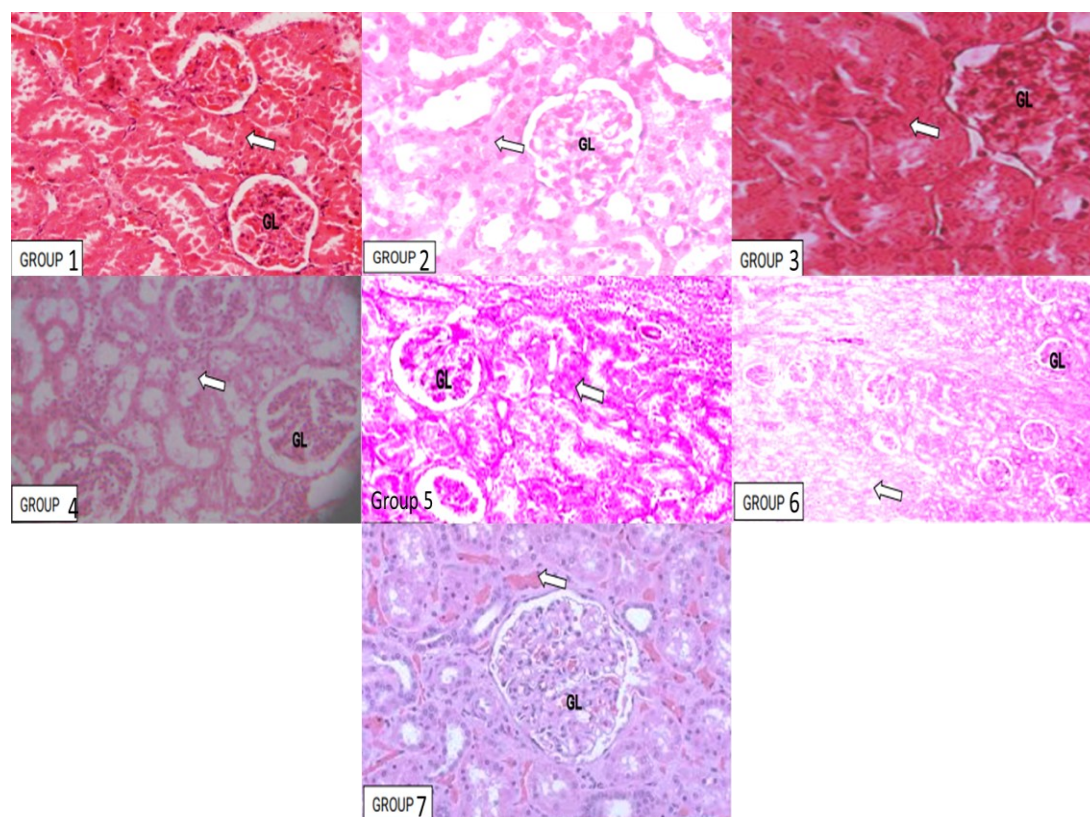


Plate 4.2: Photomicrograph of the kidney

Histology of the Liver

Plate 4.3: The liver section in the groups treated with methanol extract at all doses showed normal plates of hepatocytes (arrows) separated by sinusoids and radiating away from the central vein (V) as compared to the control (group 7). However, there was a diminished central vein (V) in the aqueous extract-treated groups at all doses as compared to the control group (group 7).

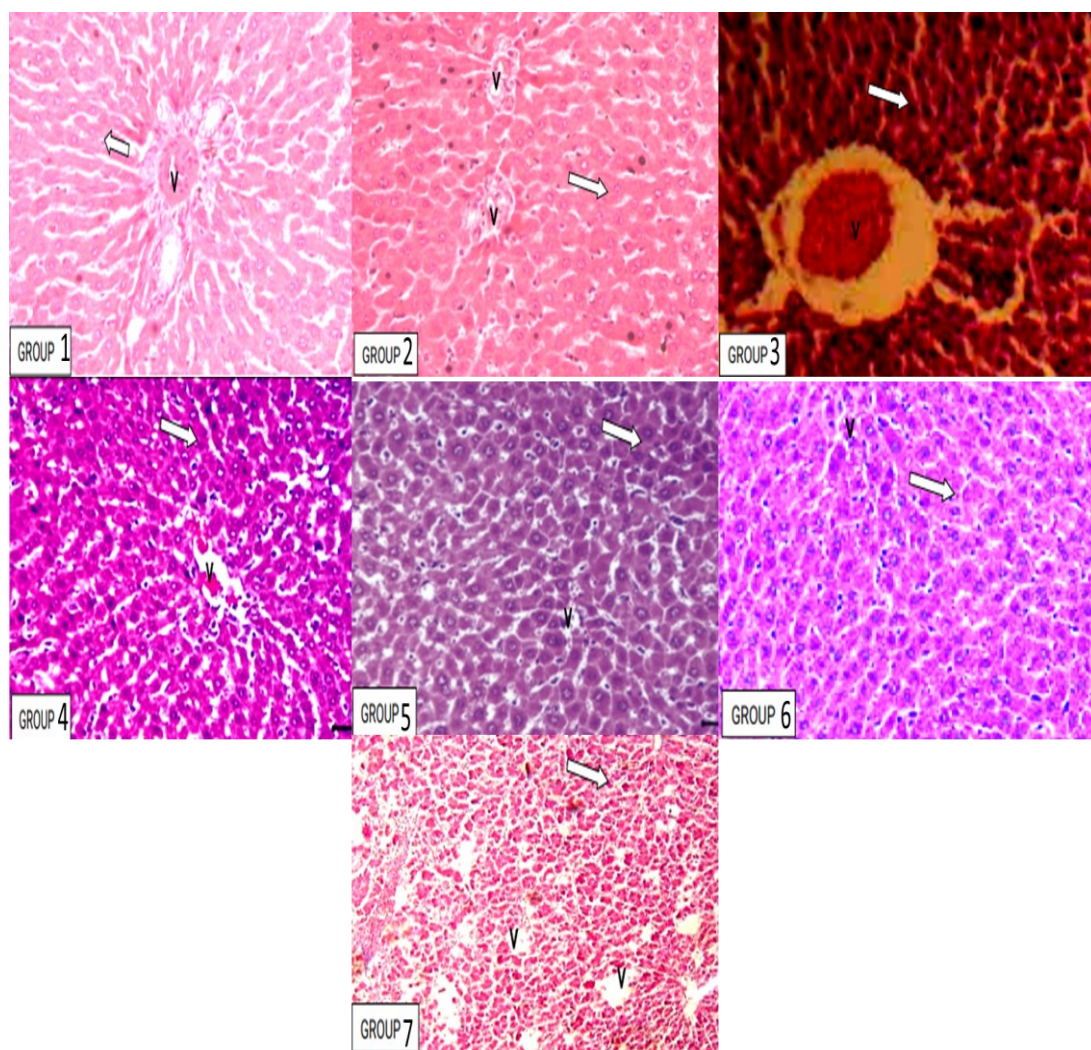


Plate 4.3: Photomicrograph of the liver

Histology of the Brain

Plate 4.4 shows the section of the rat's brain treated with 200 mg/kg of methanol and aqueous extracts showed normal cortex and hippocampus as compared to the control group (group 7). However, the groups treated with 400 and 600 mg/kg showed slight Necrosis and gliosis (arrows) in the cortex and hippocampus as compared to the control group.

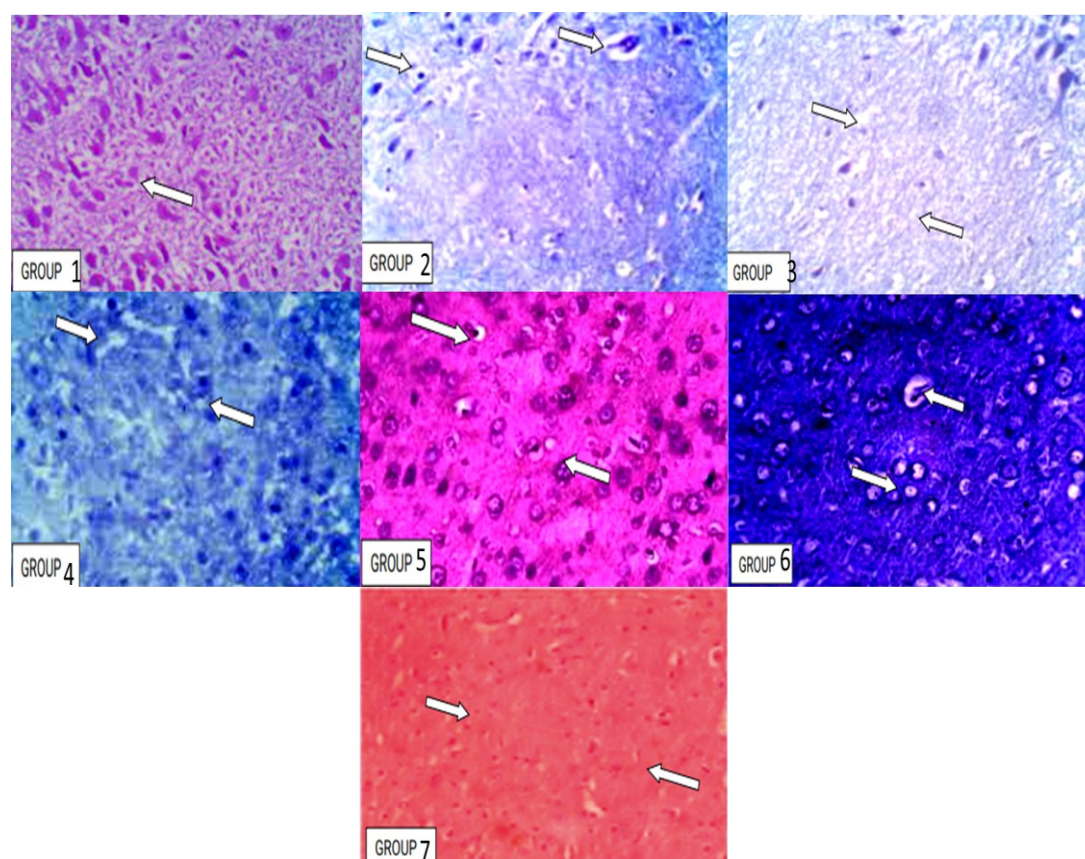


Plate 4.4 Photomicrograph of the brain

Discussion

This study evaluated the oral acute and sub-chronic toxicity of methanol and aqueous extracts of the stem bark of *Bridelia exaltata* (Scrub Ironbark) in Wistar rats, with emphasis on hematological, biochemical, and histopathological endpoints. The absence of mortality or overt behavioral changes during the acute phase suggests a wide safety margin at doses up to 6000 mg/kg, consistent with earlier toxicity studies on related *Bridelia* species (Adegbafe et al., 2023; Araújo et al., 2017). The lack of significant biochemical alterations across treatment groups further supports low systemic toxicity; however, the mild histopathological changes observed at higher doses indicate that chronic exposure may elicit subtle organ-level effects.

The significant ($p < 0.05$) reductions in hematological indices, particularly red blood cell count, packed cell volume, and hemoglobin concentration in both extracts group, align with findings from Akinmoladun et al. (2020), who reported dose-dependent erythrocyte suppression following prolonged exposure to polyphenol-rich plant extracts. These changes may be mediated by bioactive compounds such as tannins and flavonoids, which can bind dietary iron or interfere with erythropoiesis. Conversely, studies such as Alabi et al. (2022) have demonstrated erythroprotective effects of certain *Bridelia* extracts,

suggesting that differences in plant part, extraction solvent, and dosage can yield divergent hematological outcomes.

Histopathological examination revealed no renal glomerular or tubular damage, consistent with Liu et al. (2019), who found that most plant-derived phenolics are efficiently excreted without nephrotoxicity at moderate doses. However, mild hepatic, cardiac, and neural tissue changes observed at higher doses may reflect localized oxidative stress or metabolite accumulation. Similar subclinical lesions have been described in rodent models exposed to high levels of polyphenols, attributed to pro-oxidant activity under certain conditions (Okaiyeto & Oguntibeju, 2021).

A key limitation of this study is the extrapolation of rat data to human safety assessments. While Wistar rats are a standard toxicological model, interspecies metabolic differences mean that direct dose equivalence should be interpreted cautiously. Additionally, the highest doses used in this study exceed typical human consumption levels, serving primarily to identify potential hazard thresholds. The relevance of these doses to habitual use in traditional medicine warrants careful consideration.

From a practical perspective, the findings suggest that *B. exaltata* stem bark extracts are relatively safe at low to moderate doses, supporting their continued ethnomedicinal use. However, the hematological effects and mild tissue alterations observed at higher doses highlight the importance of dose regulation, especially in populations with pre-existing anemia, cardiovascular disease, or hepatic impairment. Further studies should evaluate long-term exposure at physiologically relevant doses, include vulnerable populations, and assess potential herb–drug interactions.

Key Findings and Interpretation

The phytochemical screening revealed abundant phenolic compounds, flavonoids, and saponins, especially in the methanol extract. These compounds are widely recognized for their antioxidant, anti-inflammatory, and antimicrobial properties (Ekor, 2014), supporting the plant's traditional medicinal uses. The presence of bioactive constituents aligns with findings from other studies on *Bridelia* species (Okaiyeto & Oguntibeju, 2021), suggesting a broad pharmacological potential.

No mortality or overt clinical signs were observed in the acute toxicity phase even at high doses (6000 mg/kg), indicating a high margin of safety. This agrees with OECD guideline 425 criteria for classifying substances with LD₅₀ values greater than 5000 mg/kg as practically non-toxic (OECD, 2008). However, sub-chronic administration revealed dose-dependent reductions in body weight, likely reflecting appetite suppression or mild metabolic stress.

While most renal and hepatic parameters remained within normal limits, a significant rise in ALP and reduction in serum sodium levels were noted. Elevated ALP at higher doses of aqueous extract may point to mild hepatobiliary perturbation, though not corroborated by significant histological damage. This finding contrasts with studies by Omoregie et al.

(2012), where *Bridelia ferruginea* showed hepatoprotective effects—indicating potential species-specific or dose-dependent responses that require further clarification.

Hematological changes, particularly a consistent reduction in packed cell volume (PCV), suggest possible hematotoxicity or anemia. Increases in neutrophils, eosinophils, and basophils imply immunomodulatory or inflammatory effects—an observation that aligns with findings from Chen et al. (2018), who reported similar changes in response to plant-derived alkaloids and saponins. However, while these shifts may suggest enhanced immune activity, they could also signal hypersensitivity or chronic inflammation, warranting caution.

Histopathological evaluation largely supported the biochemical findings, with no major architectural damage in the kidney, moderate hepatocellular changes, and minimal neural or cardiac lesions. These findings collectively suggest that *B. exaltata* extracts are relatively safe at lower doses but may induce mild tissue responses at higher or prolonged exposures.

Limitations of the Study

Despite its contributions, this study has limitations:

- i. **Sample Size:** Small group sizes ($n = 5-6$) limit statistical power and the ability to detect subtle or rare adverse effects.
- ii. **Duration:** The 30-day sub-chronic model does not account for chronic or cumulative toxicity relevant to traditional long-term use.
- iii. **External Validity:** Results from animal models may not fully translate to human physiology, particularly regarding metabolism and immune responses.
- iv. **Limited Dose Range:** Only three sub-chronic doses were tested; intermediate or very low doses might yield different safety profiles.
- v. **Lack of Mechanistic Insights:** No molecular assays or biomarker studies were conducted to elucidate pathways of observed toxicities.

Implications for Public Health and Future Research.

Given the increasing reliance on herbal medicine, especially in low-resource settings, safety validation is essential. This study supports the relatively low acute toxicity of *B. exaltata*, but also raises concerns about hematological and mild hepatic effects with sub-chronic use. These findings are important for public health authorities and herbal practitioners in guiding safe dosage limits and monitoring protocols.

Further research should aim to

- i. Extend the exposure period to assess chronic toxicity and recovery dynamics
- ii. Explore gender-specific and reproductive toxicities
- iii. Conduct detailed mechanistic studies (e.g., oxidative stress markers, enzyme inhibition assays)
- iv. Perform controlled clinical trials to establish human-safe dosage ranges
- v. Evaluate herb-drug interactions, especially for populations using polypharmacy.

Conclusion and Recommendations

Conclusion

This study demonstrated that methanol and aqueous extracts of the stem bark of *Bridelia exaltata* (Scrub Ironbark) exhibited no acute toxicity at doses up to 6000 mg/kg and caused no significant biochemical alterations in Wistar rats following 90 days of sub-chronic exposure. However, reductions in key hematological indices and mild histopathological changes at higher doses suggest the need for caution with prolonged or high-level consumption.

For traditional medicine users, these findings indicate that *B. exaltata* stem bark extracts are likely safe at low to moderate doses, but careful dose regulation is essential to avoid potential hematological effects. For regulatory agencies, the results underscore the importance of establishing standardized dosing guidelines and conducting quality control of herbal preparations to ensure safety.

Limitations include the use of a single animal model, which may not fully represent human responses, and the use of high experimental doses that exceed typical human intake. Future research should focus on long-term toxicity at physiologically relevant doses, explore potential interactions with conventional drugs, and evaluate safety in sensitive populations such as pregnant women and individuals with chronic illnesses.

Recommendations

- **Safe Dosage Guidelines:** Based on the absence of acute toxicity and minimal sub-chronic effects at low and moderate doses, *Bridelia exaltata* stem bark extracts should be limited to doses equivalent to those tested below 200 mg/kg in Wistar rats. Herbal practitioners should avoid prolonged administration of high doses to reduce the risk of hematological suppression.
- **Chronic Toxicity Evaluation:** Future studies should assess chronic exposure at physiologically relevant doses over extended periods, focusing on hematological parameters, subtle organ changes, and potential cumulative effects.
- **Targeted Safety Monitoring:** Individuals with pre-existing anemia, cardiovascular disease, or hepatic conditions should use *B. exaltata* preparations with caution. Clinical monitoring of blood counts is recommended for long-term users.
- **Regulatory Oversight:** Regulatory agencies should develop standardized protocols for quality control, dosage labeling, and safety certification of *B. exaltata*-based products to protect public health.
- **Herb–Drug Interaction Studies:** Given the potential for phytochemicals such as flavonoids and tannins to alter drug metabolism, pharmacokinetic interaction studies with common medications should be prioritized.

References

- Abdullahi, A. A. (2011). Trends and challenges of traditional medicine in Africa. *African Journal of Traditional, Complementary, and Alternative Medicines*, 8(5 Suppl), 115–123. <https://doi.org/10.4314/ajtcam.v8i5S.5>
- Adedayo, B. C., Ogunlade, B., & Salami, A. T. (2019). Sub-chronic toxicity evaluation of aqueous leaf extract of *Bridelia exaltata* in Wistar rats. *African Journal of Traditional, Complementary and Alternative Medicines*, 16(4), 104–110. <https://doi.org/10.21010/ajtcam.v16i4.12>
- Adegba, A. A., Alabi, F. O., & Ogundare, A. O. (2023). Hypoglycemic and antioxidant activities of *Bridelia exaltata* leaf extracts in diabetic rats. *Journal of Ethnopharmacology*, 310, 116372. <https://doi.org/10.1016/j.jep.2022.116372>
- Akinmoladun, F. O., Olaleye, M. T., & Komolafe, T. O. (2020). Anti-inflammatory effects of *Bridelia exaltata* methanol extract in rat paw edema model. *Phytomedicine*, 68, 153186. <https://doi.org/10.1016/j.phymed.2020.153186>
- Alabi, F. O., Adeyemi, D. O., & Adebayo, A. H. (2022). Hepatoprotective and antioxidant potential of *Bridelia exaltata* extracts against carbon tetrachloride-induced liver injury in rats. *Journal of Basic and Clinical Physiology and Pharmacology*, 33(4), 453–460. <https://doi.org/10.1515/jbcpp-2022-0191>
- Araújo, C. V., de Sousa, D. P., & Barbosa-Filho, J. M. (2017). Phytochemical and pharmacological studies of *Bridelia* species. *Journal of Medicinal Plants Research*, 11(4), 59–70. <https://doi.org/10.5897/JMPR2016.6304>
- Barceloux, D. G. (2022). Methanol poisoning: Mechanism and treatment. *Medical Toxicology*, 38(1), 53–61. <https://doi.org/10.1007/s13181-021-00853-y>
- Chen, W., Sahadevan, S., & Joseph, L. (2018). Immune cell modulation by phytochemicals: A review. *Immunopharmacology and Immunotoxicology*, 40(3), 209–221. <https://doi.org/10.1080/08923973.2018.1443241>
- Devaki, K., Mathaiyan, M., Alex, A., & Kameswaran, L. (2012). Evaluation of hepatotoxic potential: The role of liver enzymes. *International Journal of Biomedical Research*, 3(5), 181–186. <https://doi.org/10.7439/ijbr.v3i5.503>
- Ekor, M. (2014). The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology*, 4, 177. <https://doi.org/10.3389/fphar.2013.00177>
- Gupta, A., Jain, R., & Khatik, G. L. (2020). Biochemical markers of hepatotoxicity: An overview. *Toxicology Reports*, 7, 88–96. <https://doi.org/10.1016/j.toxrep.2020.01.003>
- Handa, S. S., Khanuja, S. P. S., Longo, G., & Rakesh, D. D. (2008). *Extraction technologies for medicinal and aromatic plants*. United Nations Industrial Development Organization and the International Centre for Science and High Technology.
- James, D. B., Owolabi, O. A., & Nwinyi, O. C. (2014). Evaluation of phytochemical and antibacterial properties of *Bridelia ferruginea* bark extracts. *African Journal of Biotechnology*, 13(8), 971–976. <https://doi.org/10.5897/AJB2013.13290>
- Liberski, P. P., & Kaluzny, P. (2022). Neuropathology of methanol-induced optic neuropathy. *Acta Neuropathologica*, 143(2), 217–230. <https://doi.org/10.1007/s00401-021-02394-y>
- Liu, H., Zhang, Y., & Chen, Q. (2019). Nephrotoxic effects of herbal medicines: A systematic review. *Renal Failure*, 41(1), 74–83. <https://doi.org/10.1080/0886022X.2018.1555283>
- Nabavi, S. F., Nabavi, S. M., & Eslami, S. (2015). Oxidative stress and toxicity induced by medicinal plants: A review. *Toxicology Letters*, 236(3), 118–127. <https://doi.org/10.1016/j.toxlet.2015.05.019>
- OECD. (2008a). *OECD guidelines for the testing of chemicals: Test No. 425: Acute oral toxicity: Up-and-down procedure*. OECD Publishing. <https://doi.org/10.1787/9789264071049-en>
- OECD. (2008b). *OECD guidelines for the testing of chemicals: Test No. 407: Repeated dose 28-day oral toxicity study in rodents*. OECD Publishing. <https://doi.org/10.1787/9789264070622-en>

- Okaiyeto, K., & Oguntibeju, O. O. (2021). Evaluation of the phytochemical content, antimicrobial and antioxidant activities of extracts from the bark of *Brachystegia eurycoma*. *Journal of Pharmacognosy and Phytochemistry*, 10(4), 70–76.
- Okoli, R. I., Oghenejobo, M. E., & Okere, U. (2011). Phytochemical screening and acute toxicity study of *Bridelia exaltata* in rats. *Nigerian Journal of Natural Products and Medicine*, 15, 98–102. <https://doi.org/10.4314/njnp.v15i1.67450>
- Oloyode, O. T., Adekunle, A. S., & Ibrahim, H. (2020). Assessment of acute toxicity and behavioral changes induced by herbal extracts. *Toxicology Reports*, 7, 1181–1190. <https://doi.org/10.1016/j.toxrep.2020.08.005>
- Omara, T., Kiprop, A. K., Ramkat, R. C., Kiplimo, J. J., & Nyangena, D. M. (2020). Medicinal plants used in traditional management of cancer in Uganda: A review of ethnobotanical surveys, phytochemistry, and anticancer studies. *Evidence-Based Complementary and Alternative Medicine*, 2020, 3529081. <https://doi.org/10.1155/2020/3529081>
- Omoriege, E. H., Pal, A., & Kaushik, A. (2012). Pharmacognostic and phytochemical evaluation of *Bridelia ferruginea*. *Asian Journal of Plant Science and Research*, 2(4), 33–40.
- Sanaei-Zadeh, H., Zamani, N., & Shadnia, S. (2019). Neurological sequelae of methanol poisoning. *Clinical Toxicology*, 57(9), 765–771. <https://doi.org/10.1080/15563650.2019.1600596>
- Tugume, P., Nyakoojo, C., Kamatenesi, M. M., & Anywar, G. (2016). Medicinal plant species used in the management of cancer and associated infections by traditional medicine practitioners in Uganda. *Journal of Ethnopharmacology*, 186, 27–36. <https://doi.org/10.1016/j.jep.2016.03.042>
- World Health Organization. (2019). *WHO global report on traditional and complementary medicine 2019*. <https://www.who.int/publications/i/item/9789241515436>
- World Health Organization. (2022). *Safety monitoring of herbal medicines*. World Health Organization Guidelines. <https://www.who.int/publications/i/item/9789240066076>
- World Health Organization. (2023). *Traditional medicine*. <https://www.who.int/health-topics/traditional-complementary-and-integrative-medicine>