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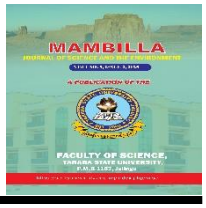

EVALUATION OF TOXICITY POTENTIAL OF *Micromeria imbricata* IN WISTAR RATS

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ABSTRACT

Micromeria imbricata (Lamiaceae) is widely employed in ethnomedicine for its antimicrobial, anti-inflammatory, and antioxidant properties. However, there is limited scientific information on its safety profile. This study evaluated the acute and sub-chronic toxicity potential of methanolic leaf extract of *M. imbricata* in albino rats. The extract was prepared by cold maceration in methanol and administered orally to Wistar albino rats. Acute toxicity was determined using Lorke's method, while sub-chronic toxicity was assessed at doses corresponding to 1/20, 1/40, and 1/80 mg/kg of the LD_{50} over 14 days. Serum biochemical markers, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, albumin, and bilirubin, were determined. The oral LD_{50} of the extract was estimated at 2154 mg/kg, indicating moderate toxicity. Sub-chronic administration significantly ($p < 0.05$) elevated liver enzyme levels compared to control. ALT increased from 90.5 ± 7.50 U/L (control) to 160.33 ± 25.00 U/L at 26.90 mg/kg, AST rose sharply from 150.00 ± 80.25 U/L to 893.33 ± 465.40 U/L at 107.00 mg/kg, while ALP increased from 349 ± 13.85 U/L to 471.33 ± 38.67 U/L at 26.90 mg/kg. Similarly, total bilirubin increased from 11.7 ± 2.02 mg/dL to 54.4 ± 1.38 mg/dL, and direct bilirubin from 7.25 ± 2.62 mg/dL to 23.05 ± 5.05 mg/dL. These alterations indicate hepatocellular leakage and impaired liver function. The study demonstrates that while *M. imbricata* possesses ethnomedicinal value, its methanolic extract exerts dose-dependent hepatotoxic effects in albino rats. Caution is advised in its traditional use, and further studies including histopathological evaluations and isolation of active compounds are recommended to establish safe therapeutic limits.

1. Introduction

Plants produce biologically active compounds which serve as the primary source of human medications as long as the history of human existence (Jamshidi-Kia *et al.*, 2017; Anand *et al.*, 2019). This implies, many varieties of plants species will routinely be used in the disease's treatment and management without much scientific documentations (Prasanth *et al.*, 2015; Moges & Moges, 2019; Lawal *et al.*, 2020). However, it is crucial to recognize that the natural origin of a plant does not guarantee its safety when consumed in its raw state (Taroncher *et al.*, 2021; Khursheed *et al.*, 2022). The therapeutic properties of medicinal plants used by traditional medical practitioners may be due to one or more of the many compounds produced by the plant (Kamil *et al.*, 2019; Awuchi *et al.*, 2023). Studies have proved the association of active pharmacological ingredients of some herbal remedies and their metabolites with adverse effects that might range from mild allergic reactions to death (Kahraman *et al.*, 2020; Kiliś-Pstrusińska *et al.*, 2021; Başaran *et al.*, 2022). *Micromeria imbricata* belongs to the mint family, Lamiaceae, that is widespread across West Africa, West Asia, with a centre of diversity in the Mediterranean region and the Canary Islands (Ahmed *et al.*, 2019; Al-Yousef *et al.*, 2021; Kremer *et al.*, 2021). *M. Imbricata* is one of the most widely used herbal plants among people of tropical and sub-tropical regions of the world (Ahmed *et al.*, 2019; Al-Yousef *et al.*, 2021; Kremer *et al.*, 2021). It has been documented in literatures that extract of *M. imbricata* leaves has antimicrobial activity (Al-Nuri *et al.*, 2022; Abu-Reidah *et al.*, 2022), larvicidal (Ahmed *et al.*, 2019; Al-Yousef *et al.*, 2021), antioxidant activity (Malayil *et al.*,

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2021; Al-Nuri *et al.*, 2022), anti-inflammatory actions, antimalarial activity, antianxiety and antidepressant activity, analgesic activity and anti-diabetic activity (Abu-Reidah *et al.*, 2022). Despite available data on activities there are scanty information relating to its toxicity and safety. This study assessed the effects of methanolic extract on liver, parameters in Wistar rats.

2. Materials and Methods

2.1 Plant Sample Collection

Leaves of *M. imbricata* were collected from Nigeria Montane Forest reserve Ngel-Nyaki, Yelwa Sardauna Local Government Area of Taraba State where it was identified and assigned voucher number (NMFP/0123/0018). The leaves were dried under ambient conditions in the shade and subsequently pulverized using a mortar and pestle and sieved with a $1mm^2$ sieve.

2.2 Crude Extraction

About 500 g of the powder of the plant material was soaked in methanol for 24 hours, after which it was filtered using a piece of clean, sterile, white Muslin cloth to remove debris and then through Whatman No. 1 filter paper. The decoction was then concentrated using a vacuum rotary evaporating system then followed by freeze drying.

2.3 Experimental Animals

Albino rats of both sexes weighing 96–114g and aged 10-12 weeks were purchased from National Veterinary Research Institute, Jos. The rats were housed in metal cages in the research laboratory of Biological Sciences Department, Taraba State University, Jalingo, Nigeria, and allowed to acclimatize for two weeks. They were kept under standard laboratory conditions at ambient temperature and fed with grower mash from vital feeds and water *ad-libitum*. The animals were handled according to the guidelines of the National Research Council's Guide for the Care and Use of Laboratory Animals.

2.4 Determination of LD_{50}

The LD_{50} was determined using the method described by Lorke (1983). In the first phase, 9 animals were grouped into 3 groups of 3 animals each. Each of the group of animals were administered with exactly 10, 100 and 1000 mg/kg per kilogram of body weight respectively of the methanolic extract of *M. imbricata*. The animals were observed at fixed intervals for 24 hours for behavioural changes, morbidity and death. In the second phase, 3 animals were placed into 3 groups with one animal each which were treated with higher doses (1600, 2900 and 5000 mg/kg of body weight) of the methanolic extract of *M. imbricata* and were then observed for 24 hours.

The LD_{50} was calculated using the formula:

$$LD_{50} = \sqrt{(D_0 \times D_{100})} \quad (1)$$

D_0 = Highest dose that gave no mortality,
D_{100} = Lowest dose that produced mortality.

2.5 Animal Grouping for Sub-Chronic Toxicity

The animals were weighed and randomly grouped into 4 groups comprising of control (given food and water *ad-libitum* with no treatment) and 3 treatment groups of three (3) rats each administered doses of 1/20 (26.90mg/kg), 1/40 (53.85mg/kg) and 1/80 (107.00mg/kg) value of LD_{50} of methanol extract of *M. imbricata* per kilogram of body weight (Diallo *et al.*, 2010). The extract was administered orally for the period of 14 days during which food consumption and water intake of the groups was equally observed together with likely physical manifestation of toxicity and mortality.

2.6 Blood Collection and Preparation of Tissue

All animals were anaesthetized humanely at the end of the experiment on the 14th day. Blood was collected into a Lithium Heparin bottle. The clotted blood samples were centrifuged in a bench top centrifuge (3000 rpm for 10 min) to obtain serum.

2.7 Biochemical Tests

Alanine aminotransferase and aspartate aminotransferase activities were assayed by the method of Reitman and Frankel (1957) using Sigma Aldrich assay kits (Merck KGaA, Darmstadt, Germany) while serum level of alkaline phosphatase was quantified by optimized standard method described by Haussament (1977) using Sigma Aldrich assay kits (Merck KGaA, Darmstadt, Germany).

Total protein was determined by colorimetric method as described by Fine, (1935) using Sigma Aldrich assay kit (Merck KGaA, Darmstadt, Germany). Serum albumin was determined by the method of Doumas *et al.* (1971) using Sigma Aldrich assay kit (Merck KGaA, Darmstadt, Germany). Serum total bilirubin (TB) concentration was

determined using Sigma Aldrich Kit (Sigma Aldrich laboratories limited UK) based on the method described by Jendrassik and Grof, (1938) and Sherlock, (1951). Total bilirubin in serum was estimated in the presence of caffeine, which liberates albumin bound bilirubin, by reacting with diazotized sulphanillic acid. The serum direct/indirect bilirubin (DB/IB) concentration was determined using the Sigma Aldrich Kit based on the method described by Jendrassik and Grof, (1938) and Sherlock, (1951).

2.8 Statistical Analysis

All assays and investigations were caried out in triplicates and data expressed as mean ± standard deviation. The results were analyzed using one-way ANOVA. Test significant were considered at $P < 0.05$.

3. Results

The oral administration of methanolic extract of *Micromeria imbricata* up to 1000 mg/kg in phase I produced no mortality in albino rats (Table 1). In phase II, mortality was observed at 2900 mg/kg, whereas no deaths occurred at 1600 mg/kg or 5000 mg/kg (Table 2). The calculated LD_{50} value was 2154 mg/kg body weight, suggesting that the extract falls within the "moderately toxic" category according to Lorke's classification. The methanolic extract significantly elevated serum levels of ALT, AST, and ALP in treated groups compared to the control ($p < 0.05$) (Table 3). The rise was dose-dependent, with the highest increase recorded in AST activity (893.33 ± 465.4 U/L at 107.00 mg/kg). There was a marked increase in serum total protein, total bilirubin, and direct bilirubin in all treatment groups compared to the control. The highest alteration was observed at the intermediate dose (53.85 mg/kg), where total bilirubin rose from 11.7 ± 2.02 mg/dL in control to 54.4 ± 1.38 mg/dL in treated rats (Table 4)

Table 1: LD_{50} determination phase I

Dose (Mg/kg)	Rats	Death	Dose Different	Mean Death	Dose Different X Mean Death
10	3	0	0	-	-
100	3	0	90	-	-
1000	3	0	990	-	-

Table 2: LD_{50} determination phase II

Dose (Mg/kg)	Rats	Death	Dose Difference	Mean Death	Dose Difference X Mean Death
1200	1	0	1200	-	-
1600	1	0	1600	-	-
2900	1	1	2900	+	-
5000	1	0	5000	-	-

LD_{50} = Maximum dose = 2154.00mg/kg

Table 3: Effect of intake of *M. imbricata* methanolic extract on liver function profiles

Parameters	Control 0.00mg/kg	Group One 26.90mg/kg	Group Two 53.85mg/kg	Group Three 107.00mg/kg
ALT(U/L)	90.5±7.50	160.33±25.00[a]	124.00±10.00[a]	131.00±28.82[a]
AST(U/L)	150.00±80.25	408.00±40.63[a]	351.50±.8.94[a]	893.33±465.4[b]
ALP(U/L)	349 ±13.85	471.33±38.67[a]	412.50±48.78[a]	448.00±144.00[a]

[a] values are significantly different from the respective control

Table 4: Effect of intake of *M. imbricata* methanolic extract on liver function parameters

Parameters	Control (0.0mg/kg)	Group One (26.90mg/kg)	Group Two (53.85mg/kg)	Group Three (107.00mg/kg)
Total Bilirubin(mg/dL)	11.7 ±2.0207	45.03±9.1170[a]	54.4±1.3856[a]	43.9±12.00[a]
Direct Bilirubin(mg/dL)	7.25±2.62	22.03±6.90[a]	23.05±5.05[a]	18.46±3.84[a]
Total Protein(g/dL)	11.7±2.02	45.03±9.11[a]	54.4±1.38[a]	43.9±12.00[a]

[a] values are significantly different from the respective control

4. Discussion

The present study assessed the toxicological potential of *M. imbricata* methanolic extract in albino rats by evaluating acute and sub-chronic toxicity indices.

The calculated LD_{50} value of 2154 mg/kg places the plant extract in the moderately toxic range, consistent with the World Health Organization (WHO) toxicity classification. This implies that while *M. imbricata* may be relatively safe at lower doses, higher concentrations can induce significant toxic effects. This agrees with previous findings that natural origin does not guarantee safety (Taroncher *et al.*, 2021; Khursheed *et al.*, 2022).

The observed elevation of ALT, AST, and ALP is a strong indicator of hepatocellular leakage and liver dysfunction. These enzymes are released into circulation when hepatic integrity is compromised (Titus *et al.*, 2023). The increase in serum bilirubin further supports impaired hepatobiliary function, possibly due to cholestatic or hepatocellular injury. This outcome is consistent with reports by Elisha (2020), who observed pharmacological activities of *M. imbricata* but also emphasized the need for toxicity evaluation to establish safe dosage ranges. Similarly, Al-Yousef *et al.* (2021) profiled the aerial parts of *M. imbricata* and identified bioactive constituents such as phenolics, flavonoids, and terpenoids with strong biological activities. These compounds, while pharmacologically beneficial, can also exert dose-dependent toxicity as seen in the present study.

Studies on related species such as *Micromeria biflora* also support this duality of activity and toxicity. Al-Samawi *et al.* (2025) demonstrated significant antioxidant and antitumor activities of *M. biflora*, but also conducted ADMET predictions which highlighted the importance of pharmacokinetic and toxicity considerations. Likewise, Al-Joufi *et al.* (2024) confirmed the therapeutic potential of *M. biflora* in arthritis models by modulating cytokine networks, yet such benefits may be offset by toxicity at higher doses. These parallels highlight that while *Micromeria* spp. are rich in pharmacologically useful phytochemicals, they require strict dosage control.

The findings also resonate with the ethnobotanical review of Ethiopian medicinal plants by Esubalew *et al.* (2017), which noted that traditional usage of plants for cancer and other chronic diseases often overlooks potential toxicity. This underscores the importance of complementing ethnomedicinal knowledge with rigorous toxicological evaluation, as provided in this study.

The significant hepatotoxic effects observed in sub-chronic dosing suggest that caution must be exercised in the traditional use of *M. imbricata*. While therapeutic effects such as antioxidant, antimicrobial, and anti-inflammatory activities are well-documented (Al-Yousef *et al.*, 2021; Elisha, 2020; Abu-Reidah *et al.*, 2022), the plant extract is not devoid of toxicological risks.

5. Conclusion

The present study demonstrated that the methanolic extract of *Micromeria imbricata* possesses a moderate level of toxicity, with an LD_{50} value of 2154 mg/kg in albino rats. Sub-chronic administration of the extract produced significant alterations in liver function biomarkers, including elevations in ALT, AST, ALP, bilirubin, and total protein, indicating hepatic stress and possible hepatocellular damage. While *M. imbricata* is widely recognized for its antimicrobial, antioxidant, and anti-inflammatory activities, the findings of this study highlight the importance of cautious use, as its therapeutic benefits may be accompanied by dose-dependent toxic effects.

Therefore, the extract should not be considered entirely safe for prolonged or high-dose use without appropriate toxicological evaluation. Further studies, including histopathological assessments, chronic toxicity testing, and compound isolation, are recommended to establish safe therapeutic ranges and identify the specific phytochemicals responsible for the observed hepatotoxicity.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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