

Acute Oral and Dermal Toxicity of *Mikania micrantha* Essential Oil in Rats

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Abstract

The safety profile of *Mikania micrantha* essential oil has not been sufficiently characterized, despite its demonstrated bioactivity. To address this, the study evaluated its acute oral and dermal toxicity in Sprague-Dawley rats weighing 190–210g. Experimental groups included a negative control (distilled water), a positive control (DMSO+PBS), and three treatment groups (300, 1000, and 2000 mg/kg doses). The evaluation was conducted in accordance with OECD guidelines Nos. 423 and 402, pertaining to acute oral toxicity and acute dermal toxicity testing in animals, respectively. These protocols are standardized, providing internationally accepted procedures for assessing potential toxic effects of substances through oral and dermal routes. No mortalities or significant clinical abnormalities were observed over the 14-day observation period, while hemato-biochemical profiles remained within normal ranges. The parasitic cyst found in histopathology, containing larval cestodes, was an incidental finding and was not attributed to *M. micrantha* essential oil exposure. All these findings suggest that *M. micrantha* essential oil is well-tolerated by rats following acute oral and dermal administration, supporting its potential safe use in preclinical studies and laying the groundwork for its future development as a therapeutic agent in broader veterinary applications.

Keywords

Essential oils, *Mikania micrantha*, Preclinical safety, Rats, Toxicity testing

Introduction

The World Health Organization (WHO, 2025) highlights that the widespread misuse of antibiotics has significantly contributed to the global treatment failures and economic burdens in common infections affecting the skin. Consequently, there is a persistent need to explore alternative, cost-effective, and sustainable therapeutic sources for wounds for long-term use. Medicinal plants, such as local weeds, have emerged as promising alternatives due to containing natural essential oils rich in phytochemicals, corresponding to the plants' antibacterial properties (Davis and Choisy, 2024). However, to ensure their safe and effective integration into clinical or commercial use, these natural products must first undergo thorough safety evaluations, particularly concerning their toxicity profiles.

Mikania micrantha, commonly known as bitter vine, is one of the local weeds traditionally used for its wound-healing, anti-inflammatory, and antibacterial properties. The plant has long been employed in folk medicine in tropical and subtropical regions for managing skin wounds, infections, and inflammatory conditions. Recent pharmacological investigations have confirmed these claims that, specifically, *M. micrantha* extracts were found to significantly enhance cutaneous wound healing by the activation of the focal adhesion kinase (FAK)/Akt/mammalian target of rapamycin (mTOR) signaling pathway, thus promoting fibroblast proliferation, collagen deposition, and angiogenesis (Das *et al.*, 2023). Moreover, an ethnopharmacological review has also identified *M. micrantha* as a plant source rich in flavonoids, terpenoids, and tannins, contributing to its therapeutic potential

against inflammatory and microbial diseases (Khan *et al.*, 2023). Additionally, comparative studies on its leaves and flowers have demonstrated strong antioxidant and antibacterial activities against *Staphylococcus aureus* and *Streptococcus pyogenes*, thereby corroborating its traditional use in the treatment of wound infections (Bera *et al.*, 2023).

Despite these promising pharmacological insights, the scientific literature remains limited regarding the safety profile of *M. micrantha*, along with its toxicological risks associated with concentrated preparations such as essential oils. While natural products are perceived as inherently safe, many bioactive plant compounds can exert adverse effects at high concentrations. Much of the current knowledge on the medicinal use of weeds like *M. micrantha* is derived from indigenous knowledge systems, with relatively few empirical studies investigating potential toxicity. Thus, a need for rigorous toxicological evaluations is crucial before recommending their use in preclinical or clinical applications.

Given this gap, the present study was designed to assess both the acute oral and dermal toxicity of *M. micrantha* essential oil in a rat model. The study monitored key indicators of toxicity and any gross or histopathological abnormalities. Furthermore, it aimed to identify potential dose-dependent effects on mortality and evaluated hematological and serum biochemical parameters to assess the impact of the essential oil on vital organ functions, particularly the liver and kidneys.

Materials and Methods

The study employed a Completely Randomized Design (CRD) and adhered to the guidelines outlined in OECD Test Guideline 423 (Acute Oral Toxicity – Acute Toxic Class Method), OECD (2002), and OECD 402 (Acute Dermal Toxicity), OECD (2017). A total of 20 healthy rats were used for each toxicity test and were randomly assigned into five treatment groups (two controls and three dose levels– 300 mg/kg, 1000 mg/kg, and 2000 mg/kg), with four animal replicates per group. Randomization was conducted using Microsoft Excel's random number generator to ensure unbiased group allocation. For OECD 423, the study applied the stepwise procedure, in which three animals were initially dosed at a specific level and were observed for 14 days. The next group of animals was dosed at a higher or lower level as per the guidelines' flowchart. On the other hand, for OECD 402, the study followed a limit test design, wherein a single dose was applied dermally to the test animals to determine whether the substance produced any toxic effects at that level.

Before testing, the collected fresh and healthy *M. micrantha* plant materials (whole plant, including the roots) were subjected to steam distillation to extract the essential oil (Nour *et al.*, 2024). Hydrosol was concentrated and separated through a rotational speed vacuum concentrator and an essential oils separator to obtain the essential oils. Acute oral toxicity test substances were administered in a single dose by oral gavage,

Results and Discussion

All test rats subjected to varying treatment doses of *M. micrantha* essential oil displayed no notable clinical signs for both toxicity tests (Table 1. Observations on

while acute dermal toxicity was initiated through fur removal from the flank area of the rats and was applied once daily for 14 days.

All rats were housed under standard laboratory conditions with free access to food and water and were monitored daily for signs of toxicity, behavioral changes, and mortality throughout the 14-day observation period. Aspects as animal handling, dosing, and sample collection were conducted with strict adherence to the principles of humane care, minimizing stress and discomfort at all times.

At the end of the observation period, blood samples were collected from representative surviving animals, before euthanasia, to evaluate the biochemical parameter components such as Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), and Blood Urea Nitrogen (BUN) and hematological profiles as hematocrit (Hct), hemoglobin (Hb) concentration, red blood cell (RBC), white blood cell (WBC) and differential leucocyte count–neutrophil (Neut %), lymphocyte (Lym%), monocyte (Mon%), eosinophil (Eos%), and basophil (Bas%) and platelet count (Plt). All rats were necropsied to identify gross and histopathological lesions and were disposed of properly after evaluation. Data were statistically analyzed using one-way analysis of variance (ANOVA), with Tukey's post hoc test applied to determine significant differences among treatment groups. A paired t-test was also used to assess the differences between pre- and post-test values.

Treatment Response of Test Animals). This implies that even at the highest dose (2000 mg/kg), *M. micrantha* essential oil did not cause acute toxicity. Moreover, all rats displayed alertness, normal grooming

behavior, and showed no signs of morbidity or mortality, supporting the stable physiological and behavioral markers across the multiple organ systems. According to OECD Guideline 423 for acute oral toxicity,

the lack of signs such as lethargy, tremors, respiratory distress, or abnormal secretions strongly suggests low acute toxicity potential (OECD, 2002).

Table 1. Observations on Treatment Response of Test Animals

Type of Test	Observations	Treatment Response (n=40)				
		(-) Control A	(-) Control B	300 mg/kg	1000 mg/kg	2000 mg/kg
Oral	Pink Skin Color	+(4/4)	+(4/4)	+(4/4)	+(4/4)	+(4/4)
Dermal	Pink Skin Color	+(4/4)	+(4/4)	+(4/4)	+(4/4)	+(4/4)
Oral	Eye: Normal	+(4/4)	+(4/4)	+(4/4)	+(4/4)	+(4/4)
Dermal	Eye: Normal	+(4/4)	+(4/4)	+(4/4)	+(4/4)	+(4/4)
Oral	Fur: Normal	+(4/4)	+(4/4)	+(4/4)	+(4/4)	+(4/4)
Dermal	Fur: Normal	+(4/4)	+(0/4)	+(4/4)	+(4/4)	+(4/4)
Oral	Coughing	-(0/4)	-(0/4)	-(0/4)	-(0/4)	-(0/4)
Dermal	Coughing	-(0/4)	-(0/4)	-(0/4)	-(0/4)	-(0/4)
Oral	Vomiting	-(0/4)	-(0/4)	-(0/4)	-(0/4)	-(0/4)
Dermal	Vomiting	-(0/4)	-(0/4)	-(0/4)	-(0/4)	-(0/4)
Oral	Wet Stool	-(0/4)	-(4/4)***	-(0/4)	-(0/4)	-(0/4)
Dermal	Wet Stool	-(0/4)	-(0/4)	-(0/4)	-(0/4)	-(0/4)
Oral	Pink mm	+(4/4)	+(4/4)	+(4/4)	+(4/4)	+(4/4)
Dermal	Pink mm	+(4/4)	+(4/4)	+(4/4)	+(4/4)	+(4/4)
Oral	Convulsion	-(0/4)	-(0/4)	-(0/4)	-(0/4)	-(0/4)
Dermal	Convulsion	-(0/4)	-(0/4)	-(0/4)	-(0/4)	-(0/4)
Oral	Alertness	+(4/4)	+(4/4)	+(4/4)	+(4/4)	+(4/4)
Dermal	Alertness	+(4/4)	+(4/4)	+(4/4)	+(4/4)	+(4/4)
Oral	Mortality	-(0/4)	-(0/4)	-(0/4)	-(0/4)	-(0/4)
Dermal	Mortality	-(0/4)	-(0/4)	-(0/4)	-(0/4)	-(0/4)

Note: Physical and Behavioral Observations in Rats Administered with Varying Doses of *M. micrantha* Essential Oil via Oral and Dermal Routes showing absence of clinical signs, except wet stool on (-) control B. (+) Normal; (-) Abnormal; (***) Wet stool; mm= mucus membrane; (-) Control A= PBS; (-) Control B= PBS + DMSO.

Additionally, no signs of neurotoxicity were observed in any of the rats administered with *M. micrantha* essential oil across all dose levels through both oral and dermal routes.

Behavioral and physical evaluations conducted throughout the 14-day observation period consistently showed normal alertness, absence of convulsions, normal gait, and

intact reflexes, without evidence of ataxia, tremors, hypersalivation, or any other neurological dysfunction. As summarized in Table 1, all treated animals ($n = 40$) maintained normal eye appearance, fur condition, skin coloration, and mucous membrane tone. Importantly, none exhibited abnormal behaviors such as lethargy, reduced responsiveness, hyperactivity, or stereotypic movements, which are commonly associated with central nervous system toxicity. Normal alertness scores were also maintained across all treatment groups (oral and dermal), and no convulsions, vomiting, or coordination deficits were detected. The only irregularity recorded was the presence of wet stool in one negative control group (PBS + DMSO), which was not observed in any of the essential oil-treated animals, further supporting the absence of treatment-related systemic or neurotoxic effects.

Taken together, these findings reinforce that even up to the highest tested dose of 2000 mg/kg, *M. micrantha* essential oil does not cause any observable neurobehavioral abnormalities, thereby supporting its lack of neurotoxic potential under acute exposure conditions. This observation aligns with established toxicological principles (OECD, 2017), which indicates that substances with neurotoxic properties typically manifest early signs of central nervous system involvement, such as tremors, convulsions, altered alertness, or impaired coordination, even at sublethal doses.

Recent toxicological studies emphasized that behavioral changes are among the earliest and most sensitive indicators of neurotoxicity, often preceding morphological or biochemical alterations. Such changes, including those of altered locomotor activity, postural instability,

and abnormal response to stimuli, can be readily observed during routine acute toxicity testing and are considered critical endpoints in early neurotoxicity screening (Thunga *et al.*, 2021). The absence of these behavioral abnormalities in the present study supports the interpretation that *M. micrantha* essential oil does not exhibit neurotoxic potential under acute exposure conditions.

The lack of any mortality or morbidity across all groups also further strengthens the implication that *M. micrantha* is well-tolerated even at doses up to 2000 mg/kg. As per the Globally Harmonized System (GHS) of Classification and Labelling of Chemicals, substances that do not produce toxic effects or death at this level may be categorized as having low acute oral toxicity (UN, 2023). The only exception observed was the presence of wet stool in all animals of Control B.

Phosphate-buffered saline (PBS) was used as a negative control in this study to represent the baseline physiological response without any vehicle influence, as it is isotonic and biologically inert. In contrast, PBS + DMSO was used as a vehicle control because dimethyl sulfoxide (DMSO) was the solvent used to dilute the essential oil for dermal and oral administration. Since DMSO can exert mild biological effects such as increased membrane permeability or gastrointestinal disturbance, its inclusion as a separate control group allows the distinction between potential effects of the vehicle itself and those of the essential oil. This ensures that any observed responses in the treatment groups can be confidently attributed to *M. micrantha* essential oil and not to the solvent. This, then, could be the possible reason for the presence of wet stool in all animals of Control B. Additionally, the normal coloration of the

mucous membrane across all test animals suggests that the compound did not impair the oxygen transport and tissue perfusion, indicating an absence of circulatory compromise or hypoxia (Rhodes and Varacallo, 2019).

In terms of body weight, animals subjected to acute oral toxicity testing exhibited a slight increase over the 14-day observation period. However, no statistically significant differences were observed among

the treatment groups ($p > 0.05$) (Table 2. Mean Body Weight of Test Animals Across Treatment Groups and Toxicity Tests). The absence of significant body weight variation is an implication that *M. micrantha* essential oil did not adversely affect metabolic function, appetite, or growth. These findings indicate that the essential oil was well-tolerated and did not induce systemic toxicity capable of disrupting normal physiological processes related to weight gain.

Table 2. Mean Body Weight of Test Animals Across Treatment Groups and Toxicity Tests.

Treatment Group	Body Weight (grams) (n= 40)					
	Acute Oral Toxicity Test			Acute Dermal Toxicity Test		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
(-) Control A	192.50±2.51	194.00±2.16	194.50±2.38	194.25±1.70	195.50±1.73	194.25±2.21
(-) Control B	192.50±1.91	193.50±1.29	193.75±2.21	194.25±1.70	194.25±2.21	196.00±2.44
300 mg/kg	195.00±3.46	196.50±3.10	197.25±2.87	194.00±1.89	194.75±2.36	195.50±2.08
1000 mg/kg	193.50±4.43	194.75±4.19	195.50±4.35	193.00±3.55	194.00±3.55	195.00±4.32
2000 mg/kg	196.00±4.00	197.50±3.69	198.00±3.46	195.50±2.64	196.75±2.87	197.25±3.30
Total/ <i>p</i>	0.520 ^{ns}	0.345 ^{ns}	0.316 ^{ns}	0.711 ^{ns}	0.594 ^{ns}	0.691 ^{ns}

Note: The Body Weight (grams) ($\pm$ SD) of Rats upon Administration with *M. micrantha* Essential Oil via Oral and Dermal Routes showing non-significant changes over the 14-day treatment period. Values are expressed as mean $\pm$ SD; SD, Standard deviation; ^{ns} = not significant; (-) Control A PBS; (-) Control B PBS + DMSO.

Relative organ weights of the heart, lungs, liver, spleen, kidneys, stomach, and intestines across treated groups (Table 3. Mean Relative Organ Weight of Test Animals Across Treatment Groups and Toxicity Tests) also showed no significant differences when compared to controls ($p > 0.05$), implying no dose-dependent organ hypertrophy or atrophy, supporting (Saleem *et al.*, 2017). Similarly, organ weight is widely recognized

as a sensitive and early indicator of target organ toxicity, often reflecting subtle biological disturbances before evident pathological changes occur. Recent studies have emphasized its diagnostic value in toxicological evaluations. For instance, Lazic *et al.* (2020) demonstrated that organ weight alterations are critical endpoints in toxicity testing and advocated for improved analytical models to enhance their interpretive value.

Table 3. Mean Relative Organ Weight of Test Animals Across Treatment Groups and Toxicity Tests.

Organ	Type of Test	Relative Organ Weight (grams) (n= 40)					p value
		(-) Control A	(-) Control B	300 mg/kg	1000 mg/kg	2000 mg/kg	
Heart	Oral	0.22 ± 0.005	0.22 ± 0.005	0.21 ± 0.009	0.21 ± 0.009	0.21 ± 0.14	0.100 ^{ns}
	Dermal	0.22 ± 0.01	0.22 ± 0.01	0.22 ± 0.005	0.22 ± 0.02	0.22 ± 0.005	0.866 ^{ns}
Lungs	Oral	0.26 ± 0.25	0.36 ± 0.20	0.47 ± 0.02	0.47 ± 0.01	0.45 ± 0.02	0.260 ^{ns}
	Dermal	0.50 ± 0.00	0.50 ± 0.00	0.50 ± 0.00	0.47 ± 0.005	0.50 ± 0.00	0.438 ^{ns}
Liver	Oral	0.26 ± 0.01	0.27 ± 0.005	0.27 ± 0.008	0.27 ± 0.008	0.26 ± 0.005	0.926 ^{ns}
	Dermal	0.28 ± 0.00	0.28 ± 0.00	0.28 ± 0.005	0.28 ± 0.005	0.28 ± 0.05	0.293 ^{ns}
Spleen	Oral	0.09 ± 0.00	0.08 ± 0.005	0.08 ± 0.005	0.08 ± 0.005	0.09 ± 0.00	0.415 ^{ns}
	Dermal	0.09 ± 0.00	0.09 ± 0.00	0.09 ± 0.00	0.09 ± 0.00	0.09 ± 0.00	1.000 ^{ns}
Kidney	Oral	0.06 ± 0.00	0.06 ± 0.00	0.05 ± 0.005	0.06 ± 0.00	0.05 ± 0.00	0.199 ^{ns}
	Dermal	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.05 ± 0.005	0.06 ± 0.00	0.438 ^{ns}
Stomach	Oral	0.15 ± 0.005	0.13 ± 0.02	0.13 ± 0.01	0.12 ± 0.01	0.14 ± 0.01	0.193 ^{ns}
	Dermal	0.16 ± 0.005	0.16 ± 0.005	0.17 ± 0.008	0.15 ± 0.01	0.16 ± 0.00	0.306 ^{ns}
Intestine	Oral	0.47 ± 0.004	0.45 ± 0.01	0.45 ± 0.009	0.45 ± 0.01	0.45 ± 0.005	0.094 ^{ns}
	Dermal	0.47 ± 0.008	0.46 ± 0.005	0.47 ± 0.01	0.45 ± 0.03	0.46 ± 0.005	0.753 ^{ns}

Note: The Relative Organ Weight (± SD) of Rats in Acute Toxicity Studies of *M. micrantha* Essential Oil showing non-significant changes after Treatment. SD= Standard deviation; *p*-value <0.05; ^{ns} = non-significant; (-) Control A= PBS; (-) Control B= PBS + DMSO.

The lack of significant variations in the relative organ weights of test animals further supports the compound's non-toxic profile, which is also evident in test animals subjected to an acute dermal toxicity test. Furthermore, the dermal responses observed in the acute dermal toxicity test (Table 4. Acute Dermal Toxicity: Draize Criteria) showed that all treatment groups exhibited mean erythema and edema scores of 0.00. According to the

OECD Test Guideline 404 for acute dermal irritation and corrosion, a Draize score of 0 indicates no observable irritation, such as redness or swelling, at the site of application. Based on these scores, *M. micrantha* essential oil is classified as non-irritant. These findings align with the OECD irritation scale, which considers a substance non-irritating when no dermal changes are observed over the 72-hour evaluation period (OECD, 2015).

Table 4. Acute Dermal Toxicity: Draize Criteria.

Dose	Mean Score for Erythema	Mean Score for Edema formation	Evaluation for Erythema	Evaluation for Edema formation
(-) Control A	0.00	0.00	Non-irritant	Non-irritant
(-) Control B	0.00	0.00	Non-irritant	Non-irritant
300 mg/kg	0.00	0.00	Non-irritant	Non-irritant

Dose	Mean Score for Erythema	Mean Score for Edema formation	Evaluation for Erythema	Evaluation for Edema formation
1000 mg/kg	0.00	0.00	Non-irritant	Non-irritant
2000 mg/kg	0.00	0.00	Non-irritant	Non-irritant

Representative samples submitted for biochemical analyses, 10 for acute oral toxicity tests, and five for acute dermal toxicity (Table 5. Mean Serum Biochemical Values $\pm$ SD of Tested Animals Before and After Treatment) were found to have stayed within the reference range and did not differ significantly ($p > 0.05$). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in the test animals showed no statistically significant elevations following exposure to *M. micrantha* essential oil. This indicates that acute administration did not result in evident hepatocellular injury. Although a slight post-test increase in ALT was observed, the values remained within the accepted physiological range (Fielder, 2022^b) and were not statistically significant. Nonetheless, given

that ALT is a sensitive marker of hepatocellular integrity, even a modest elevation, though within normal limits, could warrant further investigation in the context of repeated or chronic exposure, where cumulative effects may become more apparent. Additionally, blood urea nitrogen (BUN) levels remained within normal physiological ranges in all test groups, indicating preserved renal function following exposure to *M. micrantha* essential oil. Elevated BUN is commonly associated with impaired renal function and is a widely recognized marker for nephrotoxicity (Mahmood *et al.*, 2024). The absence of significant changes in BUN levels implies that the essential oil did not adversely affect the kidneys under the conditions of this study.

Table 5. Mean Serum Biochemical Values $\pm$ SD of Tested Animals Before and After Treatment.

Variables	Type of Test	Reference Values (Fielder 2022 ^b)	Mean ± SD		<i>p</i> value
AST (U/L)	Oral	37-205	Pre-test	101.62 ± 2.50	0.711 ^{ns}
			Post-test	106.02 ± 2.73	
	Dermal		Pre-test	105.06 ± 2.50	0.132 ^{ns}
			Post-test	107.98 ± 2.73	
ALT (U/L)	Oral	6-114	Pre-test	37.20 ± 4.54	0.361 ^{ns}
			Post-test	126.70 ± 6.74	
	Dermal		Pre-test	40.32 ± 8.33	0.089 ^{ns}
			Post-test	50.55 ± 4.82	

Variables	Type of Test	Reference Values (Fielder 2022 ^b)	Mean ± SD		<i>p</i> value
BUN (mg/dl)	Oral	13-29	Pre-test	19.68 ± 0.87	0.565 ^{ns}
			Post-test	23.070 ± 1.63	
	Dermal		Pre-test	19.41 ± 2.21	0.223 ^{ns}
			Post-test	21.98 ± 2.85	

Note: SD Standard deviation; p-value <0.05; ^{ns} = non-significant.

Similarly, hematological variables (Table 6. Mean Hematological Profiles ($\pm$ SD) of rats before and after Treatment) remained within reference ranges (Fielder, 2022^a), and no statistically significant alterations ($p > 0.05$) were observed between the pre- and post-

tests of five representative test animals, indicating an absence of hematotoxic effects. Platelet counts and differential counts did not exhibit any anomalies, further confirming hematological stability.

Table 6. Mean Hematological Profiles ($\pm$ SD) of rats before and after Treatment.

Parameters	Reference Values (Fielder 2022)	Hematological Profiles ($\pm$ SD)					
		Acute Oral Toxicity Test (n=5)			Acute Dermal Toxicity Test (n=5)		
		Pre-test	Post-test	p value	Pre-test	Post-test	p value
Hb (g/L)	12.9-15.4	13.54 $\pm$ 0.68	13.83 $\pm$ 0.87	0.114 ^{ns}	13.53 $\pm$ 0.43	14.01 $\pm$ 0.17	0.065 ^{ns}
Hct (%)	42-49	46.80 $\pm$ 1.19	47.08 $\pm$ 1.01	0.763 ^{ns}	44.36 $\pm$ 0.69	45.03 $\pm$ 0.611	0.180 ^{ns}
RBC (10 ⁶ /uL)	6.39-8.01	7.02 $\pm$ 0.15	7.40 $\pm$ 0.63	0.910 ^{ns}	7.48 $\pm$ 0.59	7.62 $\pm$ 0.23	0.115 ^{ns}
WBC (10 ⁹ /L)	3.00-9.22	8.21 $\pm$ 0.24	8.40 $\pm$ 0.35	0.442 ^{ns}	8.25 $\pm$ 0.32	8.30 $\pm$ 0.35	0.061 ^{ns}
Neut(%)	6.14-22.95	18.15 $\pm$ 0.47	18.24 $\pm$ 0.30	0.493 ^{ns}	18.69 $\pm$ 0.38	18.66 $\pm$ 0.37	0.367 ^{ns}
Lym (%)	69.88-86.89	72.01 $\pm$ 1.55	74.08 $\pm$ 3.33	0.664 ^{ns}	74.98 $\pm$ 0.56	75.44 $\pm$ 0.66	0.201 ^{ns}
Mono (%)	3.77-10.82	9.36 $\pm$ 0.43	9.32 $\pm$ 0.46	0.175 ^{ns}	7.75 $\pm$ 0.39	7.70 $\pm$ 0.97	0.691 ^{ns}
Eos (%)	0.54-3.39	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	-	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	-
Bas (%)	0.04-0.44	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	-	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	-
Plt (10 ⁹ /L)	923-1580	952.58 $\pm$ 19.06	938.76 $\pm$ 32.10	0.679 ^{ns}	977.73 $\pm$ 13.29	991.37 $\pm$ 9.18	0.945 ^{ns}

Note: SD= Standard deviation; p-value <0.05; ^{ns} = non-significant; Hemoglobin (Hb); Hematocrit (Hct); Red Blood Cell (RBC); White Blood Cell (WBC); Neutrophils (Neut); Lymphocytes (Lym); Monocytes (Mono); Eosinophils (Eos); Basophils (Baso); Platelets (Plt).

Examination of major organs revealed no treatment-related gross and histopathological

alterations, indicating that *M. micrantha* essential oil did not induce observable organ-

specific toxicity. However, in acute dermal toxicity testing, one rat from the 300 mg/kg group and another from the 2000 mg/kg group were observed to have a small white cyst protruding from the hepatic parenchyma (Figure 1. Lesions of the Right Median Lobe of Liver Found in One Rat). Histopathological examination identified this as a parasitic cyst containing larval cestodes, resembling that

being reported by the Joint Pathology Center, Veterinary Pathology Services (2019), which documented hepatic cysticercosis in wild rats, characterized by parasitic cysts compressing the liver tissue. Importantly, such lesions are unlikely to be treatment-related and are more plausibly incidental or attributable to prior environmental exposure.

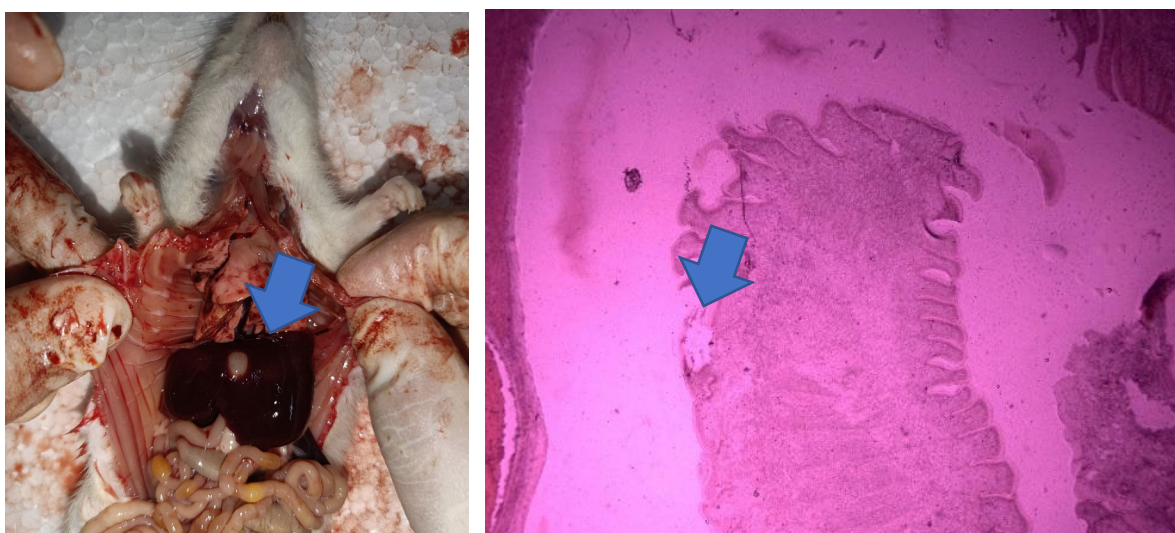


Figure 1. Lesions of the Right Median Lobe of Liver Found in One Rat. (a.) Blue arrow shows an encapsulated cyst in the right median lobe of the liver. (b.) Hematoxylin and Eosin (H&E) $\times 10$ identified as parasitic (blue arrow), containing larval cestodes. This finding is incidental, hence, not due to *M. micrantha* treatment.

Similar histopathological findings, such as mild inflammatory infiltrates or focal tissue changes, are commonly reported in laboratory rats, even in the absence of experimental treatment (Unuma *et al.*, 2019). These background lesions are well-documented in toxicological studies and are generally considered part of the spontaneous pathology spectrum in this species, thereby supporting their classification as unrelated to *M. micrantha* essential oil exposure.

Conclusion

The findings of this study demonstrate that *M. micrantha* essential oil, even at high doses up to 2000 mg/kg, has low acute oral and dermal toxicity risks in rats, supported by the absence of adverse clinical, biochemical, hematological, and histopathological effects. These findings not only proved the compounds' safety profile but also emphasized its biological potential as a

naturally derived therapeutic agent in veterinary applications. By validating the non-toxic nature of *M. micrantha* under acute exposure conditions, this study contributes to the growing body of evidence supporting the safe integration of natural product-based compounds in veterinary toxicology and pharmacology. However, the findings of the present study are limited to short-term, single-dose exposures and were conducted exclusively in female rats, which may not fully capture potential sex-related or long-term toxicological effects. Future research should therefore be employed to explore chronic and sub-chronic toxicity profiles, reproductive outcomes, and multi-generational safety assessments across both sexes to comprehensively evaluate the therapeutic potential and safety margin of *M. micrantha* essential oil.

Approval of Ethical Commission

Animals were humanely treated and managed under internationally accepted standards for the care and use of laboratory animals, as outlined by the National Research Council (2011) and the Canadian Council on Animal Care (1993), and as approved by the Institutional Animal Care and Use Committee (IACUC) of Central Mindanao University. An IACUC permit (Protocol No. 2023- 243D) was secured before the study was conducted. The CMU-IACUC operates under the guidelines of the Philippine Animal Welfare Act (Republic Act No. 8485, as amended by RA 10631) and adheres to ethical standards consistent with international norms.

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Author's Contribution

JMM, KAD, AD, TAD, and HLW designed the study. KAD and AD supervised the research process. JMM, KAD, AD, TAD, SD, HLW, ME, and AJM redefined the methods and collected, analyzed, and interpreted qualitative and quantitative data for the paper. JMM, KAD, TAD, and HLW designed the article's focus and structure and prepared the first draft of the manuscript. The manuscript was revised by KAD and AD. All the co-authors validated the manuscript.

Conflict of Interest

The authors have no conflict of interest to declare.

Data Availability Statement

The raw data will not be publicly available, and access will be granted only to those researchers listed as authors. Researchers interested in continuing research on the CMU- Natural Products Research and Development Center are welcome to contact the corresponding author.

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