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Subacute Toxicological Analysis of Hydroalcoholic Extract of *Mikania micrantha* (Asteraceae) Leaves in Wistar Rats

Soumen Dhara[1,2], Surajit Ghosh[3], Satyaki Pachal[3], Disha Saha[2], Ritu Khanra[2], Rania Indu[2], Moumita Ray[2]*

[1]Department of Pharmacology, Global College of Pharmaceutical Technology, Krishnagar, Nadia, West Bengal, 741102

[2]Department of Pharmaceutical Technology, JIS University, Agarpara, Kolkata, West Bengal, India 700109

[3]Department of Pharmaceutical Technology, NSHM Knowledge Campus, Kolkata, West Bengal, India 700053

***Corresponding Author Details:**

Dr. Moumita Ray, Assistant Professor, Department of Pharmaceutical Technology, JIS University, 81, Nilgunj Road, Agarpara, Kolkata-700109, West Bengal

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KEYWORDS

Mikania micrantha, hydroalcoholic extract, kaempferol, subacute toxicity.

ABSTRACT:

Introduction: Mikania micrantha, traditionally used in Southeast Asia and India for its antiseptic, antimicrobial, antidiabetic, anti-inflammatory, wound healing, and antidotal properties, is known to contain various bioactive secondary metabolites. Although its therapeutic benefits are well recognized, comprehensive toxicological data remain limited. This study aimed to evaluate the subacute oral toxicity of the hydroalcoholic extract of Mikania micrantha leaves (MMLE) in Wistar rats following OECD Guidelines.

Methods: A 70% hydroalcoholic extract was prepared and analyzed by phytochemical screening and HPTLC. Acute toxicity studies showed MMLE to be safe up to 2000 mg/kg. For subacute toxicity assessment, rats were orally administered MMLE (500 or 1000 mg/kg) for 28 days, with clinical signs, body weight, and food and water intake monitored. Hematological, biochemical, and histopathological analyses were performed at the end of study.

Results: MMLE comprises alkaloids, flavonoids, phenolics, tannins, and saponins, while HPTLC analysis revealed the presence of Kaempferol as the predominant component (4.58 µg/ml). No mortality or significant alterations in growth, hematological, or biochemical parameters were observed at 500 mg/kg. Histological examination of major organs (liver, kidney, brain, heart, lungs, and spinal cord) showed no remarkable changes at this dose, while mild alterations were noted at 1000 mg/kg, suggesting moderate toxicity at higher levels.

Conclusion: Overall, the findings indicate that repeated oral administration of MMLE is safe up to 500 mg/kg, whereas higher doses may induce mild organ toxicity. These results support its traditional use within a safe dosage range and underscore the need for further chronic toxicity studies.

Introduction

In many developing nations, nearly 80% of the population relies on plant-based remedies for primary healthcare needs. Over the past decade, there has been a noticeable global surge in the use of traditional, complementary, and alternative medicines. A significant number of medicinal plants have undergone pharmacological evaluation based on their traditional uses, revealing their wealth of secondary metabolites, which are often perceived as safer alternatives to synthetic drugs. These metabolites—including flavonoids, alkaloids, and polyphenols—exhibit anti-inflammatory, antimicrobial, antioxidant, and anti-obesity activities [1].

Mikania micrantha Kunth, commonly called "mile-a-minute," belongs to the Asteraceae family and is recognized as one of the top three most invasive weed species globally. Originally native to Central and South America, it now thrives in South China, Southeast Asia, and the Pacific Islands, favoring humid, nutrient-rich

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environments where it aggressively suppresses native vegetation by blocking sunlight. The genus Mikania has garnered attention for its diverse phytochemistry, and compounds like miscandenin and mikanolide contribute to its analgesic and antibacterial effects. Recent pharmacological research has demonstrated the plant's significant anti-inflammatory, cytotoxic, anticancer, antidiabetic, and wound-healing activities. As the rise of multidrug-resistant pathogens creates challenges for modern medicine, there is a growing need to explore novel antimicrobial agents, including those derived from plants [2].

Research suggests the sesquiterpene Lactones are characteristic constituents (eg. mikanolide, deoxymikanolide, scandenolide of the Asteraceae family and play a central role in the plant's biological activity. Flavonoids like quercetin, kaempferol, Luteolin are the significant polyphenolic compounds, found predominantly in the leaves [3]. These compounds having antioxidant properties by scavenging reactive oxygen species (ROS) and anti-inflammatory properties via COX inhibition. Phenolic compounds and tannins like gallic acid, caffeic acid, hydrolysable and condensed tannins contribute to the plant's antioxidant potential and protective properties. The plant's essential oil composition includes monoterpenes and sesquiterpenes like β-Caryophyllene, α-Pinene, limonene, eudesmol. The essential oils show antibacterial, antifungal, insecticidal, and anti-inflammatory properties. Volatile and non-volatile terpenoid compounds like β-amyrin, α-amyrin, friedelin, lupeol are found in both essential oils and extracts [4,5].

Despite its promising bioactivities, comprehensive subacute toxicity studies on *Mikania micrantha* remain limited. Previous research has only evaluated its acute toxicity, revealing an oral LD_{50} greater than 2000 mg/kg body weight [6]. However, acute studies alone are insufficient to establish a full safety profile, as suboptimal absorption or first-pass metabolism could obscure potential toxicities. Therefore, a repeated-dose, 28-days study is critical to understanding the potential risks associated with sustained use [7]. Guided by acute toxicity data, this study aimed to assess the *in vivo* subacute toxicity of the hydroalcoholic extract of *Mikania micrantha* leaves.

Materials and Methods

Plant Material Collection and Authentication

Fresh and matured leaves of the plant were collected from North 24 Parganas, West Bengal and authenticated by the Botanical Survey of India (BSI), as *Mikania micrantha* Kunth with authentication number CNH/Tech.II/2024/185.

Preparation of Leaf Extract

Leaves were properly cleaned and cut into small pieces, shade-dried, and powdered using a grinder. 20 grams of the dried leaf powder was macerated in a 100 ml solvent mixture of 70% hydro-alcohol for 72 hours with continuous stirring and filtered using Whatman No.1 filter paper. Maceration was repeated until the extract became colorless. All filtrates were pooled and the solvent was evaporated under reduced pressure using a rotary evaporator to obtain the hydroalcoholic extract of *Mikania micrantha* leaves and designated as MMLE [8]. The sample was stored at -20°C for further use. Percentage yield was calculated by the formula as follows [9]:

$$\text{Percentage yield (\%)} = \frac{\text{Weight of the crude extract (g)}}{\text{Weight of the powdered plant material (g)}} \times 100$$

Phytochemical analysis

The hydroalcoholic extract was evaluated for the qualitative detection of various phytochemical constituents like proteins, amino acids, phenolic compounds, alkaloids, flavonoids, reducing sugars, saponins, along with tannins [10]. The tests were performed as per standard protocol.

High-performance thin-layer chromatography (HPTLC) study

Earlier study revealed the flavonoid content present in the hydroalcoholic extract of *Mikania micrantha* Kunth leaves was notably higher compared to the phenolic constituents.[11] HPTLC was carried out on precoated silica gel 60 F_{254} plates (Merck, Darmstadt, Germany; 200 x 100 mm). Sample solutions were applied as 8 mm

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JCHR (2026) 16(2), 1317-1328 | ISSN:2251-6727

bands using a CAMAG Linomat 5 applicator under a nitrogen stream, with the application position set at 8 mm from the lower edge, an inter-track distance of 11.4 mm, and an initial track position of 20 mm. Methanol was used as the sample solvent, and 0.20 µl was applied at a dosage speed of 150 nl/s. Chromatographic development was performed in a 20 x 10 cm twin-trough chamber pre-saturated for 20 min using chloroform–ethyl acetate–formic acid (5:4:1, v/v/v) as the mobile phase. The plates were developed to a migration distance of 85 mm, air-dried at room temperature, and subjected to densitometric scanning using a CAMAG TLC Scanner 4 in absorbance mode at 254 nm with a deuterium lamp. Scanning was conducted at 100 mm/s with a slit dimension of 6.0 x 0.45 mm and a data resolution of 100 µm, and chromatographic data were processed using visionCATS software.[12]

In-vivo toxicity study

Experimental animals

The male wistar rats were 12–13 weeks old, and body weights between 180 and 200 g were selected for the experiment. All the animals were maintained as per the CCSEA guidelines keeping the temperature between 18 - 25 °C, humidity level between 40 - 70% and a 12 hrs cycle of light and dark. The animals had unrestricted access to food and water.

Acute toxicity study

The acute oral toxicity of MMLE was evaluated following OECD guideline 423 to determine the median lethal dose or LD_{50}. Non-pregnant, nulliparous female rats (n=3) received a single oral dose of 2000 mg/kg of MMLE after a 2-hour fast, with water provided ad libitum. The animals were observed for signs of toxicity including tremor, convulsion, salivation, lacrimation, diarrhea, lethargy, altered behaviour, and mortality every 30 minutes for the first 4 hours and daily thereafter for 14 days [13].

Sub-acute toxicity study

This study complied with OECD guideline 407 with minor adjustments [7]. A total of 18 rats, ranging 180–200 g, were randomly assigned to three groups for experiments, with 6 animals in each group.

Group 1: Control (Vehicle treated)

Group 2: *Mikania micrantha* leaf extract 500 mg/kg body weight

Group 3: *Mikania micrantha* leaf extract 1000 mg/kg body weight

The test groups were administered the plant-based extract at dosages of 500 mg/kg and 1000 mg/kg on a regular basis for 28 days. As a control, 1 ml/kg of body weight distilled water was given to the group at the same time. Throughout the study, the parameters like body weight, food and water intake, behavioral traits, and potential toxicity markers were carried out and documented on a daily basis. At the end of the study, the animals were fasted for overnight, euthanized, and sacrificed.

Blood was collected by heart puncture for hematological and biochemical assessments, and the desired organs were acquired for histological analysis [14]. Blood samples were centrifuged at 3000 rpm for 10 minutes to collect the serum. The hematological profiling-hemoglobin, red blood cell count, white blood cell total and differential count, platelet count and some electrolytes like sodium and chloride were assessed [15]. Along with these, the kidney function test, liver function test and lipid profile analysis were also performed [16,17].

Organs including the liver, kidneys, lungs, brain, heart, and spleen were excised and weighed [18]. For histopathological study the kidney, liver, heart, lungs, brain and spinal cord tissues were preserved in 10% v/v formalin and further hematoxylin-eosin staining was used for analysis [19].

Statistical analysis

All statistical analysis were performed using SPSS Software. Data were expressed as mean ± SEM and the data were analyzed using one-way ANOVA followed by TUKEY post-hoc test, considering the level of significant at $p<0.05$.

Results:

Phytochemical evaluation

The percentage yield of the hydroalcoholic extract of *Mikania micrantha* leaves was obtained as 15.69% w/w. According to phytochemical investigation, alkaloids,

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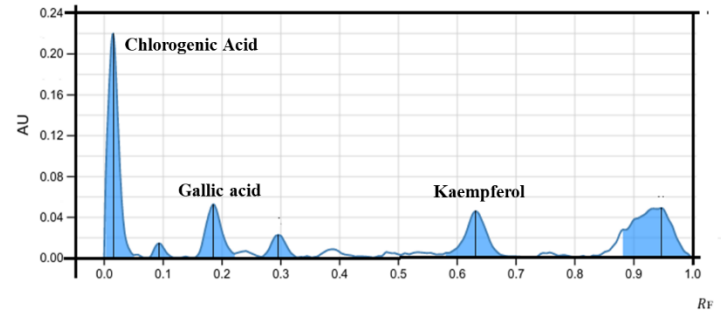
JCHR (2026) 16(2), 1317-1328 | ISSN:2251-6727

flavonoids, phenolics, tannins and saponins were present in the extract.

HPTLC study

The obtained chromatogram of MMLE was compared with thirteen polyphenolic and flavonoid standards including Trans cinnamic acid, 4-hydroxy benzoic acid, Quercetin, Caffeic acid, Gallic acid, Chlorogenic acid, Vanillic acid, Apigenin, p-coumaric acid, Myricetin, Naringenin, Caffeine and Kaempferol.



Sl. No.	Identified Phytoconstituents	Quantity (µg/ml)
1.	Chlorogenic Acid	0.32
2.	Gallic Acid	0.008
3.	Kaempferol	4.58

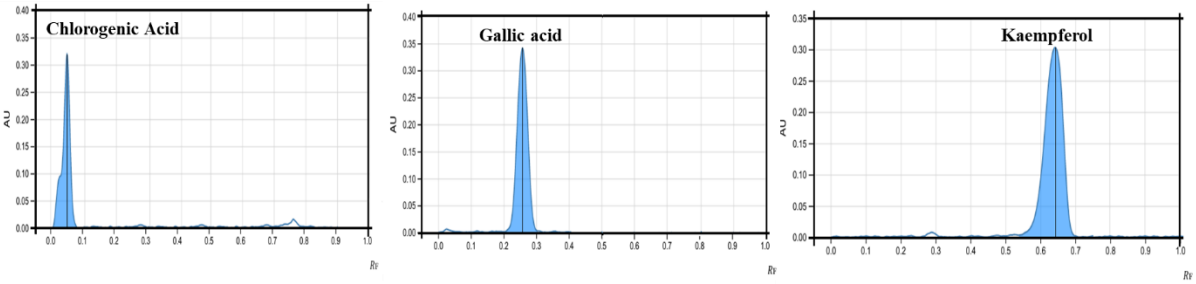


Figure 1: HPTLC chromatogram of the hydroalcoholic extract of *Mikania micrantha* Kunth leaves and standard.

The HPTLC chromatogram of MMLE indicated the presence of chlorogenic acid, gallic acid, and kaempferol, among which kaempferol was the predominant constituent (4.58 µg/ml). These findings corroborate earlier reports demonstrating the predominance of flavonoids in the extract.[11]

Acute oral toxicity study

Oral administration of MMLE at 2000 mg/kg caused no deaths or observable toxic effects in rats, indicating that its oral LD_{50} is beyond the dose level and its safe upto 2000 mg/kg body weight in rats.

Subacute oral toxicity study

In the sub-acute toxicity after 28 days no death was observed after treatment with 500 and 1000mg/kg doses of MMLE.

Body weight measurements

There were no significant changes in the body weights of the MMLE treated rats on 7th & 14th days of the study (Figure 2).

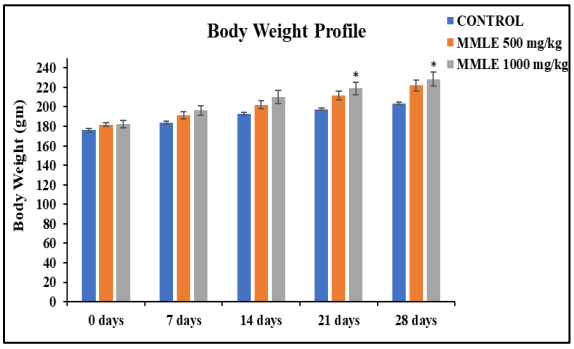


Figure 2: Body weight profile of rats following 28 days repeated administration of hydroalcoholic extract of *Mikania micrantha* leaves (MMLE)

Journal of Chemical Health Risks

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JCHR (2026) 16(2), 1317-1328 | ISSN:2251-6727

Data were analyzed using one-way ANOVA followed by TUKEY post hoc test. Data were presented as mean ± SEM (n=6), *denotes significant difference compared to the control group (p<0.05)

However, on 21st & 28th days body weights were significantly increased (p<0.05) in 1000 mg/kg treated group, compared to the control group. Normal growth of control group was observed throughout the study.

Organ weight measurement

The effect of repeated administration of MMLE on the organ weight of the liver, kidney, lungs, brain, heart, and spleen was demonstrated in table 1.

Table 1: Effect of MMLE on organ weights in sub-acute toxicity study

Organ	Control	MMLE 500 mg/kg	MMLE 1000 mg/kg
Liver	9.75±0.30	9.44±0.16	10.95±0.40*
Kidney	2.60±0.16	2.25±0.13	3.43±0.02**
Lungs	2.35±0.13	2.40±0.14	3.15±0.24*
Brain	1.96±0.04	1.86±0.11	1.82±0.06
Heart	1.54±0.11	1.51±0.13	1.36±0.07
Spleen	1.27 ±0.03	1.37±0.05	1.29±0.03

Data were analyzed using one-way ANOVA followed by TUKEY post hoc test. Data represented as mean ± SEM. (*p<0.05, **p<0.01 denotes level of significance compared to the control group).

The organ weight profile indicated administration of MMLE at 500 mg/kg revealed no significant changes, however, the higher dose 1000 mg/kg significantly increased the weights of the liver ($p < 0.05$), kidney ($p < 0.01$), and lungs ($p < 0.05$) compared to the control group, indicating potential organ-specific effects at higher doses. In contrast, there were no significant changes observed in the weights of the brain, heart, or spleen across treatment groups.

Hematological & biochemical analysis

Hematological evaluations demonstrated no adverse effects of MMLE on hemoglobin concentration, red blood cell (RBC) count, platelet count along with monocytes & lymphocytes. This indicated that MMLE administration did not affect hematopoiesis or immune cell profile.

Table 2: Hematological profile in 28 days repeated dose oral toxicity study with MMLE

Parameters	Control	MMLE 500 mg/kg	MMLE 1000 mg/kg
Hb (gm/dl)	14.68 ±0.65	14.97 ±0.11	15.47 ±0.54
RBC (mill/cu mm)	8.77 ± 0.24	8.75 ± 0.38	8.53 ± 0.26
WBC (10^3 cu mm)	8.59 ± 0.13	8.83 ± 0.11	10.93 ± 0.59***
Platelets (10^3 cu mm	740.07±19.41	756.89±3.03	754.2±2.39
Neutrophils (%)	16.72 ± 0.35	17.43 ± 0.40	17.55 ± 0.39
Lymphocytes (%)	84.35 ± 2.14	87.19±1.84	83.67 ± 2.21
Eosinophils (%)	0.74 ± 0.04	0.73 ± 0.04	0.74± 0.02
Monocytes (%)	1.67± 0.17	1.73 ± 0.14	1.86± 0.02
Sodium(mmol/l)	136.30±1.08	135.87±1.16	138.30±0.68
Chloride(mmol/l)	95.75±0.51	94.87±0.95	95.60±0.26
Fasting blood glucose (mg/dl)	106.33±1.76	104±1.57	99.16±2.07
Albumin(g/dl)	3.56±0.13	3.84±0.12	4.47±0.37*
Globulin(mg/dl)	1.6±0.01	1.71±0.06	1.63±0.05

Journal of Chemical Health Risks
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JCHR (2026) 16(2), 1317-1328 | ISSN:2251-6727

Total protein (g/dl)	8.57±0.17	7.78±0.20	7.14±0.58

Data were analyzed using one-way ANOVA followed by TUKEY post hoc test. Results are presented as mean ± SEM. *** considered statistically very significant (p<0.001) compared to the control group.

MMLE at 500 mg/kg dose was found to be safe even after 28 days repeated administration. However, the WBC (White blood cell) count was significantly increased (p<0.001) at 1000 mg/kg dose compared to the control group and this indicated that the extract at higher dose may cause some adverse response in the body (Table 2). Another finding indicated that the extract after consecutive administration for 28 days did not alter the fasting blood glucose level, it maintained the basal level throughout the study. The serum proteins (total protein, globulin) remained within normal ranges for both test groups compared to the control group. However, the albumin was significantly increased (p<0.05) at 1000 mg/kg body weight dose compared to the control group.

There was no significant alteration in the lipid profile observed after 28 days repeated administration of MMLE (Figure 3).

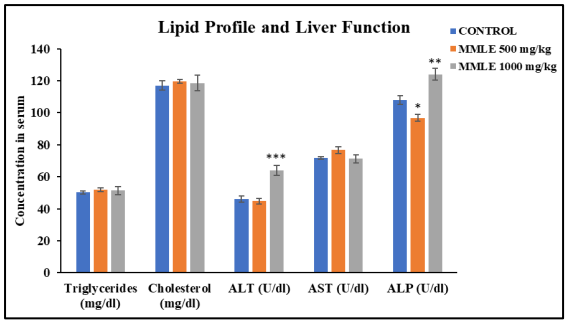

Figure 3: Effect on lipid profile and liver function of rats post-treatment with the hydroalcoholic extract of *Mikania micrantha* leaves in the repeated dose subacute toxicity study

Data were analyzed using one-way ANOVA followed by TUKEY post hoc test. Results are presented as mean ± SEM. *p<0.05, ** p <0.01 and *** p <0.001 compared to control group. ALT: Alanine Transaminase, AST: Aspartate Aminotransferase, ALP: Alkaline Phosphatase.

The liver function test suggested the toxic effect of the extract at higher dose 1000 mg/kg body weight as demonstrated in Figure 3. The liver enzymes alanine aminotransferase (ALT) (p <0.001) and alkaline phosphatase (ALP) (p <0.01) were significantly increased in 1000 mg/kg treated groups compared to the control group. However, ALP level was significantly decreased (p<0.05) in 500 mg/kg body weight dose compared to the control group.

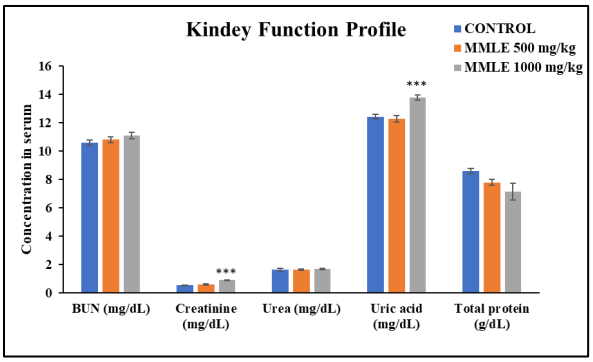

Figure 4: Effect on kidney function of rats post-treatment with the hydroalcoholic extract of *Mikania micrantha* leaves in the repeated dose subacute toxicity study

Data were analyzed using one-way ANOVA followed by "TUKEY post hoc test". Results are presented as mean ± SEM. (***p<0.001 considered statistically significant compared to the control group).

The serum samples were also assessed for the renal function markers, including urea, creatinine, uric acid, and blood urea nitrogen (BUN). The test parameters revealed no significant alterations in the treatment group 500 mg/kg compared to control group (Figure 4). However, renal markers like serum uric acid and creatinine level were significantly increased (p<0.001) in 1000 mg/kg group compared to the control group. This suggested that the plant extract 1000 mg/kg group revealed renal damage.

Histopathological assessment

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JCHR (2026) 16(2), 1317-1328 | ISSN:2251-6727

The effect of the hydroalcoholic extract of *Mikania micrantha* leaves on different organs were assessed with histological analysis with haematoxylin and eosin staining. After consecutive administration of the extract for 28 days, the gross necropsy study of the major organs like liver, kidney, brain, heart, lungs and spinal cord were performed for the morphological evaluation.

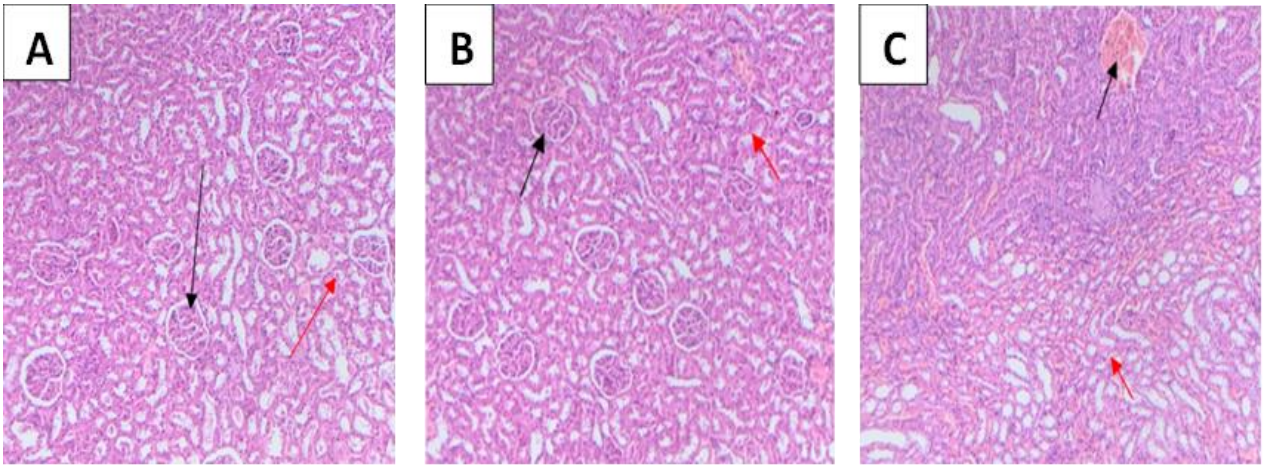

Figure 5: Photomicrographs of kidney sections of rats following 28-days repeated dose oral toxicity study. A, B, C represents control, MMLE 500mg/kg and 1000mg/kg doses respectively. Black arrows indicate the changes in glomerulus and red arrows indicating the renal tubules [Hematoxylin and eosin-stain, magnification 10X].

The histological study of the kidney revealed that the control and extract in 500mg/kg dose treated group for 28 days exhibited normal histological features of the glomerulus and renal tubules. However, the 1000 mg/kg group displayed mild necrosis of the glomerulus while the renal tubules remained normal (Figure 5).

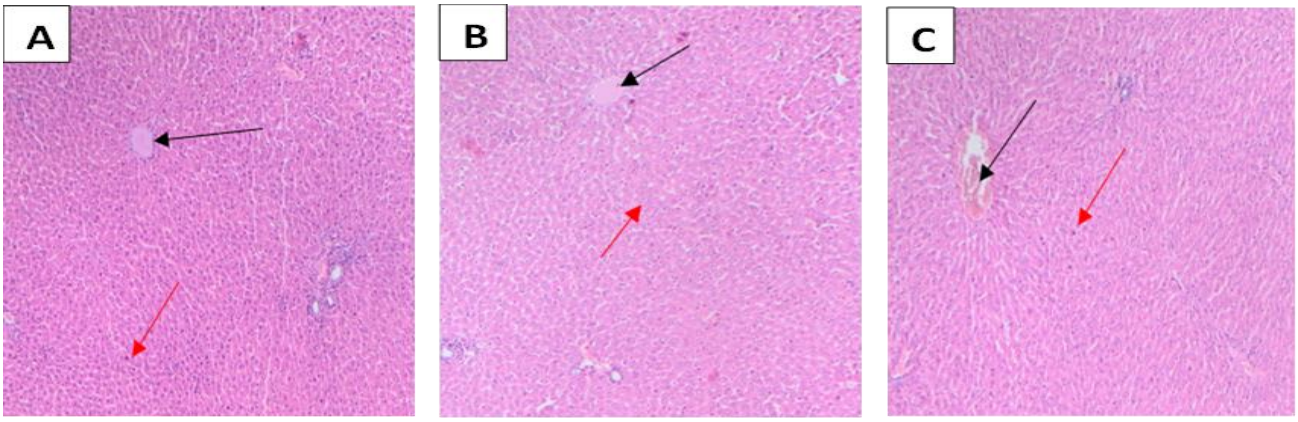

Figure 6: Photomicrographs of liver sections of rats following 28-days repeated dose oral toxicity study. A, B, C represents control, MMLE 500mg/kg and 1000mg/kg doses respectively. Black arrows indicate the central vein and red arrows indicating the changes observed in hepatocytes [Hematoxylin and eosin-stain, magnification 10X].

Figure 6 demonstrated the liver histology in both the control and 500 mg/kg groups showed a normal appearance of the central vein and hepatocytes. In contrast, the 1000 mg/kg group exhibited mild inflammation around the central vein, while the hepatocytes appeared normal.

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JCHR (2026) 16(2), 1317-1328 | ISSN:2251-6727

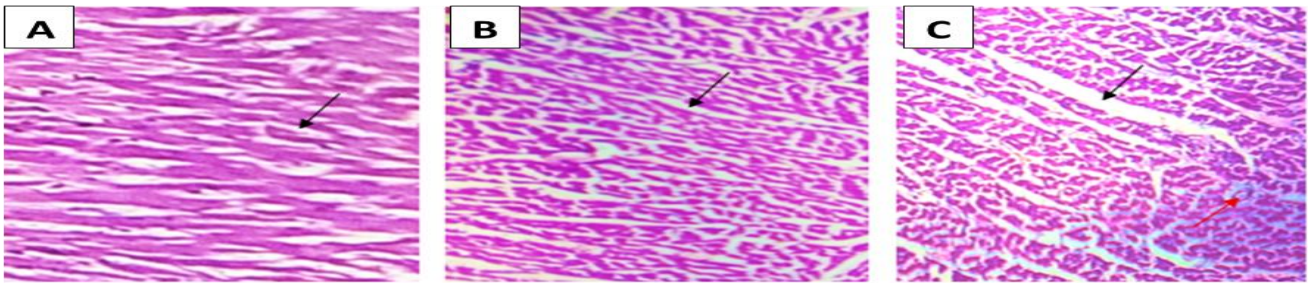

Figure 7: Photomicrographs of heart sections of rats following 28-days repeated dose oral toxicity study. A, B, C represents control, MMLE 500mg/kg and 1000mg/kg doses respectively. Black arrows indicating the myocardial fibers and its changes, red arrow indicates leukocyte infiltration [Hematoxylin and eosin-stain, magnification 10X].

Histological analysis of heart tissue sections (Figure 7) revealed normal architecture in healthy control rats, at lower dose with dense, elongated myocardial fibers, normal cardiomyocyte nuclei, and erythrocytes were observed in blood vessels. At higher dose edema, leukocyte infiltration and fragmented muscle fibers were found, indicating mild damage.

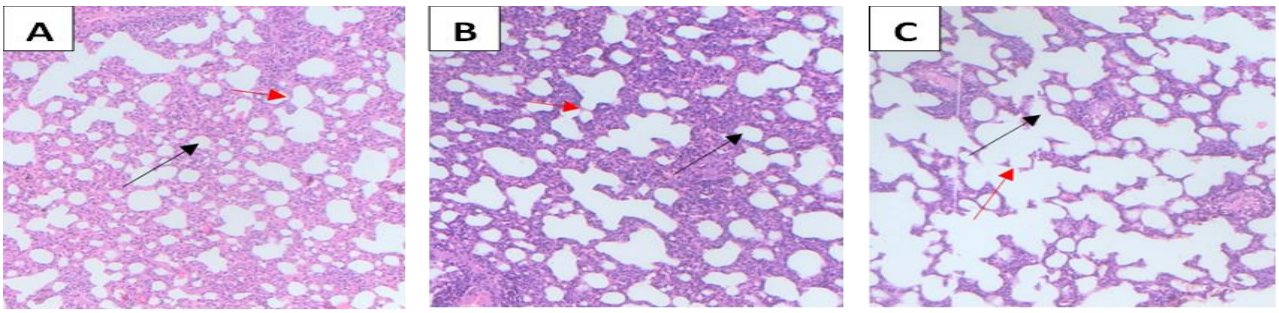

Figure 8: Photomicrographs of lungs sections of rats following 28-days repeated dose oral toxicity study. A, B, C represents control, MMLE 500mg/kg and 1000mg/kg doses respectively. Black arrows indicate the morphological changes in alveoli and red arrows indicate bronchial structure [Hematoxylin and eosin-stain, magnification 10X].

The lungs in both the control and 500mg/kg groups exhibited normal histological structures of the alveoli and lobar secondary bronchi. In contrast, the 1000mg/kg group displayed moderate congestion in the alveoli and mild necrosis in the lobar bronchi (Figure 8).

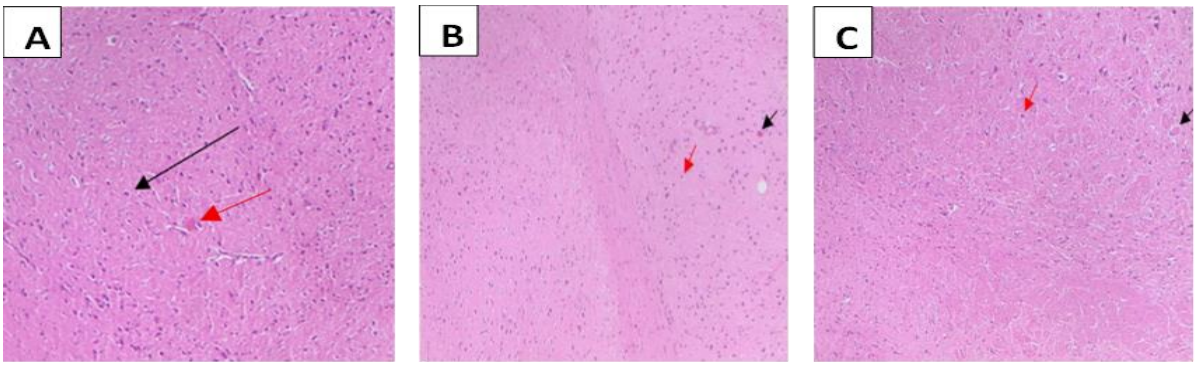

Figure 9: Photomicrographs of Brain sections of rats following 28-days repeated dose oral toxicity study. A, B, C represents control, MMLE 500mg/kg and 1000mg/kg doses respectively. Black arrows indicate the glial cells and red arrows indicate the neuronal cell bodies [Hematoxylin and eosin-stain, magnification 10X].

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Figure 9 represents the histopathological examination of the brain, which revealed normal glial cells and neuronal cell bodies in the control group. Likewise, rats treated with 500 and 1000mg/kg body weight of the hydroalcoholic extract of *Mikania micrantha* leaves showed no abnormalities in glial cells or neuronal cell bodies.

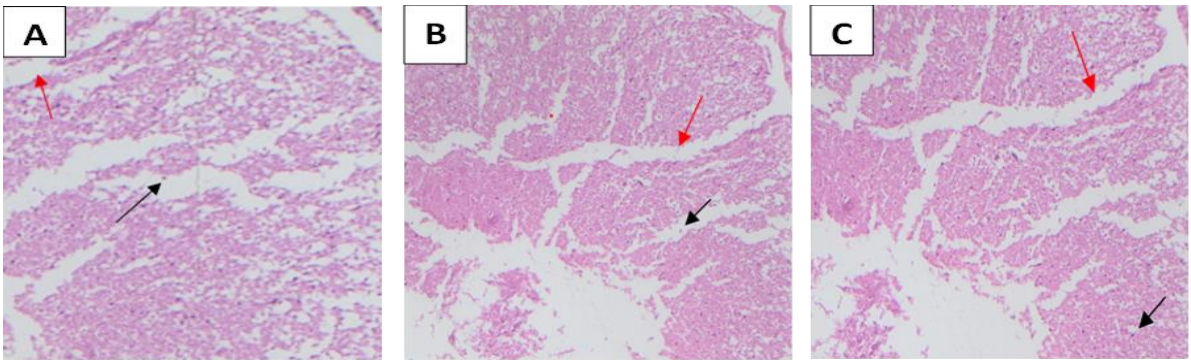

Figure 10: Photomicrographs of spinal cord sections of rats following 28-days repeated dose oral toxicity study. A, B, C represents control, MMLE 500mg/kg and 1000mg/kg doses respectively. Black and red arrows denote the changes observed in glial cells and central canal respectively [Hematoxylin and eosin-stain, magnification 10X].

The spinal cord of the control group displayed a normal central canal and glial cells (Figure 10). Similarly, rats treated with 500 and 1000 mg/kg body weight of the hydroalcoholic extract of *Mikania micrantha* leaves showed no abnormalities in the central canal or glial cells.

Discussion

The widespread use of herbal medicines, especially in developing countries, highlights the critical need for thorough scientific validation of their safety and efficacy. Although traditional remedies are widely used, the lack of comprehensive toxicological data limits their integration into mainstream therapeutic applications [20]. To address this gap, systematic toxicity assessments are essential not only to establish safe dosage ranges in preclinical studies but also to evaluate potential adverse effects prior to clinical use [21].

Mikania micrantha leaves are known for their diverse pharmacological activities and contain various bioactive secondary metabolites [22]. In the present study the hydroalcoholic extract of *Mikania micrantha* leaves was found to contain alkaloids, flavonoids, phenolics, tannins, and saponins. The HPTLC study confirmed the presence of chlorogenic acid, gallic acid, and kaempferol, among which kaempferol was present in the highest amount. Studies on acute toxicity have shown that hydroalcoholic extracts of this plant are non-toxic up to a dose of 2000 mg/kg in rats, with no observed mortality or behavioral abnormalities. However, acute toxicity findings alone are insufficient to predict the effects of repeated or long-term exposure. In the current subacute toxicity study, no mortality was recorded in any group following 28 days of treatment with 500 and 1000 mg/kg doses, indicating a favorable safety profile. Body weight measurements showed no significant changes at 7 and 14 days; however, a significant increase on day 21st and 28th, suggesting either a possible growth-promoting or adverse changes at higher doses, without adversely affecting the control group's growth pattern. The relative organ weights remained unchanged at 500 mg/kg of the extract, indicating no systemic toxicity. However, significant increases in liver, kidney, and lung weights at 1000 mg/kg suggest an organ-specific toxicity might be arising at the higher dose, potentially reflecting inflammation, or early hypertrophic changes.

Hematological analysis, a vital aspect of toxicity assessment, revealed no significant changes in red blood cell or platelet counts. However, a significant increase in WBC count at 1000 mg/kg indicates a potential inflammatory response at higher doses, aligning with histopathological evidence of mild tissue alterations. This might be regulated through immune response possibly linked to moderate alveolar congestion and minor bronchiolar necrosis, or mild hepatotoxicity [23,24]. MMLE did not alter fasting blood glucose levels

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JCHR (2026) 16(2), 1317-1328 | ISSN:2251-6727

at either dose, suggesting that the extract does not impact glucose metabolism after prolonged exposure. No significant changes were observed in serum protein levels. Liver function was further assessed through serum biomarkers and elevations in ALT and ALP at 1000 mg/kg suggest hepatocellular injury and cholestasis at higher doses, corroborated by increased liver weight and mild histological inflammation [25,26]. No significant changes were noted at 500 mg/kg; however, elevated creatinine and uric acid at 1000 mg/kg indicate mild renal impairment, supported by histological evidence of glomerular necrosis [27,28].

Furthermore, the histopathological analysis revealed slight alteration in normal morphology of organs such as heart and lungs at the 1000 mg/kg dose. This particular dose revealed moderate alveolar congestion and slight bronchi necrosis, indicating pulmonary irritation or inflammation. Mild cardiotoxic effects were also evident as edema, leukocyte infiltration, and muscle fiber fragmentation. However, no structural abnormalities were observed in the central canal or glial cells at any dose of the MMLE, suggesting neuro-safety in subsequent exposure. Additionally, both groups exhibited no histological changes in the brain or spinal cord. General health indicators like food consumption remained stable across all groups, suggesting no impairment in overall metabolism or growth. Further research is necessary to establish the long-term safety profile of *Mikania micrantha* leaf extract, with a particular focus on organ-specific effects observed at higher doses.

Conclusion

The current study demonstrated that *Mikania micrantha* leaf extract exhibits a favorable safety profile at lower doses, while early signs of organ-specific toxicity were evident at the higher dose of 1000mg/kg. Though no severe adverse effects were noted, mild to moderate alterations in hepatic, renal, pulmonary, and cardiac tissues highlight the need for further investigation. These outcomes underscore the importance of conducting chronic toxicity assessments and in-depth mechanistic studies to ensure its long-term safety. Exploring the roles of its bioactive compounds, metabolic pathways, and molecular targets will be essential to clarify both its therapeutic potential and toxicological risks. Future research will be essential in validating *Mikania micrantha* as a safe and effective herbal therapeutic within the framework of modern evidence-based medicine.

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Conflict of interests

The authors declare no conflict of interest.

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Ethical approval

All the animal experiments were approved by the Institutional Animal Ethics Committee, NSHM Knowledge Campus, Kolkata with an approval no. NCPT/IAEC-013/2025.

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