Original Research Article

Toxicological Insights into *Myrmecodia platytyrea* Tuber Aqueous Extract: An Oral Acute and Subacute Toxicity Studies in Mice

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ABSTRACT

Despite advancements in medical science, the global burden of inflammation-related diseases remains high, necessitating the exploration of novel and effective remedies that can offer relief without significant side effects. The indigenous peoples of Papua and Borneo use the decoction of *Myrmecodia platytyrea* tubers, the ant plant, for its medical benefits, particularly in cancer therapy, demonstrating the deep traditional knowledge of natural treatments. The Rubiaceae plant has been used in traditional medicine to alleviate inflammation and other conditions. Thus, this study aims to investigate the toxicological profile of the aqueous extract of *M. platytyrea* tubers (MPAE). The study conducted adhered to recognised protocols for assessing oral acute and subacute toxicity, with necessary modifications to meet the specific experimental needs. In the acute toxicity study, male and female mice received a single oral dose of MPAE at 2000 mg/kg and monitored for 14 days. In the subacute toxicity study, male and female mice received 500 mg/kg/day MPAE orally for 28 days. Physical and behavioural changes were observed. Haematological parameters and biochemical parameters were analysed, including histology of the liver and kidneys. Acute and subacute toxicity studies found no clinical signs of toxicity, mortality, or weight changes. The subacute toxicity study found no significant changes in haematological parameters or biochemical parameters for both sexes. Acute and subacute toxicity tests revealed no treatment-related necropsy, gross, or histopathological abnormalities. In conclusion, MPAE is safe to consume and supports further development of *M. platytyrea* for treating inflammation-related diseases, particularly cancer.

Keywords: *Myrmecodia platytyrea*, traditional medicine, toxicological profile, inflammation-related diseases

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1.0 Introduction

Within the area of medical science, the search for novel treatments for diseases associated with inflammation is an urgent challenge affecting people's health worldwide. Additionally, the prevalence of chronic disorders continues to place a huge burden on healthcare systems all over the world, despite the significant breakthroughs that have been made (1). Researchers are delving into the vast body of traditional medicinal knowledge, particularly the utilisation of natural plant-based therapies, because there is a pressing need to find effective treatments with minimal unwanted effects (2).

Myrmecodia platytyrea, an epiphytic plant revered by the indigenous peoples of Papua and Borneo, is one example of a natural source used as a traditional medicine (3). The plant, which is colloquially known as the ant plant, is a member of the family Rubiaceae and has been utilised in traditional medicine for the treatment of a wide variety of illnesses, the most prominent of which is cancer (3, 4). The therapeutic properties of this plant are derived from its tubers, which have traditionally been used as a decoction for the treatment of inflammation and associated disorders (3-5).

It has recently come to the attention of the scientific community that *M. platytyrea* has the potential to be a suitable candidate for developing new anti-inflammatory medicines and other inflammation-related diseases such as cancer and diabetes (3, 5-7). The need to address the limits of existing pharmacological therapies, which frequently carry the risk of major side effects, is the impetus for this interest. *M. platytyrea*, on the other hand, offers a potentially beneficial alternative based on its extensive use in traditional medicine. Moreover, this plant contains bioactive compounds, including polyphenols, flavonoids, and alkaloids, which can potentially be used in developing novel anti-inflammatory drugs with less adverse effects (5, 8). Traditional medicine often takes a holistic approach, considering not only the physical symptoms but also the overall well-being of an individual due to the modulation of immune responses and reduction of oxidative stress, promoting overall health. *M. platytyrea* fits into this paradigm, addressing inflammation-related diseases from multiple angles. For centuries, the communities have consumed it without significant adverse effects. Researchers can confidently explore the therapeutic potential of traditional medicine by validating the safety profile through rigorous scientific studies.

Hence, the purpose of this research is to conduct a comprehensive analysis of the toxicological profile of the aqueous extract of *M. platytyrea* tubers (MPAE) and further development of the extract for treating inflammation-related diseases, particularly cancer.

2.0 Materials and Methods

2.1 Plant material

The tubers of *M. platytyrea* subsp. Antionii (Becc.) Huxley & Jebb were found in the highlands of Sulawesi, Indonesia. Prof. Dr. Eko Baroto Walujo, a botanist from the Research Centre for Biology at the Indonesian Institute of Sciences in Bogor, Indonesia, verified the plant's authenticity. The plant was subsequently stored in the herbarium with voucher numbers BO 1647929 and BO 00096642.

2.2 Preparation of plant extract

Tubers were cleaned, cut into segments and air-dried right after collection. The dried *M. platytyrea* tuber was pulverised. The powder was heated at 100°C in 1:9 distilled water for 15 min and filtered with Whatman No. 1 paper. The material

processing and decoction procedure followed WHO herbal medicine's good herbal processing practices (9). A rotary evaporator (Heidolph, Germany) under reduced pressure at 100 mbar, 40 °C was used to remove the filtrate solvent. The aqueous extract powder was freeze-dried (AAPPTec, USA) from the concentrated filtrate after three days at -80°C. Subsequently, the extract was stored at 20°C until further use.

2.3 Experimental animal

Male and female ICR mice (8 weeks old, 23–30 g) were supplied by the Laboratory Animal Facility and Management (LAFAM), Faculty of Pharmacy, Universiti Teknologi MARA. Mice maintained in standard conditions in individual ventilated cages: 12 h light/dark cycle, 22 °C (± 3 °C) temperature, and 50–60% relative humidity. A commercial food pellet (Gold Coin Sdn. Bhd., Malaysia) and unlimited water were given *ad libitum*. Before the experiment started, the mice were acclimatised for at least five days. The Committee on Animal Research and Ethics (UiTM CARE) of Universiti Teknologi MARA gave its approval before the commencement of the study (UiTM CARE: 10/2012).

2.4 Acute oral toxicity study

The study was conducted as per OECD Guideline Test No. 423 with modifications required to fulfil the specific needs of the experiment (10). Female and male ICR mice were randomly divided to form two groups of female mice and two groups of male mice (n=6/group). Mice were given distilled water and MPAE via oral gavage using a metal gavage needle (0.1ml/10g body weight). The control groups (one male and one female) were orally administered with distilled water, while the treatment groups (one male and one female) received a single oral dose of 2000 mg/kg of MPAE dissolved in distilled water. The mice were observed closely during the first 30 min, periodically during the first 24 h and then, daily for a total of 14 days following dosing for mortality and any signs or symptoms of toxicity via physical and behavioural changes. Behaviour abnormalities included skin, fur, eyes, mucous membranes, secretion and excretion patterns, lacrimation, piloerection, pupil size, and atypical breathing. Changes in walking pattern, body position, sensitivity to handling, rhythmic or continuous muscular contractions, repeated behaviours (such as excessive grooming or circling), or unusual behaviours.

2.5 Subacute toxicity study

A subacute oral toxicity study was conducted following the OECD Guideline Test No. 407 with modifications (11). Mice were randomly divided into four groups of six mice each. Six males and six females were used for the control groups, and six males and six females were used for the treated groups. Group 1 received the vehicle (distilled water) and served as the control group. Group 2 received doses of MPAE at 500 mg/kg body weight daily for 28 days using an oropharyngeal cannula. To determine the reversibility or recovery from the toxic effects of the test material, the third and fourth groups, namely satellite groups were given the vehicle (distilled water) and 500 mg/kg of the extract, respectively. The test material was administered once daily for 28 days. The satellite groups were further observed for the next 14 days without the vehicle or MPAE administration.

Mortality, food consumption, water intake and observation of general toxicity signs were monitored and recorded daily throughout the study. The initial body weight of mice for all the experimental groups was recorded before the

administration of the test material and at the end of each week.

The mice were sacrificed at the end of the experimental period (on day 15 for acute oral toxicity, day 29 for subacute oral toxicity test and day 43 for the satellite groups. Blood samples were collected via cardiac puncture using a disposable syringe. The blood was transferred into EDTA tubes for haematological analysis which includes the test for red blood cells (RBCs), packed corpuscular volume (PCV), mean corpuscular volume (MCV) mean corpuscular haemoglobin concentration (MCHC) and differential white blood count (WBC) for the neutrophils, basophils, eosinophils, lymphocytes and monocytes). Haematological analyses were performed using a fully Automatic Haematology Analyzer (CellDyn 3700 Veterinary Package, USA). Meanwhile, blood samples that were transferred in Plain tubes were used for biochemical parameters including glucose level, cholesterol level, blood urea nitrogen (BUN), creatinine level, aspartate aminotransferase (AST) level, alanine transaminase (ALT) level and alkaline phosphatase (ALP) level. These analyses were performed by using the Automatic Chemistry Analyzer (Hitachi 902, Japan). Finally, the animal models were observed for gross lesions.

Necropsy was done on the acute and subacute oral toxicity test groups on days 15 and 29, respectively, as well as satellite groups, on day 43. After the mice were sacrificed, the liver and kidneys were removed through a midline incision at the mouse's abdomen and subjected to histological evaluation. The liver and kidney tissues fixed in 10% buffered formalin were processed and embedded in paraffin wax. The paraffin sections (5 µm) were cut on glass slides and stained with hematoxylin and eosin. The slides were examined blindly by a trained pathologist under a light microscope using 200x magnification power (Leica, Germany).

2.6 Statistical analysis

Data were expressed as mean ± standard error mean (SEM). A student's t-test was used to determine statistical differences between groups ($P<0.05$).

3.0 Results

3.1 Acute oral toxicity study

The effects of *M. platytyrea* tuber aqueous extract in mice after oral administration are summarised in Table 1. Throughout the 14 days of observation, mice treated with 2000 mg/kg b.w. of the extract showed no visible signs of toxicity or mortality.

Table 1: Effects of the single oral dose of MPAE administration

Gender	Group	Mice	Effects	
		Death/Total	Mortality latency (h)	Symptoms of toxicity
Male	Control (distilled water)	0/6	No	None
	MPAE (2000 mg/kg)	0/6	No	None
Female	Control (distilled water)	0/6	No	None
	MPAE (2000 mg/kg)	0/6	No	None

Values represent the proportion of deaths to the total number of animals in each gender group (n=6/group)

3.2 Subacute toxicity study

The mice in the subacute toxicity study received repeated doses of MPAE at 500 mg/kg daily for 28 days. No signs of toxicity and mortality were observed in the treated group compared to the control group (Table 2). Both male and female mice of the control and treated groups gained weight compared to day 0 (Table 3).

The status of bone marrow activity and intravascular effects were monitored by haematological examination as summarised in Table 4 and Table 5. In the male satellite-treated group, the red blood cell (RBC) and haemoglobin (Hb) were significantly lower than the satellite-control group (P<0.05).

Blood biochemistry of both male and female treated groups was as shown in Table 6. The treated male group showed a significant decreased in ALP levels when compared to the control group, 60.40 ± 5.72 and 81.17 ± 1.31 U/L, respectively. The ALP level was found not to be in the range of the normal value. However, the effect was reversible as ALP level in the satellite male group did not show any significant difference compared to the control group.

Table 2: Effects of 500 mg/kg of MPAE oral administration daily for 28 days

Sex		**Group**	**Mice**	**Effects**	
			Death/ Total	Mortality latency (h)	Symptoms of toxicity
Male	Subacute 28 days	Control (distilled water)	0/6	No	None
		MPAE (500 mg/kg)	0/6	No	None
	Subacute satellite 42 days	Control (distilled water)	0/6	No	None
		MPAE (500 mg/kg)	0/6	No	None
Female	Subacute 28 days	Control (distilled water)	0/6	No	None
		MPAE (500 mg/kg)	0/6	No	None
	Subacute satellite 42 days	Control (distilled water)	0/6	No	None
		MPAE (500 mg/kg)	0/6	No	None

Table 3: Body weights (g) of mice treated with MPAE (500 mg/kg, p.o.) for 28 days

	Male		**Female**	
	Control	MPAE (500 mg/kg)	Control	MPAE (500 mg/kg)
Day 0	30.52 ± 0.88	30.90 ± 0.37	24.32 ± 1.26	24.08 ± 1.47
Day 14	31.30 ± 1.23	31.55 ± 0.64	25.60 ± 0.53	24.85 ± 2.14
Day 28	33.22 ± 1.11	34.48 ± 0.92	25.53 ± 0.78	25.35 ± 1.35
Weight gain after 28 days (g)	2.75 ± 0.29	2.67 ± 0.55	1.27 ± 0.42	1.27 ± 0.59

Day 42	34.50 ± 0.28	33.57 ± 2.00	25.20 ± 0.83	25.75 ± 2.05
Weight gain after 42 days (g)	3.98 ± 0.14	3.67 ± 0.28	0.88 ± 0.25	1.67 ± 0.39

Values are mean ± standard error mean (SEM)

Table 4: Effects of oral administration of MPAE on haematological values

Male	**Subacute 28 days**		**Satellite subacute 42 days**		**Normal value**
	Control	Treated	Control	Treated	
RBC (× 10^{12})	8.39 ± 0.15	8.01 ± 0.24	8.99 ± 0.36	7.95 ± 0.29*	7.60 ± 1.17
Hb (g/L)	138.50 ± 2.54	130.33 ± 3.09	140.00 ± 2.63	126.50 ± 2.03*	172.70 ± 29.40
PCV (L/L)	0.41 ± 0.01	0.38 ± 0.01	0.54 ± 0.01	0.47 ± 0.13	0.51 ± 0.08
MCV (fL)	49.12 ± 0.52	47.68 ± 0.27	60.22 ± 0.87	61.10 ± 1.55	56.10 ± 4.59
MCHC (g/L)	336.36 ± 0.75	341.62 ± 1.30	258.86 ± 4.04	275.57 ± 3.01	338.400 ± 26.80
Thrombo (× 10^9/L)	200.60 ± 65.64	134.50 ± 25.69	524.38 ± 56.86	575.13 ± 66.66	344.00 ± 73.00

Female	**Subacute 28 days**		**Satellite subacute 42 days**		**Normal value**
	Control	Treated	Control	Treated	
RBC (× 10^{12})	8.34 ± 0.25	8.06 ± 0.09	8.31 ± 0.22	8.33 ± 0.14	7.37 ± 1.09
Hb (g/L)	142.83 ± 4.85	137.83 ± 2.71	140.33 ± 4.49	141.17 ± 2.94	165.20 ± 27.20
PCV (L/L)	0.43 ± 0.01	0.41 ± 0.01	0.43 ± 0.01	0.42 ± 0.01	0.49 ± 0.07
MCV (fL)	51.56 ± 0.49	50.21 ± 0.67	51.37 ± 0.75	50.63 ± 0.44	56.39 ± 3.75
MCHC (g/L)	332.23 ± 2.79	340.35 ± 1.26	328.70 ± 2.16	334.90 ± 4.20	341.00 ± 23.40
Thrombo (× 10^9/L)	200.60 ± 160.79	134.50± 62.94	218.83 ± 66.89	277.13 ± 59.50	344.00 ± 73.00

Values are mean ± standard error mean (SEM). *Significant with control ($p < 0.05$). [§]Normal range values were adopted from Charles River Laboratories International. Abbreviations: RBC, red blood cells; Hb, haemoglobin; PCV, packed corpuscular volume; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; thrombo, thrombocyte.

Table 5: Effects of oral administration of MPAE on WBC and differential WBC count values

Male	**Subacute 28 days**		**Satellite subacute 42 days**		**Normal value**
	Control	Treated	Control	Treated	
WBC (10^9/L)	2.54 ± 0.41	2.80 ± 0.46	4.11 ± 0.85	2.59 ± 0.53	10.83 ± 3.84
B Neutrophils (%)	1.00 ± 0.07	0.83 ± 0.04	2.17 ± 0.31	1.67 ± 0.41	-
S Neutrophils (%)	10.50 ± 1.26	10.00 ± 1.97	17.50 ± 1.48	14.00 ± 0.96	30.54 ± 15.40
Lymphocytes (%)	81.00 ± 2.63	83.00 ± 2.68	71.00 ± 2.03	76.83 ± 1.10	62.05 ± 23.40
Monocytes (%)	4.33 ± 0.31	5.17 ± 0.54	6.17 ± 0.40	5.17 ± 0.35	6.20 ± 3.05
Eosin (%)	1.83 ± 0.67	1.00 ± 0.10	3.17 ± 0.70	2.33 ± 0.48	1.20 ± 1.20
Basophils (%)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Female	**Subacute 28 days**		**Satellite subacute 42 days**		**Normal value**
	Control	Treated	Control	Treated	
WBC (10^9/L)	2.54 ± 1.01	2.80 ± 1.13	2.62 ± 0.51	2.93 ± 0.41	10.17 ± 3.72
B Neutrophils (%)	1.00 ± 3.08	0.83 ± 0.98	2.17 ± 0.48	1.83 ± 0.40	-
S Neutrophils (%)	10.50 ± 3.08	10.00 ± 4.82	19.50 ± 3.82	16.50 ± 1.36	25.80 ± 12.20

Lymphocytes (%)	81.83 ± 5.56	83.00 ± 6.57	66.17 ± 4.85	73.83 ± 4.26	66.20 ± 26.00
Monocytes (%)	4.83 ± 0.75	5.17 ± 1.33	6.67 ± 0.67	5.17 ± 0.65	6.10 ± 2.80
Eosin (%)	1.83 ± 2.14	1.00 ± 0.63	5.50 ± 1.38	2.67 ± 0.67	1.50 ± 1.60
Basophils (%)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Values are mean ± standard error mean (SEM). Abbreviation: WBC, white blood cells.

Table 6: Effects of MPAE on the biochemical blood parameters

Male	**Subacute 28 days**		**Satellite subacute 42 days**		**Normal value**
	Control	Treated	Control	Treated	
ALT (U/L)	37.22 ± 2.27	39.02 ± 4.41	44.48 ± 7.37	39.82 ± 4.16	65.01 ± 32.28
ALP (U/L)	81.17 ± 1.31	60.40 ± 5.72*	66.83 ± 6.55	68.33 ± 5.15	154.63 ± 50.03
AST (U/L)	106.23 ± 21.19	173.03 ± 14.08	77.95 ± 6.46	81.10 ± 13.52	113.67 ± 64.46
Chol (mmol/L)	3.97 ± 0.18	3.88 ± 0.20	3.20 ± 0.34	2.84 ± 0.20	5.70 ± 1.5
Creat (umol/L)	28.50 ± 1.34	30.83 ± 1.17	32.50 ± 1.54	30.67 ± 1.36	39.79 ± 9.73
Gluc (mmol/L)	9.62 ± 1.23	10.92 ± 0.81	12.82 ± 0.51	12.93 ± 1.16	12.60 ± 4.80
BUN (mg/dL)	9.58 ± 0.40	10.58 ± 0.60	10.68 ± 0.69	10.73 ± 0.92	14.19 ± 4.29

Female	**Subacute 28 days**		**Satellite subacute 42 days**		**Normal value**
	Control	Treated	Control	Treated	
ALT (U/L)	46.17 ± 4.19	60.18 ± 11.64	39.17 ± 8.01	36.88 ± 7.80	56.72 ± 32.40
ALP (U/L)	125.00 ± 8.33	95.17 ± 8.62	81.17 ± 7.19	81.83 ± 6.81	156.54 ± 51.10
AST (U/L)	142.50 ± 26.20	194.08 ± 25.18	160.82 ± 23.66	149.42 ± 31.15	111.88 ± 65.11
Chol (mmol/L)	2.78 ± 0.22	2.52 ± 0.20	2.64 ± 0.21	2.56 ± 0.16	5.30 ± 1.20
Creat (umol/L)	36.00 ± 2.48	35.83 ± 2.60	32.17 ± 1.08	32.33 ± 0.95	41.56 ± 8.84
Gluc (mmol/L)	10.90 ± 0.82	9.68 ± 0.36	9.20 ± 0.62	10.00 ± 0.38	13.90 ± 5.50
BUN (mg/dL)	9.67 ± 0.73	9.92 ± 0.68	9.38 ± 0.42	10.03 ± 0.58	13.45 ± 4.19

Values are mean ± standard error mean (SEM). *Significant with control ($p < 0.05$) Abbreviations: ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; Chol, cholesterol; Creat, creatinine; Glu, glucose; BUN, blood urea nitrogen. §Normal range values were adopted from Charles River Laboratories International.

Daily treatment of mice with 500 mg/kg of *M. platytyrea* tuber aqueous extract for 28 days did not cause significant histological changes in both livers (Figure 1) and kidneys (Figure 2).

4.0 Discussion

During the 14-day observation, mice given a single dose each of MPAE (2000 mg/kg) orally did not exhibit any overt toxicity or mortality symptoms. Generally, the reduction in body weight gain and relative organ weight is an important sign of deterioration of health and an indicator of adverse effects of the drugs and chemicals (12, 13). In the current study, MPAE did not produce any statistically significant difference among the three groups in both parameters (data not shown). Furthermore, gross examination of the internal organs of all rats revealed no detectable abnormalities, indicating MPAE is nontoxic.

Compared to the control groups, the 28 days of MPAE consumption did not result

in appreciable changes in physical appearance, gross examination, or relative organ weight (data not shown). All treatment groups consumed the same amount of food and water as the control group. Given the importance of these nutrients in a range of physiological processes in the body, these results indicated the appropriate processing of lipids, carbs, and protein metabolism within the body (12). Even though the red blood cell (RBC) and haemoglobin (Hb) in the male satellite-treated group were significantly lower than in the satellite control group, no significant differences were observed in other parameters. Iron is a component of numerous enzymes in cells and is also a component of haemoglobin protein (14).

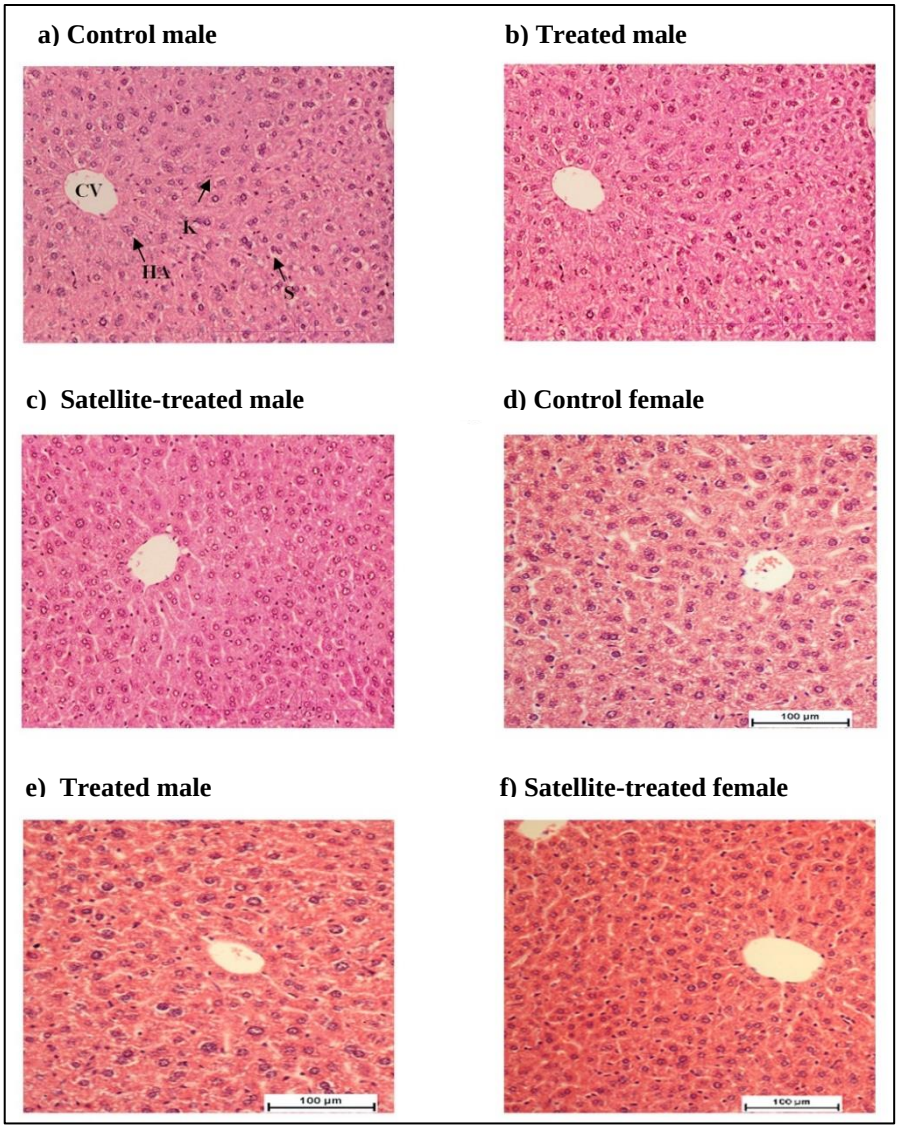

Figure 1: Photomicrographs of the liver from male and female mice of control and treated with 500 mg/kg in a 28-day subacute oral toxicity evaluation of MPAE (a-f). Cross-sections were stained with hematoxylin and eosin. The observation was made at 200× magnification. The liver cross-section shows a normal structure of central vein (CV), sinusoid (S), Kupffer cells (K) and hepatocytes (H). No significant damage was detected in any treatment group.

Much of the body's iron is stored in RBC and is essential for Hb synthesis (14). The reduction in Hb and RBC might have occurred due to lysis of the blood cells (14). It may be due to the incorrect needle size, improper tube mixing, incorrect filling of tubes, excessive suction and difficulty in blood collection during phlebotomy (15). Remarkably, the values of RBC still fall within the normal range, however, the value of Hb is lower than the normal range. The results of other haematological values show no significant differences from the control group. The variations can be due to the heterogeneous sensitivity of the test animals. Thus, such changes do not suggest that the MPAE produces toxicity subacutely.

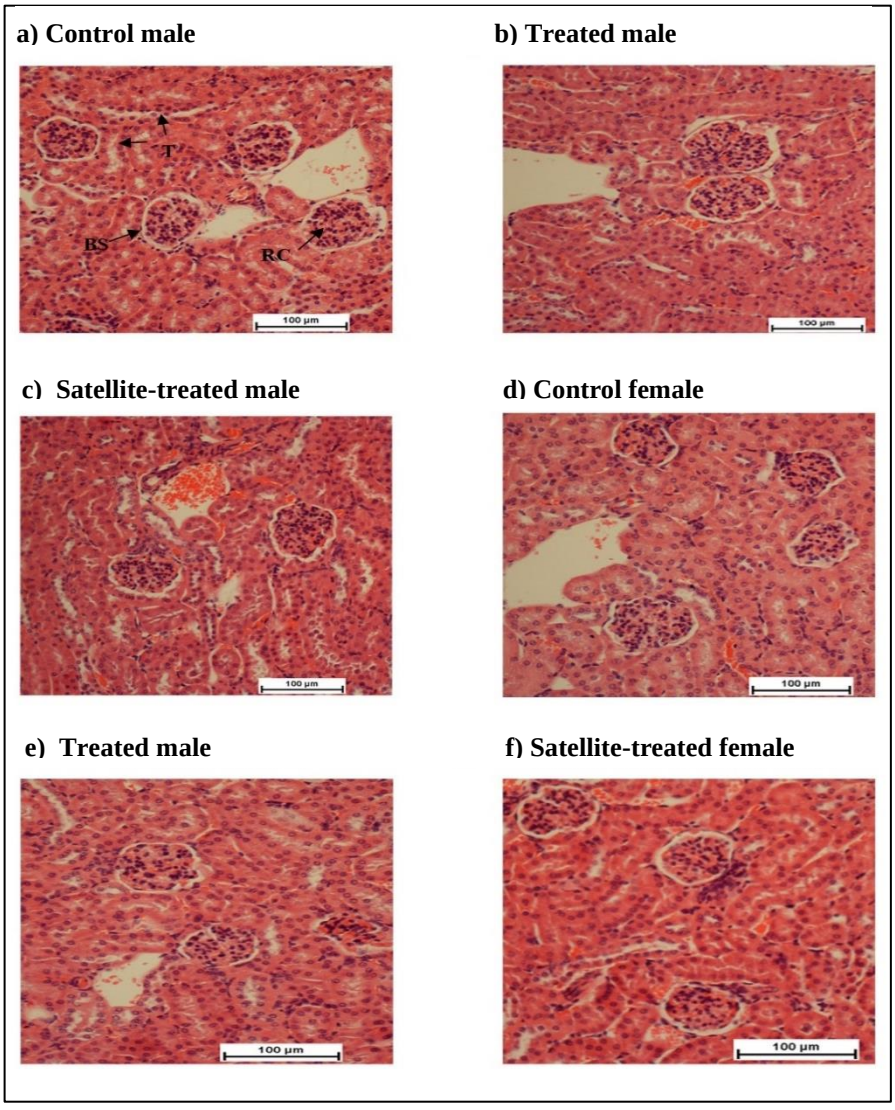

Figure 2: Photomicrographs of the kidney from male and female mice of control and treated with 500 mg/kg in a 28-day subacute oral toxicity evaluation MPAE (a-f). Cross-sections were stained with hematoxylin and eosin. The observation was made at 200× magnification. The kidney cross-section shows renal corpuscles (RC), tubules (T) and Bowman's space (BS), all of which were found to be conserved. No significant damage was detected in any treatment group.

Compared to the control group, the treated male group's ALP levels were significantly lower, but the ALP level was not in the normal value range (16). However, the effect is reversible as the ALP level in the male satellite group showed no significant difference compared to that of the control group. The reduction in ALP levels might be attributed to a decrease during the transition of ALP to the enzymatic circulation by rapid metabolising cells or due to intestinal mucosa injury (17). Since there were no significant changes in both male and female glucose levels, cholesterol and creatinine levels, this suggests that the extract did not affect the lipid and carbohydrate mechanisms and the normal hepatic and renal functions (12). This indicates that the effects of *M. platytyrea* extract are reversible and showed a tendency to recover at the end of the subacute exposure.

Mice treated with 500 mg/kg of MPAE for 28 days daily demonstrated an absence of histological lesions in the liver and kidneys, which is further evidence of the non-toxicity of the extract. In a 13-week repeated dose study, no adverse effects were detected in rats even when they were given doses of up to 5000 mg/kg/day of the herbal combination HemoHIM G, which is composed of *Angelica sinensis*, *Ligusticum chuanxiong*, and *Paeonia lactiflora* (18). This research provides evidence for the safe use of HemoHIM G as a functional food component. Most people assume medicinal plants are nontoxic, and their usage as folk medicine is not preceded by mandatory pre-clinical testing (19). Most of the safety proof comes from long-term traditional use. Since there is no available information on the extract's safety in the literature, there are safety requirements for herbal medicines, necessitating both pre-clinical and clinical testing (20). Testing must be verified and approved by that country's national health surveillance agency (20, 21). Traditional medicines, including those from plants, might induce side effects; thus, pre-clinical investigations and clinical trials are needed to ensure their safety.

The toxicity of the compounds in plant extracts could be related to the metabolic activation by the phase I drug metabolising enzymes (22, 23). Biotransformation of endobiotic compounds by metabolising enzymes, primarily cytochrome P450 superfamily, can produce reactive metabolites (23, 24). Accumulation of reactive metabolites contributes to the toxic effects; thus, the concentration of a substance, duration of treatment, and route of administration are crucial in conducting a toxicity study. Thus, the dose of a test substance, the duration of treatment and the route of administration are crucial determinants of a toxicity study. Interestingly, the results obtained from this study indicated that MPAE is regarded to be safe. No untoward effect was observed following daily exposure to the extract for 28 days. The mice recovered well after cessation of treatment in the 28-day repeat dose study. No delayed toxic effect was observed.

Plants belonging to the Rubiaceae family have several bioactive compounds that possess antioxidant, anti-inflammatory, and regulatory effects on metabolic tissues (24, 25). These compounds can help in the prevention and progression of non-communicable chronic diseases (26). Myrmecophytes have such therapeutic potential in this family, especially *M. platytyrea*. As mentioned earlier, recent studies have provided insight into the therapeutic potential of the tuber extract of *M. platytyrea*. Extracts from the tubers of *M. platytyrea* demonstrate anti-inflammatory effects, both *in vitro* and *in vivo* (3-8). It efficiently suppresses the production of biomarkers that cause inflammation, indicating its ability to modulate the immune system and decrease inflammation (3). The high presence of iridoid glycosides, phenolics and flavonoids in MPAE is likely responsible for its beneficial effects (4, 5, 7, 8, 26).

The findings in this study have significant ramifications since they imply that MPAE is safe to consume and has the potential to be developed further as a therapeutic agent to treat disorders associated with inflammation, including cancer. This aligns with the worldwide effort to incorporate traditional medicine into modern healthcare, providing a comprehensive approach to managing diseases.

5.0 Conclusion

Myrmecodia platytyrea aqueous tuber extract demonstrated no toxicity at doses of 2000 mg/kg and below. There were no general behavioural abnormalities, and the extract did not cause any deaths. Additionally, it had no significant effect on body weight or haematological/blood chemistry levels in treated mice compared to the control group. These data indicate that MPAE is safe to consume and may have pharmacological benefits, such as anti-cancer, anti-diabetic, and anti-inflammatory effects that recognise the knowledge and insights passed down through folklore.

Authorship contribution statement

MMZ: Experimented, data analysis, writing–original draft. **IAW**: Visualisation, methodology, draft corrections. **MM**: Visualisation, methodology, draft corrections. **AHJ**: Visualisation, methodology, draft corrections. **TP**: Visualization, resources, devised the initial concept. **MHH**: Designed and directed the project, Supervised, funding acquisition, writing – review & editing. All authors discussed the results and contributed to the final manuscript.

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Conflict of Interest

The authors declared that they have no conflicts of interest to disclose.

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