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Acute and Sub-acute toxicity of the aqueous leaf extract of *Lantana trifolia* (Verbenaceae) in experimental rodents

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ABSTRACT

Lantana trifolia, a plant of the Verbenaceae family, is traditionally used to treat several diseases; however, empirical data to validate its toxicity profile and safety is lacking. Thus, this study investigated the qualitative phytochemical composition, acute and sub-acute toxicity of the aqueous leaf extract of *L. trifolia* to validate its ethnomedicinal usage. **Methods:** Qualitative phytochemical analysis of the studied plant extract was performed based on standard procedures to appraise its pharmacological value. Acute oral toxicity of the study extract was investigated at dose levels of 300 mg/Kg BW and 2000 mg/Kg BW according to guideline 423 described by the Organization for Economic Co-operation (OECD) for 14 days. Sub-acute oral toxicity of the studied plant extract was investigated at three dose levels (250 mg/Kg BW, 500 mg/Kg BW, and 1000 mg/Kg BW) in Swiss albino mice based on the OECD guideline number 407 for 28 days, after which haematological, biochemical, and histological traits were determined. **Results:** Phytochemical screening revealed the presence of tannins, saponins, phenolics, terpenoids, flavonoids, alkaloids, and reducing sugars. In an acute oral toxicity study, the aqueous leaf extract of *L. trifolia* demonstrated a median lethal dose (LD_{50}) of >2000 mg/Kg BW, depicting its safety. Following sub-acute oral toxicity, the urea levels in female mice which received 1000 mg/Kg BW of the aqueous leaf extract to *L. trifolia* were significantly elevated compared to those of the control group mice ($P<0.05$). Also, significantly higher platelet counts were observed in all the extract-treated mice compared with those of the control group mice ($P<0.05$). Additionally, the mice administered with 1000 mg/Kg BW of the studied plant extracts demonstrated diffuse tubular epithelium degeneration, indicating nephrotoxicity and a dose-related hepatocyte degeneration, indicating hepatotoxicity. **Conclusions:** The aqueous leaf extract of *L. trifolia* may be relatively non-toxic when administered orally for a short period. The aqueous leaf extract of *L. trifolia* induces nephrotoxicity and hepatotoxicity in experimental mice when administered sub-acutely at a dose of ≥1000 mg/Kg BW.

Keywords: *Lantana trifolia*; Acute oral toxicity; Sub-acute oral toxicity; Haematological, Biochemical, and histological traits.

INTRODUCTION

Medicinal plants have been used to manage different human and animal diseases by approximately 80% of the global populations in developing nations [1, 2]. Over the last two decades, the demand and access to traditional medicine have grown exponentially [3]. Despite the widespread use of medicinal plants, there is a lack of precise chemical classification, dosage regimens for various diseases, toxicity profiles, and safety data to appraise their usefulness [4]. Therefore, this study was designed to investigate the qualitative phytochemical composition, acute and sub-acute oral toxicity of the aqueous leaf extract of *Lantana trifolia* to appraise its safety.

Lantana trifolia is a three-leaved, scrambling, an evergreen herbaceous shrub of the Verbenaceae family, which grows uprightly up to 3 metres tall [5]. It grows in the subtropical and tropical regions. Especially on disturbed forests, abandoned cultivated lands, and even roadsides [6]. In Kenya, it is known as "*Shimenenwa*" among the Luhya community and "*Bekaptarit*" among the Marakwet community [7,8]. Some ethnomedical claims associated with various parts of *L. trifolia* include treatment of asthma, common cold, malaria, epilepsy, eye cataracts, conjunctivitis, among other maladies [7, 9]. In addition, it is claimed to boost lactation in breastfeeding mothers [8].

Previous studies reveal that extracts of *L. trifolia* have antimicrobial effects against *Mycobacterium fortuitum* and *Mycobacterium tuberculosis* and anti-inflammatory and antinociceptive efficacy [10, 11]. Besides, some major essential oils, including germacrene D, (E)-caryophyllene, bicyclogermacrene, and alpha-humulene, have been isolated [10]. However, there is insufficient empirical data to validate the ethnopharmacological applications and safety of *L. trifolia*, hence the present study.

MATERIALS AND METHODS

Plant material

The leaves of *Lantana trifolia* were collected from Cherangany Sub-County, Trans-Nzoia County in Kenya, in their natural habitat. A plant taxonomist did plant identification and authentication at the Department of Land Resource Management and Agricultural Technology Herbarium, University of Nairobi. The collected leaves were slightly washed with clean tap water and rinsed with distilled water to remove dust, and then spread on a wooden bench in a well-ventilated, insect, rodent, and dust-free room, then air-dried at room temperature for two weeks. After that, the dried leaves were ground to a uniform powder using an electric mill and stored in sachets on a cool, dry shelf awaiting extraction.

Extraction procedure

The ground material was extracted by the cold maceration method. One hundred (100) grams of the dried *L. trifolia* powder was accurately weighed using an analytical balance and soaked in 1litre of distilled water for 72 hours. The mixture was then filtered through a Whatman filter paper. The filtrate was transferred into freeze-drying flasks and lyophilised for 48 hours. The powdered extract was weighed, transferred into air-tight sealed containers, and stored in the refrigerator at 4 ^{0}C awaiting analysis. The percentage yield of the extract was calculated using the formula shown below.

$$Percentage\ yield = \frac{Weight\ of\ the\ extract}{Weight\ of\ the\ macerated\ material} \times 100$$

Qualitative phytochemical screening

Qualitative analysis of various phytochemical constituents, including tannins, saponins, phenolics, terpenoids, cardiac glycosides, flavonoids, alkaloids, reducing sugars, and anthraquinones in the aqueous leaf extract of *L. trifolia*, was done using standard procedures [12].

Acute and sub-acute toxicity studies

Experimental animals, husbandry, and ethical clearance

The acute and sub-acute oral toxicity of the aqueous leaf extract of *L. trifolia* were investigated using female Wistar rats (180-220g) aged 8-12 weeks and Swiss albino mice (male and female) weighing 20-30 grams and aged 4-6 weeks, respectively. The experimental animals were obtained from the animal breeding unit of the Department of Public Health, Pharmacology, and Toxicology, University of Nairobi. They were housed in polypropylene cages in the research laboratory at standard conditions; temperature of 25±3°C; 56- 60% relative humidity and a photoperiod of 12 hours light and 12 hours darkness. The animals were fed on standard rodent pellets from a commercial supplier (Unga Group Plc, Kenya) and clean water *ad-libitum.* They were allowed to acclimatize for ten days prior to experimentation. Ethical clearance was sought and granted from the Faculty of Veterinary Medicine Biosafety, Animal use and ethics committee (REF: FVM BAUEC/2018/176).

Acute oral toxicity study

The acute oral toxicity study was conducted according to the Organization for Economic Co-operation and Development (OECD) test guideline 423 with slight modification [13]. Nine (9) healthy female Wistar rats were randomly assigned into three groups (1, 2, and 3), individually weighed, and marked for easy identification. Food was withheld overnight, but the drinking water was provided ad libitum before dosing. Rats in group 1 were orally administered with 10 ml/Kg BW of distilled water and served as the control. The rats in groups 2 and 3 were orally administered 300 mg/Kg BW and 2000 mg/Kg BW, respectively, of the aqueous leaf extract of *L. trifolia.* The animals were observed individually at least once during the first 30 minutes after dosing, then periodically during the first 24 hours, with special attention in the first 4 hours. All the still alive animals were observed up to the 14th day for any abnormal behaviour or signs of toxicity. The observations included changes in eyes and mucous membranes, skin and fur, circulatory, respiratory, central nervous system, autonomic nervous systems, Somatomotor activity, behaviour patterns, and weekly body weight. Other observations included convulsions, tremors, salivation, diarrhoea, lethargy, and sleep. After 14 days, the surviving rats were euthanised using diethyl ether, and necropsy was performed.

Sub -Acute Toxicity Study

The sub-acute oral toxicity study was carried out according to OECD guideline 407 [14]. Ten experimental mice (5 male and 5 female) were randomly allotted four groups in this study. The control group mice (Group I) received distilled water (10 ml/Kg BW) orally, once daily, for 28 days. Mice in experimental groups II, III, and IV received 250 mg/Kg BW, 500 mg/Kg BW, and 1000 mg/Kg BW in distilled water, respectively, of the aqueous leaf extract of *L. trifolia* orally once daily for 28 days. The body weights of the animals were measured weekly using an electronic balance (Mettler PM 4600, Germany) and recorded. The animals were observed daily for any physiological and behavioural changes.

Haematological and biochemical assays

On the 29th day, blood samples were collected from all surviving mice, which were used for sub-acute toxicity study after anaesthesia and decapitation. Blood samples were collected into heparinised (EDTA) tubes for haematological assay and into plain tubes (non-heparinised) for biochemical assay. The blood in non-heparinised tubes was left to coagulate and thereafter centrifuged at 3000 rpm for 10 minutes to extract serum stored at -20 ^{0}C awaiting biochemical analysis. Biochemical traits including Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), creatinine, blood urea, and total proteins were determined. Besides, various haematological traits including packed cell volume (PCV), haemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and thrombocytes in the heparinised blood samples were measured.

Histopathological study

The animals were dissected immediately after blood sample collection, whereby various organs (heart, liver, spleen, and kidney) were removed, observed macroscopically, weighed separately, and the percentage organ: body weight ratios determined. The organs were then preserved in 10 % buffered formalin solution in labelled bottles before histopathological processing and investigation. The sections of the organs, which had been excised, were processed and embedded in paraffin wax and sliced using a microtone. After that, the tissue slices were affixed on glass slides, stained using haematoxylin and eosin

(H&E), and observed under the light microscope for any histological aberrations.

Statistical analysis

Qualitative phytochemical data was tabulated. Quantitative data were descriptively expressed as mean± standard deviation of respective group animals. One-Way analysis of variance was performed to determine the significance of differences among means, followed by Tukey's *post hocs* test for pairwise comparison and separation of means. The IBM Statistical Package for the Social Sciences (SPSS version 21.0) software was used for analysis. $P<0.05$ was considered significant.

RESULTS

Extract yield and qualitative phytochemical composition

The yield of the aqueous leaf extract of *L. trifolia* was about 5.2 %. Qualitative phytochemical screening revealed the presence of tannins, saponins, phenolics, terpenoids, flavonoids, alkaloids, and reducing sugars, and the absence of cardiac glycosides and anthraquinones in the aqueous leaf extract of *L. trifolia* (Table 1).

Table 1: Qualitative phytochemical composition of the aqueous leaf extract of *L. trifolia*

Phytochemical	Test method	Observation
Tannins	Ferric chloride test	+ve
Saponins	Foam test	+ve
Phenolics	Ferric chloride test	+ve
Terpenoids	Salkowski's test	+ve
Cardiac glycosides	Keller-killiani test	-ve
Flavonoids	Alkaline reagent test	+ve
Alkaloids	Dragendorrf's test	+ve
Reducing sugars	Fehling's test	+ve
Anthraquinones	Bontrager's test	-ve

Key: + ve: present; -ve: Absent

Acute and sub-acute oral toxicity

No mortality was observed within the first 4 hours of continuous observation, after 24 hours, and thereafter throughout the 14-day acute oral toxicity study period. Besides, the general behaviour of the extract-treated animals remained normal, and their body weights did not change significantly across the 14-day acute oral toxicity study period ($P>0.05$; Table 3). The LD_{50} of the aqueous leaf extract of *L. trifolia* was >2000 mg/Kg BW.

Table 2: Acute oral toxicity effects of the aqueous leaf extract of *L. trifolia* in Wistar rats

Observation	Control (10 ml/Kg BW distilled water)	300 mg/Kg BW of extract	2000 mg/Kg BW of extract
Respiratory changes	-	-	+
Circulatory changes	-	-	-
Gripping strength	+	+	+
signs of aggression	-	-	-
Skin and fur changes	-	-	-
Response to sound	+	+	+
Salivation	-	-	-
Urinary incontinence	-	-	-
Defaecation	+	+	+
Diarrhoea	-	-	-
Sedation	-	-	+
Convulsions	-	-	-
Mortality	-	-	-
Post mortem changes	-	-	-

Key: + = Present - = Absent

Table 3: Effect of the aqueous leaf extract of *L. trifolia* body weights of Wistar rats

Treatment group	Initial weight	7th Day	14th Day	P-value
Control (10 ml/Kg BW)	203.04±18.22	229.14 ±43.03	240.59±41.90	-
300 mg/Kg BW extract	209.33±1.04	232.71±14.49	247.12±15.99	0.99
2000 mg/Kg BW extract	205.09±19.97	219.64±17.82	223.70±17.79	1.00

All values are expressed as mean ± standard deviation of three animals.

Sub-acute oral toxicity

Effects of the aqueous leaf extract of L. trifolia on body weights and relative organ weights of Swiss albino mice

There were no dose-related significant changes in mean body weights of female and male mice treated with aqueous extract of the studied plant compared with the control throughout the 28-day study period ($P>0.05$; Table 4). Moreover, no significant differences in relative organ: body weights were observed in mice treated with the studied plant extract at all dose levels were observed (Table 5).

Table 4: Effects of the aqueous leaf extract of *L. trifolia* on body weights of Swiss albino mice

Day	Sex	Control	250mg/kg Bwt	P -Value	500mg/kg Bwt	P- Value	1000mg/kg Bwt	P -Value
0	**F**	28.54±1.84	24.87±2.92	0.09	24.79±2.05	0.08	24.61±2.14	0.06
	M	23.99±1.72	28.38±1.63	0.11	24.28±1.61	0.99	27.60±0.82	0.08
7	**F**	26.87±3.33	24.56±2.58	0.56	24.90±2.26	0.68	25.51±2.76	0.86
	M	25.00±1.42	28.77±2.78	0.13	26.13±3.01	0.89	27.16±2.69	0.55
14	**F**	26.66±3.72	24.86±3.71	0.77	24.90±2.29	0.62	26.66±3.72	0.78
	M	25.50±1.20	29.40±2.45	0.08	27.92±1.68	0.39	27.48±3.45	0.56
21	**F**	25.52±3.08	24.39±3.39	0.91	25.79±1.22	1.00	23.54±2.65	0.66
	M	25.78±1.92	29.80±3.10	0.14	25.40±3.12	1.00	27.79±2.82	0.67
28	**F**	25.59±3.06	24.19±3.64	0.83	25.65±0.73	1.00	23.11±2.33	0.48
	M	26.26±1.98	30.84±3.07	0.12	25.17±3.94	0.94	26.58±2.79	1.00

Note: All values are expressed as mean ± standard deviation of five animals.

Table 5: Effect of the aqueous leaf extract of *L. trifolia* on relative organ weights of experimental mice

Organ Weight (grams)	Sex	Control	250mg/kg	P value	500mg/kg	P value	1000mg/kg	P value
Liver	**M**	5.72±1.06	5.13±0.61	0.56	4.50±0.53	0.43	5.26±0.48	0.73
	F	5.88±0.33	5.51±0.20	0.82	5.89±0.86	1.00	5.87±0.78	1.00
Kidneys	**M**	1.56±0.13	1.48±0.11	0.82	1.39±0.20	0.31	1.60±0.15	0.96
	F	1.24±0.12	1.41±0.10	0.63	1.35±0.14	0.98	1.48±0.14	0.19
Heart	**M**	0.51±0.06	0.50±0.02	1.00	0.50±0.08	1.00	0.59±0.08	0.23
	F	0.55±0.09	0.56±0.06	1.00	0.54±0.08	1.00	0.62±0.15	0.69
Spleen	**M**	0.84±0.15	0.73±0.14	0.81	1.00±0.33	0.42	0.99±0.35	1.00
	F	0.94±0.07	0.83±0.32	0.90	1.02±0.15	1.00	0.85±0.18	0.56

All Values are expressed as mean ± standard deviation, n= 10 (5 female and 5 male mice).

Haematological parameters

The effects of the aqueous leaf extract of *L. trifolia* at the studied dose levels on haematological parameters of mice following a 28-day sub-acute oral toxicity study are presented in Table 6. No significant differences among the RBC, WBC, Haematocrit, and MCV, respectively, were observed in experimental mice administered with 250 mg/Kg BW, 500 mg/Kg BW, and 1000 mg/Kg BW of the aqueous leaf extract of *L. trifolia* compared with those of the control group mice (P>0.05; Table 6). However, mice that received the studied plant extract posted significantly higher platelet counts than those recorded in the negative control group mice (P<0.05; Table 6). Besides, the mice that received the studied plant extract at doses of 250 mg/Kg BW and 500 mg/Kg BW had significantly lower MCH levels than those obtained in the control group mice (P<0.05; Table 6). Moreover, the mice administered with 1000 mg/Kg BW of the studied plant extract had significantly lower MCHC levels than those of the control group mice (P<0.05; Table 6).

Table 6: Effects of the aqueous leaf extract of *L. trifolia* on Haematological parameters of experimental mice following Sub-Acute toxicity study

Parameter	Sex	Control	250mg/kg	P value	500mg/kg	P value	1000mg/kg	P value
RBC (10^6/uL)	M	9.52±0.96	11.25±0.56	0.06	11.08±0.59	0.09	9.95±1.50	0.90
	F	8.39±3.60	10.34±0.54	0.12	10.46±0.74	0.35	10.02±0.93	0.55
WBC (10^3/uL)	M	12.06±3.72	13±6.35	0.96	11.20±3.21	0.99	7.21±2.74	0.31
	F	5.96±2.25	12.05±4.27	0.62	13.8±11.09	0.42	17.74±10.14	0.13
Platelets (10^3/uL)	M	326±122.75	847±138.20	0.00	849±73.24	0.00	980±290.75	0.00
	F	312±226.66	792±203.66	0.02	895±118.76	0.03	1129±295.19	0.00
Haematocrit (%)	M	40.34±3.48	45.56±0.52	0.10	46.24±2.90	0.06	43.10±4.83	0.57
	F	35.74±14.39	45.12±0.69	0.23	43.54±1.41	0.37	42.82±2.98	0.45
Haemoglobin (g/dL)	M	15.28±0.99	15.32±0.39	0.10	15.86±1.00	0.91	14.04±2.41	0.52
	F	12.66±5.11	15.82±0.46	0.30	14.54±0.49	0.70	14.10±1.83	0.84
MCV (fL)	M	42.48±1.33	40.56±1.92	0.40	40.06±1.85	0.22	43.54±2.33	0.81
	F	43.54±3.33	40.04±2.10	0.15	41.74±2.68	0.66	42.96±1.26	0.98
MCH (pg)	M	16.20±2.39	13.64±0.59	0.03	13.72±0.50	0.03	14.08±0.61	0.08
	F	15.46±1.20	14.00±0.31	0.04	13.94±0.64	0.04	14.08±0.74	0.06
MCHC (g/dL)	M	37.64±4.97	33.62±0.65	0.15	34.30±0.98	0.27	32.38±2.29	0.04
	F	35.56±1.84	35.08±1.18	0.97	33.38±0.65	0.19	32.82±2.34	0.07

All Values are expressed as mean± standard deviation, n= 10 mice (5 female and 5 male); RBC: Red blood cells; WBC: White blood cells; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin (MCH); MCHC: Mean corpuscular haemoglobin concentration (MCHC).

Biochemical parameters

The biochemical traits of experimental mice treated with the aqueous leaf extract of *L. trifolia* are shown in Table 7. No significant differences (P>0.05) among the ALT, AST, and Total protein levels of experimental mice treated with the studied plant extracts at all three dose levels compared with those of the control group mice were observed (Table 7). However, blood urea levels obtained in female mice which received 1000 mg/Kg BW of the aqueous leaf extract of *L. trifolia* were significantly higher than those of the control group mice (P<0.05; Table 7). Moreover, the results showed significantly higher serum creatinine levels in mice treated with 1000 mg/Kg BW than those of the control group mice (P<0.05; Table 7).

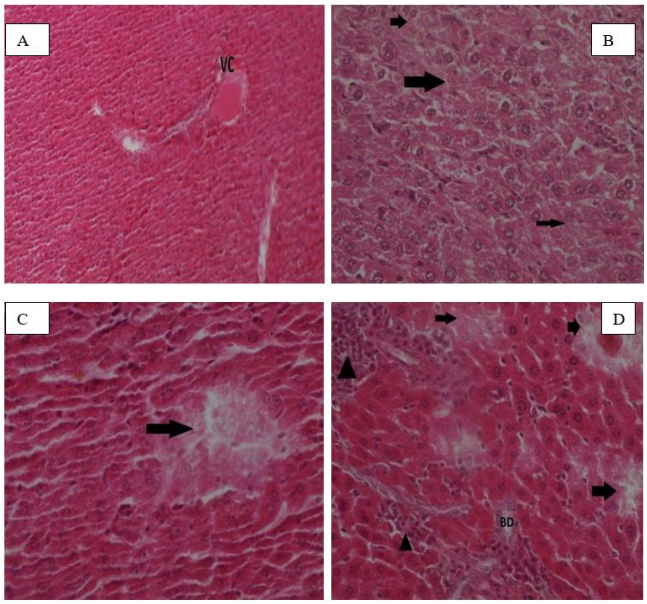
Table 7: Effects of the aqueous leaf extract of *L. trifolia* on biochemical parameters of Swiss albino mice following sub-Acute toxicity study

Parameter	Sex	Control	250mg/kg	P value	500mg/kg	P value	1000mg/kg	P value
ALT (IU/L)	M	156.29±112.06	67.76±18.78	0.45	92.48±37.08	0.70	157.34±141.67	1.00
	F	85.70±8.23	112.02±26.82	0.77	81.51±8.32	1.00	135.50±81.79	0.30
AST (IU/L)	M	526.00±382.16	349.40±27.97	0.78	414.32±153.92	0.93	553.72±419.57	1.00
	F	214.28±42.39	293.96±97.46	0.96	305.02±60.65	0.93	499.05±484.51	0.31
UREA (mg/dl)	M	7.30±1.51	8.52±1.71	0.80	7.67±1.86	0.99	10.10±3.01	0.20
	F	6.81±1.05	8.06±1.72	0.76	7.76±0.80	0.88	10.79±3.41	0.03
Creatinine (mg/dl)	M	35.60±3.13	37.00±3.00	0.59	43.80±1.92	0.07	52.80±5.07	0.00
	F	40.00±6.65	35.60±2.61	0.42	43.40±4.09	0.63	40.20±3.49	1.00
T.P (g/L)	M	50.59±5.62	54.64±5.86	0.84	57.81±8.13	0.48	59.58±10.57	0.30
	F	59.48±2.09	51.92±5.35	0.31	56.21±6.17	0.86	67.62±10.20	0.25

All Values are expressed as mean± standard deviation, n= 10 mice (5 female and 5 male); ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; TP: Total protein.

Histopathological examination

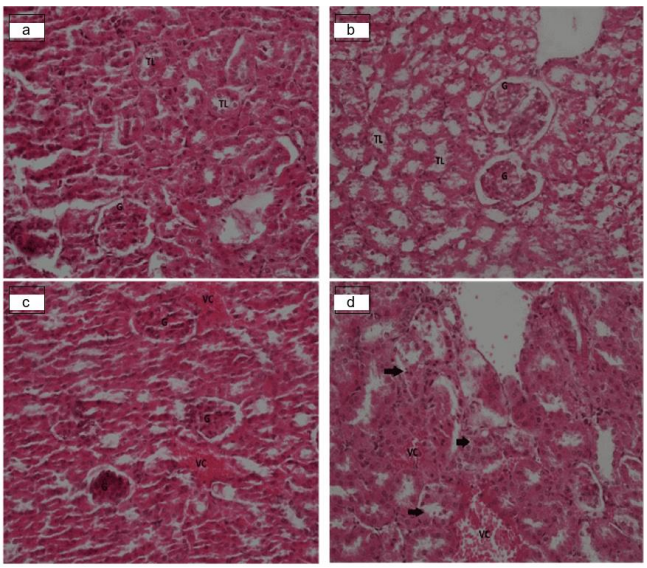
The histological micrographs of the liver sections are shown in Plate 1 (A-D). Plate 1A showed a liver section derived from the control group mouse and was characterised by normal parenchymal architecture with normal hepatic cells that were evenly distributed and separated by sinusoids. The liver section shown in Plate 1B was obtained from mice treated with 250 mg/Kg BW of the studied plant extract and was characterised with focal hepatocyte swelling. Besides, the liver section shown in Plate 1C was obtained from a mouse administered with 500 mg/Kg BW of the studied plant extract and was characterised by hepatocyte necrosis and focal hepatocyte swelling. Plate 1D shows a liver section derived from a mouse treated with 1000 mg/Kg BW of the plant extract and characterised with kupffer cell proliferation, neutrophil proliferation, diffuse hepatocyte necrosis, and karyolysis.



VC: portal vein; **BD**: Bile duct proliferation; **A:** Liver section from a mouse administered with distilled water (control) **B:** Liver section from a mouse dosed with 28-day repeated dose of 250 mg/Kg BW of the aqueous leaf extract of *L. trifolia.* **C**: Liver section from a mouse dosed with 28-day repeated dose of 500 mg/Kg BW of the aqueous leaf extract of *L. trifolia;* **D**: Liver section from a mouse dosed with 28-day repeated dose of 1000 mg/Kg BW weight of the aqueous leaf extract of *L. trifolia.*

Plate 1: Photomicrographs showing liver sections from mice treated with different doses of *L. trifolia* for 28 days (H&E stained; X400).

Plate 2 (a-d) shows histopathological features of kidney sections of experimental mice treated with various doses of the aqueous leaf extract of *L. trifolia* post-sub-acute oral toxicity study. Kidney sections in Plate 2 (a, b, and c were) were derived from the control group mice, and mice treated with 250 mg/Kg BW and 500 mg/Kg BW, respectively of the studied plant extract, and were all characterised with normal tubules and regular glomeruli. The kidney section in plate 1 (d) was obtained from mice treated with 1000 mg/Kg BW of the studied plant extract showing diffuse tubular epithelium degeneration characterised with cell swelling and necrosis (Arrow G).



G; glomerulI; VC: Vascular congestion; TL- Tubular lumen; a. Kidney section from a mouse administered with distilled water (control); b. Kidney section from a mouse treated with a 28-day repeated dose of 250 mg/Kg BW of the aqueous leaf extract of *L.* trifolia; c. Kidney section from a mouse treated with a 28-day repeated dose of 500 mg/Kg BW of the aqueous leaf extract of *L. trifolia*; d. Kidney section from a mouse treated with a 28-day repeated dose of 1000 mg/Kg BW of the aqueous leaf extract of *L. trifolia.*

Plate 2: Photomicrographs showing kidney sections from mice treated with different doses of the aqueous leaf extract of *L. trifolia* for 28 days (H&E X400).

DISCUSSION

Lantana trifolia is widely used in traditional medicine in Eastern Africa to treat various ailments. Despite the ethnopharmacological benefits of *L. trifolia*, detailed knowledge about its phytochemical

composition and toxicity profile is scanty. Therefore, the current study was performed to determine the qualitative phytochemical profile and acute and sub-acute oral toxicity of the aqueous leaf extract of *L. trifolia* in experimental rodent models.

In the present study, various phytocompounds detected in the plant extract have been associated with various pharmacological properties [14,15]. However, the absence of anthraquinones, which are associated with toxicity, may be responsible for the extracts' safety. Besides, the toxicity-associated phytocompounds may be present in low levels to induce any observable signs of toxicity [16].

In the 14-day acute toxicity study, the aqueous leaf extract of *L. trifolia* did not elicit any significant clinical signs of toxicity or death at all the studied doses and depicted an LD_{50} greater than 2000 mg/Kg BW thus considered safe as previously demonstrated [17]. This finding corroborates previous scholarly reports [18-20]. An adverse change in body weight is a key indicator of the toxicological effects of extracts or drugs [21]. In this study, the aqueous leaf extract of *L. trifolia* did not produce significant changes in relative liver, kidney, heart, and spleen weights, indicating its safety.

One of the most sensitive targets of toxic compounds is the haematopoietic system, and it serves as an important index of physiological and pathological response to toxic insults [22]. The haematological parameters such as RBC, WBC, HB, haematocrit, and MCV of the extract-treated mice and those of the control group were comparable, depicting the extract's safety. The differences in MCHC levels in male mice which received 1000 mg/Kg BW of the studied plant extract may be due to analytical errors as described [23]. A non-significant increase in red blood cells and haemoglobin shows that the extract did not induce anaemia. Moreover, there was no change in WBC count, which is known to rise in response to toxic environments [24].

The liver is the body's major organ for detoxification, and its damage may result from the accumulation of toxic compounds due to inefficient metabolism and excretion. This damage is usually assessed by determining serum transaminases (ALT and AST) and total protein in serum samples [21, 25]. In our study, there were no significant alterations in the levels of alanine aminotransferase, aspartate aminotransferase, and total protein, which are indicators of liver function. However, the urea levels in female mice and creatinine levels in male mice, which received 1000 mg/Kg BW of the aqueous leaf extract of *L. trifolia*, implying a possible impaired kidney function [18; 26].

Histology remains the gold standard diagnostic tool for structural-related organ damage [27]. In our study, histological analysis of the liver revealed varying degrees of hepatocyte swelling and hepatocyte necrosis, which could be attributed to alkaloids in the plant, as reported in a previous study [28]. The kidney is highly susceptible to damage caused by various toxic amalgams since a large volume of blood flows through it, and the toxins are typically filtered and concentrated in the kidney tubules [29]. The histo-architecture of the kidney from mice dosed with 1000 mg/Kg BW of the studied plant extract showed diffuse tubular epithelium degeneration, which was characterised by cell swelling and necrosis, suggesting kidney damage. Such structural changes typical of adaptive responses to pathological stimuli have been reported in the liver and kidney of rats treated with high doses of ethanolic leaf extract of *Lantana camara* [30]. This implies that the liver and kidney functions should be monitored in cases of long-term administration of high doses of the studied plant extract.

CONCLUSIONS AND RECOMMENDATIONS

Based on the study findings, short-term use of the aqueous leaf extract of *Lantana trifolia* at doses of 300 Kg BW and 2000 mg/Kg BW may be considered non-toxic in the animal model. The extract is neither nephrotoxic nor hepatotoxic at lower doses, but liver and kidney functions should be monitored, particularly in cases of high-dose administration. Chronic toxicity studies, including genotoxicity and carcinogenicity of the studied plant extracts, and the determination of specific mechanisms of toxicity should be performed to appraise its safety. Considering that the leaves of this plant have various ethnopharmacological uses, including the management of tonsilitis, eye infections, ear infections, chronic cough, malaria, and the common cold, our findings lay a framework for further empirical investigations to elucidate its pharmacologic efficacy and potential.

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Authors' contributions

Ronny Ivayo Musinya - Conceptualization, investigation, methodology, project administration, resources, visualization, writing the original draft, writing-review, and editing

James Mucunu Mbaria – Conceptualization, investigation, methodology, supervision, validation, writing- review and editing

Isaac Ole-Mapenay - Conceptualization, investigation, methodology, supervision, validation, writing- review and editing

Competing interests

All the authors declare no competing interests.

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