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Acute and Sub-Acute Toxicity of Ethanolic Leaf Extracts *of Rumex abyssinica* Jacq. (Polygonaceae) and *Mentha spicata* L. (Lamiaceae)

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Abstract

Rumex abyssinica Jacq. (Polygonaceae) is locally used in management of allergies and female reproductive healthcare; whereas _Mentha spicata_ L. (Lamiaceae) is used to treat gastrointestinal and respiratory distress, dandruff, halitosis and malaria in Uganda. Owing to the paucity of data on their safety profiles, this study evaluated the acute and sub-acute toxicities of 70% ethanolic leaf extracts of both plants in mice and Wistar albino rats. The oral acute toxicity of both plants was evaluated in Swiss mice of 7 - 8 weeks old (16 - 22 g) body weight and LD_{50} determined. Sub-acute toxicity was evaluated in Wistar albino rats (6 per group) at dose rates of 500, 1000 and 1500 mg/kg for 28 days. The LD_{50} of _R. abyssinica_ and _M. spicata_ in mice was 7727 mg/kg and 13,606 mg/kg body weight, respectively. General signs of toxicity due to large doses of both plants extract included hyperurination, abdominal muscle twitches and convulsions. In the sub-acute toxicity test, rats treated with both extracts did not exhibit any clinical signs of toxicity; no mortality and changes in body weight were observed. _R. abyssinica_ did not cause significant changes in haema-

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tological indices, except a significant increase in HCT ($p < 0.05$). However, a dose dependant significant decrease in HCT ($p < 0.05$) and a significant increase ($p < 0.05$) in the levels of WBC, LMY and MCHC were observed in rats treated with *M. spicata* extract. Biochemical test showed that both extracts caused a significant dose dependent increase ($p < 0.05$) in levels of ALT and ALP. Marked increase in the levels of AST was also observed in rats treated with *M. spicata* extract. Of the two extracts, only rats treated with *R. abyssinica* revealed congestion, hemorrhages and cellular infiltration in vital organs. In conclusion, the LD50 values of both plant extracts were above 5000 mg/kg suggesting that they are experimentally safe, thus justifying their use in traditional medicine. However, prolonged exposure to higher doses may cause observable alterations in histopathological, biochemical, and haematological parameters, particularly with *R. abyssinica.*

Keywords

Histopathology; *Mentha spicata*; *Rumex abyssinica*; Toxicity

1. Introduction

Traditional medicine has maintained greater popularity all over the world and the use is rapidly on the increase [1] [2]. The use of herbs in treatment of disease has declined in the west, but it continues to exist throughout the developing countries [3]. Uganda is well endowed with diversity of medicinal plant resources that serve as both alternative and complementary medicine in various communities. Plants which are commonly used in traditional medicine are frequently promoted as natural and, therefore, harmless [3] [4]. However, some medicinal plants must be used with caution since they can be potentially harmful at high doses and can interact with modern drugs [5] [6]. As such, there is a growing public demand for research that focuses on bridging knowledge gaps on safety of medicinal plants [7] [8], that are used as complementary or alternative medicine (CAM) [9]. Such studies can inform scientists, health practitioners and policy makers of safety of herbal remedies derived from particular plants to humans. In Uganda, the use of medicinal plants is an important and essential part of the culture and of traditional healthcare system. However, the production, prescription, packaging, distribution and use of these plants are still poorly regulated. This predisposes users of herbal products to potential toxicities. Thus, there is a need for evaluating the safety profile of plants with medicinal value since such inventory will be useful for future reference by medical and herbal practitioners. Spearmint or *M. spicata* is a species of mint plant native to Europe and Asia that grows well in nearly all temperate climates. In folk medicine, spearmint has been used for gastrointestinal and respiratory distress, reproductive healthcare, halitosis and malaria across Africa [10]. Some human research suggests that drinking spearmint tea may help reduce excessive hair growth (hirsutism) in women with polycystic ovarian syndrome (PCOS). *R. abyssinica* on the other hand is traditionally used in Uganda for management of allergies and female reproductive healthcare. Despite the extensive use of *M. spicata* and *R. abyssinica* in traditional medicine in Uganda, their toxicological profiles are not known. Therefore, the present study evaluated the LD_{50}, oral acute and sub-acute toxicity effects of *M. spicata* and *R. abyssinica* in Swiss albino mice and Wistar rats.

2. Materials and Methods

This study was conducted in Pharmacology and Toxicology Research Laboratory (PTRL), Department of Veterinary Pharmacy, Clinics and Comparative Medicine, College of Veterinary Medicine, Animal resources and Biosecurity, Makerere University-Kampala.

2.1. Collection and Extraction of Plant Materials

Fresh leaf samples of *M. spicata* and *R. abyssinica* were collected from Western Uganda. Valid samples of *M. spicata* and *R. abyssinica* were submitted in Makerere University Hebarium, Department of Botany and voucher specimen deposited for authentification under the reference MK001 and MK002, respectively. The plant voucher specimens were referenced based on International Plant Naming Index (IPNI) database. The leaf samples were

washed with clean running tap water, shade dried and ground into fine powder using an electrical grinder (Brook Crompton series 2000, Germany). The powder was soaked in ethanol-water (70-30: v/v) for 72 hrs with twice daily manual shaking to enhance solvent particles interaction. The mixture was filtered using cotton wool fixed in a glass pyrex funnel and the filtrate concentrated using a rotary evaporator (Rota vapor Re111, Germany) set at 65˚C. The concentrate was further dried in an oven at 50˚C into a semi-solid extract and percentage yield determined.

2.2. Experimental Animals

Both Male and female Wistar albino rats (n = 42, weighing 150 - 170 g, 8 weeks old) and Swiss albino mice (n = 50, weighing 16 - 22 g, 7 - 8 weeks old) were purchased from PTRL. The animals were housed in a normal environmental condition (temperature 24˚C ± 1˚C; 12:12 day/night; relative humidity of 60% ± 5%), fed with standard rodent pellet (Engaano limited-Kampala), and water was given *ad libitum*. Animal care and handling conformed to international guidelines [11] [12].

2.3. Acute Toxicity Test

This study was conducted according to Organisation For Economic Co-operation and Development (OECD) guidelines 420 [11] where the limit test dose of 5000 mg/kg was used. The mice were divided into 6 groups (5 test groups and 1 control group for each plant extract) of 6 animals each (3:3, males:females). The extract stock solutions of 300 mg/ml was prepared by dissolving 3 g to 10 ml of normal saline and used on the same day. The mice were fasted overnight and their body weights determined. Both extracts were administered orally using intragastric tube (size 4). The dose rate for *R. abyssinica* for the different groups were 4500, 6500, 8500, 10,500 and 12,500 mg/kg body weight while for *M. spicata* were 10,000, 12,000, 14,000, 16,000 and 18,000 mg/kg body weight. The negative control group received normal saline at a dose rate of 10 ml/kg body weight. signs of toxicity and any mortality was recorded between 4 hrs to 72hrs post treatment. Finally, percentage mortality was determined and transformed into probits and LD_{50} calculated from the log dose-Probit curve (**Figures 1(a)** and **(b)**).

2.4. Sub-Acute Toxicity Test

Fourty two rats were divided into seven groups of 6 animals each (3:3, males:females). The rats were grouped according to the dose rates (500, 1000 and 1500 mg/kg body weight) of *M. spicata* and *R. abyssinica* ethanolic extracts and treated orally with a single dose daily for 28 days. Another group received 10 ml/kg of normal saline as negative control. The repeated doses for this study were carried out according to OECD guideline 407 [12]. Signs of toxicity due to the treatments, were monitored daily and the body weights of the rats were recorded on days 0, 14 and 28 of the experiment.

2.4.1. Haematological and Biochemical Analysis

On day 29, rats were humanely sacrificed and blood samples were collected in both heparinised and non-heparinised vacutainers for hematological and biochemical analysis respectively, in the Central Diagnostic Laboratory, College of Veterinary Medicine, Animal Resources and Biosecurity. The hematological parameters such as white blood cells count (WBC), lympocytes (Lym), red blood cells count (RBC'S), hemoglobin(HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and platelet count were determined using automatic counter Sysmex (K21. Tokyo, Japan). Serum was also obtained for biochemical analysis after centrifugation of the collected blood at 2500 rpm for 15 min. The biochemical indices for liver function tests (LFT's) (aspartate transferase (AST), alanine transferase (ALT), alkaline phosphatase (ALP)) and renal function test (creatinine and urea) were analysed using standardized diagnostic kits (Labkit®) and a Biotron® spectrophotometer [13].

2.4.2. Histopathological Studies

Tissue necroscopy was carried out on 2 randomly selected rats (1:1, male:female) per treatment group; the liver, kidney, lung and small intestine were collected, labeled and fixed in 10% formaldehyde. The tissues were dehydrated with increasing concentrations of ethanol and cleared with xylene and paraffin blocks were made. Then, thin sections of about 5 to 7 μm were made and stained with hematoxylin and eosin stains. Histopathological

examination of the slides was carried out by a veterinary pathologist and the images were captured using a computerized imaging system (Canon power short A640, 10 megapixels camera (Japan) mounted to a carl zeiss microscope (Germany).

2.5. Statistical Analysis

To determine the LD_{50}, the percentage mortalities and dose rates were transformed into probits and log dose respectively, and plotted as log dose *vs* Probit response curves. Results for heamatological and biochemical analyses were expressed as Mean ± Standard Error of the Mean (SEM) in tables and graphs. Statistical analysis was performed by oneway analysis of variance (ANOVA) with Dunnet test to evaluate significant mean differences between treatment groups. Values of $p < 0.05$ were considered significant. All statistical analysis was carried out using Graph Padprism software 5.01 (Inc., USA).

3. Results

3.1. Acute Toxicity of *R. abyssinica*

The dose of *R. abyssinica* with non-observable effect level (NOEL) was 4,500 mg/kg, while at 12,500 mg/kg the mortality was 100% (**Table 1**). The calculated LD_{50} value from the log dose-Probit curve (**Figure 1(a)**) was 7,727 mg/kg body weight. The signs of toxicity observed were in doses ranging from 6,500 to 12,500 mg/kg body

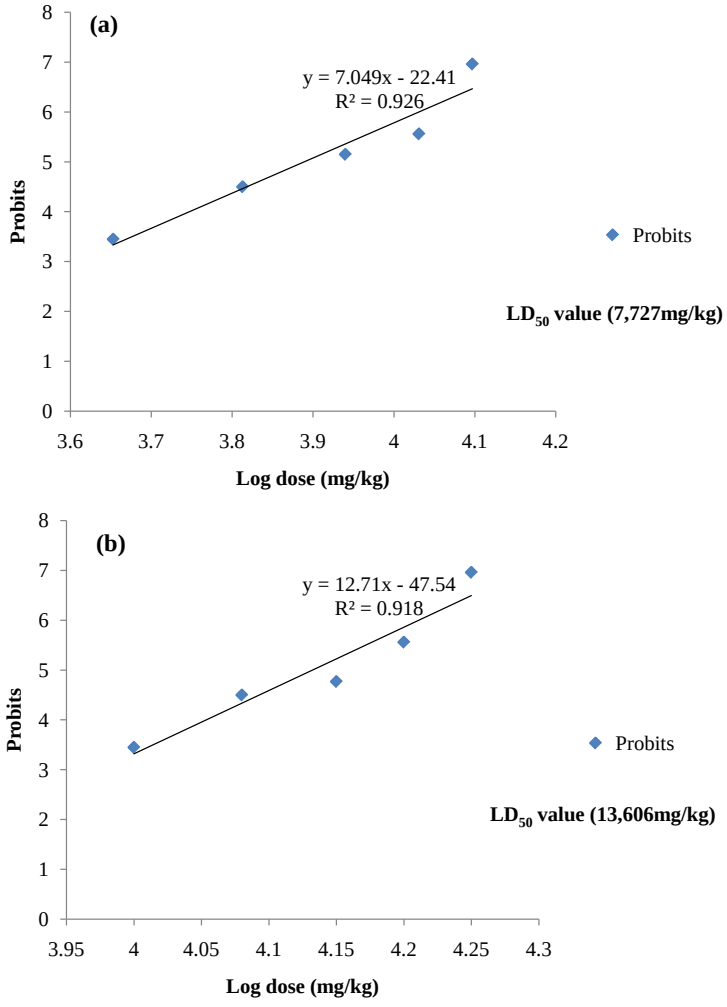


Figure 1. Log dose-Probit curve for LD_{50} determination for *R. abyssinica* (a) and *M. spicata* (b).

weight. These signs included hyperurination, abdominal muscle twitches and with convulsions were observed at 12,500 mg/kg body weight.

3.2. Acute Toxicity of *M. spicata*

The NOEL dose for *M. spicata* was 10,000 mg/kg per body weight; however, 100% mortality was recorded at 18,000 mg/kg body weight (**Table 2**). The LD_{50} value derived from the Log dose-Probit response curve (**Figure 1(b)**) was 13,606 mg/kg body weight. The signs of toxicity observed included hyperurination, abdominal muscle twitches and convulsions at dose rates of at least 12,000 mg/kg body weight.

3.3. Sub-Acute Toxicity Profile of *R. abyssinica* and *M. spicata*

At a dose rate of 500, 1000 and 1500 mg/kg over 28 days period, rats treated with both extracts did not show any signs of toxicity (physical and behavioral); no mortalities were also recorded. There were no significant changes in the body weights of the treated rats when compared to the control group (data not shown).

3.3.1. Effect of Treatment with *R. abyssinica* and *M. spicata* on Haematological Parameters

R. abyssinica did not have any significant effect on WBC, Lym, RBC, HGB, MCV and MCHC, although HCT significantly increased ($p < 0.05$) at 1500 mg/kg body weight compared to the control group (**Table 3**). Treatment with *M. spicata* ethanolic extract caused significant increase ($p < 0.05$) in the levels of WBC, Lym and MCHC. However, significant reduction ($p < 0.05$) was observed for HCT level (**Table 3**).

3.3.2. Effect of Treatment with *R. abyssinica* and *M. spicata* on Biochemical Parameters

Liver function test revealed a dose dependant increase ($p < 0.05$) in ALP and ALT enzymes in the rats treated with both extracts, although the levels were more pronounced with *M. spicata*. Similarly, a significant increase ($p < 0.05$) in the levels of AST was observed in the groups of rat treated with *M. spicata* extract. Kidney function test showed that serum creatinine and urea were not affected by both plant extracts. However, a slight

Table 1. Mortality of mice in the various dose groups of *R. abyssinica.*

Group	Dose (mg/kg)	Log dose	% dead	Probits
1	4500	3.653	0	3.45
2	6500	3.813	16.56	4.50
3	8500	3.940	50.3	5.15
4	10,500	4.031	66.56	5.56
5	12,500	4.097	100	6.96

Number of mice per group (n = 6).

Table 2. Mortality of mice in the various dose groups of *M. spicata.*

Group	Dose (mg/kg)	Log dose	% dead	Probits
1	10,000	4	0	3.45
2	12,000	4.08	16.57	4.50
3	14,000	4.15	33.33	4.77
4	16,000	4.20	66.57	5.56
5	18,000	4.25	100	6.96

Number of mice per group (n = 6).

insignificant increase and decrease was observed in the levels of urea and creatinine respectively, although the values were within the normal range (**Table 4**).

3.4. Histopathology Findings

M. spicata did not cause any significant histopathological changes at various dose used. However, at 1000 and 1500 mg/kg body weight, *R. abyssinica* caused gross histopathological changes in the liver, kidney, lung and small intestinal tissues (**Figure 2**). Focal cellular necrosis, congestion and haemorrhages were observed in both the liver and kidney. The major lesions that were observed in the lung included cellular infiltration and tissue degeneration (**Figure 2**).

4. Discussion

Acute toxicity tests, with appropriate animal model may be used to satisfy the requirements for classification of the level of safety of xenobiotic substances in humans and the environment [12]. This formed the basis for evaluation of the toxicities for ethanolic extracts of *R. abyssinic*a and *M. spicata* in both mice and rats. The LD_{50} values for *R. abyssinica* and *M. spicata* were above 5000 mg/kg body weight. According to OECD classification of toxic substances [11] [14] the high LD_{50} values of both extracts are considered to be experimentally safe. However, at higher doses of both extracts, mild signs of toxicity such as abdominal muscle twitches, hyperventilation, hyperurination and hypoactivity were observed. Convulsions were seen in mice that eventually died. The above signs were considerably more pronounced in the animals treated with *R. abyssinica* than *M. spicata*. This suggests that at low dose (<4000 mg/kg body weight), the above plant extracts may be safer when used as herbal remedies. However, taking large doses of the extracts (above the LD_{50} values) should be discouraged since signs

Table 3. Hematological parameters effects of *R. abyssinica* and *M. spicata and in wistar albino rats.*

Parameters	Control group	*R. abyssinica*			*M. spicata*		
	(Normal saline)	500 mg/kg	1000 mg/kg	1500 mg/kg	500 mg/kg	1000 mg/kg	1500 mg/kg
WBC	9.313 ± 0.47	11.79 ± 1.473	12.58 ± 1.06	12.26 ± 1.82	17.74 ± 0.758[a]	19.12 ± 1.32[a]	22.38 ± 1.24[a]
LYM	5.027 ± 0.31	5.980 ± 1.23	8.052 ± 1.123	9.467 ± 0.539	10.50 ± 0.561[a]	10.57 ± 0.93[a]	16.12 ± 1.37[a]
RBC'S	9.947 ± 0.620	9.730 ± 0.25	8.473 ± 0.216	8.405 ± 0.176	8.31 ± 0.08	7.980 ± 0.77	8.143 ± 0.34
HGB	13.63 ± 0.201	13.48 ± 0.88	12.83 ± 0.87	12.07 ± 1.017	13.17 ± 0.26	12.80 ± 0.16	10.82 ± 1.05
HCT	42.87 ± 0.658	43.02 ± 1.049	48.23 ± 3.025	50.47 ± 3.67[a]	41.37 ± 0.663	38.36 ± 1.701[a]	37.66 ± 2.03[a]
MCV	47.89 ± 0.293	50.25 ± 0.966	51.42 ± 1.307	52.42 ± 5.17	47.90 ± 0.70	49.05 ± 1.14	51.79 ± 0.49
MCHC	27.30 ± 2.256	29.52 ± 1.643	31.63 ± 0.272	32.58 ± 0.431	31.34 ± 0.28[a]	32.12 ± 0.28[a]	32.33 ± 0.39[a]

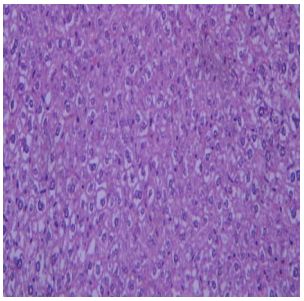
Key: Values expressed as Mean ± SEM (n = 6) [a]p < 0.05, White blood cells (WBC), lymphocytes (Lym), red blood cells (RBCs), hemoglobin (HGB), Hematocrit (HCT), Mean corpuscular volume (MCV) and Mean corpuscular haemoglobin concentration (MCHC).

Table 4. Biochemical parameters for rats treated with *R. abyssinica*, *M. spicata* and control.

Parameters	Control group	*R. abyssinica*			*M. spicata*		
	(Normal saline)	500 mg/kg	1000 mg/kg	1500 mg/kg	500 mg/kg	1000 mg/kg	1500 mg/kg
ALP(u/l)	117.2 ± 2.845	118.2 ± 9.92	196.2 ± 5.39	204.8 ± 17.18[a]	178.60 ± 37.93[a]	202.00 ± 19.06[a]	211 ± 6.25[a]
ALT(u/l)	124.0 ± 18.07	142.9 ± 8.31	182.6 ± 42.25	205.6 ± 30.33[a]	284.80 ± 23.30	294.80 ± 36.33[a]	365 ± 81.34[a]
AST(u/l)	282 ± 37.84	290.7 ± 5.68	341.4 ± 23.00	357.30 ± 48.79	385.90 ± 43.69	533.50 ± 213.9[a]	600.2 ± 127.5[a]
Creatinine (umol/L)	87.0 ± 7.474	57.50 ± 4.67	63.70 ± 4.29	83.00 ± 5.79	71.17 ± 5.21	68.62 ± 4.25	58.83 ± 1.62
Urea (mmol/l)	6.40 ± 0.99	6.65 ± 0.26	7.60 ± 0.15	6.75 ± 0.34	7.87 ± 0.65	7.68 ± 1.58	5.55 ± 0.05

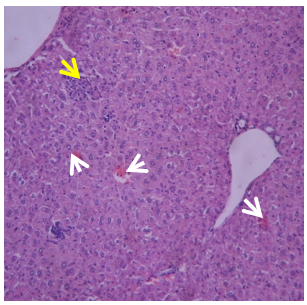
Key: Values expressed as Mean ± SEM (n = 6) [a]p < 0.05, Standard error of mean (SEM), Alkaline phosphatase (ALP), Alanine aminotransferase (ALT), Aspartate transferase (AST).

Liver (Normal Control)



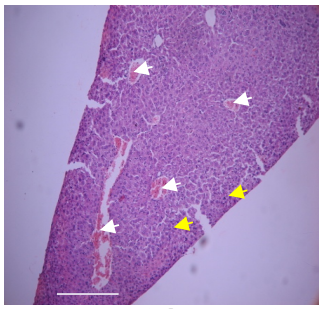
No observable changes (x200) , the scale bar is 100µm

Liver (1,000 mg/kg)



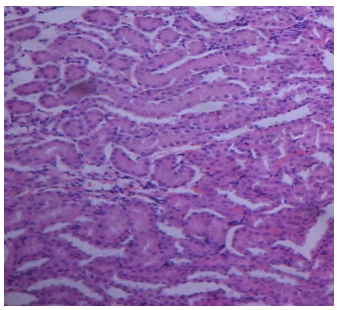
Focal area of hepatic hemorrhages (white arrows) and degeneration (yellow arrow): x200 , the scale bar is 100µm

Liver (1,500 mg/kg)



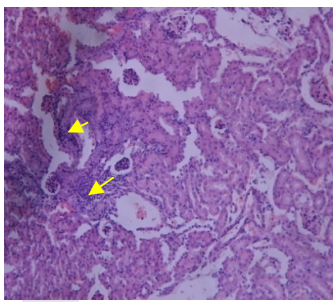
Congestion(white arrow) and degeneration (yellow arrow): x200,the scale bar is 100µm

Kidney (Normal control)



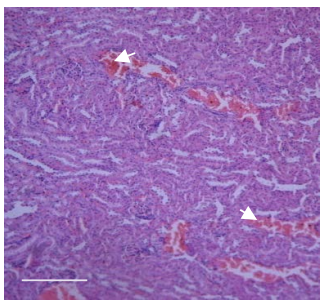
No observable changes: x200, the scale bar is 100µm

Kidney (1,000 mg/kg)



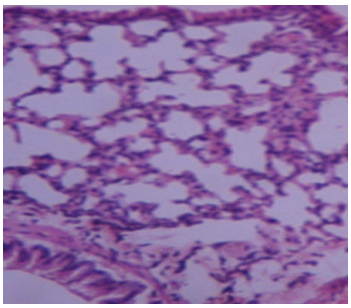
Cellular degenerations (yellow arrows): x200, the scale bar is 100µm

Kidney (1,500 mg/kg)



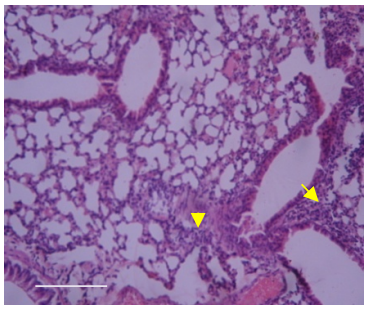
Hemorrhages (white arrows) and tissue degeneration (yellow arrow): ×200, the scale bar is 100µm

Lung (Normal control)



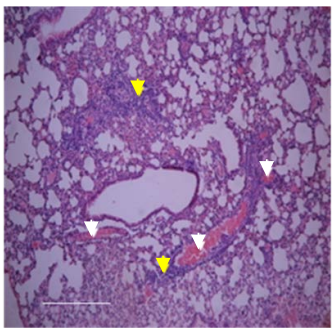
No observable change(×200), The scale bar is 100µm

Lung (1,000 mg/kg)



Tissue infiltration (yellow arrows): ×200 , the scale bar is 100µm

Lung (1,500 mg/kg)



Hemorrhages (white arrows) an tissue degeneration (yellow arrow): ×200, the scale bar is 100µm

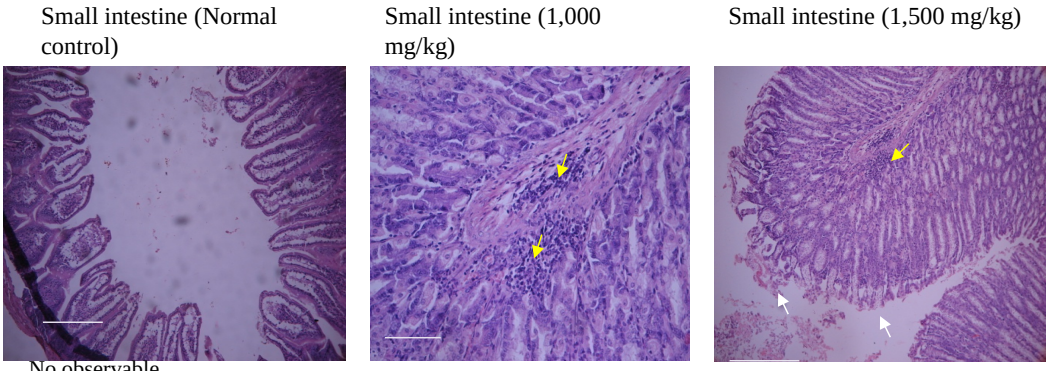


Figure 2. Key histopathological findings in liver, kidney, lung and small intestinal tissues of rats treated with *R. abyssinica*.

of toxicity may be eminent as described above. The current study did not elucidate the toxic principles and their mechanism of toxicity; however, the above signs indicate that the autonomic and central nervous systems were dominantly affected.

Sub-acute and chronic toxicity studies are essential in assessing the bioaccumulative effects of xenobiotics in biological systems. Assessment of liver and kidney function is very important in evaluating toxicity of modern and traditional medicines since these organs play major roles in metabolism of xenobiotics in the body. In sub-acute toxicity *M. spicata* extract caused significant increase in ALT and AST although there was no gross histopathological changes. The lack of visible changes may be attributed to relatively shorter duration of exposure to *M. spicata* which appeared to be safe. *R. abyssinica* on the other hand caused significant increase in LFTs (ALT and ALP) which agrees with the histopathological findings (**Figure 2**). Moreover, the ratio of AST:ALT was above 1 in all the treatment groups. A mild or higher level of AST indicates liver injury or myocardial infarction [15] [16], and the ratio of AST/ALT may be employed in disease diagnosis. An AST/ALT ratio greater than 1 suggests myocardial infarction, while a ratio less than 1 may be due to the release of ALT from the affected liver [17]. This implies that use of large doses of *R. abyssinica* for a longer period of time could cause significant damage to the liver.

Assessment of the haematological indices showed that *R. abyssinica* did not cause any significant effect on WBC, Lym, RBC, HGB, MCV and MCHC, although HCT was significantly increased at 1500 mg/kg body weight (**Table 3**). This implies that *R. abyssinica* could be having minor effect on erythropoiesis considering the significant increase in the level of HCT. However, the normal levels of MCV and MCHC indicates that the morphology and osmotic fragility of the red blood cells were not affected [18]. Treatment with *M. spicata* ethanolic extract caused a significant increase in the level of WBC, Lym, HCT and MCHC across all treatment groups (**Table 3**). The significant increase of WBC and Lym by the *M. spicata* extract suggests that it may contain biologically active compound(s) that can boost the immune system [7]. The above increase could also be due to an imbalance in the rate of hematological parameter synthesis and catabolism [11]. The significant reduction in HCT observed in the rats treated with *M. spicata* extract is indicative of aneamia that may be attributed to the presence of steroid saponins that may be known to have hemolytic activity [19].

The sub-acute toxicity of the extracts on kidney function was evaluated by using serum urea and creatinine, as markers. The result showed that serum creatinine and urea were not affected by sub-acute exposure to the two extracts, although mild cellular infiltration, degeneration and congestions were observed with *R. abyssinica* on the kidney tissues (**Figure 2**). This observation further suggests that the plant extract may not cause significant kidney damage when used at lower clinical doses as herbal remedy. Similarly, *M. spicata* did not cause any significant histopathological changes to both the lung and small intestinal tissues. However, *R. abyssinica* caused cellular infiltration and congestion in the lungs as well as mucosal sloughing in the intestine. The above observations could be attributed to induction of inflammatory process by active chemicals in the extract.

5. Conclusion

The higher LD_{50} values (>5000 mg/kg) for ethanolic extract of both plants suggest that they are experimentally safe, thus justifying the use of these plants as herbal remedies. However, prolonged exposure at higher doses may cause observable changes in biochemical, haematological and histopathological changes, particularly with *R. abyssinica*. Therefore, we recommend that high exposure to the extracts for prolonged period of time should be avoided. However, further studies on chronic tocixicity of the two plants and phytochemical screening to determine the active compounds responsible for the observed changes in the above organs are needed.

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Conflict of Interest

The authors hereby declare that there was no conflict of interest in this study.

Ethical considerations

The experimental procedure was approved by the ethical committee of College of Veterinary Medicine, Animal Resources and Bio-security, Makerere University. Animal care and handling conformed to OECD guideline [11] [12].

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