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Acute and subacute toxicity profile of ethanolic stem bark extract of *Albizia coriaria* Welw. ex Oliv. in Wistar albino rats

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ABSTRACT

Albizia coriaria (Fabaceae) crude extracts are key ingredients of several licensed and unlicensed herbal products in East Africa. However, there is limited and often contradicting information regarding its toxicity. We therefore evaluated the acute and subacute toxicity of the ethanolic stem bark extract of *A. coriaria* in mature healthy Wistar albino rats following Lorke's method and OECD guidelines 407. The LD_{50} of the ethanolic stem bark extract of *A. coriaria* was 2000 mg/kg. The acute toxicity signs observed included piloerection, hyperventilation, lethargy, and loss of righting reflex. There was a significant increase in aspartate aminotransferase, alkaline phosphatase, red blood cells and haemoglobin in rats after 28 days at the dose of 500 mg/kg. Histological analyses revealed multifocal random parenchymal necrosis and scattered periportal mononuclear inflammatory cells infiltration in the liver, interstitial nephritis in the kidney and multifocal lymphoid accumulation in the peribronchiolar and perivascular lung tissue at 500 mg/kg. The ethanolic stem bark of *A. coriaria* was therefore moderately toxic to the rats when administered in a single high oral dose within 24 h. The extract caused a dose dependent toxicity with significant damage to the kidney, liver and lung tissues at a dose of 500 mg/kg after 28 days. Herbal medicines containing *A. coriaria* extracts should be consumed cautiously due to likelihood of toxicity particularly at higher doses greater than 500 mg/kg.

1. Introduction

Traditional and complementary medicine (TCM) is an integral component of Uganda's health care system with over 60 % of the population relying on it for primary health care [41]. However, there are unresolved debates about the quality, safety and efficacy of several TCM in the country with some reported instances of toxicity and even death being attributed to TCM use [3,14]. The toxicity of TCM could be attributed to presence of inherent toxic phytochemicals from poisonous plants used, contamination during the harvesting, preparation and distribution process, misidentification of the plant species and adulteration [29]. Although there is a general belief that traditional medicines are

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relatively safe due to their long-term use, the World Health Organization (WHO) recommends that their toxicity be extensively studied to protect the public against exposure to potential toxic chemicals that may be present in TCM [46].

Albizia coriaria Welw. ex Oliv. (Fabaceae), is a medicinal tree that is widely distributed in several African countries. It is among the common trees used locally to prepare herbal therapies for management of various ailments [35]. The plant is commonly found in the savannah regions of Uganda and is known by different local names such as Itek, Bata (Lango), Ober, Ayekayek (Acholi), Musita (Lusoga), Mugavu (Luganda), Etek, Etekwa (Ateso), Musiisa (Lukiga and Lutoro), and Murongo (Lunyankore) ([31,35]. In Uganda, decoctions of the stem bark of *A. coriaria* are used to treat cough, flu, fever, headache, pain, inflammation, postpartum haemorrhage, stomachache, snake bites, diarrhoea, tuberculosis, malaria and syphilis [2,27,30,42,43]. The leaves are used to treat pain, inflammation, diabetes and sleep disorder while seeds are used for the treatment of cough and diarrhea [39]. Some of these traditional claims have been scientifically validated with extracts, fractions and compounds showing promising pharmacological activity. For instance, the ethyl acetate and ethanol flower extract of *A. coriaria* showed moderate antibacterial activity against *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli* and *Staphylococcus aureus* with diameters of inhibition ranging from 7.00 ± 0.00 to 22.00 ± 1.73 mm [36]. The minimum inhibitory concentration of the acetone and methanol stem bark extracts of *A. coriaria* against M. *tuberculosis was* 937.0 ± 442.0 μg/mL and 2500.0 ± 0.0 μg/mL respectively [31]. Other bioactivities of this plant that have been proved are anticancer [28], antioxidant [36] and anti-inflammatory [40].

Phytochemical analysis of *A. coriaria* has shown the presence of various classes of bioactive compounds such as terpenoids, alkaloids, flavonoids, tannins, and saponins [36]. Two oleanane-type saponins (coriariosides A and B) along with gummiferaoside C (saponin), betulinic acid, lupeol, and catechin have been isolated from different extracts of *A. coriaria* [10,34]. These compounds have demonstrated several pharmacological activities such as analgesic, anti-inflammatory, anti--tumor, anti-diabetic, and anti-microbial activities [10,23,35]. Whereas there is sufficient efficacy data on *A. coriaria*, scientific evidence on its safety is limited, incomplete and often contradictory. For example, the stem bark contains alkaloids that have been reported to cause respiratory distress and even death in some animals [35]. Additionally, the saponins in the seeds of *A. coriaria* have been reported to be toxic and caused vomiting and diarrhoea in humans and animals [37]. In Malawi, incidences of animal death after consumption of leaves of this plant have been reported [37]. It was also reported that *A. coriaria* and other *Albizia* species are among the fish–poisoning plants with potent neurotoxicity [37]. The ethanol and DMSO stem bark extracts of *A. coriaria* harvested in central Uganda had high cytotoxicity against the human glioblastoma cell line (U87. CD4. CXCR4) with CC_{50} of 6.4 and < 4 μg/mL respectively [4]. However, the stem bark extracts (hexane, chloroform, acetone and methanol) of *A. coriaria* harvested from western Kenya were non-toxic on Vero E6 Cells with all their cytotoxic concentrations greater than 500 μg/mL [31]. Also, the methanol and water stem bark extracts of *A. coriaria* were non-toxic against human embryonic lung fibroblast (HEL) cells [21]. Since *in vitro* studies may not sufficiently predict toxicity in humans, this study sought to evaluate the acute and sub chronic toxicity effects of the ethanolic stem bark of *A. coriaria* in Wistar albino rats to obtain a better understanding of its toxicity.

2. Materials and methods

2.1. Sample collection, authentication and processing

A. coriaria stem barks were collected in January 2020 from their natural habitats in Katakwi district Eastern Uganda (N02° 05'18''E034°03'49''). The stem barks were harvested from mature and healthy plants following good harvesting practices with the help of a plant taxonomist [45]. Voucher specimens of *A. coriaria* (OSB/003/2020) were prepared and deposited at the Makerere University Herbarium, at the Department of Plant Sciences, Microbiology & Biotechnology for reference purposes. The stem barks were harvested, packed in zip lock bags and transported to the Busitema University Natural Products Research and Innovation Centre laboratory for processing. The stem barks were cut into small pieces and air-dried for 28 days at room temperature (25.0 ± 2.0 °C) in the shade. Using an electric grinder (NutriBullet® 600 Series), the dried samples were pulverized and stored in clean labeled paper envelopes at room temperature until extraction.

2.2. Extraction

Analytical grade ethanol was purchased from Sigma - Aldrich (Germany) and diluted with distilled water to make a 70 % ethanol which was used for extraction. 300 g of dried *A. coriaria* stem bark samples were macerated for 72 h with intermittent shaking in 1000 mL of ethanol (70 %) to obtain crude ethanolic extracts. The mixture was double filtered using cotton wool first and lastly with Whatman No. 1 filter paper to obtain a solution. Using a rotary evaporator (DLAB: RE100-Pro) at 40 °C and reduced pressure (70 −100 mBar), the solution was concentrated to a minimal volume. The concentrated crude extracts were dried to constant weight in a desiccator over anhydrous copper (II) sulfate, at room temperature. The extracts were kept in clean, labeled bottles in a refrigerator at 4 °C for further use.

2.3. Experimental animals and handling

Healthy inbred Wistar albino rats (100–200 g) of 8 to 10 weeks old were purchased from the Makerere University College of Veterinary Medicine, Animal Resources, and Biosecurity (CoVAB) Animal facility. The rats were kept in standard wooden cages (25cmx40cmx50cm; Height*Width*Length) with wood chips for bedding and nipple watering systems. A maximum of 3 rats of the same sex were kept in each cage. The animals were fed on standard pellets (NUVITA Rat Pellets) supplied by an authorized feeds supplier (NUVITA Poultry and Animal feeds Limited) and allowed free access to water *ad libitum.* The animals were kept in clean cages with bedding changed three times a week. The animal facility was maintained at a temperature of 23 to 27 °C with a 12-hour cycle of light and darkness. Prior to the start of the investigation, the animals were acclimatized for two weeks and handled in accordance with standard laboratory animal care protocols [22]. All animals at the end of the experiment were sacrificed under general anesthesia using an overdose of pentobarbitone sodium solution. All animals which died before the end of the experiment and those sacrificed were pooled in a bio-hazard container and stored at −20 ℃ (for approximately 24 h) before being incinerated [44].

2.4. Preparation of extract solutions

The concentrated ethanolic stem bark extract (1000 mg) of *A. coriaria* was dissolved in 10 mL of 1 % tween 80 in distilled water at room temperature (25.0 ± 2.0 °C) to make an extract suspension of 100 mg/mL. The suspension was vortexed (Analog Vortex mixer OHAUS) for 20 min and after digitally shaken (VWR – digital shaker) for two hours to allow maximum dissolution. The prepared extract solution was then poured into a clean labeled flask for administration to the animals. All the extract solutions were freshly prepared every day for use.

2.5. Acute toxicity assessment

Female rats were used for acute toxicity oral testing because they are more susceptible to xenobiotics than male rats and provide more accurate and reliable results (OECD, 2001). Lorke's method (1983) was used to determine the median lethal dose with some minor adjustments.

Instead of the 1600, 2900, and 5000 mg/kg doses proposed in Lorke's method, 1600, 2500, 5000 mg/kg were used in the second phase. Wistar albino female rats (n = 9) were randomly divided into three treatment groups in the first phase (3 rats in each group). The rats were fasted overnight and then orally administered with determined doses of the extracts (100, 500, and 1000 mg/kg) using a feeding cannula depending on their body weight. The injectable volume administered was calculated using equation:

$$Volume\ \ of\ \ extract\ \ administered(ml) = \frac{Body\ \ weight\ \ (g) \times Dose(\frac{mg}{kg})}{concentration\ \ \left(\frac{mg}{ml}\right) \times 1000}$$

The animals were observed for manifestation of toxic symptoms which were recorded at time intervals of 30 min, 1, 2, 3, 4, 6, 9, 12, and 24 h after administration. The animals that survived were observed for an additional 14 days and daily observations made.

In the phase II, three treatment groups each having one rat were administered with extracts at doses of 1600, 2500, 5000 mg/kg. Again, observations of toxic symptoms including death were recorded at the time 30 min, 1, 2, 3, 4, 6, 9, 12, and 24 h after administration interval. The highest dose that produced no mortality (D_0) and the lowest dose that caused mortality (D_{100}) was determined. A repeat was done on the lowest dose that caused mortality using two rats to ensure that it caused mortality of either both or one rat. The median Lethal dose (LD_{50}) was then calculated using the equation.

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

2.6. Sub-acute toxicity evaluation of the ethanolic stem bark extract of A. coriaria

The OECD 407 guidelines for oral repeated toxicity testing of compounds in rodents were used to assess the sub-acute toxicity profile of the ethanolic stem bark extract of *A. coriaria* [32]. Wistar Albino rats (24) of both sexes were randomized based on their body weights to four groups (100, 200, 500 mg/kg and 1 % tween 80); 6 rats per group). The doses for subacute toxicity evaluation were calculated based on the predetermined median lethal dose and they correspond roughly to $^1/_{20}$, $^1/_{10}$, and $^1/_4$ of the median lethal dose (2000 mg/kg). The animals were orally administered the extracts using a feeding cannula daily for a period of 28 days. Weights of the rats were measured and recorded on day 0, 7, 14, 21 and 28. On the 29th day, the rats were sacrificed under general anesthesia using an overdose of pentobarbitone sodium solution.

2.7. Biochemical analysis

Blood (2 mL) was collected by cardiac puncture from each rat into non-heparinized vacutainers using syringes. Blood was centrifuged at 3000 rpm for 5 min to obtain serum which was assayed using an automated chemistry analyzer (HumaStar 200) for levels of different biochemical parameters. Test kits for measurement of different parameters were purchased from Sigma-Aldrich and used according to the manufacturer's instructions. The parameters measured included blood proteins, bilirubin, triglycerides, total cholesterol, low-density lipoproteins (LDL), high-density lipoproteins (HDL), electrolytes (Na^+, K^+, Ca^{2+}, Cl^-, and H^+) and serum diagnostic enzymes (ALP, AST and ALT).

2.8. Hematological analysis

Blood (2 mL) was collected by cardiac puncture from each rat into heparinized vacutainers using syringes and analyzed using an analyzer (Sysmex 1000i) for hematological counts of different parameters. These included white blood cells (WBC), basophils (BASO), lymphocytes (LYMP), neutrophils (NEUT), monocytes (MONO), eosinophil (EO), red blood cells (RBC), hematocrit (HCT), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelet count (PCH).

2.9. Histopathological evaluation

The rats were also dissected to obtain vital organs (liver, kidney, heart, and spleen) for histopathological analyses. The organs were evaluated for any visible histomorphological changes, and the weight of each organ was determined using a digital weighing scale. The isolated organs were preserved for 72 h in buffered, 10 % (v/v) formalin-labeled bottles. The tissues were cut and put into cassettes after fixation in order to be processed by an automated tissue processor (Leica 40). They were initially dehydrated by being placed in tissue cassettes containing varying amounts of alcohol (70 %, 80 %, 90 %, and 96 %, v/v), which they were then withdrawn from and submerged in xylene solution baths to remove the alcohol. Then, melted wax was impregnated into them, and were left to dry. The tissues were then sectioned by use of Rotary microtome (at 5 μm thickness) and then stained with hematoxylin and eosin (H & E). Slides were prepared and then examined using a research light microscope connected to a computerized camera (Lieca LB_2 – image analyzer). Photomicrographs were captured and then examined for histopathological changes by two independent pathologists who were not aware of the biochemical and hematological data.

2.10. Data analysis

The means and standard error of the means of quantitative data were computed using Microsoft Excel 2013's features. The results were presented as means ± standard error of mean. Statistically significant differences were determined using one-way analysis of variance (ANOVA) and/or Student's *t*-test followed by Dunnett's post hoc test using Graph Pad Prism version 5.01 (Graph Pad software, San Diego, California, U.S.A). Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Acute toxicity study

At doses less than 1000 mg/kg, the ethanolic stem bark extract caused mild to moderate acute toxicity which manifested as piloerection (30 min), hyperventilation (45 min) and lethargy (90 min) but with no mortalities recorded after 24 h and after 10 days. However, at higher doses (greater than 1000 mg/kg), the animals were moribund at 1600 mg/kg (D_0) but later recovered whereas mortality was recorded at 2500 mg/kg (D_{100}) and 5000 mg/kg doses. The additional signs of toxicity observed were loss of righting reflex (150 min), coma (240 min) and death (300 min). Therefore, the calculated median lethal dose (LD_{50}) = $\sqrt{(1600 \times 2500)}$ =2000 mg/kg. Fig. 1.

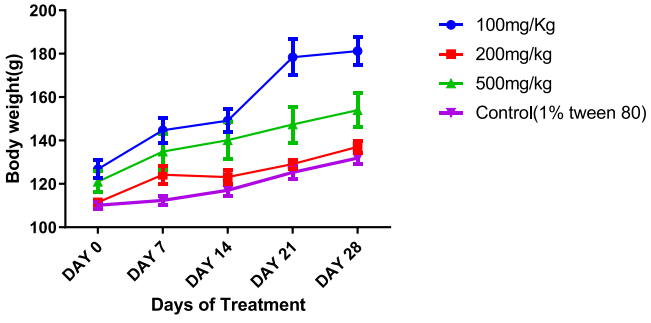

Fig. 1. The changes in body weight with time (days) of rats administered with 1 % tween 80 and different doses of *A. coriaria* (n = 6 animals per group).

3.2. Effect of the ethanolic stem bark extract of A. coriaria on body weight

There was progressive increase in the body weight of the experimental animals throughout the 28-days ($P < 0.05$), with no significant differences amongst the treatment groups (Table 1).

3.3. Effect of the ethanolic stem bark extract of A. coriaria on biochemical parameters

Administration of the extract for a period 28 days significantly increased alkaline phosphatase (ALP) and aspartate aminotransferase (AST) at a dose of 500 mg/kg and significantly decreased low density lipoproteins (LDL) at 200 and 500 mg/kg ($p < 0.05$). All the administered doses significantly decreased the triglycerides ($p < 0.05$). The rest of the biochemical parameters were not significantly changed by extract administration ($p > 0.05$). The details are presented in Table 2.

3.4. Effect of the ethanolic stem bark extract of A. coriaria on electrolytes

Administration of the ethanolic stem bark extract of *A. coriaria* at all doses for 28 days did not cause significant changes in the electrolytes of the animals (Table 3).

3.5. Effect of the ethanolic stem bark extract of A. coriaria on haematological parameters

The ethanolic stem bark extract of *A. coriaria* did not significantly alter most of the haematological indices as shown in Table 4 ($P > 0.05$). However, it significantly increased the red blood cell count at all doses and hemoglobin content at 500 mg/kg ($p < 0.05$).

3.6. Effect of the ethanolic stem extract A. coriaria on histology of the major organs

Histopathological changes were analyzed at the different extract concentrations (100, 200, and 500 mg/kg) across five selected organs (liver, heart, spleen, kidney, jejunum and lungs). Our results showed that, after 28 days of daily administration of *A. coriaria* ethanolic stem bark extract, there were no significant lesions in the histological architecture of the selected organs observed at 100 mg/kg in all organs and in the heart, kidney and jejunum at 200 mg/kg (Fig. 2). At 500 mg/kg extract group, multifocal random parenchymal necrosis and scattered periportal mononuclear inflammatory cells infiltration were observed in the liver tissue, with multifocal lymphoid accumulation in the peribronchiolar and perivascular tissue with moderate type II pneumocytes proliferation in the lungs and kidneys showed evidence of focal interstitial nephritis. No lesions were observed in the heart, spleen, and jejunum even at increased extract doses.

4. Discussion

Acute toxicity includes the adverse effects experienced in animals within 24 h following administration of a single high dose of xenobiotic.

Table 1
Mean body weights of the rats at different days of the experiment.

Dose (mg/kg)	Mean body weights (g) at different days			
	100	200	500	1 % tween 80
DAY 0	126.8 ± 4.1	111.4 ± 1.5	121.0 ± 4.9	110.1 ± 1.6
DAY 7	144.7 ± 5.8	124.2 ± 4.3	134.9 ± 8.2	112.4 ± 2.3
DAY 14	149.2 ± 5.2	123.1 ± 3.2	140.2 ± 8.5	117.1 ± 2.6
DAY 21	178.4 ± 8.4	129.2 ± 2.1	147.4 ± 8.3	125.3 ± 3.1
DAY 28	181.2 ± 6.4	137.1 ± 2.6	154.0 ± 7.9	131.9 ± 2.5

Data were expressed as mean ± SEM, n = 6

Table 2
Mean levels of biochemical parameters after 28 days at different doses of the extract.

Biochemical parameters	Mean Levels of Biochemical parameters at different dose				
	1 % tween 80	100 mg/kg	200 mg/kg	500 mg/kg	p-value
Alb (g/dl)	3.597 ± 0.122	3.26 ± 0.05	3.343 ± 0.083	3.302 ± 0.058	0.3103
Total Protein (g/dl)	7.443 ± 0.136	7.322 ± 0.13	7.56 ± 0.119	7.442 ± 0.137	0.6549
ALP DEA(U/L	255.7 ± 43.84	437 ± 70.09	344.3 ± 67.06	701.3 ± 86.6*	0.0012
AST/GOT(U/L)	119.7 ± 12.93	142.5 ± 6.307	157.7 ± 13.77	174.5 ± 9.969*	0.016
ALT/GPT(U/L)	91.67 ± 6.76	119 ± 4.531	106.7 ± 12.14	104.5 ± 8.35	0.1854
Bilirubin total (mg/dl)	0.2583 ± 0.039	0.3233 ± 0.018	0.3633 ± 0.0241	0.2717 ± 0.042	0.1166
Bilirubin direct (mg/dl)	0.06333 ± 0.013	0.09 ± 0.009	0.1017 ± 0.007	0.13 ± 0.036	0.1542
Creatinine (mg/dl	0.6167 ± 0.008	0.7017 ± 0.029	0.6567 ± 0.23	0.74 ± 0.053	0.0726
Urea(mg/dl)	42.85 ± 1.56	40.28 ± 0.846	37.17 ± 1.641	38.17 ± 1.958	0.0791
Cholesterol (mg/dl)	46.17 ± 3.93	50.83 ± 1.956	46.33 ± 3.353	52.5 ± 3.481	0.43
LDL (mg/dl)	7.25 ± 0.856	4.86 ± 2.71	3.983 ± 0.821*	3.477 ± 0.632*	0.0295
Triglyceride (mg/dl)	93.17 ± 7.332	84 ± 14.17*	83 ± 12.02*	59.5 ± 5.638*	0.0486
HDL (mg/dl)	38.88 ± 4.804	49.42 ± 12.28	44.03 ± 9.012	53.17 ± 4.944	0.6489

Data were expressed as mean ± SEM, n = 6, * significant at $p < 0.05$, Alkaline phosphatase (ALP), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Low Density Lipoprotein (LDL) and High-Density Lipoprotein (HDL)

Table 3
Mean levels of electrolytes after 28 days at different doses of the *A. coriaria* ethanolic stem bark extract.

Dose (mg/kg)	Mean concentration of electrolytes					
	K^+ (mmol/L)	Na^+ (mmol/L)	Cl^- (mmol/L	ICa^{2+} (mmol/L)	TCa^{2+} (mmol/L)	pH
Control	4.2 ± 0.02	146.3 ± 0.62	107.6 ± 0.23	0.555 ± 0.08	1.083 ± 0.16	7.28 ± 0.17
100	4.3 ± 0.22	153.4 ± 0.33	103.2 ± 0.54	0.282 ± 0.10	0.55 ± 0.19	7.028 ± 0.02
200	4.5 ± 0.21	148 ± 0.42	105.2 ± 0.3	0.438 ± 0.04	0.852 ± 0.08	7.07 ± 0.02
500	4.3 ± 0.03	147.6 ± 0.19	103.5 ± 0.5	0.407 ± 0.05	0.793 ± 0.10	7.77 ± 0.02

Key: Data expressed as mean ± SEM, n = 6, Intracellular calcium (ICa^{2+}), Total calcium (TCa^{2+}), Chloride ion (Cl^-), Sodium ion (Na^+), Potassium (K^+)

On the other hand, subacute toxicity includes adverse effects experienced in animals following repeated administration of xenobiotics in small doses for a period of 30 days [32]. The Loomis & Hayes, [24] criteria uses the median lethal dose (LD_{50}) to classify chemicals into different toxicity categories as follows; highly toxic ($LD_{50} \leq 50$ mg/kg), toxic (LD_{50} = 50 - 300 mg/kg), moderately toxic (LD_{50} = 301 - 2000 mg/kg), slightly/weakly toxic (LD_{50} = 2001 - 5000 mg/kg) and non-toxic ($LD_{50} > 5000$ mg/kg) [15,24]. The median lethal dose of 2000 mg/kg indicates the ethanolic extract was moderately toxic to the animals. The displayed toxicity signs such as piloerection, hyperventilation, lethargy, loss of righting reflex, coma, and ultimately death imply a disturbance in the autonomic and central nervous system [12,19,9]. This suggests that the ethanolic stem bark extract probably contains phytochemicals which interfere with the normal functioning of the

Table 4
Mean levels of haematological parameters after 28 days at different doses of the *A. coriaria* ethanolic stem bark extract.

Hematological Parameters	Mean level of Haematological parameters at different doses				
	Control	100 mg/kg	200 mg/kg	500 mg/kg	p-values
WBC (10*3/UL)	12.3 ± 1.372	12.19 ± 1.626	12.12 ± 1.156	11.08 ± 0.823	0.575
NEUT (10*3/uL)	1.575 ± 0.283	1.803 ± 0.377	1.733 ± 0.512	1.422 ± 0.309	0.895
LYMPH (10*3/uL)	9.728 ± 1.172	9.263 ± 1.23	9.702 ± 0.616	8.378 ± 0.969	0.3779
MONO (10*3/uL)	0.555 ± 0.109	0.6833 ± 0.202	0.655 ± 0.102	0.9233 ± 0.203	0.4409
EO (10*3/uL)	0.185 ± 0.06	0.2617 ± 0.045	0.1817 ± 0.029	0.2033 ± 0.031	0.8189
BASO (10*3/uL)	0.2583 ± 0.06	0.175 ± 0.027	0.49 ± 0.325	0.15 ± 0.034	0.4744
NEUT (%)	12.48 ± 1.43	14.63 ± 2.036	11.72 ± 2.038	13.97 ± 3.754	0.8305
LYMPH (%)	79.03 ± 2.807	76.28 ± 1.495	80.48 ± 3.758	74.6 ± 4.375	0.593
MONO (%)	4.867 ± 1.03	5.25 ± 1.3	5.383 ± 1.021	8.15 ± 1.301	0.2081
EO (%)	1.533 ± 0.426	2.367 ± 0.597	1.433 ± 0.1892	1.883 ± 0.344	0.4011
BASO (%)	2.083 ± 0.411	1.467 ± 0.246	3.383 ± 1.971	1.4 ± 0.375	0.5109
IG (10*3/uL)	0.04 ± 0.008	0.0283 ± 0.012	0.0117 ± 0.004	0.015 ± 0.005	0.0887
IG (%)	0.3333 ± 0.078	0.2333 ± 0.095	0.085 ± 0.031	0.15 ± 0.056	0.0954
RBC (10*6/Ul)	7.01 ± 0.312	8.247 ± 0.284*	7.428 ± 0.214*	8.825 ± 0.126*	0.0002
HGB(g/dL)	13.47 ± 0.223	13.17 ± 0.392	14.38 ± 0.324	15.32 ± 0.162*	<0.0001
HCT (%)	52.83 ± 1.322	47.4 ± 0.716	55.4 ± 0.868	48.52 ± 1.06	<0.0001
MCV(FL)	69.92 ± 3.45	64.3 ± 1.956	63.92 ± 1.484	62.83 ± 1.22	0.1346
MCH (pg)	19.38 ± 0.866	17.5 ± 0.432	17.95 ± 0.355	17.38 ± 0.265	0.0558
MCHC(g/dL)	27.78 ± 0.218	27.23 ± 0.297	27.58 ± 0.296	27.65 ± 0.219	0.5025
RDW-SD (fL)	35.23 ± 4.16	38.97 ± 3.928	31.22 ± 1.341	31.65 ± 2.22	0.2926
RDW-CV (%)	14.68 ± 0.69	19.38 ± 1.195	15.47 ± 0.572	17.28 ± 0.531	0.0022
PLT (10*3/uL)	774.5 ± 29.42	736 ± 75.12	727.5 ± 25.37	808.2 ± 33.84	0.5819
PDW (fL)	8.567 ± 0.149	8.417 ± 0.631	8.983 ± 0.515	8.667 ± 0.297	0.8272
MPV (fL)	8.15 ± 0.108	7.8 ± 0.308	8.4 ± 0.279	7.867 ± 0.163	0.2609
P-LCR (%)	10.98 ± 0.759	9.417 ± 2.378	11.87 ± 1.945	9.667 ± 1.09	0.7049
PCT (%)	0.6333 ± 0.02	0.5667 ± 0.045	0.6083 ± 0.014	0.6383 ± 0.024	0.3573

Data were expressed as mean ± SEM, (n = 6), *significant at $p < 0.05$

different neurotransmitters and / or their receptors [20,37]. In a different study, the administration of an aqueous stem bark extract of *A. coriaria* at a dose of 2000 mg/kg caused death of one rat out of five [33]. Like in this study, it was also concluded that the stem bark extract of *A. coriaria* was toxic at a dose of 2000 mg/kg with excessive urination and defecation, hard breathing and lethargy [33]. The acute toxicity observed is of great significance because many herbal products that contain this plant are not standardized in terms of dosage [17]. Hence there is likelihood of poisoning occurring when such herbal products are consumed in relatively large single oral doses within 24 h. This confirms reports of toxic side effects experienced by humans such as vomiting, dizziness and body weakness upon consumption of *A. coriaria* containing herbal medicines in amounts that exceed those prescribed by experienced traditional medicine practitioners in Uganda [3]. These findings are also in agreement with the highly cytotoxic effects of *A. coriaria* extracts against the U87. CD4. CXCR4 cell lines [4]. Although a plant may be relatively safe during acute toxicity testing, this does not guarantee its safety during sub-acute toxicity studies. This is because over time the xenobiotic may be metabolized to toxic metabolites that cause damage to tissues in vital organs. But also, the xenobiotic or its metabolites may bioaccumulate with in tissues and cause toxicity [18,26]. Therefore, acute toxicity results are not evident enough to confirm the safety of xenobiotics in animals [1]. Other toxicity tests such as subacute, subchronic and chronic toxicity tests are used to conclusively assess the toxicity of substances in animal models.

A. coriaria stem bark is used in the preparation of herbal remedies for management of chronic ailments such as tuberculosis, cancers and diabetes mellitus which require the consumption of these products for a long period of time ranging from weeks to months [35]. Therefore, assessment of the changes in the levels of haematological, biochemical and histopathological indices after repeated administration of this plant extract for a given period of time provides useful insight into the potential long-term toxicity associated with this plant extract. A dose dependent toxicity pattern was observed during subacute toxicity evaluation with no significant toxicities occurring at the lowest dose of 100 mg/kg but instead moderate to severe toxicity occurring at the highest dose of 500 mg/kg. Repeated administration of the ethanolic stem bark extract of *A. coriaria* for 28 days did not result into significant alterations of the various hematological parameters except for the red blood cells and hemoglobin which were significantly increased at doses of 200 and 500 mg/kg ($P < 0.05$). The increase in red blood cell and hemoglobin could be due to presence of phytochemicals in the extract that activate the production of erythropoietin which stimulates the stem cells of the haemopoietic tissue to synthesize more red blood cells [5]. Additionally, it could be due to the presence of phytochemicals which inhibit hemolysis of red blood cells. Hemolysis of red blood cells elevates the serum levels of bilirubin indicating harmful effects on the hematological system [16]. The nonsignificant change in the levels of bilirubin therefore further shows that the extract did not have a deleterious impact on the erythropoietic system. Flavonoids which have been reported to dose dependently inhibit hemolysis of RBCs due to their antioxidant effect [6].

Significant increase in the levels of alkaline phosphatase (ALP) and aspartate aminotransferase (AST) ($P < 0.05$) after 28 days of administration of the ethanolic extract of *A. coriaria* at 500 mg/kg implied toxicity to vital organs where these enzymes reside. Their increase in serum indicates damage of the membranes (loss of cellular integrity) of the organs that leads to leakage of these enzymes into blood. Since AST and ALP are nonspecific enzymes present in various tissues, the increase could imply damage to the bile duct, lungs, kidneys and liver [8,25]. The aforementioned observations are consistent with the histological findings on the liver, lung and kidney tissues which showed that at 500 mg/kg, the extract caused multifocal random parenchymal necrosis and scattered periportal mononuclear inflammatory cells infiltration in the liver, nephritis in the kidney and multifocal lymphoid accumulation in the peribronchiolar and perivascular lung tissue. Treatment of animals with crude ethanolic extracts of *A.coriaria* did not cause significant effect on the histology of the heart, spleen and the jejunum. The liver and kidney being major organs involved in drug metabolism and elimination are prone to toxic effect of xenobiotics [7,8] The ethanolic extracts of *A. coriaria* have been reported to contain abundant tannins and terpenoids. Yet studies have reported that some tannins and terpenoids are potentially nephrotoxic and can cause interstitial nephritis, renal tubular necrosis, acute kidney injury, oxalate nephropathy and glomerulonephritis [11,13]. Hence, it is highly probable that the observed nephrotoxicity is due to terpenoids and tannins present in this plant (). The pneumotoxicity observed could be attributed to hydroquinone that has been reported to be present in the stem barks of *A. coriaria* [35]. Hydroquinone produces reactive oxygen species which

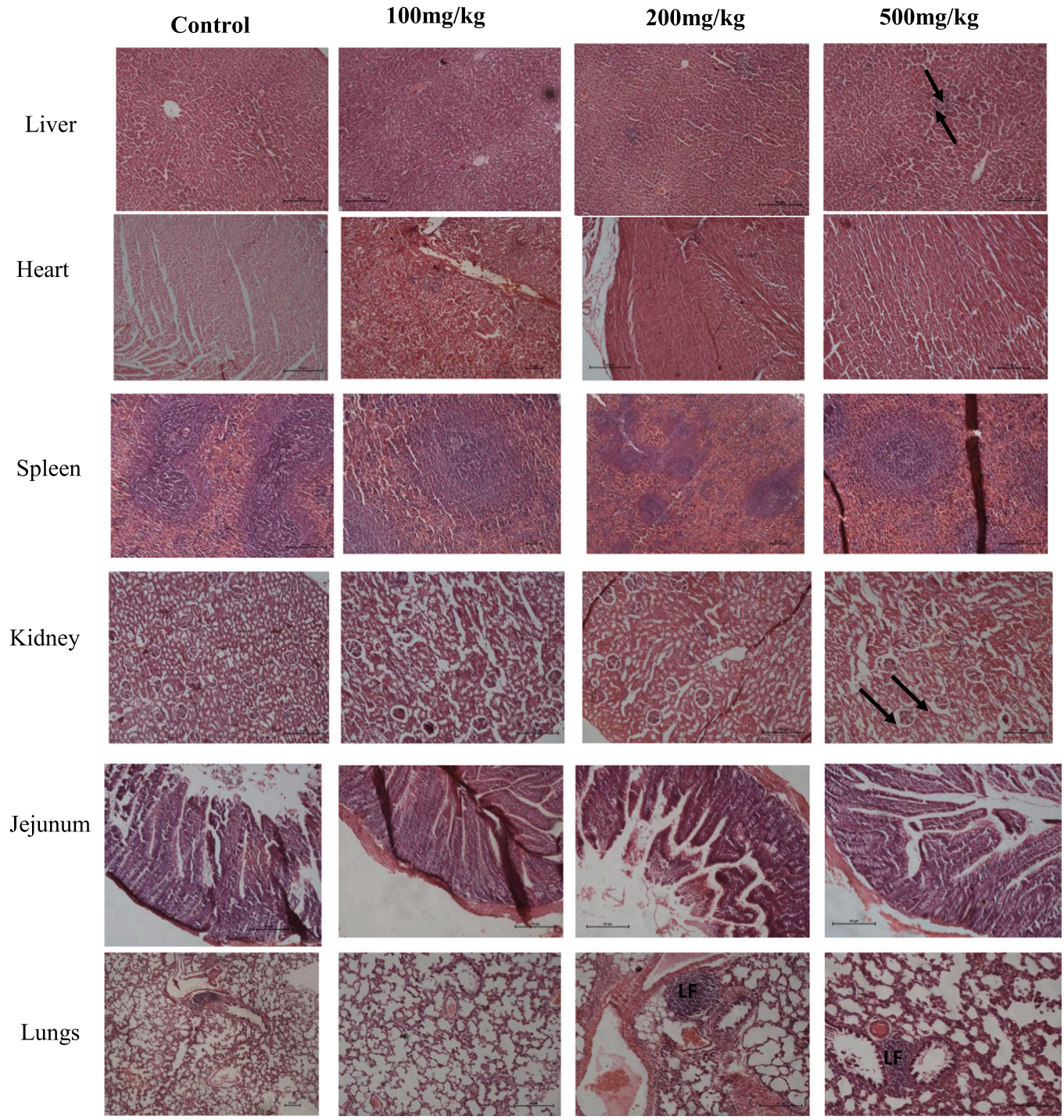

Fig. 2. Histopathology of selected organs showing a dose dependent toxicity, characterized by multifocal random parenchymal necrosis and scattered periportal mononuclear inflammatory cells infiltration in the liver at 500 mg/kg (arrow heads in liver slides), nephritis at 500 mg/kg (arrow heads in kidney slides), and Multifocal lymphoid accumulation in the peribronchiolar and perivascular lung tissue at 500 mg/kg (LF).

cause oxidative stress that leads to lung damage. It has also been reported to increase the production of inflammatory cytokines in lung cells, contributing to lung inflammation and damage [38]. However, it is also possible that other unknown secondary metabolites present in the plant extracts are responsible for the observed organ toxicity.

5. Conclusion

The ethanolic stem bark of *Albizia coriaria* was moderately toxic to the Wistar albino rats when administered in a single high oral dose within 24 h. Repeated administration of lower doses for a period of 28 days caused a dose dependent toxicity to the kidney, liver and lung tissues significant at a dose 500 mg/kg in inbred Wistar Albino rats. Herbal medicines containing *A. coriaria* extracts should be consumed cautiously due to likelihood of toxicity particularly at higher doses greater 500 mg/kg. More studies to investigate the toxicity of *Albzia coriaria* in outbred wistar rats, a single dose exposure of *Albzia coriaria* at 2000 mg/kg (LD_{50}) following the OECD acute oral toxicity tests (Guideline 423) and the phytochemicals responsible for the toxicity of this plant are needed. Herbal medicine regulatory authorities need to be

more vigilant when licensing and monitoring *A. coriaria* containing products.

Ethics approval

The protocol for this study was reviewed and approved by Cure Children's Hospital Uganda -Research and Ethics Committee (CCHU-REC/11/020) and registered by the Uganda National Council of Science and Technology (HS1222ES).

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CRediT authorship contribution statement

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Samuel Baker Obakiro reports financial support was provided by Government of Uganda through the Presidential Scientific Initiative on Epidemics.

Data availability

Data will be made available on request.

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