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Evaluation of acute toxicity and sub-acute toxicity of the methanolic extract of *Aloe rabaiensis* Rendle in BALB/c mice

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This study was undertaken to evaluate preclinical acute and sub-acute toxicity of *Aloe rabaiensis* leaf methanolic extract (ARLME) on BALB/c mice following OECD guidelines 423 and 407, respectively. In an acute oral toxicity test, ARLME was administered to the mice by oral gavage at a single dose of 1000, 2000, 3000, 4000 and 5000 mg/Kg body weight. The mice were observed for toxic signs for 14 days. In sub-acute oral toxicity test, ARLME was administered to the mice by oral gavage at 500, 800 and 1000 mg/Kg body weight daily up to 28th day. At the end of the test, haematological and biochemical analyses of the collected blood sample were carried out as well as gross and microscopic pathology. The control group (F) received a single oral dose of 0.5 mL of 1% DMSO in normal saline. In acute oral toxicity, no treatment-related death or toxic signs at the dosage below 4000 mg/Kg was observed. Nevertheless, at the dosage of 4000 and 5000 mg/Kg, drowsiness and sedation were observed. It was, therefore, revealed that ARLME could be tolerated up to the dose of 3000 mg/Kg body weight and may be classified as category 5. Sub-acute toxicity study at dosage 500 and 800 mg/Kg displayed no adverse changes in the haematological parameter, body weights and histopathological examination. However, at a dosage of 1000 mg/Kg, the serum biochemical aspartate transaminase and alanine transaminase increased, and in histopathological examination of liver and kidney, there was a proliferation of bile duct and leucocytes infiltration respectively. Thus, observations from this study indicate that oral administration of ARLME had no adverse toxic effects in BALB/c mice at the dosage below 1000 mg/Kg, hence supports the use of *Aloe rabaiensis* in drug formulations.

Key words: Acute toxicity, sub-acute toxicity, *Aloe rabaiensis*, BALB/c mice.

INTRODUCTION

Medicinal plants are potential resources of health care services to the majority of people around the world. The African, Ayurvedic, Naturopathic, Unani, Chinese and Native American are the most well-known systems of

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herbal therapy (Chevallier, 2016).

In Africa, people use medicinal plants as part of the culture and civilisation which were recognised before the introduction of conventional medicine (Kayombo et al*.,* 2013). There is a strong belief to people on medicinal plants that they do not induce toxic side effects. However, scientific reports have proven to the contrary, when herbal medicines are used above a certain tolerance level (Patel et al., 2012). Different scholars have also reported on some medicinal plants to exhibit higher toxicity levels harmful to human and animal life (Botha and Penrith, 2008; Wagstaff, 2008; Frohne and Pfänder, 2005).

On the other hand, the evaluations of the toxic effects of most of the medicinal plants used by the majority in rural areas are limited (Ekor, 2014). Despite insufficient data on the toxicity of medicinal plants used in rural areas, the identification of toxicity levels is essential for developing pharmaceutical products of plant origin (Singh, 2015; Gilani, 2005). Therefore, there is a high necessity for evaluating the toxic components and toxicity levels of medicinal plants that may be used for drug formulations

The *Aloe rabaiensis* Rendle (family *Asphodelaceae*) is a drought tolerant succulent, an erect evergreen shrub during wet seasons and deep maroon-aubergine during dry seasons. The leaves grow up to 30-45 cm long and 4-5 cm wide with a softer toothed margin. The stem grows up to 2 m long. The *A. rabaiensis* is geographically distributed in Northern Tanzania, Kenya and Southern Somalia (Carter, 1994). The plant has various pharmacological actions including treating diseases of bacterial infections reported around Kenya and Tanzania.

In Kenya, the leaf decoction of *A. rabaiensis* is traditionally claimed by Maasai to heal enlarged spleen (Bjorå et al., 2015). For instance, a survey conducted on the ethnomedical practice by inhabitants around Lake Jipe in Tanzania revealed that *A. rabaiensis* sap is used to treat diarrhoea related diseases in human and livestock. The excess intake of the sap of *Aloe* spp. results in vomiting and diarrhoea due to anthraquinone derivatives with laxatives action (Kaur et al., 2013; Kwon et al., 2006). The anthraquinones are the largest group of plant quinones, responsible for loosening stools and increasing bowel movement (Maan et al., 2018).

A. rabaiensis and other species in genus *Aloe* produce concentrated exudates known as bitters with active ingredient Aloin. The bitter is highly demanded in international trade (Chen et al., 2012), and exported from Tanzania and other *Aloe* producing countries to Europe for the manufacture of different *Aloe* products of pharmaceuticals such as lotion, toothpaste, drugs, soap and dietary juices (Fanali et al., 2010). Due to cytotoxicity, mutagenicity and carcinogenicity of anthraquinones reported from *Aloe vera* (Guo and Mei, 2016), the need to establish the tolerance level of *A. rabaiensis* therefore, becomes crucial*.* This study reports the toxicity profile of *A. rabaiensis* leaf methanolic extract (ARLME) and what the knowledge would contribute towards investigations in drug formulations.

MATERIALS AND METHODS

Plant materials collection

A. rabaiensis leaves were collected from around Lake Jipe in Northern Tanzania (3.34882 S and 37.44202 E at altitude 718 m). A Botanist from Tanzania Pesticides Research Institute (TPRI) identified the plant and a voucher specimen number ARH 403 was found in the herbarium at TPRI.

Plant material preparation

Dust and soil on leaves were removed by washing with running tap water and finally with distilled water. The clean, fresh leaves were chopped into small pieces with the use of a sharp knife and dried at room temperature with enough ventilation for four weeks. After drying, leaves were pulverised into powder by mill machine (Swinging Traditional Chinese Machine Pulverizer Diaxiang electronic equipment (DXF- 20D). The powder was later weighed and stored in food bags until use.

Methanolic crude extraction process

The pulverised *A. rabaiensis* leaf (250 g) was soaked in methanol (1000 mL) (RFCL Limited, Haryana-India) while shaking after every 5 h interval for 48 h. Then, the extract was filtered using cotton wool and Whatmans No 1 filter paper. The rotary evaporator (Heidolph, Germany) at 40°C and vacuum pressure of 120 psi concentrated the filtrate. Further evaporation of the extract was made by placing in a water bath at 40°C for 48 h. Following this, the weighed dry extract was then kept in an air-tight bottle and stored at 4°C until use.

Experimental animals

The female adult albino mice aged eight weeks and weighing 35 to 48 g purchased from TPRI, Plant Protection Division (PPD) in the Agriculture Extension Department, Arusha, Tanzania were used in the study. Mice were allowed to acclimatise for seven days in the animal house at a controlled temperature of 25 ± 2°C and relative humidity 60 ± 10% with the natural lighting of 12 h light/dark cycle. Mice were provided free access to standard broiler mash and water *ad libitum*. The experimental procedures complied with the health research ethics committee approved by Kibong'oto Infectious Diseases Hospital, Nelson Mandela African Institution of Science and Technology, and Centre for Educational Development in Health, Arusha with ethical clearance reference number KNCHREC006

Acute toxicity study

The oral acute toxicity study was conducted according to the procedures of Organization for Economic Co-operation and Development (OECD) guidelines number 423 of 2001 on BALB/c mice with the limit test dose of 5000 mg/Kg body weight. The eighteen healthy mice were divided into six cages with three mice per cage in sawdust litters. The cages were labelled A, B, C, D, E and F. In preparing for dosing, the mice were starved for 4 h with free access to water. Before treatment, the weight of each mouse

was taken, and the doses were calculated per body weight. The treatment groups A, B, C, D and E received a single oral dose of 1000, 2000, 3000, 4000 and 5000 mg/Kg, body weight respectively of ARLME that was dissolved in 1% DMSO in normal saline. The control group (F) received a single oral dose of 0.5 mL of 1% DMSO in normal saline. Mice were starved for 1 h with constant supply of drinking water. The mice were strictly observed individually for the first 4 h after the treatment period and later once for 14 days for mortality or any sign of toxicity including change in body weight, fur and skin, eyes, mucus secretion, food and water intake, urination, colour of the faecal, respiratory effect, convulsion and diarrhoea.

Sub-acute toxicity study

The evaluation of oral sub-acute toxicity study was according to OECD guidelines number 407. The study used a total of twenty female mice (35-48 g) with five mice per cage in sawdust litters. The cages were labelled L, M, N and O. The mice in cage L, M and N were orally administered dosage of 500, 800 and 1000 mg/Kg body weight of ARLME respectively once daily for 28 days. The mice in cage O received 0.5 mL of normal saline by oral gavage. The mice were then observed daily for any sign of toxicity and the body weights recorded every seven days up to the 28^{th} day.

Termination of the experiment

On the 29^{th} day of the experiment, mice were starved for 4 h then subjected to chloroform anaesthesia. Before death, the blood via cardiac puncture was collected and placed in labelled tubes with and without ethylene diamine tetra acetic acid (EDTA) for haematological and biochemical analyses, respectively. The blood in the tubes without EDTA was allowed to coagulate and centrifuged at 4000 r/min for 5 min, and the serum was collected and stored at -20°C for analysis of biochemical parameters. After collecting blood, the mice were dissected for the collection of organs such as liver, kidney, spleen and heart. The organs of extract treated mice were observed for any gross lesions and compared with the control group. Afterwards, the organs were aseptically excised and weighed on an electronic balance (Series FA-N, China). The average and relative organ weights of control and extract treated mice to a body weight of mice on the day of sacrifice were calculated and compared. The organs were then kept in 10% neutral buffered formalin for histopathological examinations.

Calculation of organ to body weight ratio

The calculation of the relative organ weight (ROW) ratio was as follows:

$$ROW = \frac{weight\ of\ an\ organ\ from\ sacrificed\ mice}{weight\ of\ mice\ on\ sacrificed\ day} \times 1oo$$

Haematological parameters

Red blood cell count (RBC), haemoglobin (HGB), haematocrit (HCT), mean corpuscular haemoglobin (MCH), red blood cell distribution width (RDW), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean platelet volume (MPV), white blood cell count (WBC), monocyte (MON), neutrophil (NEU), lymphocyte (LYM) and platelet (PLT) count of the control and extract treated groups were evaluated and compared using an automatic haematology analyzer (Abbott Emerald 22, USA)

Biochemical parameters

The serum collected after centrifugation of blood in plain tubes was subjected to biochemical analysis for parameters including creatinine, aspartate transaminase (AST), alanine transaminase (ALT) and Urea. The analysis was evaluated for control and extract treated groups using clinical chemistry analyser (Erba Mannheim XL 180, Germany).

Histopathological examination

The liver and kidneys preserved in 10% neutral buffered formalin were processed following routine tissue processing, embedded in paraffin wax and sectioned in 5 µm thickness using microtome. The sectioned organs were mounted on the glass slides and stained with haematoxylin and eosin and cover-slipped using standard methodologies. The slides with stained organs were subjected to a light microscope, and the photomicrographs of the tissue samples were taken for documentation.

Statistical analysis

The data collected from haematological and biochemical parameters were analysed using STATISTICA Version 10 and expressed as mean ± SEM (n=5). One-way ANOVA was used to test the means at 95 % CI. Values were considered statistically significant at P = 0.05. The Tukey's test was used to locate significant differences between means.

RESULTS AND DISCUSSION

Acute toxicity of *A. rabaiensis* leaf methanolic extract (ARLME) performed according to OECD guideline 423 on BALC/mice with the limit dose of 5000 mg/Kg body weight. It was observed that the behaviour of treated mice and the control group in the first 4 h and later once for 14 days did not show any severe clinical signs of drug-related changes. However, the patient characteristics of drowsiness occurred at both doses of 4000 and 5000 mg/Kg body weight, while sedation occurred at a dose of 5000 mg/Kg in the first 4 h of observation. There were no death or signs of decrease water and food intake, no mucus secretion, no diarrhoea, no change in skin or eye colour. These observations showed that ARLME could be tolerated up to the dose of 3000 mg/Kg body weight when administered at a single dose and thus classified as Category 5. Category 5 is the lowest toxicity class, generally regarded as safe when dealing with acute toxic effects without considering repeated exposure (Haschek et al., 2013). Therefore, the ARLME is considered safe up to a dose of 3000 mg/Kg body weight and the lethal dose (LD50) was evaluated to be above the limit dose of 5000 mg/Kg body weight used in this study.

Table 1. General macroscopic and behavioural observations of acute toxicity study of control and ARLME treated groups.

Observation	Normal (0 mg/Kg)	1000 (mg/Kg BW)	2000 (mg/Kg BW)	3000 (mg/Kg BW)	4000 (mg/Kg BW)	5000 (mg/Kg BW)
Food/water consumption	Normal	Normal	Normal	Normal	Normal	Normal
Mucus secretion	Not occurred	Not occurred	Not occurred	Not occurred	Not occurred	Not occurred
Colour of faecal	Normal	Normal	Normal	Normal	Normal	Normal
Diarrhoea	Not occurred	Not occurred	Not occurred	Not occurred	Not occurred	Not occurred
Sedation	Not observed	Not observed	Not observed	Not occurred	Not occurred	Occurred
Eyes colour	Normal	Normal	Normal	Normal	Normal	Normal
Convulsion	Not occurred	Not occurred	Not occurred	Not occurred	Not occurred	Not Occurred
Drowsiness	Not occurred	Not occurred	Not occurred	Not occurred	occurred	Occurred
Skin change	Normal	Normal	Normal	Normal	Normal	Normal
Urination	Normal	Normal	Normal	Normal	Normal	Normal
Coma	Not observed	Not observed	Not observed	Not observed	Not observed	Not occurred
Loss of life	Alive	Alive	Alive	Alive	Alive	Alive

A study by Panel (2007) on different *Aloe* spp. observed no toxicity of the diet of about 50,000 ppm or 4.1 to 4.6 g/Kg day^{-1} of *Aloe* polysaccharide on mice. The toxicity of the plant extracts, therefore, depends on factors such as cultivar, plant part ingested, age of the part used, growing conditions (soil pH and availability of water), light, and concentration of glycoalkaloid present (Folashade et al., 2012).

In a similar study by Almança et al. (2011) the hydroalcoholic extract of *Solanum cernuum* was used up to 25 g/Kg body weight in acute toxicity and 0.1 to 1.4 g/Kg body weight in sub-acute toxicity using mice without exhibiting any toxicity. Despite higher doses and the plant species exhibiting no toxicity to mice, other Solanaceae family induce toxic effects on man and animals (Barceloux, 2009) Therefore, in order to suggest whether the multiple doses of *A. rabaiensis* will cause effects to biochemical, haematological parameters and organs weight of the host, sub-acute toxicity study is recommended. Table 1 present the general characteristics and behaviour observations during acute toxicity study

The slight increase in average body weight of mice on day 14 to all treated groups and the control group were observed. It is therefore indicative that *A. rabaiensis* extracts did not interfere with the physiological process of mice because drinking and feeding intakes of mice were normal up to the 14th day of the treatment period and there was no feeding suppression induced by ARLME.

The evaluation of the sub-acute toxicity study of ARLME was according to OECD guidelines number 407. The treated mice received oral doses of ARLME at 500, 800 and 1000 mg/Kg body weight survived up to the 28th day of the experiment. On the 29th day, the anaesthetised mice with chloroform were used to collect the blood through cardiac punctual for haematological and serum biochemical parameters. The organs collected were used for weights, macroscopic and microscopic examination. According to Sudasinghe and Peiris (2018), the traditional practices of administering herbal drugs advise consuming natural remedies early in the morning. This practice is supported by this study which administered *A. rabaiensis* extract to mice starved for 4 h with *ad libitum* to drinking water.

The increase in weight of methanolic *A. rabaiensis* treated mice was observed in this study. However, there are plant extracts with critical bioactive compounds that induce low food intake causing low caloric value and finally decrease in weight (Yun, 2010). A study by Provenza et al. (2003) observed that increase or decrease in weight of an animal is scientifically evidenced to be associated with physiological adaptation responses to the plant extract rather than the toxic effect of the drug. The ARLME had no adverse effects on physiological processes in the bodies of mice. The macroscopic observation of organs such as liver, heart, spleen and kidney revealed no lesion or necrosis observed. However, further investigation through histopathological examination is advised for detailed information on the toxicity of *A. rabaiensis* in vital organs.

Results in Tables 2 and 3, showed that there was no statistical difference between average and relative organ weights of the extract treated mice and control group ($P > 0.05$). Similarly, the average body weights (BW) of mice on the sacrificed day was not statistically significant between plant extract treated mice and the control ($P > 0.05$). Thus this report supports the safety of *A. rabaiensis* in drug use.

Tested haematological parameters include total red blood cells (RBC), haemoglobin (HGB) content, haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), platelet (PLT), mean platelet volume (MPV), white blood cell count (WBC), neutrophil, lymphocyte and monocyte. Changes in blood parameters were considered

Table 2. Effect of oral administration of ARLME on average organ weight of mice (g).

Organ	Average organ weight			
	Normal (0 mg/Kg)	500 (mg/Kg BW)	800 (mg/Kg BW)	1000 (mg/Kg BW)
Liver	1.389 ± 0.156	2.577 ± 0.500	2.445 ± 0.335	1.899 ± 0.227
Heart	0.133 ± 0.010	0.141 ± 0.007	0.137 ± 0.007	0.169 ± 0.009
Spleen	0.230 ± 0.063	0.510 ± 0.164	0.641 ± 0.149	0.248 ± 0.010
Kidney (R)	0.168 ± 0.017	0.210 ± 0.019	0.271 ± 0.002	0.263 ± 0.044
Kidney (L)	0.172 ± 0.028	0.264 ± 0.016	0.265 ± 0.020	0.240 ± 0.022
BW on sacrificed day	45.433 ± 4.480	49.866 ± 1.266	54.466 ± 0.328	49.466 ± 3.678

Values are expressed as mean ± SEM (n= 5). P > 0.05 when compared to the normal group.

Table 3. Effect of oral administration of ARLME on relative organ weight of mice (g).

Organ	Relative organ weight			
	Normal (0 mg/Kg)	500 (mg/Kg BW)	800 (mg/Kg BW)	1000 (mg/Kg BW)
Liver	3.057 ± 0.156	5.167 ± 0.500	4.489 ± 0.335	3.839 ± 0.227
Heart	0.292 ± 0.010	0.282 ± 0.007	0.251 ± 0.007	0.341 ± 0.009
Spleen	0.506 ± 0.063	1.022 ± 0.164	1.176 ± 0.149	0.501 ± 0.010
Kidney (R)	0.369 ± 0.017	0.421 ± 0.019	0.497 ± 0.002	0.531 ± 0.044
Kidney (L)	0.378 ± 0.028	0.529 ± 0.016	0.486 ± 0.020	0.485 ± 0.022

Values are expressed as mean ± SEM (n=5). P > 0.05 when compared to the normal group.

as an indicator of stress, infectious diseases or intoxication (Lykkesfeldt and Svendsen, 2007). Etim et al. (2014) indicated that haematological indices are essential in understanding the physiological and pathological position of an animal.

The ARLME showed no haematological effects up to the 28th day of the treatment period. There were no signs of anaemia which indicated defects to stem cells of bone marrow to extract treated mice when compared to control (P > 0.05) at the dosage of 500, 800 and 1000 mg/Kg body weight, respectively. The ARLME was found to be safe in maintaining haematological parameters within reasonable limits as observed in Table 4.

Table 5, summarises the results of extract treated mice and control group on serum biochemical parameters. The parameters tested were creatinine, urea, AST and ALT. The oral administration of plant extracts at doses 500, 800 and 1000 mg/Kg per body weight did not reveal significant changes on urea and creatinine when compared to the control group. Urea is among nitrogenous compound eliminated by the kidney. The increase or decrease of this biomarker beyond the normal range in the blood circulatory system indicates kidney dysfunction (Gowda et al., 2010). The kidney decreases the elimination of waste products after leakage of biochemical indices like urea in the blood circulatory system. In the present study, there is no significant increase in urea from extract treated mice compared to control group (P > 0.05) at doses of 500, 800 and 1000 mg/Kg body weight.

Similarly, the serum creatinine of treated mice with ARLME was non-significant (P > 0.05) at all doses of 500, 800 and 1000 mg/Kg body weight. The increase in serum creatinine beyond the reasonable limits is a sign of renal failure and in the chronic condition, the secretion of creatinine by the glomerulus and tubules decreases (Amin et al., 2010). However, the elevated creatinine may also be observed in animals with anaemia, leukaemia, muscular dystrophy paralysis, and hyperthyroidism (Gowda et al., 2010).

On the other hand, liver hepatic enzymes SGOT (AST) and SGPT (ALT) were statistically significant at doses of 500, 800 and 1000 mg/Kg body weight (P = 0.05) for AST and P = 0.001 for ALT. The increased levels of AST and ALT in the blood of mice might be associated with the activity of phytochemical compounds with hepatotoxic effects. The liver is a target site for drug metabolism, transport and toxicological actions (Kitamura and Sugihara, 2014). Thus, the significant elevation of AST and ALT in the blood is due to the inflammatory response of liver membrane that alters membrane permeability and finally leakages of cellular constituents. However, observations from Amat et al. (2010) on the activity of aqueous extract of *Artemisia absinthium* in repeated doses (P < 0.001) prevented serum increase of hepatic liver enzymes by chemical and immunological induced responses when used for the treatment of an injured liver. The *A. absinthium* has hepatoprotective effects. The study corroborates with the work of Feng et al. (2019) who administered *Pueraria lobata* and *Silybum marianum*

Table 4. Effect of oral administration ARLME on haematological parameters.

Parameter	Normal (0 mg/Kg)	500 mg/Kg BW	800 mg/Kg BW	1000 mg/Kg BW
Total RBC (10^6/µL)	8.430 ± 0.276	7.126 ± 0.342	5.896 ± 1.109	8.703 ± 0.739
HGB(g/L)	13.733 ± 0.895	9.900 ± 0.404	8.866 ± 1.905	12.266 ± 1.690
HCT (%)	41.266 ± 2.640	31.100 ± 0.862	25.966 ± 5.852	37.500 ± 4.158
MCV (fL)	42.566 ± 0.425	44.066 ± 0.581	44.100 ± 0.929	45.033 ± 0.578
MCH (pg)	15.633 ± 0.352	15.433 ± 0.466	15.666 ± 0.384	16.366 ± 0.569
MCHC (g/dL)	34.400 ± 1.644	31.700 ± 0.680	34.100 ± 1.058	32.900 ± 1.006
RDW (%)	15.033 ± 0.726	12.466 ± 1.041	13.733 ± 0.768	14.666 ± 0.866
PLT (10^3/µL)	798.333± 134.191	745.666± 70.965	587.333 ± 81.685	632.000± 79.431
MPV (fL)	8.566 ± 0.317	8.966 ± 0.545	10.966 ± 2.372	10.833 ± 1.682
WBC (10^3/µL)	13.600 ± 1.115	12.133 ± 0.676	10.933 ± 1.407	10.333 ±2.718
Neutrophil (%)	20.833 ± 1.140	18.466 ± 2.310	19.166 ± 5.503	21.166 ±2.862
Lymphocyte (%)	89.100 ± 0.577	75.933 ± 2.784	76.833 ± 3.331	88.200 ± 3.839
Monocyte (%)	3.800 ± 0.642	2.500 ± 0.416	2.600 ± 0.702	2.866 ± 0.656

Values are expressed as mean ± SEM (n=5). P > 0.05 when compared to the normal group.

Table 5. Effect of oral administration of ARLME on serum biochemical parameters.

Parameter	Normal (0 mg/Kg)	500 (mg/Kg BW)	800 (mg/Kg BW)	1000 (mg/Kg BW)
Creatinine(mg/dl)	0.366 ± 0.033	0.400 ± 0.057	0.333 ± 0.033	0.433 ± 0.033
Urea (mg/dl)	22.766 ± 2.051	22.833 ± 0.821	27.033 ± 2.066	28.300 ± 2.193
SGOT (AST) (U/L)	131.000 ± 13.279	213.333 ± 12.666*	202.333 ± 8.412*	218.666 ± 28.788*
SGPT (ALT) (U/L)	28.666 ± 2.728	45.333 ± 4.910***	56.000 ± 2.081***	58.666 ± 2.848***

Values are expressed as mean ± SEM, *** and *, significant at P < 0.001 and P≤ 0.05 when compared to the normal group.

extracts to mice with the injured liver that occurred after being exposed to excessive alcohol. The extracts showed the most effective protection of alcoholic liver disease. The protection was associated with reducing alcohol-induced hepatic steatosis via upregulating LKB1/AMPK/ ACC signaling and inhibiting hepatic inflammation via LPS-triggered TLR4-mediated NF-κB signaling pathway.

The observations of liver sections using a light microscope (magnification 200×) revealed normal hepatocytes with kupffer cells lining the wall of sinusoids Figures 1A, C and E that received 0.5 mL normal saline, 500 and 800 mg/Kg of extract, respectively. However, the accumulation of lymphocytes in the portal triad (Figure 1G) that received 1000 mg/Kg was an indication of the immune response against the intoxication of *A. rabaiensis*. A study by Ali and Hamed (2006) observed that the accumulation of lymphocytes in a cell is a response of immunological functions. The ARLME (1000 mg/Kg body weight) has an alteration in lymphocytes count (Figure 1G) and possibly caused leukopoietic and immunomodulatory effects. From this observation, the plant extract may consist of bioactive compounds that have hepatotoxic effects that caused inflammation in the liver and resulted in leukocytes infiltration around portal triad and congestion of blood in the hepatic sinusoids.

The light microscopic observations of kidney section (magnification 200×) of the mice which were administered with no extract (Figure 1B) that received 0.5 mL of normal saline, revealed normal glomeruli, tubules and interstitial tissues. Likewise, the kidneys of extract treated groups showed no degeneration in spaces between Bowman's capsule, glomeruli and in tubules (Figure 1D, F) that received 500 and 800 mg/Kg body weight of extract, respectively. However, the kidney of mice (Figure 1H) that received 1000 mg/Kg showed leukocytes infiltration which is an indication of kidney intoxication. The ARLME is safe when used below tolerance dosage; however, above that dosage it has revealed hepatotoxic and renal toxic effects when used with long term treatment at the concentration equal or above 1000 mg of extract per kilogram body weight.

Conclusion

The *Aloe* species have been traditionally used worldwide as a folk remedy for various diseases because of their multiple biological activities. However, not all species have been investigated for toxicity profile. The findings from this study demonstrate that *A. rabaiensis* leaf extract is safe and can be used to develop drugs to manage infectious and chronic diseases. The use of *A. rabaiensis*

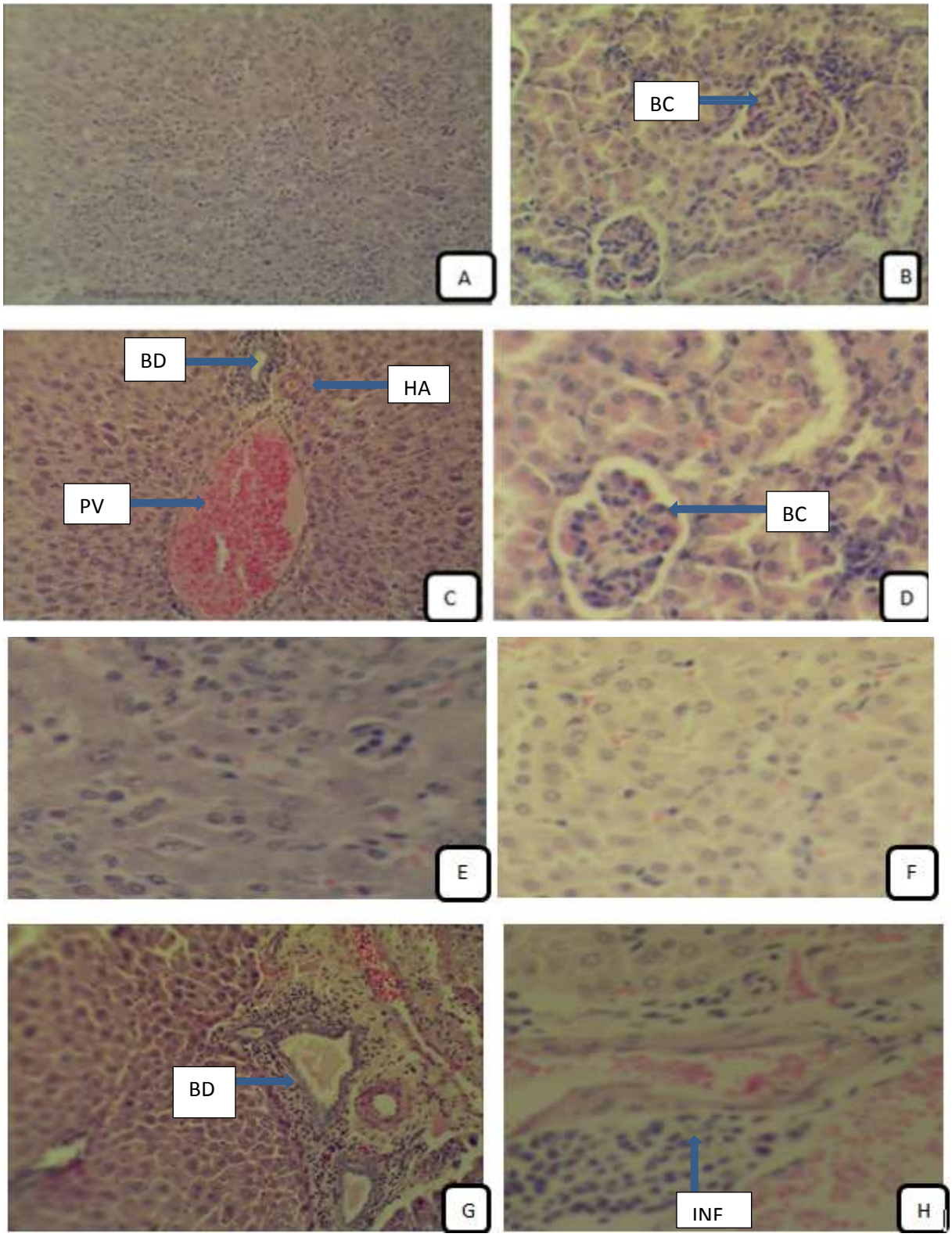

Figure 1. Photomicrographs of mice liver (A, C, E and G) and kidney (B, D, F and H) sections. Liver A and C have normal hepatocytes with liver C having normal portal triad with bile duct (BD), portal vein (PV) and hepatic artery (HA). The glomeruli in the Bowman's capsule (BC) in B and D are normally distributed. The distal convoluted tubules in F are normal while in H there is leukocytes infiltration (INF) and congestion of blood in tubules. Liver E has normal hepatocytes though liver G has abnormal portal triad with the proliferation of bile ducts (BD) and accumulation of lymphocytes indicating injury in the liver due to the intoxication of plant extract.

should not exceed a dosage of 1000 mg of extract per kilogram body weight. The significant alterations in biochemical and histopathological examinations are responses toward toxic effects of *A. rabaiensis* leaf extract on animal species when used above its tolerance limit. Therefore, this study supports the cautious use of *A. rabaiensis* for further investigation in drug formulation.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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