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Acute and repeated-dose toxicity of *Echinops kebericho* Mesfin essential oil

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ABSTRACT

Echinops kebericho Mesfin is used for the management of various diseases and fumigation during child birth. This study investigated acute and repeated-dose toxicity of *E. kebericho* M. essential oils (EOs). The study was conducted in Swiss albino mice. Organ weight, histopathology and clinical chemistry were analyzed. The dose and duration of treatment were defined in accordance with Organization for Economic Co-operation and Development (OECD) guideline.

No mortality was observed in acute oral dose toxicity study up to 2000 mg/kg per body weight. Compared to control group, treated groups did not show significant abnormalities in body weight and most parameters of clinical chemistry parameters and relative organ weight in repeated-dose toxicity study. However, urea, albumin, aspartate aminotransferase, and relative organ weight of right kidney showed variations in treated groups compared to control group. All treated groups and control group showed normal histology except lymphocytic infiltrates observed on the kidney with 200 mg/kg treated female group. The current study revealed that EO of *E. kebericho* M. could be considered well tolerated in acute and repeated-dose exposure. Further, teratogenic, mutagenic, carcinogenic, and sub-chronic and chronic toxicity studies are warranted.

1. Background

Medicinal plants contributed to the treatment of many common diseases and are sources of new modern medicines. Their use is increasing owing to perceived low cost, alignment with sociocultural, religious and spiritual values, emergence of resistance, and dissatisfaction with conventional healthcare [1]. Secondary metabolites within the plants exert biological activities with potential therapeutic effects [2] and adverse effects. Essential Oils (EOs) are among the many secondary metabolites produced by plants, and are commonly employed in the arena of health and health-related care for many years. Essential oils possess significant commercial, environmental, agricultural, dietary, medical, perfumery and aromatherapy applications [3]. Essential oils are complex natural mixtures containing about 20–60 components of different concentrations and are characterized by one or two major components at high concentrations (20–70 %) compared to the other components present in trace amounts [4]. Essential oils' major use is in fragrances where approximately about 300 EOs are in use from 3000 known EOs sources [5]. Essential Oils' includes flavoring in foods, fragrances in perfumes and cosmetics and food preservatives due to their antimicrobial activity. Their direct addition to food stuff exerts antimicrobial, antioxidant and radical scavenging effect [6]. A recent systematic review and meta-analysis revealed that EOs could be viable alternatives for the decolonization of methicillin resistant *Staphylococcus aureus* (MRSA), acne treatment, and topical antifungal infections [7].

Essential oils are traditionally used commonly in food processing or as medicines in Ethiopia [8]. Fresh or dried leaves of thymus species are used as condiments and ingredients in the preparation of "*berbere*" and

Abbreviations: EO, essential oil; *E. kebericho*, *Echinops kebericho*; AST, aspartate transaminase; ALT, alanine transaminase; ALP, alkaline phosphate; TG, triglycerides; Cr, creatinine.

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"shirro" (pepper and bean/pea flour) as well as Metata ayb (traditionally fermented cottage cheese) in Ethiopia [9]. *Echinops kebericho* Mesfin (family, Asteraceae) is among the commonly used EO-producing endemic medicinal plants in Ethiopia [10]. Ethno-pharmacological survey showed *E. kebericho*'s tremendous use in the treatment of infectious disease, stomachache, headache, heart disease, migraine, mental illness, kidney disease, where the smoke is inhaled in most cases [11–17]. The tuber is also smoked to repel snakes from the vicinity and for fumigation during child birth [18]. The traditional use of inhalation could imply volatile component's therapeutic effect. It has been confirmed that the major constituent of *E. kebericho* EO is dehydrocostus lactone [19] which was proved to exert anticancer activity [20–22] and antimicrobial activities [23,24]. *In vitro* and *in vivo* studies revealed various pharmacological activities of *E. kebericho* EOs such as antibacterial and antifungal activities [25], anti-mycobacterial activity (against *Mycobacterium smegmatis*) with significant resistance modulatory effects [26], stronger activity against leishmania [19], insect repellent activity [27], and mosquito larvicidal activity [28].

Previous toxicity studies established relative safety of the EOs *in vitro* model [19] and decoction in rats [29]. Extrapolation of *in vitro* toxicity to human is less reliable and there is a need for animal model toxicity study. Given the numerous studies confirming the medicinal use and wide traditional use of *E. kebericho* as inhalation, characterization of its EO toxicity profile is required in order to protect and promote public health. Hence, this is the continuation of our previous study [29] to establish the acute and repeated-dose toxicities of *E. kebericho* EOs. The study predicted the safety of the EO in human use, ensure the public safety, and promote further development.

2. Methods

2.1. Ethics statement

The experimental procedures were conducted in accordance with Organization for Economic Co-operation and Development (OECD) guidelines [30,31], complied to the ethical standards of EU Directive 2010/63/EU for animal experiments, and was conducted with proper animal handling under well-founded conditions in accordance with the recommendations of guide for the care and use of animals [32]. The protocol was approved by the Research Ethics Committee of Mbarara University of Science and Technology and registered with Uganda National Council for Science and Technology (UNCST).

2.2. Extraction and formulation of the essential oil

Echinops kebericho M. was collected from Andracha woreda, Sheka zone, Southwest, Ethiopia. The identification of the plant material was previous described [29]. The collected fresh tuber was first washed by tap water and then by distilled water to remove dirt and debris. Pounded material of the plant was added to 5 L of distilled water in 1/5 (w/v) ratio in a round bottom glass flask and subjected to hydro-distillation for 3 h using Clevenger type apparatus at Pharmacognosy Department, School of Pharmacy, Addis Ababa University. After extraction, the EO was collected; its volume and weight were measured. The EO was dissolved in 4% Tween 80 solutions for administration. The volume of solution administered was 1 ml/100 g and the concentration was adjusted to volume needed for administration.

2.3. Study design, sample size and allocation

This was a laboratory-based experimental study in Swiss albino mice and the results of the study were reported in accordance with ARRIVE guidelines [33]. The sample size was determined in accordance with OECD guideline. The dose level for repeated-dose toxicity study were determined based on the acute toxicity and ethno-pharmacological claims [34]. Graded doses, 100 mg/kg, 200 mg/kg and 400 mg/kg, of the EOs and the vehicle were administered by oral gavage once daily for 28 consecutive days every morning. The control groups received the same volume of vehicle, 4% tween 80, as the test group. In acute toxicity study, female mice were used as per OECD-423 guideline [30]. In repeated-dose toxicity study, ten mice of five from female and male were included. Total number of groups in acute toxicity study was three while in repeated-dose toxicity study it was four as outlined in Fig. 1.

The mice were allocated into experimental groups by simple randomization technique using lottery method and were marked using permanent marker for identification purpose. The animals were treated in rotation every other day from control to highest dose and vice versa.

2.4. Experimental animals and husbandry

Swiss albino mice weighing 18–26 g were obtained from Ethiopian Public Health Institute. The mice were kept in plastic cages in environmental conditions (22–24 °C, 12 h: 12 h dark/light cycle), fed a rodent pellet diet and allowed to drink water *ad libitum*. All mice were housed in standard cages in groups and acclimatized for two weeks before experiment. In acute toxicity study three female mice of the same group were housed together. In repeated-dose toxicity study five per group were housed together.

2.5. Experimental procedures in acute toxicity study

Acute toxicity study was conducted and results were interpreted before the commencement of repeated-dose toxicity study. In acute toxicity study, nine mice grouped in three groups were employed as recommended in OECD guideline [30]. The first group received 4% Tween 80, the second group 300 mg/kg EO and the third group 2000 mg/kg EO after 24 h. The mice were fasted overnight and provided with water only, and then EO was administered via oral gavages. Observations were made closely for the first 4 h, 24 h and up to 14 days. The number of death within the study period was recorded. At the end of the experiment, the mice were sacrificed for gross anatomy investigation and organ weight measured. The LD_{50} values of the EOs were determined based on the Globally Harmonized System of Classification and Labeling of Chemicals (GHS) [35].

2.6. Experimental procedures in repeated-dose toxicity study

Ten mice, five male and five female, were employed in accordance with the repeated-dose 28-day oral toxicity study OECD guideline in rodents [31]. Parameters measured include: food consumption, body weight, relative organ weight, clinical chemistry (aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphate (ALP), total protein (TP), total cholesterol (TC), blood urea nitrogen (BUN), creatinine (Cr), total bilirubin (T-Bili), direct bilirubin (Bili-dir), albumin (Alb), triglycerides (TG), and glucose (Glu)) and histopathology as described previously [29]. The relative organ weight, defined as the organ weight per 100 g of body weight at sacrifice was calculated.

2.7. Statistical analysis

Statistical analyses were performed using GraphPad Prism version 5 software. Data were presented as mean ± (Standard deviation, SD). ANOVA assumptions were checked using Bartlett's test for equality of variance and D'Agostino & Pearson omnibus normality test. ANOVA was performed; if assumptions were met otherwise Kruskal-Wallis analyses. For significant values post-hoc analysis was performed using Tukey for ANOVA or Dunn's Multiple Comparison Test for Kruskal-Wallis analysis. The $p < 0.05$ was considered significant.

3. Results

The yield of the essential oil was 0.18 % per dry weight of

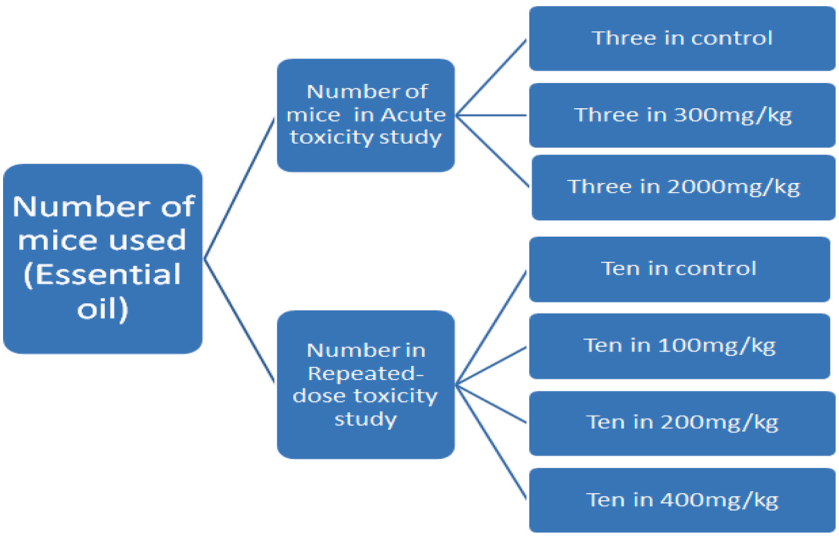

Fig. 1. Number of animals used and dose level in acute and repeated-dose toxicity study.

E. kebericho M. All mice were naïve to the treatment gievn. All mice completed treatment in good health condition and were included in the analyses.

3.1. Acute toxicity of EO of Echinops kebericho

Single oral dose of *E. kebericho* M. EO up to 2000 mg/kg did not show treatment-related morbidity in mice. Treated mice did not show significant treatment related morbidity: yet short term disturbances including piloerection, muscle spasm and apathy were observed immediately after administration of 2000 mg/kg and ended in 12 h' time. The changes in body weights were not statistically significant (slope of linear regression, P = 0.5231). Better increase in body weight was observed in control group compared to 300 mg/kg and also 300 mg/kg compared to 2000 mg/kg treated (Fig. 2). Mean body weight showed non-significant variation between 300 mg/kg treated group versus 2000 mg/kg, p = 0.2950.

3.2. Sub-acute toxicity of essential oil of Echinops kebericho

3.2.1. Body weight, food consumption and relative organ weight

Body weight and food consumption remained constant for combined groups but significant variations when male and female groups were analyzed separately. Control and 100 mg/kg EO treated female groups showed constant weight, while 200 mg/kg and 400 mg/kg EO treated female groups showed significant loss in weight. All male groups showed significant weight gain, increased food consumption pattern in controls and those treated with 100 mg/kg EO, but constant consumption pattern in medium and high dose treated male groups. However, combined groups showed constant weight and food consumption pattern throughout the treatment period (Table 1)

No statistical significant variations in food consumption and weight were observed between treated groups and the control group (p = 0.3518 and p = 0.6942, respectively). However, significant variations in food consumption were observed between females and males (p = 0.0001). Fig. 3 shows a trend of similar food consumption and weight among high dose treated groups compared to other groups as a function of time (in days) though the difference is non-significant (Fig. 3).

No significant difference in relative organ weight (left kidney, lung, heart, spleen) was observed in treated groups compared to controls. However, there was a significant difference in mean relative organ weight of right kidney treated with highest dose of EO (400 mg/kg) compared to control group (mean difference 0.1896, 95 % CI: 0.04638, 0.3329, *P* = 0.0093). Significant variations in relative organ weight of spleen, left and right kidney was also observed between male and female groups (Table 2).

3.2.2. Clinical chemistry

Except for albumin and AST, no significant variations in biochemical values were observed between male and female groups. However, significant variations in urea, albumin and AST levels were observed between males and females (Table 3).

3.2.3. Post-hoc analysis

Relative right kidney weight, AST and albumin values showed significant variations between treated and control groups. Groups treated with highest dose of the EO showed elevated level of AST value compared to control (mean difference of controls versus 400 mg/kg = -315.6, 95 % CI: −610.3 to −20.83). Significant difference in mean albumin level was observed among different treated groups. Albumin

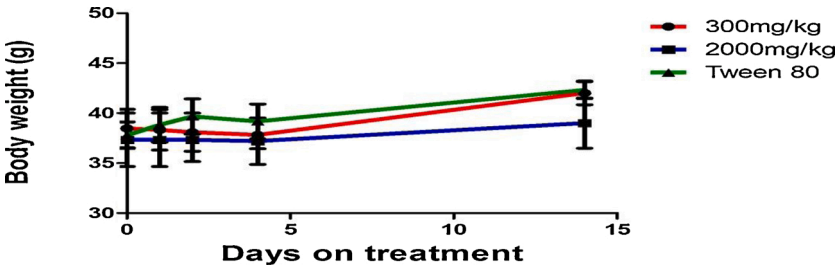

Fig. 2. Effect of *E. kebericho* EO on body weight as function of time (in days) after single dose exposure in 300 mg/kg and 2000 mg/kg treated groups compared to controls.

Table 1
Linear regression of weight gain in different groups of *E. kebericho* EO treated and control group.

Group	Weight gain				Food consumption			
	Slop (95 %CI)	p-value	R^2	Interpretation	Slop (95 %CI)	p-value	R^2	Interpretation
Males and females treated separately								
Control-Female	−0.1040 ± 0.04773	0.0721	0.4419	No change	0.07349 ± 0.1533	0.6420	0.02246	No change
100 mg/kg-Female	−0.05847 ± 0.02829	0.0842	0.4160	No change	0.06484 ± 0.1358	0.6432	0.02230	No change
200 mg/kg-Female	−0.1610 ± 0.03272	0.0027	0.8014	Loss	0.1427 ± 0.1730	0.4290	0.06363	No change
400 mg/kg-Female	−0.1098 ± 0.04439	0.0482	0.5048	Loss	0.1547 ± 0.1077	0.1816	0.1709	No change
Control-Male	0.4130 ± 0.01038	< 0.0001	0.9962	Gain	0.3833 ± 0.1336	0.0167	0.4516	Increased
100 mg/kg-Male	0.4623 ± 0.03946	< 0.0001	0.9581	Gain	0.4025 ± 0.1490	0.0222	0.4220	Increased
200 mg/kg-Male	0.4249 ± 0.03962	< 0.0001	0.9504	Gain	0.4433 ± 0.2160	0.0672	0.2964	No change
400 mg/kg-Male	0.3757 ± 0.02571	< 0.0001	0.9727	Gain	0.07637 ± 0.1991	0.7094	0.01449	No change
When male and females combined								
Control	0.1545 ± 0.1030	0.1559	0.1384	No change	0.2248 ± 0.1302	0.0984	0.1193	No change
100 mg/kg	0.2019 ± 0.1126	0.0945	0.1868	No change	0.2157 ± 0.1143	0.0723	0.1394	No change
200 mg/kg	0.1320 ± 0.1104	0.2520	0.09255	No change	0.2394 ± 0.1668	0.1653	0.08560	No change
400 mg/kg	0.1330 ± 0.1274	0.3142	0.07221	No change	0.1152 ± 0.1175	0.3375	0.04187	No change

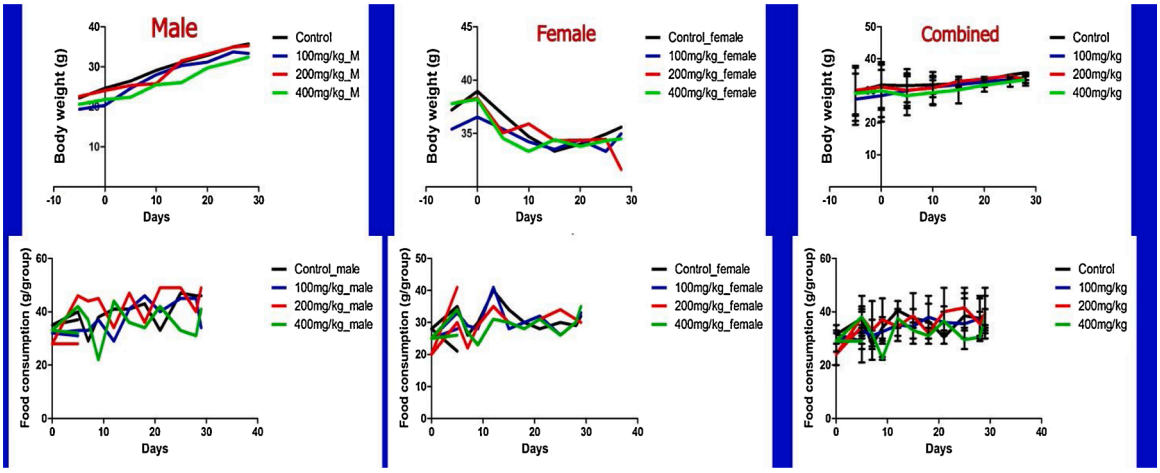

Fig. 3. Food consumption pattern among different groups of male, female and male and female merged together. Body weight pattern among different groups of male, female and male and female merged together.

Table 2
Effects of oral administration of *E. kebericho* essential oil on organ weight.

Organs	Male				Female				P-value
	control	100 mg/kg	200 mg/kg	400 mg/kg	Control	100 mg/kg	200 mg/kg	400 mg/kg	
Relative weight = Organ weight in gram/body weight of the mice (%)									
Liver	5.94 ± 0.63	6.12 ± 0.76	6.57 ± 0.43	6.54 ± 0.68	5.85 ± 0.84	4.66 ± 0.60	5.08 ± 0.74	4.98 ± 0.41	0.0014/0.6172
Right kidney	0.82 ± 0.07	0.87 ± 0.08	0.82 ± 0.15	0.76 ± 0.07	0.91 ± 0.15	0.74 ± 0.14	0.72 ± 0.04	0.59 ± 0.07	0.0121/0.0093
Left kidney	0.81 ± 0.13	0.86 ± 0.03	0.83 ± 0.06	0.80 ± 0.05	0.65 ± 0.05	0.63 ± 0.05	0.65 ± 0.05	0.58 ± 0.05	< 0.0001/0.7258
Spleen	0.40 ± 0.09	0.37 ± 0.06	0.55 ± 0.16	0.65 ± 0.33	0.85 ± 0.63	0.43 ± 0.04	0.48 ± 0.06	0.42 ± 0.04	0.0304/0.0677
Lung	0.90 ± 0.27	0.84 ± 0.19	0.94 ± 0.30	0.95 ± 0.14	0.91 ± 0.15	0.88 ± 0.24	1.00 ± 0.35	0.88 ± 0.17	0.9129/0.7411
Heart	0.66 ± 0.30	0.60 ± 0.10	0.55 ± 0.10	0.58 ± 0.10	0.50 ± 0.16	0.53 ± 0.10	0.57 ± 0.09	0.49 ± 0.03	0.6719/0.8303
Absolute weight (in grams)									
Liver	2.14 ± 0.40 g	2.05 ± 0.42	2.31 ± 0.17	2.11 ± 0.20	2.09 ± 0.38	1.64 ± 0.37	1.60 ± 0.17	1.72 ± 0.26	
Right kidney	0.29 ± 0.05	0.29 ± 0.02	0.29 ± 0.06	0.25 ± 0.04	0.33 ± 0.07	0.26 ± 0.03	0.23 ± 0.02	0.21 ± 0.04	
Left kidney	0.29 ± 0.06	0.29 ± 0.04	0.29 ± 0.02	0.26 ± 0.03	0.23 ± 0.02	0.22 ± 0.04	0.21 ± 0.02	0.20 ± 0.03	
Spleen	0.14 ± 0.04	0.12 ± 0.03	0.19 ± 0.04	0.21 ± 0.10	0.31 ± 0.25	0.15 ± 0.02	0.15 ± 0.02	0.14 ± 0.02	
Lung	0.33 ± 0.12	0.28 ± 0.08	0.33 ± 0.08	0.31 ± 0.04	0.33 ± 0.07	0.32 ± 0.13	0.32 ± 0.12	0.30 ± 0.05	
Heart	0.23 ± 0.12	0.20 ± 0.05	0.20 ± 0.04	0.19 ± 0.03	0.18 ± 0.05	0.18 ± 0.03	0.18 ± 0.03	0.17 ± 0.01	

Note: The first serious of the p-value column shows ANOVA/ Kruskal-Wallis analysis result when male and female groups are grouped separately while the second when both are grouped together.

concentration in those treated with 200 mg/kg EO was significantly lower compared to all other treatment groups (Table 4).

3.2.4. Sex variations

Food consumption, clinical chemistry, liver weight, relative left kidney weight, relative right kidney weight, and urea as well as albumin levels were significantly higher in males compared to females (Supplementary file 1).

3.2.5. Effect on histopathology

Gross anatomical examination of the vital organs (liver, kidney, heart, lung and spleen) did not show gross pathological lesions in both

Table 3
Clinical chemistry parameters in repeated dose oral toxicity study of E. kebericho essential oil treated mice.

Parameter	Male				Female				P-value
	Control	100 mg/kg	200 mg/kg	400 mg/kg	Control	100 mg/kg	200 mg/kg	400 mg/kg	
AST (U/L)	406.4 ± 297.7	237.0 ± 111.1	229.6 ± 60.54	711.3 ± 184.0	209.6 ± 92.13	379.0 ± 215.4	669.8 ± 324.9	553.4 ± 365.9	0.0086*/0.0394*
ALT (U/L)	163.4 ± 188.0	77.00 ± 50.20	51.80 ± 22.31	104.8 ± 44.89	55.00 ± 24.05	71.40 ± 35.08	151.2 ± 93.45	104.8 ± 44.89	0.1195/0.3339
ALP (U/L)	128.6 ± 16.92	188.0 ± 138.1	186.8 ± 108.4	109.4 ± 62.68	245.2 ± 179.2	69.40 ± 21.82	50.40 ± 44.65	165.8 ± 90.73	0.0588/0.5238
Creatinine (mg/dl)	0.32 ± 0.19	0.22 ± 0.11	0.28 ± 0.08	0.22 ± 0.08	0.20 ± 0.10	0.26 ± 0.05	0.32 ± 0.39	0.45 ± 0.31	0.6380/0.7947
Urea (mg/dL)	57.04 ± 10.24	48.86 ± 19.47	64.80 ± 27.33	56.40 ± 6.98	51.23 ± 8.97	77.20 ± 32.23	102.6 ± 25.71	74.83 ± 25.67	0.0104*/0.0817
Protein-T (g/dl)	6.38 ± 0.34	6.66 ± 2.07	5.98 ± 2.89	5.74 ± 0.39	5.90 ± 1.06	5.88 ± 0.24	14.04 ± 4.79	5.90 ± 0.97	0.9880/0.9312
Albumin (g/dl)	2.72 ± 0.13	2.86 ± 0.19	2.18 ± 0.16	2.72 ± 0.08	2.82 ± 0.11	2.84 ± 0.09	2.42 ± 0.50	2.92 ± 0.38	0.0006*/< 0.0001*
Glucose (mg/dl)	235.6 ± 111.0	290.4 ± 136.6	188.0 ± 40.79	175.8 ± 24.67	162.6 ± 33.25	151.2 ± 18.20	163.2 ± 66.84	186.6 ± 26.54	0.0630/0.5758
Bil-total (mg/dl)	0.45 ± 0.17	1.62 ± 2.08	0.68 ± 0.26	1.10 ± 0.54	0.34 ± 0.23	0.94 ± 0.55	2.90 ± 3.83	1.08 ± 0.65	0.2987/0.3310
Bil-dams (mg/dl)	0.60 ± 0.34	1.34 ± 1.69	0.32 ± 0.28	1.06 ± 0.38	0.16 ± 0.15	0.72 ± 0.40	2.08 ± 2.23	1.00 ± 0.74	0.1472/0.3799
TG (mg/dl)	120.4 ± 18.72	130.4 ± 49.15	106.8 ± 28.92	116.8 ± 10.35	106.9 ± 37.42	110.0 ± 26.69	128.2 ± 45.10	87.00 ± 23.01	0.5148/0.6195
Cholesterol (mg/dl)	149.2 ± 20.43	158.6 ± 23.66	142.8 ± 34.11	149.6 ± 28.36	127.2 ± 30.06	115.4 ± 17.21	131.6 ± 55.39	121.0 ± 17.00	0.3004/0.9978

Note: The first serious of the p-value column shows ANOVA/ Kruskal-Wallis analysis result when male and female groups are grouped separately while the second when both are grouped together.

Table 4
Post-hoc test for the sub-acute toxicity study.

Parameters	Mean Diff.	95 % CI of diff
Relative right kidney weight		
Control vs 400 mg/kg	0.1896	0.04638, 0.3329
AST		
Control vs 400 mg/kg	−315.6	−610.3, −20.83
Albumin		
Control vs 200 mg/kg	0.4778	0.1628, 0.7927
100 mg/kg vs 200 mg/kg	0.5500	0.2434, 0.8566
200 mg/kg vs 400 mg/kg	−0.5200	−0.8266, −0.2134

acute and repeated-dose oral toxicity studies. Histopathological examinations revealed mild alterations in the kidney lymphocytic infiltrates at medium dose of EO. Photomicrographs of the liver and spleen showed normal morphological architecture in all different treatment groups (Fig. 4).

4. Discussion

The present study revealed the relative safety of EO of *E. kebericho* in a mouse model. Acute toxicity and repeated-dose 28-day toxicity studies did not show significant physiological alterations. No death was observed up to the 2000 mg/kg dose, and hence *E. kebericho* EO could be considered Category 4 according to GHS classification [35]. Changes observed in body weight, relative organ weight, food consumption, clinical chemistry parameters and histopathology were not significant in 28-day repeated-dose toxicity studies. However relative right kidney weight, AST and albumin levels in treated groups showed significant variations compared to controls. Food consumption, relative liver weight, relative left kidney weight, relative right kidney weight, and urea as well as albumin concentrations were observed to be higher in males compared to females.

Single oral dose of *E. kebericho* EO up to 2000 mg/kg did not show significant treatment-related morbidity in female mice, though piloerection, muscle spasm and apathy were observed immediately after administration. Body weight increment was observed in those treated with 300 mg/kg EO compared to those which received 2000 mg/kg. The LD_{50} of *E. kebericho* EO could be considered greater than 2000 mg/kg as no death was noted up to this dose. This LD_{50} is lower compared to *Ocimum gratissimum* EO (1410 mg/kg [36] but higher to that of *Eucalyptus citriodora* EO (2337.9–2967.5 mg/kg) [37]. On the other hand, *Litsea cubeba* EO (used as ingredient of food, cosmetics, and cigarettes) showed oral LD_{50} of approximately 4000 mg/kg [38]. No histopathological changes, or biochemical alterations or skin irritation were not observed Cymbopogon citratus EO up to dose of 2000 mg/kg [39]. Similarly, black caraway essential oil did not affect the immune, blood system, body enzymes and vital organs of the body [40]. On the other hand, Citrus aurantifolia EO showed mild hematotoxicity, nephrotoxicity and hepatotoxicity among the 100 mg/kg and 500 mg/kg dose groups [41].

Constant body weight and food consumption pattern was observed for combined groups with significant variations in gender groups which is in line to previous findings [42]. Constant weight pattern was observed in control and 100 mg/kg EO treated female groups. Significant weight loss was seen in 200 mg/kg and 400 mg/kg EO treated female groups indicating reduced tolerability to increased dose. Significant weight gain in all male groups, increased food consumption pattern in controls and those treated with 100 mg/kg, and constant consumption pattern in medium and high dose treated male groups could explain less sensitivity of males to toxicants compared to females [43,44]. The present finding is in agreement with international guidelines and results of most studies which revealed that males tolerate better compared to females [30]. Relative body weight in most of the tested organs did not show significant variations except mean relative organ weight of right kidney where highest dose treated groups varied significantly compared to controls in concentration dependent toxicity fashion.

Most clinical chemistry values did not show significant variations among different treatment groups, except albumin concentration and AST values. Elevated AST level was observed in high-dose treated group compared to control group. AST and ALT levels are widely used as sensitive markers to evaluate toxic manifestations of liver [45]. Increased serum ALT level could be attributed to hypertrophy and other disorders of the liver yet with possible extra hepatic sources [45], while an increased AST level could indicate hepatic cell damage. Elevated AST level in this study was observed without histological abnormalities. ALT is a cytoplasmic enzyme present in the liver, and it significantly increased during hepatocellular damage [46], while AST is present extra-cellularly in various tissues including heart, skeletal muscles, liver,

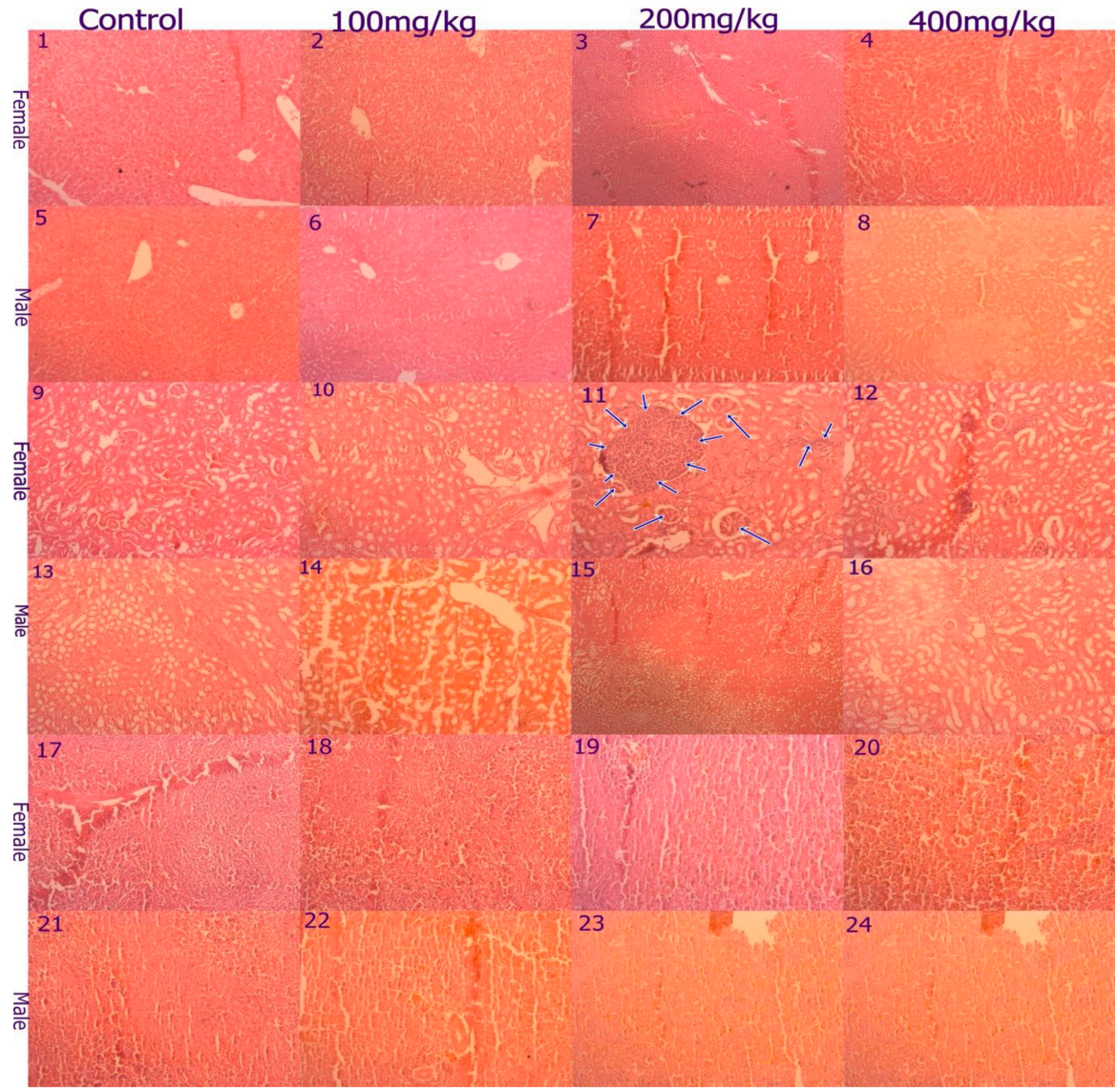

Fig. 4. Photomicrograph of liver, kidney and spleen in repeated-dose *E. Kebericho* EO oral toxicity study. All liver including female (from control to highest dose, 1–4) and male (from control to highest dose 5-8) 'showed normal morphology architecture. 'All kidneys (9-16) showed normal morphology except medium dose treated female (200 mg/kg). 'Spleen of all groups (17–24) showed normal morphology, H&E, magnification 10×.

kidneys, pancreas, and erythrocytes [47]. AST is released from multiples of cells in response to cellular damage or change in membrane permeability. Hence, as ALT is a more sensitive biomarker of hepatic damage compared to AST, an elevated AST without change in ALT level could not necessarily indicate an insult in the liver.

Lower albumin concentration observed among medium dose treated groups may be associated with the observed lymphocytic infiltrates which could confer some level of renal toxicity in medium-dose treated group. Renal inflammatory responses could increase renal excretion of albumin [48] consequently decreasing serum albumin level. Renal toxicity dose-response curve in this study appears to be concentration-dependent peaking in the middle, while toxicity observed with middle-dose compared to a higher or a lower dose requires confirmation from a large sample size.

Sex variations on food consumption, relative liver weight, relative left kidney weight, relative right kidney weight and urea and albumin concentrations could not be explained based on this study. Most parameters showed higher values in males compared to females. Similarly, higher TG, ALP, ALT, and glucose levels were reported in male Sprague-Dawley rats compared to females [49,50]. Male mice had also been reported to have higher TG, cholesterol, high-density lipoprotein cholesterol, glucose, and amylase values [51].

Compounds with similar chemistry can trigger common mechanisms of toxicity and toxicity profile at the level of organism, organ, tissue, and cell [52]. In the current study, the EO largely displayed similar toxicity profile to most EOs from other plant EOs. Turmeric EO oral administration up to the dose of 5 g/kg body weight is safe to rats [53]. Thymus vulgaris L EO was well tolerated up to the dose of 2000 mg/kg in acute exposure, and the 28-day sub-acute toxicity study revealed that the no-observed-adverse effect level (NOAEL) was greater than 250 mg/kg/day [54]. *Citrus aurantifolia* posed mild hematotoxic, nephrotoxic and hepatotoxic effects in doses of 100 mg/kg and 500 mg/kg in 60-day repeated dose study [41]. Mild toxicity observed in the latter case could be attributed to longer treatment; 28 days versus 60 days. Long term exposure to moderate concentrations of mixtures of terpenes could cause possible adverse health effect [55]. Ursolic acid a pentacyclic triterpenoid which can be isolated from different plant EO did not cause significant toxicity [56]. The biological effects (either therapeutic or toxic) of EOs could be because of the pro-oxidant effect in cells [4] and display more or less similar effect because of their structural

similarity [57].

Plant medicines are blindly considered safe without any scientific evidence but could cause significant damage to public health. Equating "natural to safe" does not work where dozens or more poisons are coming from natural compounds [58]. In most developing countries, people use plants from their courtyards as well as street markets without any valid knowledge of their safety. Despite significant numbers of people using herbal medicine for their primary health care, there is paucity of studies on safety of herbal medicine. Clinical studies can be commenced in humans, after assessment of quality, efficacy and safety of herbal medicines in animal models [59]. With growing use of natural derived substances from plants all over the world, it is now wise not to rely only on the traditional beliefs; explanatory and reasonable pre-clinical and clinical studies, similar to the current one, are required and should be considered mandatory in gaining trustworthy findings.

5. Conclusion

This study provided comprehensive evidence on the safety of *E. kebericho* EO and confirmed the relative safety in acute and repeated-dose conditions. No significant morbidity or mortality was observed up to 2000 mg/kg. The repeated-dose toxicity study demonstrated no observable behavioral adverse effect or damages on gross anatomy. Histological changes did not occur in the liver and spleen. The indifferent finding of AST and histopathology at highest dose and the observation of some level of renal adverse effect at low dose but not higher or lower dose warrants further investigation in larger sample sizes. In general, EO of *E. kebericho* M. could be considered well tolerated in acute and repeated-dose exposure. Further, teratogenic, mutagenic, carcinogenic, and sub-chronic and chronic toxicity studies are warranted.

Authors' statement

Serawit Deyno conceived the research idea and drafted the manuscript. Mesfin Asefa Tola read and interpreted the histopathological findings. Joel Bazira, Eyasu Makonnen, Paul E. Alele supervised and mentored the research work. All authors revised, edited and approved the final manuscript.

Ethics approval and consent to participate

The experimental protocol was approved by the Research Ethics Committee (REC) of Mbarara University of Science and Technology, Uganda. The protocol was approved and registered with Uganda National Council for Science and Technology (UNCST) with reference number HS398ES.

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Authors' contributions

SD conceived the research idea. MAT read and interpreted the histopathological findings. JB, EM and PEA supervised and mentored the research work. SD drafted the manuscript. All authors revised, edited and approved the final manuscript.

Consent for publication

Not applicable.

Availability of data and materials

The data supporting the conclusions of this article are included within the article.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.toxrep.2020.12.027.

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