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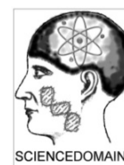

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Toxicological Studies of Ethanolic Extract of *Emilia praetermissa* Milne-Redh (Asteraceae) in Rats

Ndji Otto Gustave Lebeau[1], Amang André Perfusion[2], Mezui Christophe[3], Nkwengoua Zodengoumba Ernestine[4], Enow-Orock Enonchong George[5], Tan Paul Vernyuy[1*] and Nyasse Barthelemy[4]

[1]Department of Animal Biology and Physiology, Faculty of Science, University of Yaoundé I, P.O.Box 812, Yaoundé, Cameroon.
[2]Department of Biological Sciences, Faculty of Science, University of Maroua, P.O.Box 46, Maroua, Cameroon.
[3]Department of Biological Sciences, Higher Teachers' Training College (ENS), University of Yaoundé I, Yaoundé, Cameroon.
[4]Department of Organic Chemistry, Faculty of Science, University of Yaoundé I, P.O.Box 812, Yaoundé, Cameroon.
[5]Department of Biomedical Sciences, Faculty of Health Science, University of Buea, Buea, Cameroon.

Authors' contributions

This work was carried out in collaboration between all authors. Authors NOGL and TPV designed the study and wrote the protocol. Authors NOGL, AAP and MC managed the biochemical analysis. Authors TPV and NOGL did the literature search and statistical analysis. Author NOGL wrote the first draft. Authors TPV and EOEG supervised the study and interpreted the histology slides. Authors NZE and NB did the phytochemical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The objective of the present study was to determine the acute and subchronic toxicity of the ethanolic extract of *E. praetermissa* in rats.

Place and Duration of Study: Department of Animal Biology & Physiology (Animal Physiology Laboratory) and Department organic chemistry (Laboratory of medicinal chemistry), Faculty of Science, University of Yaoundé I. Between January and November 2016.

**Corresponding author: E-mail: pvernyuy@yahoo.com;*

Methodology: Acute toxicity (single administration, 2000 mg/kg) and sub chronic toxicity (28 days: 10 rats per group 5 males and 5 females) given, respectively, distilled water, 250, 500 and 1000 mg/kg of extract every 24 h orally) were conducted according to OECD guidelines No. 423. Satellite controls and satellite test groups received, respectively, distilled water and extract (1000 mg/kg). Body weight was recorded on day 1 and once a week for six weeks. The animals were sacrificed on the 29th day and blood biochemical and hematological parameters were measured. Histological examination of tissue specimens of liver, kidney and lungs was performed after hematoxylin-eosin staining.

Results: Extract showed significant increase in relative weights of liver in males at 500 and 1000 mg/kg. Dose 1000 mg/kg showed significant increase in relative kidney weights in both sexes. Extract-treated males showed significant increases (500 and 1000 mg/kg) of total white blood cell and platelet counts, while female rats showed no significant increase of total WBC counts. Biochemical study showed, in male rats, significant decreases in HDL-cholesterol (at 1000 mg/kg). Serum transaminases (ASAT, ALAT), total protein and creatinine also increased in male rats at 1000 mg/kg. In female rats, biochemical study revealed significant decreases in HDL-cholesterol at 1000 mg/kg and significant decreases in HDL-cholesterol, and increased levels of ASAT, ALAT, total protein and creatinine at 1000 mg/kg (vs. control female rats). Microscopically, there were hepatic (parenchymal leucocyte infiltration, nuclear enlargement and intense margination) and renal (mesengeal hyperplasia) and lung (atelectasis) tissue lesions at high doses supporting by the hematological observations.

Conclusion: Acute toxicity study of the extract revealed that its LD50 is above 2.0 g/kg bw in rats. Subacute toxicity study suggests that high doses of the extract taken for long periods can result in toxic effects in liver, lungs and kidneys.

Keywords: Emilia praetermissa; acute and subchronic toxicity; Asteraceae; OCDE.

1. INTRODUCTION

Plants have always been an important source of drugs. A large number of the world's populations, especially in developing countries, depend upon medicinal plants as an alternative and complimentary therapy for various ailments. Some of the most common practices involve the use of crude plant extracts, which may contain a broad diversity of molecules often with unknown biological effects [1]. Since the medicinal plants are being used indiscriminately without notifying to their possible unhealthy or toxic effects, the World Health Organization has recommended that traditional plants used for the treatment of diseases need further scientific investigation on their toxic side effects [2]. Plants produce bioactive compounds which act as defense mechanisms against any disease, and at the same time, may be toxic in nature [3]. However, the general acceptability of herbal medicines has been limited by a lack of defined chemical characterization, dose regimen, and adequate toxicity data to evaluate their safety [4]. Therefore, it has become essential to assess the safety of plants used for medicinal purposes for possible toxicity.

Emilia praetermissa was originally described from Sierra Leone and Nigeria and was subsequently found in other West African countries, including, Ivory Coast, Ghana, Guinea and Liberia [5,6]. It has similar uses as *Emilia lisowskiana* in Western Africa and the Democratic Republic of Congo, the leaves are often eaten like vegetable to make salads [6]. In Nigeria, Cameroun and Gabon, the leaves are used to treat eye disorders and filariasis [7]. In Gabon, the macerated leaves are used to treat heart problems and the crushed leaves mixed with copper filings are used to treat ulcers. In Nigeria, a decoction of leaves is employed as febrifuge. In Congo, the sap of the leaves is used to treat various skin problems including abscess, ulcers and leprous affections. It is also employed to treat the lice, hernia, back ache, syphilis, gonorrhea, convulsions, enlarged spleen, menstrual vertigo and epilepsy.

A recent study revealed the presence of saponins, flavonoids, oils, phenols, coumarins, sterols, triterpenoids and polysaccharides in *Emilia praetermissa*. The evaluation of ethanolic extract of *Emilia praetermissa* showed significant gastric cytoprotective and antisecretory effects using different gastric ulcer models [8]. Since the literature provides no reference about the safe dosage of *Emilia praetermissa* in traditional medicine, the present study was carried out on the toxicity profile of the ethanolic extract.

2. MATERIALS AND METHODS

2.1 Plant Collection and Extraction

The leaves of *E. praetermissa* were harvested in Yaoundé, Center region of Cameroon, in January 2016. The plant was identified in comparison with the existing voucher specimen N° 32105/SRF/HNC at the Cameroon National Herbarium. The dried leaves of *E. praetermissa* were crushed, powdered and extracted with 95% ethanol (10% w/v) for 48 hours. After filtration through Whatman filter paper No. 3, the filtrate was concentrated using a rotavapor, and then evaporated at 40℃ using a *Raven* convection air oven (Jencons PLS, UK). The pasty, green extract obtained (4.8% yield) was stored at 4℃ and used for further experiments.

Fig. 1. Photograph of *Emilia praetermissa*

2.2 Experimental Animals

Healthy young female Wistar albino rats (115 - 125 g) were used for acute toxicity and young Wistar rats of both sexes (85-115 g) were used for subchronic toxicity. The female rats were nulliparous and non-pregnant. Animals were maintained under standard husbandry conditions (temperature 25 ± 2℃, 12 h light/12 h dark cycle) and fed with standard pellet diet and water *ad-libitum*. All animal experiments were handled according to the Cameroon National Ethics Committee (Ref. N° FWIRB 00001954) and all experiments have been examined and approved.

2.3 Acute Oral Toxicity Study

The rats were divided into one control group and two treated groups, each group consisting of three animals. The control group received vehicle and the first treated group received, by oral route, a unique dose of the extract at 2000 mg/kg. Another dose of 2000 mg/kg was administered to the confirmation group 48 h later. The animals were observed continuously for two hours and then they were observed during 24 hours to determine any changes in the behavioral responses and also for tremors, convulsion, salivation, diarrhea, lethargy, sleep and coma and monitored for any mortality [9].

2.4 Subchronic Oral Toxicity Study

Toxicity studies were performed according to the OCDE guideline N° 407 [10]. (Results of acute toxicity studies in female Wistar rats indicated that the LD50 was greater than 2000 mg/kg body weight). The doses selected for the subchronic toxicity study were 250 mg/kg, 500 mg/kg and 1000 mg/kg. The oral route was selected for use because oral route is considered to be the proposed therapeutic route. The Animals were divided into 6 groups of 10 animals each with 5 males and 5 females per group. Vehicle and *E. praetermissa* extract were administered daily for 28 days as shown below:

Group 1 (Control): received distilled water in 4% of tween 20 (10 ml/kg b.w)

Group 2: received *E. praetermissa* (250 mg/kg b.w)

Group 3: received *E. praetermissa* (500 mg/kg b.w)

Group 4: received *E. praetermissa* (1000 mg/kg b.w)

The remaining 20 rats were distributed into 2 satellite groups that were observed for an extra 14 days at the end of the 28 days study. The satellites groups (groups 5 and 6) were orally treated respectively with distilled water in 4 % of Tween 20 and extract at dose of 1000 mg/kg/day. The rats were maintained under identical conditions with food and water *ad libitum* for the entire period with close observation.

2.5 Hematological Parameters

Hematological analyses were performed on total blood collected in tubes with EDTA. White blood cell count (WBC), red blood cell count (RBC), hemoglobin (Hb), hematocrit (HCT), platelets count, mean corpuscular volume and mean platelet volume were determined using an automatic analyzer (Hospitex Diagnostics Hema Screen 18).

2.6 Biochemical Parameters

Biochemical analyses were performed on serum obtained after centrifugation of total blood without anticoagulant at 2400 rpm for 15 min. The analysis of total protein, ASAT, ALAT, creatinine, total bilirubin, direct bilirubin, total cholesterol, HDL cholesterol and triglycerides were estimated in serum using Commercial kits (Fortress and GCM).

2.7 Relative Organ Weights and Histopathology

After sacrifice, organ weights (heart, kidney, liver, stomach, spleen, lungs, ovary, testicles and adrenals) were recorded and relative organ weights (ROW) were calculated as follows.

$$ROW = \frac{\text{Absolute organ weight (g)}}{\text{Body weight on the day of sacrifice (g)}}\ X\ 100$$

Tissue pieces of vital organs (lungs, liver and kidneys) were fixed in 10 % formalin for paraffin histology and processed in paraffin embedding as per the standard protocol. 5 µm thick sections of each tissue were stained with hematoxylin and eosin, and observed for possible histopathological damages.

2.8 Statistical Analysis

Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Dunnett's test. The results were expressed as mean ± SEM, with *p* values < 0.05 considered as statistically significant.

3. RESULTS

3.1 Acute Toxicity

In acute toxicity study, there were no signs of mortality and behavioral changes even after the oral administration of extract at 2000 mg/kg body weight. The LD50 of *E. praetermissa* extract in rats was estimated above 2.0 g/kg. The result of body weight is tabulated (Table 1).

3.2 Subchronic Toxicity Study

Subchronic toxicity studies were performed to check the overall toxicity of *E. praetermissa*. During the period of experiment, there was no mortality in the extract-treated as well as the control groups.

3.3 Body Weight

E. praetermissa extract did not exhibit any significant changes in body weight of test male and female rats in comparison with the controls. Graphical representations of the changes in body weights of the male and female rats are shown in Figs. 2 and 3, respectively.

3.4 Relative Organ Weights

In male rats treated with *E. praetermissa* at 500 and 1000 mg/kg, there were significant increases in liver and kidney weights compared with the controls, but these effects disappeared in the satellite groups (Fig. 4).

In female rats, the internal organ weights were not significantly changed due to extract treatment compared with the controls (Fig. 5).

3.5 Hematological Parameters

Hematological parameters of extract-treated groups of male and female rats are shown in Tables 2 and 3, respectively. The results of the hematological study in male rats (Table 2) indicated a significant increases ($p<0.05$) in platelet count at the doses of 500 and 1000 mg/kg, although there was no dose-dependent effect. The extract also caused a significant increase in the total WBC count at the dose of 500 mg/kg ($p<0.01$) and 1000 mg/kg ($p<0.00$), and significant increase ($p<0.001$) in the total WBC count in the satellite group (vs. control group). The results of the hematological study of the female rats as shown in Table 3, indicate a significant increase ($p<0.01$) in the platelet count at doses of 500 and 1000 mg/kg (vs. control group).

Table 1. Body weight change of rats during acute toxicity study

Treatment	Dose (mg/kg)	Body weight (g)	
		Initial	2 weeks later
Control	-	119.0 ± 3.79	175.4 ± 1.81
E. praetermissa	2000	119.3 ± 3.76	174.5 ± 6.36

N = 3 animals in each group; Values are expressed as Mean ± SEM

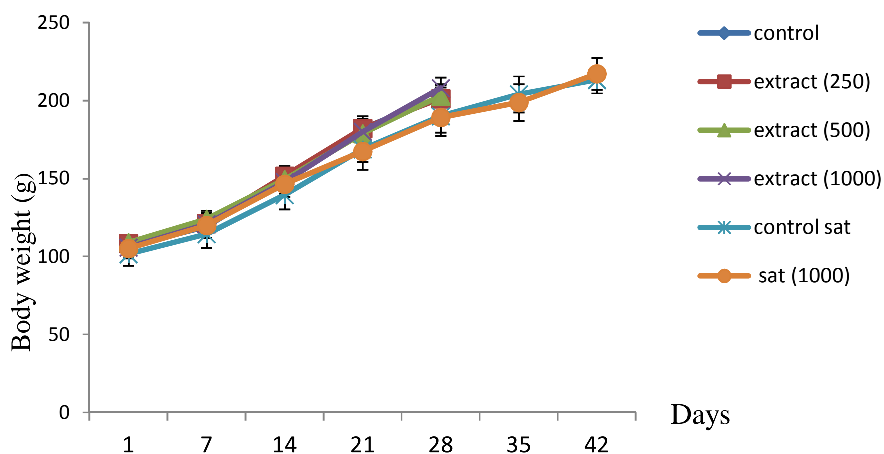

Fig. 2. Effects of *E. praetermissa* on body weight in male rats

Values were expressed as Mean ± SEM. (n=5)

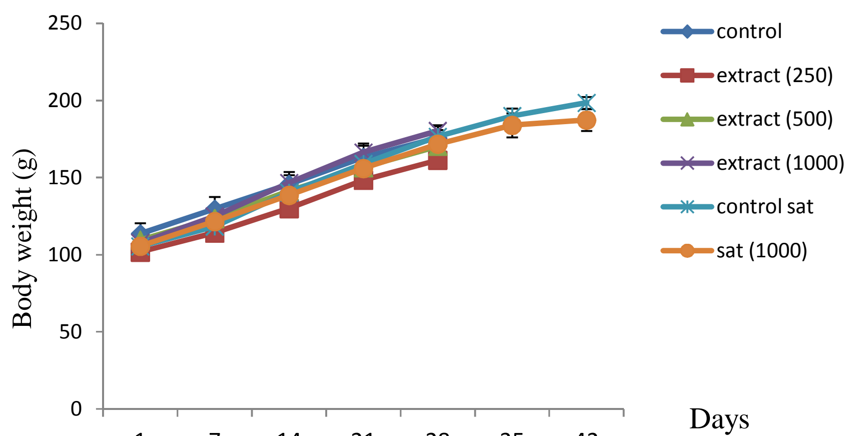

Fig. 3. Effects of *E. praetermissa* on body weight in female rats

Values were expressed as Mean ± SEM. (n=5)

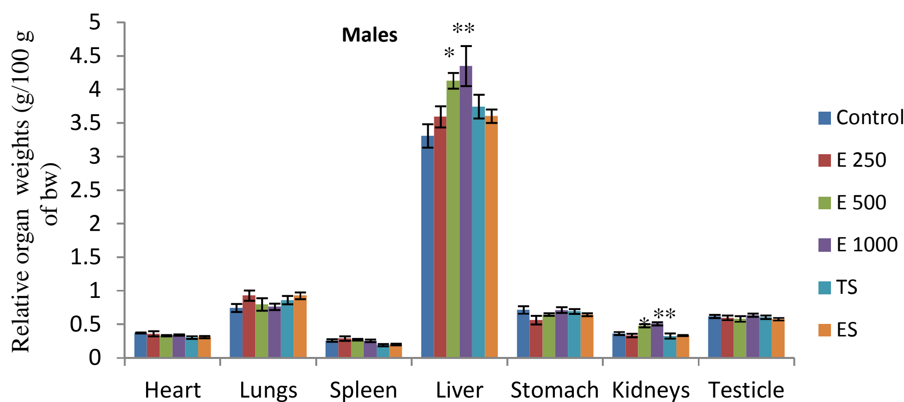

Fig. 4. Effects of ethanolic extract of *E. praetermissa* on relative organ weights in male rats

*Values are expressed as Mean ± SEM of five animals, * = significantly different from control, p< 0.05; ** = significantly different from control, p< 0.01*

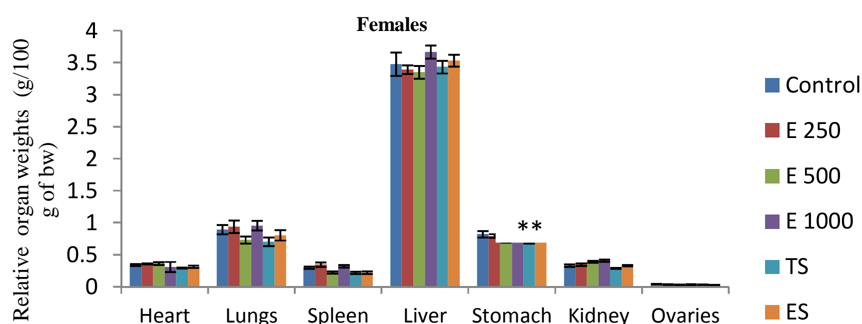

Fig. 5. Effects of ethanolic extract of *E. praetermissa* on relative organ weight in females rats
Values were expressed as Mean ± SEM of five animals (n=5) = significantly different from control, p< 0.05*

3.6 Biochemical Parameters

The effects of graded doses of ethanolic extract of *E. praetermissa* on serum biochemical parameters of male and female rats are shown in Table 4. The ethanolic extract of *E. praetermissa* induced various changes in biochemical parameters according to sex group. In both males and females, the extract significantly reduced HDL cholesterol levels (p<0.01) at the dose of 1000 mg/kg. These changes persisted in the 1000 mg/kg satellite groups for both sexes. In the male rats, *E. praetermissa* induced a significant (p<0.01) increase in ASAT levels at dose 1000 mg/kg and extract-treated satellite group 1000 (p<0.05), a significant (p< 0.001) increase in ALAT at the dose 1000 mg/kg, and a significant decrease of total cholesterol in the extract-treated satellite group (p<0.05). In female rats, *E. praetermissa* induced a significant (p<0.05) increase in ASAT and ALAT. Levels 1000 mg/kg dose, but the values dropped following cessation of treatment. Serum creatinine values increased at the 1000 mg/kg dose in both sexes (p<0.05), and did not revert after treatment stoppage.

3.7 Histopathological Analysis

Microscopic examination of liver sections from vehicle control rats showed normal liver architecture (Fig. 6A), with patent central vein to lower left of male control section. The section shows normal liver traberculae (columnar arrangement of hepatocytes), with no infiltration, inflammation or necrosis. Section from satellite control shows a normal typical portal triad (central vein, an artery to the right and a bile duct underneath).

Liver sections in males and females from dose 250 treatment show central venous congestion (blood in vein lumen), with banal enlargement of hepatic nuclei, but without necrosis or infiltration.

At the dose of 500 mg/kg, there was venous congestion, nuclear enlargement and hyperchromatism (densely colored nuclei). Margination of leucocytes was evident (white blood cells move to the periphery of central vein and line along the endothelium), as well as leucocytes emigration from the vein lumen into the parenchyma. Female section shows no emigration, but with local (non central) interstitial edema and cellular nuclear enlargement.

At the dose of 1000 mg/kg, margination was intense in male rats, with persistence of parenchymal leucocyte infiltration and nuclear enlargement, less hyperchromatism but still no signs of necrosis. Females showed no signs of margination.

Liver sections from male extract satellite animals showed reduced margination and regression of infiltration, but nuclear enlargement and hyperchromatic nuclei persisted. Female extract satellite section shows no margination or infiltration, but have hyperchromatic nuclei and edema.

Lung sections of male and female rats showed similar reactions to *E. praetermissa* treatment (Fig. 7). Sections from control rats show normal lung tissue, with central bronchioles, alveoli and alveolar walls. At dose 250 mg/kg, there was thickening of alveolar walls, and this effect persisted at the dose of 500 mg/kg. The 1000 mg/kg dose provoked visible atelectasis (collapse of the alveoli). This condition did not reverse in the satellite extract group but deteriorated into intense alveolar collapse and intrabronchial hemorrhage, and lung fibrosis was also visible.

Table 2. Effect of the ethanolic extract of *E. praetermissa* on hematological parameters in male rats

Parameters	Control	Extract (250 mg/kg)	Extract (500 mg/kg)	Extract (1000 mg/kg)	Satellite (Control)	Satellite (1000 mg/kg)
RBC (10^6/mm^3)	5.30 ± 0.47	6.19 ± 0.30	6.05 ± 0.62	6.19 ± 0.29	5.55±0.37	6.44±0.37
White blood cells (10^3/mm^3)	7.45 ± 0.32	8.49 ± 0.82	10.31±0.10**	11,58± 0.57***	7.70± 0.23	10.83 ± 0. 33***
Platelet (10^3/mm^3)	166.5 ± 4.84	199.3±17.89	232.0±18.71*	233.3 ± 18.43*	159.0± 0.71	213.3± 3.82
Hemoglobin (g/dL)	10.55 ± 1.30	10.95 ± 1.66	12.63 ± 1.18	12.25 ± 0.99	10.05± 1.05	11.75 ± 0.72
Hematocrit (%)	36.63 ± 0.80	34.75 ± 1.30	39.68 ± 2.32	38.83 ± 1.70	36.38±0.73	39.58±1.07
Mean corpuscular volume (µm^3)	84.00 ±5.31	76.50 ± 4.87	82.75 ± 1.44	84.50 ± 1.19	86.50±3.80	83.75± 1.31
Mean platelet volume (µm^3)	7.75 ± 0.34	7.80 ± 0.31	7.75 ± 0.46	8.08 ± 0.32	8.05 ± 0.23	8.33 ± 0.14

*Values are expressed as Mean ± SEM (n= 5 rats); significantly different from control: *p < 0.05; **p<0.01, ***p<0.001*

Table 3. Effect of the ethanolic extract of *E. praetermissa* on hematological parameters in female rats

Parameters	Control	Extract (250 mg/kg)	Extract (500 mg/kg)	Extract (1000 mg/kg)	Satellite (Control)	Satellite (1000 mg/kg)
RBC (10^6/mm^3)	5.17± 0.41	5.73 ± 0.38	5.83 ± 0,36	6.16 ± 0.19	4.92 ±0.17	5.91±0.14
White blood cells (10^3/mm^3)	8.63 ±0.58	9.22 ± 0.96	11.52 ± 0.87	11.56 ±0.76	8.38±0.22	10.81± 0.31
Platelets (10^3/mm^3)	177.0± 9.81	205.5±10.15	237.3±16.95**	241.8±15.46**	168.5±4.33	211.8±2.39
Hemoglobin (g/dL)	8.88 ±1.09	11.05 ± 1.48	11.38 ± 1.18	13.95 ± 0.35*	12.95± 0.47	12.70± 0.24
Hematocrit (%)	35.20 ±1.12	36.90 ±1.87	36.00 ± 3.67	40.78 ± 1,51	34.20±0.42	41.78±1.05
Mean corpuscular volume (µm^3)	83.00±3.49	79.00 ±3.87	82.00 ± 3.19	83.00 ± 2.12	84.50 ± 2.63	85.50 ± 2.02
Mean platelet volume (µm^3)	6.83 ± 0.29	8.03 ± 0.40	7.20 ± 0.42	7.80 ± 0.25	7.05 ± 0.30	7.63 ± 0.15

*Values are expressed as mean ± SEM (n= 5 rats); significantly different from control: *p < 0.05; **p<0.01*

Table 4. Effects of subchronic toxicity study on biochemical parameters in rats

	Control	Treatment (250 mg/kg)	Treatment (500 mg/kg)	Treatment (1000 mg/kg)	Control satellite	Satellite (1000 mg/kg)
			Male			
ASAT (U/L)	97.96 ± 4.70	109.4±12.73	109.9 ± 1.69	137.4 ± 10.51**	93.96 ±1.59	131.6 ±3.20 *
ALAT (U/L)	36.10 ± 0.66	36.78 ±0.65	37.01 ± 1.80	47.00 ± 1.69 ***	36.60±0.57	48.25 ± 0.86 ***
Total protein (mg/mL)	9.69 ± 0.69	10.57 ± 0.55	10.95 ± 0.33	11.42± 0.26*	9.09 ± 0.29	9.67± 0.34
Total bilirubin (mg/dL)	3.36 ± 0.14	4.00 ± 0.36	3.63 ± 0.16	3.48 ± 0.20	3.39 ± 0.13	3.37 ± 0.11
Bilirubin direct (mg/dL)	0.68 ± 0.01	0.70 ± 0.01	0.94 ± 0.01	0.97 ± 0.01	0.74 ± 0.03	0.85 ± 0.02
Total cholesterol (mg/dL)	114.9 ± 2.02	109.8 ± 1.03	105.3 ± 5.68	105.3 ± 1.60	112.4 ± 2.02	103.3 ± 0.67 *
HDL cholesterol (mg/dL)	69.97 ± 3.55	65.00 ± 1.92	59.78 ± 2.93	55.53 ± 2.72**	71.47 ±2.93	56.03 ± 2.30**
LDL cholesterol (mg/dL)	26.27 ± 2.12	28.00 ± 2.09	29.45 ± 3.52	30.79 ± 2.88	27.77 ± 1.60	32.29 ± 1.89
Triglycerides (mg/dL)	93.53 ±3.15	84.07 ± 5.27	80.31 ± 3.88	94.63 ± 4.72	92.03 ± 3.06	95.38 ±4.39
Creatinine (mg/dL)	0.53 ± 0.03	0.61 ± 0.06	0.49 ± 0.05	0.70 ± 0.02*	0.55 ± 0.04	0.72 ± 0.03*
			Female			
ASAT (U/L)	116.8 ±6.10	128.8±10.69	112.4 ±7.59	149.9 ± 7.90 *	139.4 ± 8.75	140.3± 3.11
ALAT (U/L)	34.38 ± 1.42	35.31 ± 1.07	36.55 ± 0.38	40.22 ± 2.11 *	36.13 ± 0.55	37.97 ± 1.46
Total protein (mg/mL)	9.03 ± 0.80	9.87± 0.35	9.21 ± 0.21	11.34 ± 0.28*	9.21 ± 0.92	9.960 ± 0.68
Total bilirubin (mg/dL)	3.55 ± 0.25	3.09 ± 0.46	3.51 ± 0.76	4.31 ± 0.31	3.60 ± 0.22	4.11 ± 0.27
Bilirubin direct (mg/dL)	1.07 ± 0.05	1.24 ± 0.17	1.45 ± 0.15	1.07 ± 0.08	1.21 ± 0.11	1.00 ± 0.04
Total cholesterol (mg/dL)	113.4 ± 2.36	108.7 ± 2.74	106.9 ± 1.56	107.6 ± 1.19	111.0±1.42	105.3 ±1.40
HDL cholesterol (mg/dL)	67.55 ± 1.15	63.84 ± 2.18	62.04 ± 2.16	59.51 ± 1.06 **	68.30± 1.25	60.51 ± 0.48
LDL (mg/dL)	27.42 ± 1.96	30.97±1.26	30.47 ± 2.41	31.59 ± 1.79	28.42 ±1.60	32.09 ± 1.57
Triglycerides (mg/dL)	92.22 ± 4.22	69.69±8.84*	71.83 ± 7.63	82.48 ± 2.77	89.47± 3.02	83.73 ±1.82
Creatinine (mg/dL)	0.37 ± 0.04	0.51 ± 0.02	0.52 ± 0.09	0.59 ± 0.04*	0.37 ± 0.04	0.59 ± 0.01*

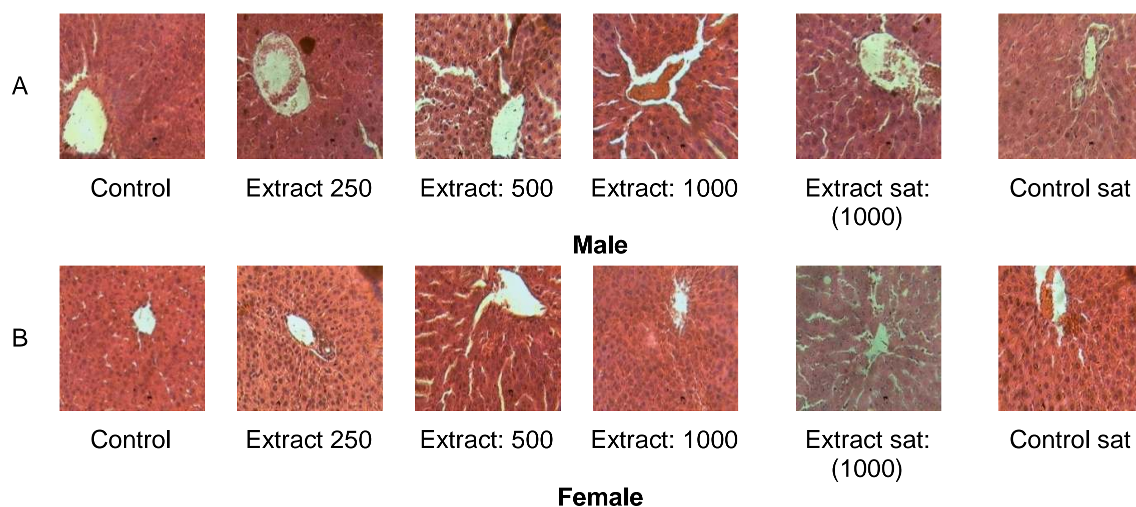

Fig. 6. Histology of liver (H&E, x 400) of control and *E. praetermissa* treated animals. (A) Sections of liver from male animals and (B) Sections of liver from female animals

Sat = Satellite

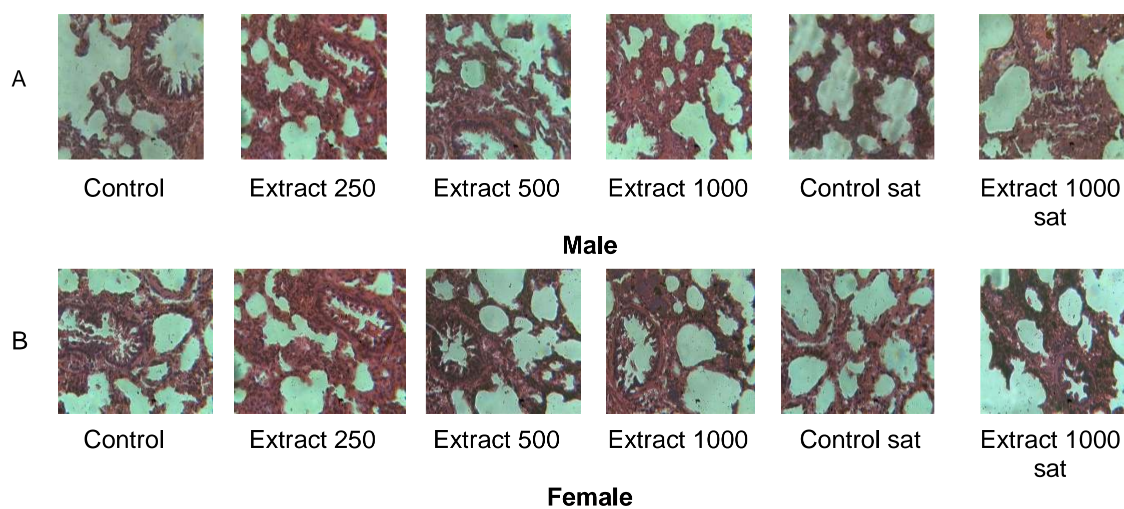

Fig. 7. Histology of lungs (H&E, x400) of control and *E. praetermissa* -treated animals. (A) Section of lungs from male animals and (B) Section of lungs from females animals

Sat = Satellite

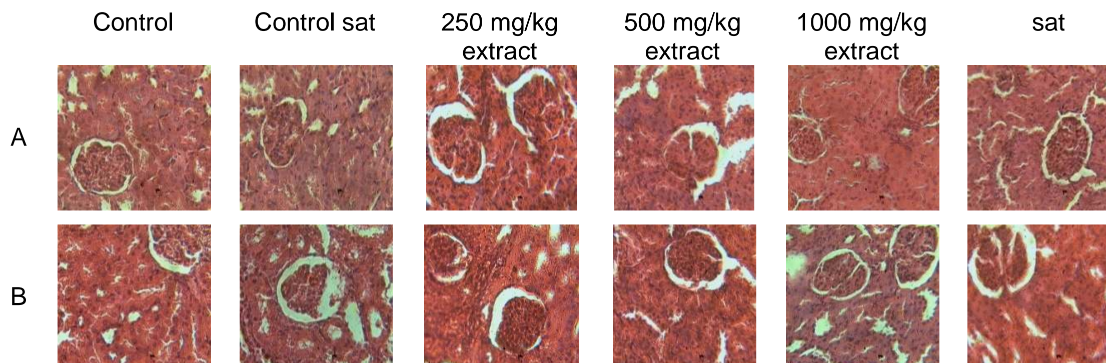

Fig. 8. Histology of kidneys (H&E, x400) of control and *E. praetermissa* treated animals. (A) Section of kidneys from male animals and (B) Section of kidneys from females animals

Sat = Satellite

Examination of kidney sections showed normal kidney architecture in normal rats, but the dose of 250 mg/kg of extract caused mesengeal hyperplasia in males but not in the females. The mesengeal hyperplasia persisted at 500 mg/kg and was present in females. At the dose of 1000 mg/kg, there was obliteration of the Bowman's space in both males and females. This condition showed signs of recovery in the 1000 mg/kg satellite controls (Fig. 8).

4. DISCUSSION

The present study was carried out to evaluate the acute and subchronic toxicity of leaves ethanolic extract of *E. praetermissa* in male and female rats. The results of the acute toxicity study showed that extract doses up to 2000 mg/kg did not result in death of the animals. In addition, no changes attributable to extract treatment were found in body weight, respiratory rate and general behavior. Similar observations were made in sub chronic oral toxicity study. Generally, reductions in body weight gain and internal organ weights are simple and sensitive indices of toxicity after exposure to toxic substances. Organ weight is also an important index of physiological and pathological status in animals. The heart, liver, kidney, spleen, and lungs are the primary organs affected by metabolic reaction caused by toxicants [11]. The extract-treated females rats showed no significant differences in body weight gain and internal organ weight except for kidneys. However, the male rats given 500 and 1000 mg/kg of extract showed significant increase in the relative weights of liver and kidneys Satellite (1000 mg/kg) rats did not show any change internal organ weights. The liver, being a key organ in the metabolism and detoxification of xenobiotics, is vulnerable to damage induced by a huge variety of chemicals [11]. Thus, the observed significant ($P<0.05$ and $P<0.01$) increase in liver weight of the group administered orally with 500 mg/kg and 1000 mg/kg body weight of *E. praetermissa* after 28 could be attributed to high rate of metabolism of the liver resulting from the brief exposure to extract (containing several constituents). In general, increase in relative liver weight is due to inflammation [12]. The kidney is the primary organ for plasma clearance, homeostasis and excretion of xenobiotics including drugs and drug products from the body [13]. Nonetheless, all observed changes were slight and the differences could have been due to variation in size of internal organs of the animals [14].

The hematopoietic system is very sensitive to toxic compounds and serves as an important index of the physiological and pathological status for both animals and humans [15]. Analysis of blood parameters is relevant to risk evaluation and the changes in the hematological system have a higher predictive value for human toxicity, when the data are translated from animal studies [16]. The assessment of hematological parameters could be used to reveal the deleterious effect of foreign compounds including plant extracts on the blood constituents of animals. Hematological parameters provide vital information regarding the status of bone marrow activity and intravascular effects such as hemolysis and anemia [17]. In our study, *E. praetermissa* ethanolic extract at 500 and 1000 mg/kg increased the level of total WBC count $P<0.001$ in male rats. The increase of total WBC count may be a consequence of inflammatory reactions produced in damaged tissues of extract-treated animals [18]. The significant increase in WBC was persisted in the male satellite rats but not in the females. Increase in WBC may indicate the impact of *E. praetermissa* in boosting the immune system of treated animals. However, these values were within the normal range [19,20], suggesting that the extract does not affect hematopoiesis and leukopoiesis in rats at the low dose. A significant increase of blood platelets was observed in rats treated with the extract at doses of 500 and 1000 mg/kg. Usually significant decrease in blood platelet count reduces the blood clotting ability, which would lead to severe and prolonged bleeding [21,22]. This present results suggest no risk of coagulation inhibition due to prolonged administration of the plant extract.

Serum biochemical markers are generally employed to asses liver function. Serum enzymes, ASAT and ALAT, are quantitative markers for the determination of various liver and body diseases. It is established that ASAT can be found in the liver, cardiac and skeletal muscle, whereas ALAT is predominantly present in the liver [23,24]. In the present study, the significant changes in ALAT and ASAT in both male and female rats at the dose of 1000mg/kg (Table 4) suggests that the extract may have deleterious effects on liver function. Generally, damage of the liver parenchyma induces elevation of transaminases in blood. Thus any increase is a sign of first cell damage that induces the reflux of these enzymes in serum [25]. This effect was more pronounced in male rats especially at high

doses. This is confirmed by the fact that the liver of satellite group rats also presented a significant changes in ALAT. This finding was confirmed by the histology of the liver which revealed the presence of inflammatory cells in some rats treated with the dose of 1000 mg/kg/day. Almost all of the plasma proteins are synthesized in the liver. Thus, low plasma protein levels would indicate either liver disease or nephrotic syndrome in which there is excessive shedding of plasma proteins by the kidneys, while high levels suggests liver disease or an inflammatory or immune response in general [12]. Serum protein levels increased significantly (P <0.01) in 1000 mg/kg group but returned to normal within two weeks following discontinuation of treatment, which shows that any inflammatory effect of extract at high doses is transient and reversible.

The decrease in plasma total cholesterol levels observed in satellite male rats given the extract may be attributed to the presence of hypolipidemic agents (terpenoids and flavonoids) in the extract [26]. The general lack of significant changes in HDL and LDL levels indicate that the extract had no effect on lipid metabolism of animals. However, the non-significant decrease in HDL-cholesterol levels and the increase in LDL-cholesterol levels observed in rats administered 250 mg/kg and 500 mg/kg is an indication that low doses of the extract may not increase cardiovascular risk factors which contribute to death of diabetic subjects [27]. In this study, the extract significantly decreased plasma HDL cholesterol levels at 1000 mg/kg, suggesting that prolonged intake of high doses may pose the risk of cardiovascular disease [28, 29] often found in hypertension [30], obesity [31] and diabetes mellitus [32]. Clinical data shows that increase in plasma HDL cholesterol concentration increases cardiovascular risk [33] and increases in plasma HDL cholesterol have been considered to reduce the risk of coronary heart disease [33]. High HDL exerts its protective effect by decreasing the rate of entry of cholesterol into the cell via LDL and by increasing the rate of cholesterol release from the cell [34].

Bilirubin is formed by the breakdown of hemoglobin in the liver, spleen and bone marrow. An increase in tissue or serum bilirubin concentrations occurs as a result of increased breakdown of RBC (hemolysis) or liver damage e.g., hepatitis or bile duct obstruction. The normal levels of serum bilirubin concentrations at all doses of the extract used in this study are indicative of non-adverse effects of the extract on hemoglobin metabolism pathways.

Creatinine levels provide information on the degree of renal filtration. It is a constituent of muscle proteins which is eliminated only by the kidney, and is thus a marker of renal function [35]. Increases in serum creatinine levels reflect a functional defect at the level of the nephron [36]. Serum creatinine values may also vary as a result of extra kidney factors such as excessive protein intake in the diet and high muscle catabolism related to body mass and age. The extract induced dose-dependent increases in creatinine levels which were significant at the 1000 mg/kg dose and persisted following stoppage of treatment. This suggests that high doses of extract may cause irreversible kidney damage, especially renal filtration mechanism [37].

The histopathological observations of liver and kidney samples provided supportive evidence for the hematological and biochemical analysis. The enlarged nuclei represent a dystrophic lesion, simple hypertrophy which, associated with cellular edema, can transform into hepatomegaly (liver enlargement). Leucocytes margination, venous congestion and vasodilation are reactions characteristic of the inflammation process, which can translate as toxic hepatitis i.e., hepatitis due to chemical injury induced by high doses of extract. However, the absence of margination in females at this dose suggest that they do not show the same inflammatory reaction as the males. The observed mild hepatitis at 500 mg/kg and with persistence of parenchymal leucocyte infiltration, nuclear enlargement and intense margination at 1000 mg/kg suggest that the extract of *E. praetermissa* may be toxic if taken for prolonged periods at elevated doses.

In lung tissue, the thickening of alveolar walls thickened at low doses and collapsed alveoli at 1000 mg/kg (atelectasis). This condition which did not reverse in the satellite group but deteriorated into intense alveolar collapse with intrabronchial hemorrhage and fibrosis is referred to as lung consolidation, can cause the lung tissue to become solid and affected patients would cough up blood.

In kidney tissue, the observed mesengeal hyperplasia can lead to increased tubular

reabsorption with consequent increase in blood volume and possibility of hypertension.

5. CONCLUSION

The present study was conducted to verify the toxicity of *E. praetermissa*. Acute toxicity study of the extract as per the latest OECD guidelines revealed that its LD50 is more than 2.0 g/kg body weight in albino rats. The results of subacute toxicity study of 28 days duration suggest that high doses of the extract taken for long periods can result in toxic effects in liver, lungs and kidneys.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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