

Studies on sub-acute oral toxicity of ethanolic extract of aerial parts of *blumea laevis* (EABL) in sprague dawley rats

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Received: 19.12.2022; Accepted: 20.2.2023

ABSTRACT

Blumea genus belonging to *Asteraceae* family consists of more than 80 species, among which *Blumea laevis* is found abundantly sprouting during the post monsoon season and available full-fledged with flowers and fruiting from December to June in different regions of Kerala, the present study was undertaken as several veterinarians reported of high mortality in goats ascribing of ingesting *Blumea laevis* plant. This present study was envisaged to ascertain sub-acute toxic effects by evaluating the haemato-biochemical and pathomorphological alterations in rats fed with aerial parts of *Blumea laevis*. In the repeated dose 28-day oral toxicity study (OECD407) the extract administered at dose rates of 250, 500, 1000 mg/kg when compared to control group of animals, showed a significant increase ($p < 0.05$) in total leucocyte count (TLC) alongside significant increase ($p < 0.05$) in serum biochemical parameters of ALT, AST, ALP and BUN in both male and female treatment groups. On gross examination no specific lesion was observed albeit on microscopic examination showed minimal coagulative necrosis, vacuolar degeneration of hepatocytes and inflammatory cell infiltration in the liver and tubular epithelial cell degeneration and hyperplasia of the tubules were evident in the kidneys of rats receiving highest dose of EABL extract which clearly is conclusive of minimal toxicity in rats fed with EABL extract for 28 days.

Keywords: *Blumea laevis*, body weight, EABL, Pathology, sub-acute, toxicity

INTRODUCTION

Nature has been a great source for the reapeutics for thousands of years and plant-based products continue to play a key role in the primary health care of about 80-85% of the world's population¹. Medicinal plants have increasingly gained public and professional acceptance due to progress in the understanding of the mechanisms by which these plants positively influence health and quality of life². Medicinal plants are widely used for the treatment of a variety of diseases and assumed to be safe; Lack of awareness on toxic metabolites of medicinal herbs and their haphazard use can result in toxicity associated health problems. Plants with secondary metabolites such as cyanide, alkaloids, tannins, saponins, phenolic compounds and terpenoids, when ingested can cause acute or chronic illness affecting normal physiology and productivity leading to severe livestock loss³. These medicinal plants can potentially be harmful at excessive doses or can interact with modern drugs⁴. According to the World Health Organization (WHO), there is an increasing public demand for research on bridging knowledge gaps about safety of medicinal plants⁵.

Furthermore, Ethnoveterinary medicines are being used for folk skills, beliefs, knowledge, practices and methods related to animals' health, and to cure various ailments in the ethnic group areas⁶. Ethnoveterinary medicine is the overall scientific term for traditional animal health care, which provides low-cost alternatives to allopathic drugs. The utilization of traditional ethnoveterinary remedies provides a cheaper, easier, and more sustainable alternative to synthetic drugs and pharmaceuticals⁷. Ethnoveterinary medicine has been used for a long time, especially in countries with more developed animal husbandry, such as Italy⁸, Spain⁹, Navarra¹⁰, East Africa¹¹, Pakistan¹², Brazil¹³, India¹⁴, and China¹⁵. Traditional and low-cost methods to treat animal diseases instead of synthetic

How to cite this article : Kumar, Y.R., Nair, D.N., Prasanna, K.S., Raj, A.R., Palekkodan, H., George, A.J. and Rani, S. 2023. Studies on sub-acute oral toxicity of ethanolic extract of aerial parts of *blumea laevis* (EABL) in sprague dawley rats. Indian J. Vet. Pathol., 47(2) : 142-149.

drugs are often desired.

Blumea is an erect glabrous shrub in *Asteraceae* family consists of more than 80 species. This plant is commonly grown in tropical and sub-tropical zones of Asia, especially the Indian Subcontinent and Southeast Asia¹⁶. Although several literatures are available with regard to *Blumea* genus for its medicinal properties, very less is known for its toxicity. Since the information regarding *Blumea laevis* is scanty or absent this study is undertaken to contribute scientific data on safety of the ethanol extract of aerial parts of *B. laevis* (EABL) in rats. Hence, in

the current study we aimed to determine the sub-acute oral toxicity of ethanolic extract of aerial parts of *B. laevis* (EABL) in Sprague Dawley rat model study.

MATERIALS AND METHODS

The study was performed as per the Organization for Economic Cooperation and Development (OECD) Guidelines for the Testing of Chemicals No. 407¹⁷. The study was approved by Institutional Animal Ethics Committee of Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Pookode.

Procurement and Preparation of Plant Material

Aerial parts of *B. laevis* plant in its flowering stage was collected in the month of December 2016 from Malappuram district of Kerala. The plant was identified and authenticated by plant taxonomist and plant specimen was assigned with accession no. 88480. Herbarium and passport data sheets of plant were prepared accordingly and voucher specimen was deposited in the Department of Botany, University of Calicut. The plant materials were cleaned, shade dried and ground to coarse powder using blender. Powdered samples were collected and stored in a closed container until required for extraction.

Preparation of Ethanol Crude Extract

The 100 grams of the powdered sample was extracted by continuous hot extraction using the Soxhlet apparatus with 95% ethanol. The extract was then concentrated and reduced under pressure through Rotary vacuum evaporator (M/s. Buchi, Switzerland) at temperature ranging from 40-50°C. The concentrated extract was air dried at room temperature for about 24 h and placed in suitable containers and preserved in refrigerator for further studies.

Experimental Animals

Twenty healthy young male and twenty healthy non-pregnant, nulliparous Sprague-Dawley male and female rats, 6 to 8 weeks old and weighing between 130 to 200 gm obtained from the Small Animal Breeding Station of College of Veterinary and Animal Sciences, Mannuthy, Kerala were used for the study. The rats were kept in polypropylene cages in the animal house with an ambient temperature of 25°C, 12 h light and 12 h dark periodicity. The rats were fed with standard diet and water *ad libitum*

and allowed to acclimatize for seven days before the procedure. They were randomly assigned to the cages and the individual animal was further marked with felt tip marker pen. The veterinary examination was done before the allocation of animals to groups and after the completion of acclimatization period.

Experimental Design

The experiment was performed as per OECD guidelines 407 for sub-acute oral toxicity study. Animals were divided into 4 groups of 5 male and five female rats. The animals were fasted for 12 hr prior to the experiment with free access to water. Prior to dosing each animal was fasted overnight and weighed prior to extract administration. The procedure was performed by following the OECD guideline (2001) and WHO guideline (2000)^{18,19}. The animals were orally given a single dose of the EABL with a volume of 2 mL/100g body weight. The control group was treated with distilled water and the other 3 groups were daily treated by oral administration of EABL at different doses of 250, 500, and 1000 mg/kg bwt for 28 days. All the experimental animals were observed daily for any abnormal clinical signs, body weight change and mortality for 28 days. The body weight of the experimental animals was recorded at weekly intervals (Table 1). On day 28, the animals were subjected to overnight fasting and later sacrificed under mild anesthesia.

Haematology and Biochemical Analysis

Blood was collected from animals during the terminal sacrifice in EDTA containing vials (EDTA 2 mg/mL blood). Hematological parameters such as PCV (Wintrobe method), Hemoglobin (Sahli's Acid hematin method), total leucocyte count (Hemocytometer method) Differential leucocyte count (Wright's staining method) were performed. During terminal sacrifice, 2 mL of blood was collected from each animal in a clean non-heparinized vial and were allowed to clot. Sera were separated by centrifuging the clot at 2000 rpm for 20 minutes, Estimation of Glucose (GOD/POD method), ALT (IFCC method), AST (IFCC method), ALP (P-Nitrophenyl phosphate, kinetic method), Creatinine (Jaffe's kinetic and end point method), BUN (GIDH.UV enzymatic method), Total Protein (Biuret method) were carried out using kits procured from Euro diagnostics.

Histopathological examination

Table 1. Experimental design.

Group	Treatment	No. of Animals		Study Duration
		Male	Female	
Group I	Animals receiving vehicle (Control group)	5	5	28 Days
Group II	Animals receiving EABL at 250 mg/kg bwt.	5	5	
Group III	Animals receiving EABL at 500 mg/kg bwt.	5	5	
Group IV	Animals receiving EABL at 1000 mg/kg bwt.	5	5	

All the animals were subjected to overnight fasting and on the day, (29th day of the experiment) animals were weighed and humanely sacrificed. The organs were carefully observed for any gross lesions. The weights of the organs *viz.* liver, adrenal, kidney, epididymis/uterus, spleen, heart, thymus, brain, testis/ovary were recorded. The relative organ weights were calculated and expressed in percentage as given below:

Relative organ weight (%) =

$$\frac{\text{Weight of the organ (g)} \times 100}{\text{Body weight (g)}}$$

Organs were collected in 10% neutral buffered formalin (NBF) for histopathology. Formalin fixed paraffin embedded tissues were sectioned to 5 μ m thickness and stained by Haematoxylin & Eosin (H&E) for histopathological examination.

Statistical analysis

The data on different parameters obtained from distinct groups were subjected to one-way analysis of variance (ANOVA) and paired t-test followed by multiple range test by Duncan's for grouping means having significant difference was carried out using software SPSS version 16.0.

RESULTS AND DISCUSSION

Sub-acute oral toxicity study

The study was designed to determine the sub-acute oral toxicity profile of EABL in female Sprague-Dawley rats. Rats are one of the recommended rodent species by the regulatory authorities for conducting preclinical toxicity study among rodents. In the sub-acute oral toxicity study as per the OECD Test Guideline 407, the animals fed with EABL at different doses of 250, 500, and 1000 mg/kg bwt for 28 days.

Body Weight

The mean body weights of animals receiving EABL at

different dose levels at weekly intervals are presented in the Table 2. Results depicted that there was no significant differences in body weight between different treatment groups in both male and female at any of the time interval.

Hematological parameters

The mean values of various hematological parameters analyzed such as hemoglobin (Hb), packed cell volume (PCV), total leucocyte count (TLC) and differential leucocyte count (DLC) in both male and female rats are represented in Table 3. Results depicted that there was a significant increase ($p < 0.05$) in the TLC in Group-IV (1000 mg/kg bwt) as compared to control and other treatment groups *viz.* Group II & Group III in both male and female animals. Comparison of the mean values of DLC, the neutrophils, monocytes and eosinophils showed no-significant difference between groups in male and female rats, whereas there was a significant reduction ($p < 0.05$) in lymphocyte count when compared with the control rats.

Serum Biochemical Parameters

Glucose

There was no significant difference between control and treatment groups in male rats, whereas in female rats, a significant increase ($p < 0.01$) in blood glucose level was observed in group IV when compared to group II and group III animals; but there was no significant difference observed between control, group II and group III animals (Table 4).

Liver function test

In male rats mean values of ALT, was found to be highly significant increase ($p < 0.01$) in all treatment groups as compared to control rats. No significant difference in ALT values was observed among the treatment groups in male rats. In female rats mean ALT values of the treatment groups increased significantly high ($p < 0.01$) in comparison with the group-I animals. There was a significant difference in ALT values between group-II and group-IV, where as no significant difference in ALT values was observed between group-III and group

Table 2. Effect of 28-day sub-acute oral administration of EABL on bodyweights (g).

Body Weight	Sex	Group I (Control)	Group II (250 mg/kg)	Group III (500 mg/kg)	Group IV (1000 mg/kg)	F-value
0 day	Male	191.80 $\pm$ 12.24	195.00 $\pm$ 11.89	190.00 $\pm$ 6.40	195.40 $\pm$ 13.23	0.05 ^{ns}
	Female	183.00 $\pm$ 12.47	187.40 $\pm$ 7.89	190.00 $\pm$ 6.40	186.67 $\pm$ 4.17	0.11 ^{ns}
7 th day	Male	221.40 $\pm$ 11.30	211.80 $\pm$ 8.29	206.80 $\pm$ 6.47	199.00 $\pm$ 13.24	0.85 ^{ns}
	Female	189.40 $\pm$ 11.64	195.00 $\pm$ 8.90	204.20 $\pm$ 6.80	191.33 $\pm$ 8.45	0.51 ^{ns}
14 th day	Male	228.80 $\pm$ 9.97	224.50 $\pm$ 12.82	202.67 $\pm$ 9.77	212.00 $\pm$ 15.57	0.85 ^{ns}
	Female	200.40 $\pm$ 12.91	206.60 $\pm$ 15.74	211.00 $\pm$ 1.82	192.33 $\pm$ 18.85	0.28 ^{ns}
21 st day	Male	242.40 $\pm$ 8.68	230.75 $\pm$ 12.95	220.50 $\pm$ 0.50	210.00 $\pm$ 15.83	1.41 ^{ns}
	Female	210.80 $\pm$ 12.23	224.25 $\pm$ 21.45	212.00 $\pm$ 3.00	197.33 $\pm$ 22.01	0.43 ^{ns}
28 th day	Male	247.40 $\pm$ 8.86	232.50 $\pm$ 12.62	224.00 $\pm$ 12.0	208.25 $\pm$ 16.39	1.83 ^{ns}
	Female	214.00 $\pm$ 12.42	225.00 $\pm$ 24.31	213.25 $\pm$ 8.41	188.67 $\pm$ 19.87	0.67 ^{ns}

Values were expressed Mean $\pm$ SEM; NS - Non-Significant

Table 3. Effect of 28-day repeated sub-acute oral toxicity study of EABL on hematology.

Hematology Parameters	Sex	Group I (Control)	Group II (250 mg/kg)	Group III (500 mg/kg)	Group IV (1000 mg/kg)	F-value
Hb (g/dl)	Male	14.08 ± 0.17	13.05 ± 0.37	13.25 ± 0.55	13.77 ± 0.58	2.54 ^{ns}
	Female	12.96 ± 0.95	12.15 ± 0.27	12.45 ± 0.28	12.20 ± 0.41	1.11 ^{ns}
PCV (%)	Male	42.80 ± 0.86	41.75 ± 0.85	41.50 ± 1.50	42.00 ± 1.08	0.095 ^{ns}
	Female	41.20 ± 0.37	39.00 ± 0.47	40.50 ± 0.28	40.33 ± 0.33	2.65 ^{ns}
TLC×10 ³ /mm ³	Male	8.22 ± 0.20 ^b	7.65 ± 0.37 ^{bc}	7.00 ± 0.10 ^c	9.13 ± 0.23 ^a	8.99 ^{**}
	Female	7.56 ± 0.22 ^c	8.15 ± 0.20 ^{bc}	8.40 ± 0.14 ^{ab}	8.80 ± 0.11 ^a	6.92 ^{**}
Lymphocytes (%)	Male	70.20 ± 2.13	61.25 ± 2.50	65.00 ± 9.00	73.50 ± 2.02	3.36 ^{ns}
	Female	70.40 ± 1.50 ^a	64.00 ± 1.47 ^{ab}	65.50 ± 1.85 ^{ab}	60.33 ± 4.48 ^b	3.58 [*]
Neutrophils (%)	Male	24.60 ± 0.92	24.50 ± 0.95	26.00 ± 1.00	27.25 ± 0.62	1.67 ^{ns}
	Female	25.60 ± 1.50	33.00 ± 1.96	33.00 ± 2.52	35.67 ± 4.84	3.152 ^{ns}
Monocytes (%)	Male	1.40 ± 0.40	1.25 ± 0.25	1.00 ± 0.00	1.00 ± 0.48	0.27 ^{ns}
	Female	2.00 ± 0.32	1.50 ± 0.29	2.00 ± 0.00	1.33 ± 0.67	0.94 ^{ns}
Eosinophils (%)	Male	1.80 ± 0.20	1.75 ± 0.25	1.50 ± 0.50	2.00 ± 0.00	0.62 ^{ns}
	Female	2.00 ± 0.32	2.00 ± 0.71	1.27 ± 0.25	2.00 ± 0.58	2.38 ^{ns}

NS - Non-Significant, *Significant at 0.05 level, **Significant at 0.01 level. Means having different letters as superscripts are significantly different within row.

IV animals (Table 4).

The mean values of AST increased significantly high ($p < 0.01$) in group-IV compared to control. The mean values of AST were non-significantly higher in group-III than group-II. But in female, no significant difference was observed between control, group-II and group-III animals (Table 4).

Comparison of ALP mean values in both male and female rats revealed a significantly high ($p < 0.01$) increase ($p < 0.01$) of ALP level in treatment groups with respect to control groups. In male rats, ALP level differed highly significantly ($p < 0.01$) between group-I and group-IV

animals while no significant difference was observed between group-II and group-III animals. In female rats significantly high ($p < 0.01$) increase ($p < 0.01$) in the ALP level in group IV animals in comparison with group II was observed (Table 4). However, Comparison of mean values of total protein (TP) did not show any significant difference between control and treatment groups (Table 4).

Kidney function test

Analysis of mean values of BUN revealed a significant difference ($p < 0.01$ & $p < 0.05$) between control and group-IV animals in both male and female rats. There was a highly significant increase ($p < 0.01$) in the

Table 4. Effect of 28-day repeated sub-acute oral toxicity study of EABL on biochemical parameters.

Biochemical Parameters	Sex	Group I (Control)	Group II (250 mg/kg)	Group III (500 mg/kg)	Group IV (1000 mg/kg)	F-value
Glucose (IU/L)	Male	98.40 ± 4.57	102.75 ± 14.79	142.50 ± 13.50	112.92 ± 15.40	1.77 ^{ns}
	Female	79.14 ± 3.54 ^b	81.47 ± 4.38 ^b	77.25 ± 14.40 ^b	124.73 ± 0.37 ^a	6.81 ^{**}
BUN (mg/dl)	Male	21.79 ± 0.59 ^b	19.96 ± 0.81 ^{ab}	25.27 ± 3.23 ^{ab}	25.99 ± 0.53 ^a	6.39 ^{**}
	Female	20.12 ± 0.303 ^b	23.60 ± 1.72 ^{ab}	22.33 ± 1.32 ^b	25.30 ± 1.55 ^a	3.80 [*]
Creatinine (mg/dl)	Male	0.56 ± 0.07	0.49 ± 0.05	0.49 ± 0.03	0.48 ± 0.13	0.18 ^{ns}
	Female	0.52 ± 0.02	0.55 ± 0.08	0.51 ± 0.09	0.57 ± 0.03	0.17 ^{ns}
ALT (IU/L)	Male	69.80 ± 4.73 ^b	98.24 ± 7.25 ^a	108.81 ± 2.57 ^a	122.70 ± 6.68 ^a	8.25 ^{**}
	Female	65.09 ± 4.17 ^c	90.45 ± 0.82 ^b	93.44 ± 2.00 ^{ab}	103.15 ± 4.21 ^a	26.35 ^{**}
AST (IU/L)	Male	109.99 ± 3.59 ^c	167.43 ± 5.04 ^b	172.73 ± 13.24 ^b	202.51 ± 6.09 ^a	54.29 ^{**}
	Female	182.21 ± 5.59 ^b	174.56 ± 6.11 ^b	170.10 ± 5.38 ^b	210.40 ± 6.77 ^a	7.54 ^{**}
ALP (IU/L)	Male	241.60 ± 13.6 ^c	402.07 ± 54.63 ^b	461.16 ± 6.37 ^{ab}	526.94 ± 3.83 ^a	18.03 ^{**}
	Female	228.98 ± 7.09 ^c	329.61 ± 5.34 ^b	328.68 ± 18.60 ^b	460.86 ± 19.41 ^a	52.28 ^{**}
Total Protein (g/dl)	Male	5.90 ± 0.18	4.38 ± 0.19	5.62 ± 0.20	5.29 ± 0.45	8.24 ^{ns}
	Female	4.94 ± 0.77	4.19 ± 0.16	5.52 ± 0.18	5.91 ± 0.97	2.75 ^{ns}

Values are expressed as Mean ± SEM; NS - Non-Significant, *Significant at 0.05 level, **Significant at 0.01 level. Means having different letters as superscripts are significantly different within row.

Table 5. Effect of 28-day repeated sub-acute oral toxicity study of EABL on male organ weights.

Male Organ	Group I (Control)	Group II (250 mg/kg)	Group III (500 mg/kg)	Group IV (1000 mg/kg)	F-value
Brain	0.63 ± 0.01	0.61 ± 0.00	0.65 ± 0.03	0.66 ± 0.01	2.55 ^{ns}
Heart	0.27 ± 0.00	0.32 ± 0.02	0.28 ± 0.01	0.31 ± 0.00	2.65 ^{ns}
Liver	2.66 ± 0.06	3.16 ± 0.23	2.86 ± 0.43	3.22 ± 0.26	1.69 ^{ns}
Kidney	0.61 ± 0.01	0.62 ± 0.00	0.65 ± 0.02	0.66 ± 0.02	1.79 ^{ns}
Adrenal	0.008 ± 0.00	0.009 ± 0.00	0.008 ± 0.00	0.010 ± 0.00	1.54 ^{ns}
Spleen	0.24 ± 0.02	0.28 ± 0.01	0.27 ± 0.04	0.24 ± 0.01	0.58 ^{ns}
Thymus	0.06 ± 0.00	0.06 ± 0.00	0.07 ± 0.03	0.08 ± 0.00	1.09 ^{ns}

Values are expressed as Mean ± SEM; NS - Non-Significant

Table 6. Effect of 28-day repeated sub-acute oral toxicity study of EABL on female organ weights.

Female Organ	Group I (Control)	Group II (250 mg/kg)	Group III (500 mg/kg)	Group IV (1000 mg/kg)	F-value
Brain	0.63 ± 0.01	0.67 ± 0.01	0.67 ± 0.00	0.64 ± 0.02	1.55 ^{ns}
Heart	0.24 ± 0.01	0.25 ± 0.01	0.27 ± 0.00	0.26 ± 0.01	1.30 ^{ns}
Liver	3.40 ± 0.11	3.44 ± 0.22	4.00 ± 0.16	3.49 ± 0.53	1.28 ^{ns}
Kidney	0.59 ± 0.04	0.58 ± 0.01	0.58 ± 0.011	0.55 ± 0.05	0.20 ^{ns}
Adrenal	0.01 ± 0.00	0.009 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	1.47 ^{ns}
Spleen	0.28 ± 0.00	0.27 ± 0.01	0.27 ± 0.01	0.26 ± 0.00	0.39 ^{ns}
Thymus	0.08 ± 0.00	0.07 ± 0.00	0.08 ± 0.00	0.08 ± 0.00	1.20 ^{ns}

Values are expressed as Mean ± SEM; NS - Non-Significant

BUN level in group IV male rats when compared to group I male rats. However, there was no significant difference between group-I, group-II and group-III animals. Comparison of mean values of BUN in female rats revealed a significant increase ($p<0.05$) in the BUN

level in group-IV animals with respect to group-I and group-III animals. No significant difference was observed between group-I, group-II and group-III animals. Statistical analysis showed no significant difference in serum creatinine level between control and treatment

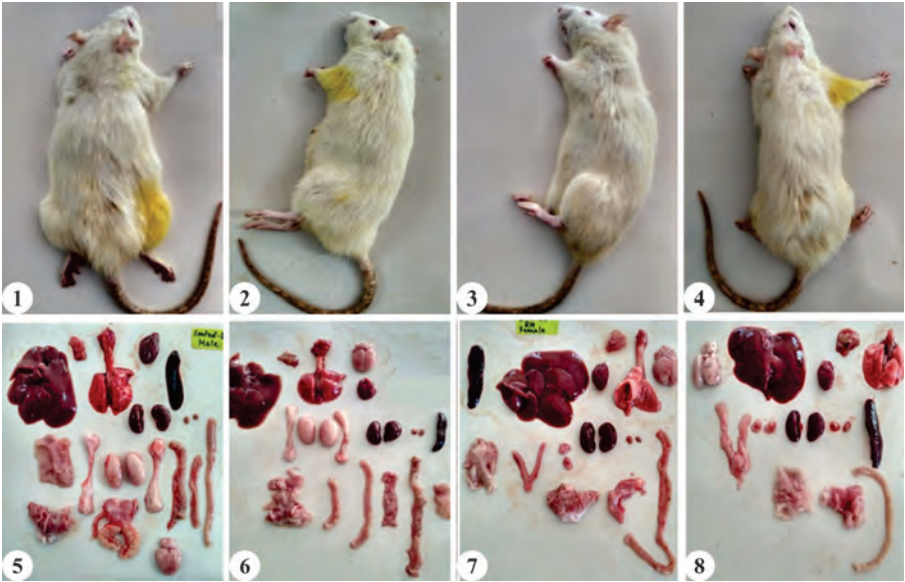


Plate 1. Male rat, Group I-Control; **Plate 2.** Male rat, Group IV (1000 mg/kg). Body condition comparable to Group I animals; **Plate 3.** Female rat, Group I-Control; **Plate 4.** Female rat, Group IV (1000 mg/kg). Body condition comparable to Group I animals; **Plate 5.** Male rat, Group I-Control. No gross lesions in visceral organs; **Plate 6.** Male rat, Group IV (1000 mg/kg). No gross lesions in visceral organs; **Plate 7.** Female rat, Group I-Control. No gross lesions in visceral organs; **Plate 8.** Female rat, Group IV (1000 mg/kg). No gross lesions in visceral organs.

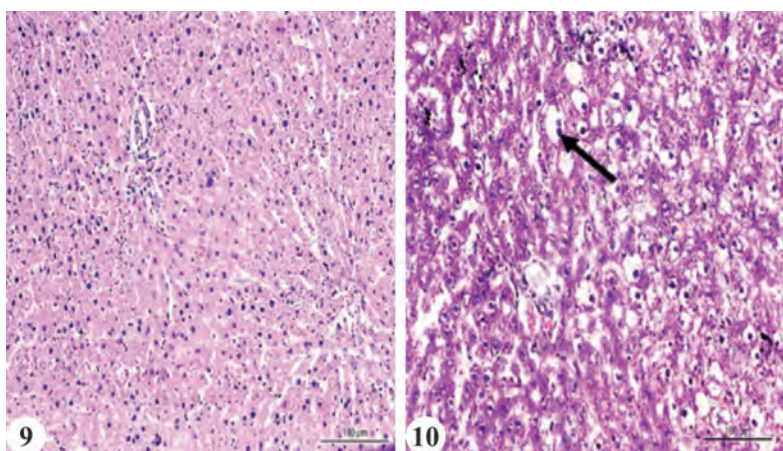


Plate 9. Female rat-Liver, Group III (500 mg/kg), Vacuolar degeneration of hepatocytes-Ballooning of hepatocytes (arrow) (H&E 200X); **Plate 10.** Female rat-Liver, Group III (500 mg/kg), Vacuolar degeneration of hepatocytes-Ballooning of hepatocytes (arrow) (H&E 200X).

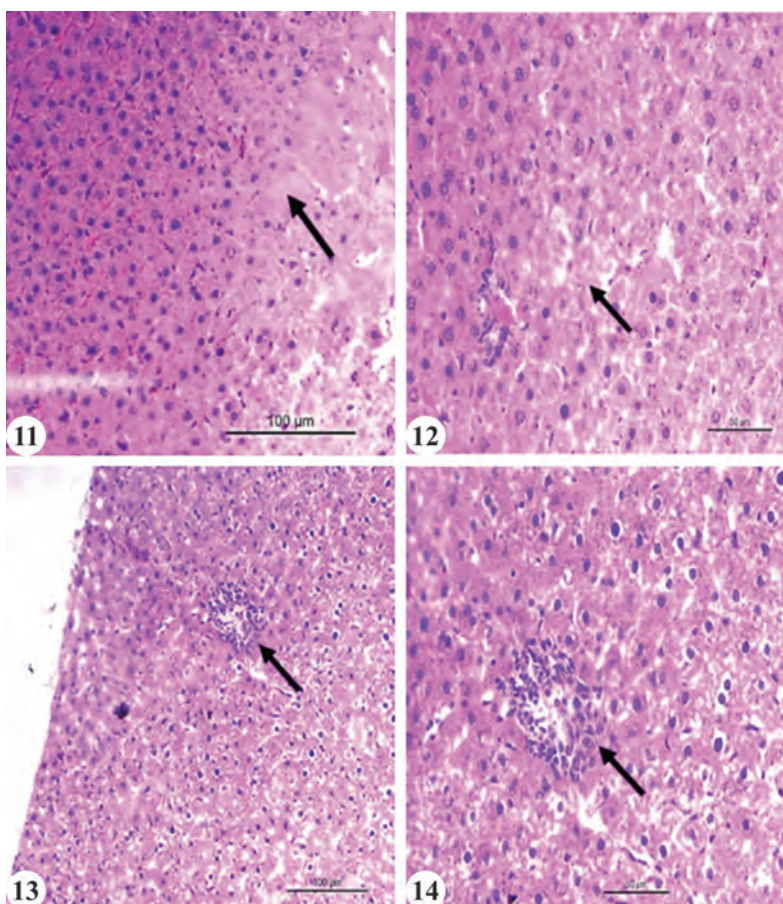


Plate 11. Male rat-Liver, Group IV (1000 mg/kg), Mild coagulative necrosis of hepatocytes (arrow) (H&E 200X); **Plate 12.** Male rat-Liver, Group IV (1000 mg/kg), Mild coagulative necrosis. Loss of nuclei & homogeneous eosinophilic cytoplasm (arrow) (H&E 400X); **Plate 13.** Female rat-Liver, Group IV (1000 mg/kg), Inflammatory mononuclear cell infiltration in necrotic foci (arrow) (H&E 200X); **Plate 14.** Female rat-Liver, Group IV (1000 mg/kg), Inflammatory mononuclear cell infiltration in necrotic foci (arrow) (H&E 400X).

groups (Table 4).

A significant increase in the TLC, BUN, ALT, AST, ALP was observed in both male and female rats at highest dose level (Group IV 1000 mg/kg bwt) indicating that ethanolic extract of EABL at dose level of 1000 mg/kg

bwt induces nephrotic and hepatotoxicity.

Relative Organ Weight

The mean values of relative organ weights of male and female rats are shown in Table 5 and Table 6 respectively. Comparison of mean values of relative

organ weights of different organs showed no significant difference between control and treatment groups in both male and female rats.

Pathology

The animals were subjected to systematic necropsy at the end of the study. Detailed gross examination showed no specific external lesions (Plates 1-4). No pathological changes could be detected in the visceral organs (Plates 5-8). In both male and female rats, microscopic examination of liver showed apparently normal hepatic architecture in Group I (Plate 9) and II. Mild vacuolar degeneration of hepatocytes with a focal distribution was observed in Group-III animals (Plate 10). Coagulative necrosis involving small group of hepatocytes (5-6 hepatocytes) with loss of nuclei and homogeneously eosinophilic cytoplasm was observed at 2-3 foci in the liver of rats in the treatment Group-III. Similar lesions were observed in liver of Group-IV animals but with increased severity. Sinusoidal congestion, mild degree of vacuolar degeneration of hepatocytes and mild multifocal areas of coagulative necrosis (Plates 11, 12) with minimal inflammatory cell infiltration (Plates 13, 14) was observed in group-IV animals.

In male rats, histopathological examination of the kidneys showed apparently normal architecture with intact glomerulus and tubular epithelium in control (Plate 15) and group-II animals. Microscopic examination of kidneys of group-III animals showed multifocal tubular epithelial cell degeneration (Plate 16) with loss of brush border. Tubular epithelial cell degeneration and tubules with necrotic desquamated epithelial cells (Plate 17) were observed in the kidneys of group-IV animals. Apart from these lesions, there was marked congestion in intra tubular blood vessels.

Microscopically normal architecture was observed in kidneys of group-I female rats (Plate 18). Focal to multifocal tubular epithelial cell degeneration was observed in group-II female rats. Histopathological examination of kidneys of group-III animals revealed multifocal areas of tubular epithelial cell hyperplasia (Plate 19). The lumen of the affected tubules was obliterated with hyperplastic epithelial cells. There was crowding of tubular epithelial cell nuclei in the affected part. Similar lesions were observed in kidneys of group-IV female rats, but with increased severity. Multifocal areas of tubular epithelial cell degeneration were also

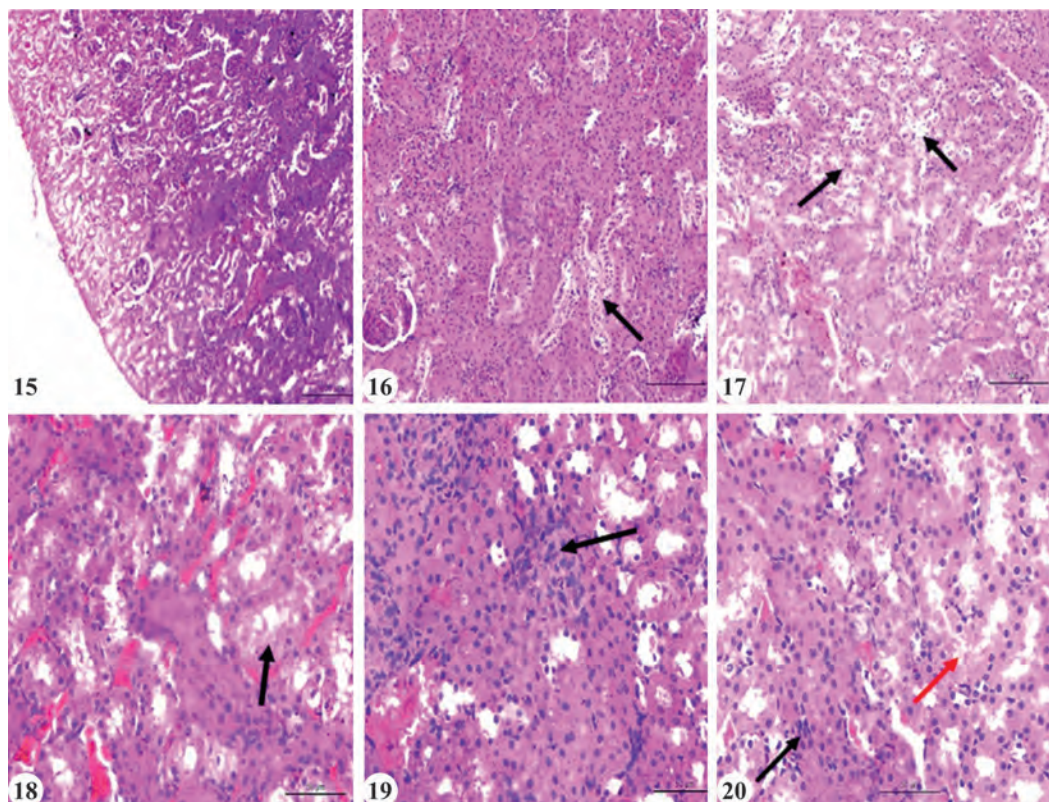


Plate 15. Male rat-Kidney, Group I-Control. Normal renal architecture (H&E 100X); **Plate 16.** Male rat-Kidney, Group III (500 mg/kg), Tubular epithelial cell degeneration (arrow) (H&E 200X); **Plate 17.** Male rat-Kidney, Group IV (1000 mg/kg), Multifocal tubular epithelial cell degeneration and necrosis (arrows) (H&E 200X); **Plate 18.** Female rat-Kidney, Group II (250 mg/kg), Focal mild tubular epithelial cell degeneration and necrosis (arrow) (H&E 400X); **Plate 19.** Female rat-Kidney, Group III (500 mg/kg), Hyperplasia of tubular epithelial cells (arrow) (H&E 400X); **Plate 20.** Female rat-Kidney, Group IV (1000 mg/kg), Tubular epithelial cell hyperplasia (black arrow) & degeneration (red arrow) (H&E 400X).

observed in these animals (Plate 20). The toxicity, if any, could be attributed to the presence of phytoconstituents of the plant under investigation since literature reports evidenced that many of the phytoconstituents like steroids, alkaloids, triterpenes and glycosides in plant have been associated with toxicity²⁰. Similar findings on sub-acute oral toxicity study of ethanol extract of *Cosmos caudatus*, a member of the Asteraceae family were reported²¹.

CONCLUSION

There was no significant difference in the relative organ weights of the animals treated with EABL in comparison to control group of animals. Gross examination of the organs revealed no specific lesions, whereas on microscopic examination revealed congestion, minimal coagulative necrosis, vacuolar degeneration and presence of inflammatory cells in liver and kidneys showed multifocal degeneration of the tubular epithelial cell degeneration at 500 mg/kg bwt and 1000 mg/kg body weight in both male and female rats. Kidneys at 500 and 1000 mg/kg bwt in female rats also showed tubular epithelial cell hyperplasia of varying degrees. Microscopic examination of other organs such as heart, spleen, brain, stomach and intestine revealed no specific lesions. In conclusion, EABL at dose levels of 500 and 1000 mg/kg bwt was found to be mildly hepatotoxic and nephrotoxic to rats.

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