RESEARCH ARTICLE

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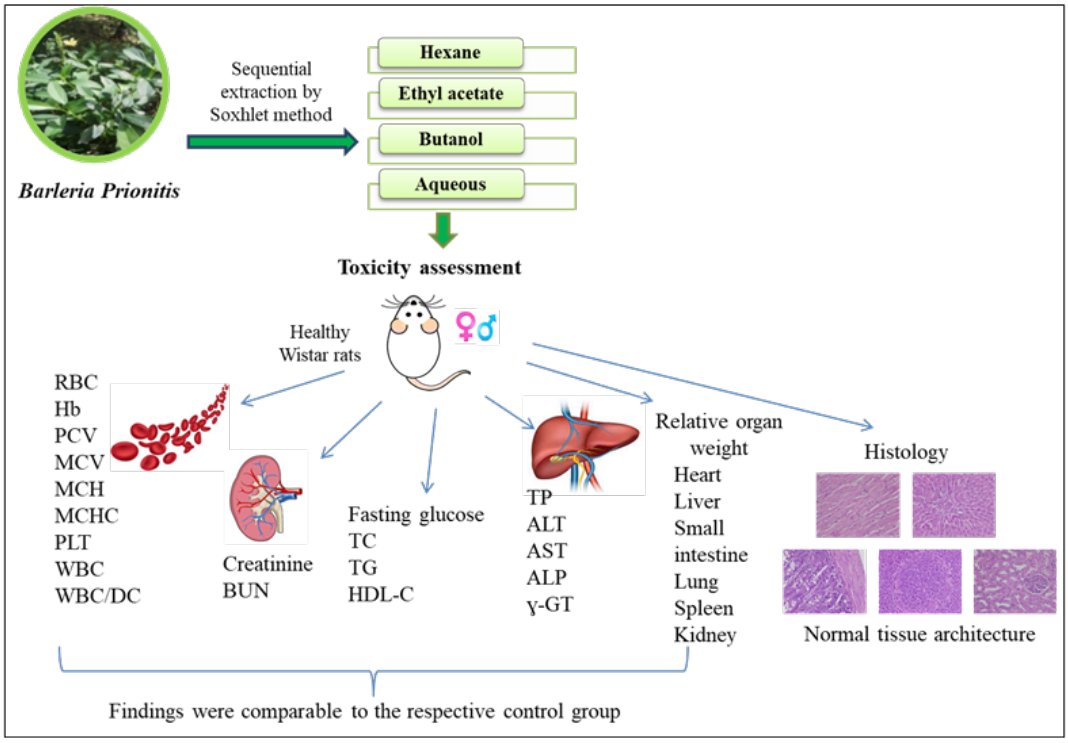

Highlights

- *Barleria prionitis* is a medicinal plant with a wide usage in traditional medicine
- Acute and sub-acute toxic effects of the plant were evaluated using an animal model
- Absence of significant changes in hematological and biochemical parameters in both male and female rats excludes potential toxic effects of *B. prionitis* extracts

Ceylon Journal of Science 52 (4) 2023: 445-454
DOI: https://doi.org/10.4038/cjs.v52i4.8255

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Acute and sub-acute toxicological evaluation of *Barleria prionitis* Linn. in Wistar albino rats

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Received: 11.04.2023; Accepted: 19.09.2023

Abstract: Despite the wide usage of *Barleria prionitis* Linn. (Acanthaceae) in traditional medicine, controversial findings have been reported on possible toxic effects of the plant. The present study evaluated the acute and sub-acute toxic/adverse effects of *B. prionitis* using a healthy Wistar albino rat model. Repeated oral administration of the hexane, ethyl acetate, butanol, and aqueous extracts of the whole plant at therapeutic doses (25, 80, 70, and 120 mg/kg) revealed no mortality or treatment-related hematological and biochemical changes in experimental rats. However, statistically significant changes were observed in a few red cell and white cell parameters, suggesting increased erythropoiesis and immunity in experimental rats treated with *B. prionitis* ($p<0.05$). The findings on total cholesterol, glucose, and liver function tests revealed potential changes in lipid and carbohydrate metabolism and liver functions after treatments. However, the absence of statistically significant changes in the plant treated groups was noted compared to their respective vehicle control groups ($p>0.05$), and the lack of those changes in male and female rats excludes potential toxic effects from *B. prionitis* extracts. The findings on the relative weight of organs and histopathology further corroborated the safe use of the selected extracts at doses in therapeutic applications. In conclusion, the findings on acute and 28-day repeated dose oral toxicity assessments of the whole plant extracts resulted in neither mortality nor treatment-related hematological, biochemical, and histopathological changes in healthy Wistar albino rats.

Keywords: *Barleria prionitis,* animal models; toxicity; hematological changes; biochemical changes; histology changes

INTRODUCTION

Medicinal plants, being reserves of curative phytoconstituents, often dominate the pharmacological industry (Naryan & Nikam, 2018). During the past three decades, the majority of newly approved drugs for cancer and infectious diseases were derived from natural bioactive compounds of ethnobotanical origin (Joana Gil-Chavez et al., 2013). Hence, the search for natural products with the potential for treatment and prevention of human diseases has become a key topic in the present era.

Barleria prionitis Linn. (Acanthaceae) commonly known as Vajradanti in Ayurveda, is one of those medicinal plants which received the exclusive attention of the scientific community. Almost every part of the plant is being used in the traditional system of medicine for remedial measures against a variety of human diseases (Choudhary et al., 2014). The plant is a diuretic and is being used in the management of urinary infections, renal dropsy, etc. Root decoction is often used for rat bite poisoning, toothache, and scrotum enlargement. The dried bark and leaves are used in the treatment of whooping cough and catarrhal affections respectively (Talukdar, 2015). The use of this plant in the management of fever, tonic, inflammation, glandular swelling, expectorant, gastrointestinal disorders, boils, and ulcers are a few more therapeutic applications (Choudhary et al*.,* 2014).

Despite the substantial use of the whole plant of *B. prionitis* in traditional medicine, a complete understanding of the plant in terms of its identity, properties, and applications is essential, especially during the use of more concentrated forms of the plant in therapeutic applications (Kumar et al*.*, 2017). In this context, the toxicological evaluation of the plant is crucial. Interestingly, numerous studies have been conducted to evaluate the potential toxic/adverse effects of the plant, fortifying the therapeutic value of *B. prionitis*. Several studies carried out on ethanol extract of roots and leaves, methanol leaf extract, and aqueous extract rich in iridoid glucosides of *B. prionitis* on different models of rats and mice revealed that single administration of the plant extract for 14 days was toxicologically safe (Singh et al*.,* 2005; Dheer & Bhatnagar, 2010; Choudhary et al*.,* 2014). However, no single *in vivo* study has been reported on the sub-acute or chronic toxic effect of the plant to date. Moreover, controversial findings have been reported on the potentially toxic effects of the plant *in vitro*. Sawarkar et al. (2016) revealed significant cytotoxic effects of ethanolic leaf extract on human gingival and human dermal fibroblast cell lines by the MTT assay. These findings were further corroborated by the findings on the brine shrimp lethality test on methanol, ethyl acetate, ethanol, and aqueous extracts of *B. prionitis,* which disclosed potential toxic effects of the plant (Honmane et al., 2021). However, the brine shrimp lethality test is considered a preliminary toxicity assay and is fairly ineffective in terms of clarifying the mechanisms of toxic effects (Honmane et al., 2021). Hence, there is a compelling need to evaluate the long-term toxic effects of the whole plant using an *in vivo* animal model simulating the traditional practice, to collect precise data that could be inferred from humans.

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MATERIALS AND METHODS

Plant collection

The whole plant of *B. prionitis* was collected from the Southern region, of Sri Lanka in March 2019. The authentication of the plant was carried out at the National Herbarium, Department of National Botanic Garden, Peradeniya, Sri Lanka. A voucher specimen of the plant (Ref No. PG/2016/55/03) was preserved in the mini herbarium, Department of Biochemistry, Faculty of Medicine, University of Ruhuna, Sri Lanka for future reference.

Preparation of extracts

The whole plant was thoroughly washed to remove visible contamination and oven-dried at 40°C. The dried crude plant materials placed in an extraction thimble were subjected to successive sequential extraction with hexane (yield: 1.96 % w/w), ethyl acetate (yield: 6.51 % w/w), butanol (yield: 5.47 % w/w), and water (yield: 9.32 % w/w) by hot Soxhlet extraction method. The extraction with each solvent was continued until the leachate became colorless. The subsequent solvents were added following the complete evaporation of the previous. Different solvent extracts were collected separately and concentrated in a rotary evaporator (Heidolph Instrument, Laborota 4000, Germany) and dried in a vacuum oven (Thermo Scientific, USA). The dried extracts were preserved in an airtight glass container at 4-8°C.

Dose and route of administration

The human equivalent therapeutic dose in rats was calculated based on the percentage yield of each whole plant extract. Accordingly, the doses selected for hexane (BPHE), ethyl acetate (BPEE), butanol (BPBE), and aqueous (BPAE) extracts were 25 mg/kg, 80 mg/kg, 70 mg/kg, and 120 mg/kg respectively. Fresh treatment regimens were prepared in corn oil (BPHE and BPEE), polyvinylpyrrolidone (3% v/v; BPBE), and water (BPAE) at the time of administration and administered oral route using a gavage tube specific for rats.

Animals

Healthy Wistar albino rats (150–200 g) of either sex purchased from Medical Research Institute, Colombo (Sri Lanka), were housed in the animal house, Faculty of Medicine, University of Ruhuna, Sri Lanka. The animals were fed with commercially available feed, provided water *ad libitum,* and were maintained under standard laboratory conditions of relative humidity (55 ± 10%), temperature (25°C ± 5°C), and 12/12 h light/dark cycle. The animals were cared for according to the guide for the care and use of laboratory animals (Council NR, 2010). The study was approved by the Ethics Committee of Faculty of Medicine, University of Ruhuna, Sri Lanka (Register Number: 14.12.2015:3.1).

Grouping of animals was done as follows including five animals of either sex into each group.

Group I: vehicle control I (distilled water)
Group II: vehicle control II (corn oil)
Group III: vehicle control III (polyvinylpyrrolidone (3% v/v)
Group IV: BPHE (25 mg/kg body weight/rat)

Group V: BPEE (80 mg/kg body weight/rat)
Group VI: BPBE (70 mg/kg body weight/rat)
Group VII: BPAE (120 mg/kg body weight/rat)

The animals were administered with a single dose of the treatment regimens and observed for three hours and then daily for 14 days for signs of toxicity, per the OECD guideline 420, fixed-dose procedure (OECD, 2001).

Assessment of sub-acute toxicity in Wistar albino rats

The experimental groups and the treatment doses were the same as in the acute toxicity assessment. After the initiation of treatment regimens, all groups were kept on vehicles/plant extracts for 28 more days. On day 29, blood was drawn by cardiac puncture for the assessment of selected hematological and biochemical parameters. Animals were sacrificed under CO_2 anesthesia and vital organs were excised for the estimation of relative organ weight and assessment of histopathology. Body weight, food consumption, and water intake were measured daily over 28 days and averaged into weekly intervals.

Hematological assessment

Blood (0.5 mL) was collected into a pediatric tube containing an anticoagulant (5% EDTA) and hematological parameters were determined using the automated hematology analyzer (Mindray BC 5150, China).

Biochemical assessment

Blood (3 mL) was collected into serum collecting gel tubes and the serum was separated. Investigations were carried out to assess serum glucose, lipid profile parameters, liver, and kidney function tests using spectrophotometric assay kits.

Relative weight of organs

Relative weights of heart, lung, small intestine, liver, spleen, and kidneys of each the animal was calculated and presented as a percentage of the body weight.

Histological analysis

The tissues collected were fixed in 10% formalin and processed for paraffin embedding. Tissue sections (5 µm thick) were cut and stained with hematoxylin and eosin (H and E). The stained slides were observed under a light microscope for any pathological alterations of the vital organs in a random manner without taking into consideration the different treatment regimens.

Statistical analysis

The results were expressed as mean ± SEM. Statistical comparison was made in between the plant-treated groups and plant treated groups with their respective vehicle control groups. Statistical difference between the two means was determined by one-way ANOVA followed by Tukeys' multiple comparisons *posthoc* test using SPSS statistical computer software. Mean values with a statistical difference of $p<0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Acute toxicity effects of *B. prionitis* extracts in Wistar albino rats

Administration of a single dose of BPHE, BPEE, BPBE, and BPAE orally did not cause mortality or treatment-related signs of toxicity/adverse effects in Wistar albino rats during a 14-day study period. There were no significant changes in the average body weight, food consumption, and water intake of rats administered with plant extracts compared to the respective vehicle control groups.

Sub-acute toxicity effects of *B. prionitis* extracts in Wistar albino rats

Food consumption, water intake, and change in body weight

Daily oral administration of BPHE, BPEE, BPBE, and BPAE for 28 consecutive days did not cause mortality or obvious signs of toxicity in Wistar albino rats. No significant changes were observed in food consumption and water intake ($p>0.05$). Figure 1 shows the change in body weight of experimental rats treated with different extracts of *B. prionitis* during the study period of 28 days. No significant changes in body weight were observed in any group of rats treated with plant extracts compared to their respective vehicle control groups and compared to the vehicle control group I ($p>0.05$).

Effect of selected plant extracts on hematological parameters

The measured hematological parameters of Wistar albino rats administered with BPHE, BPEE, BPBE, and BPAE are given in Table 1. A significant increase in white blood cell counts was observed in male rats treated with BPEE compared to the respective vehicle control group (vehicle II) as well as the vehicle control I ($p<0.05$). Additionally, an increase in red blood cell count, packed cell volume, and hemoglobin concentration was observed in several groups of male rats treated with *B. prionitis* extracts compared to the respective control groups. Moreover, a reduction in monocyte counts could be observed with both male and

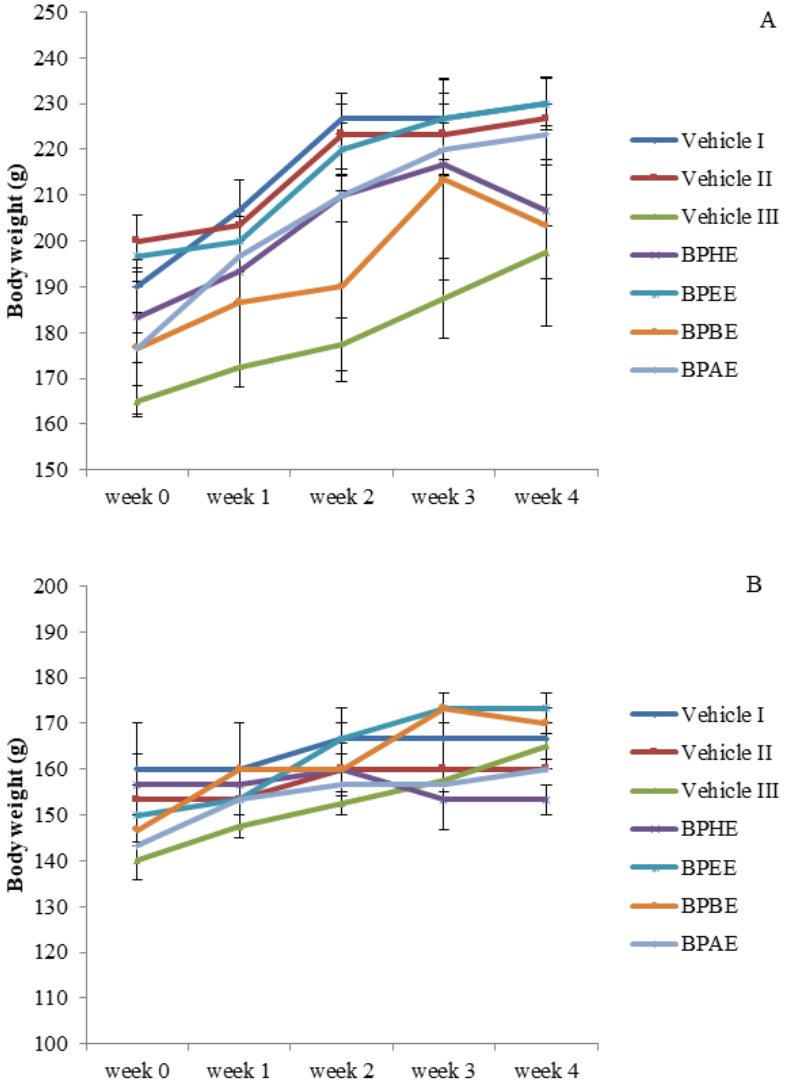

Figure 1: Change in body weight (g) of male (A) and female (B) Wistar albino rats. BPHE: *Barleria prionitis* hexane extract, BPEE: *Barleria prionitis* ethyl acetate extract, BPBE: *Barleria prionitis* butanol extract, BPAE: *Barleria prionitis* aqueous extract, the vehicle I: distilled water, vehicle II: corn oil, vehicle II: polyvinylpyrrolidone (3% v/v).

female groups of Wistar albino rats administered plant extracts, compared to the vehicle control I. The changes were not significant for the rats treated with *B. prionitis* extracts considering the red cell indices, platelet count, and WBC differential count ($p>0.05$). Yet, a significant increase in hemoglobin concentration could be observed in male rats treated with BPEE ($p<0.05$).

Effect of selected plant extracts on biochemical parameters

28-day repeated dose oral toxicity assessment of selected extracts of *B. prionitis* in Wistar albino rats showed comparable findings in test groups compared to the vehicle control I as well as with their respective vehicle control groups, concerning renal and liver function parameters, and serum glucose measurements. No significant difference in either parameter was observed except in the ALP levels of male rats treated with the BPHE and BPBE. Moreover, the activity of AST and ALT was decreased in rats treated with plant extracts of the same groups, and BPAE compared to the untreated control group. Similarly, a reduction in fasting serum glucose concentrations could be observed in rats treated with *B. prionitis* extracts ($p>0.05$). The findings are shown in Table 2.

Effect of selected plant extracts on relative organ weight

Treatment with plant extracts did not modify the relative weights of the heart, liver, small intestine, lungs, spleen, and kidneys of the experimental rats in the treatment groups. The results are shown in Table 3.

Effect of selected plant extracts on histopathology of vital organs

Figure 2 shows photomicrographs of tissue sections stained with H and E from vital organs of the rats of different experimental groups. The heart, liver, small intestine, spleen, and kidney tissues of experimental rats treated with BPHE, BPEE, BPBE, and BPAE for 28 consecutive days showed normal tissue architecture with no signs of cell injury, necrosis, congestion, hemorrhages, or inflammatory infiltrations.

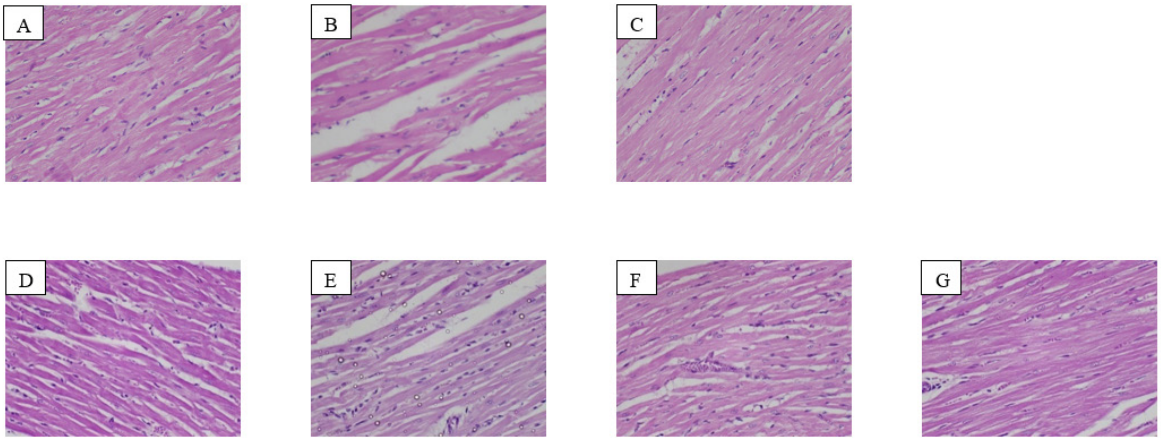

Figure 2(a): Histopathological examination of cardiac tissues (×400). A; vehicle I (distilled water), B; vehicle II (corn oil), C; vehicle III (polyvinylpyrrolidone (3% v/v), D; experimental rats administered with *Barleria prionitis* hexane extract, E: experimental rats administered with *Barleria prionitis* ethyl acetate extract, F: experimental rats administered with *Barleria prionitis* butanol extract, G: experimental rats administered with *Barleria prionitis* aqueous extract.

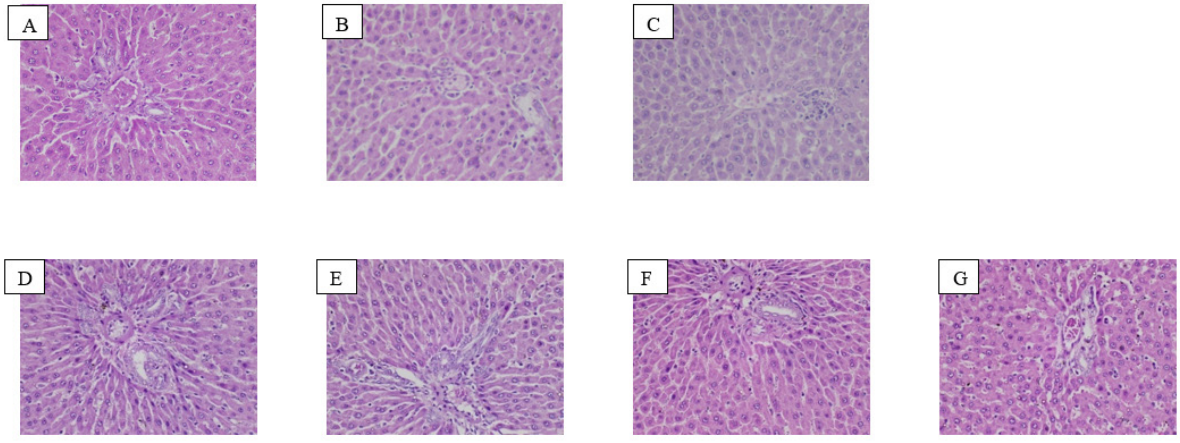

Figure 2(b): Histopathological examination of liver tissues (×400). A; vehicle I (distilled water), B; vehicle II (corn oil), C; vehicle III (polyvinylpyrrolidone (3% v/v), D; experimental rats administered with *Barleria prionitis* hexane extract, E: experimental rats administered with *Barleria prionitis* ethyl acetate extract, F: experimental rats administered with *Barleria prionitis* butanol extract, G: experimental rats administered with *Barleria prionitis* aqueous extract.

Table 1: Effect of selected *Barleria prionitis* extracts on hematological parameters.

Parameters	Vehicle I	Vehicle II	Vehicle III	BPHE	BPEE	BPBE	BPAE
Male							
RBC (× 10^6/μL)	7.40±0.18	8.23±0.39	8.74±0.41	8.65±0.95	8.87±0.09	8.54±0.72	8.32±0.02
Hb (g/dL)	13.64±0.44	14.50±0.26	14.37±0.49	14.30±0.60	16.07±0.09*▲	15.03±0.88	14.76±0.07
Hct (%)	50.30±1.55	55.53±2.13	57.43±2.99	57.35±5.85	57.80±0.53	57.37±4.33	54.87±0.18
MCV (fL)	66.74±0.24	67.43±0.91	65.67±0.33	66.75±0.05	65.63±0.58	67.17±1.08	65.97±0.09
MCH (pg)	18.14±0.25	17.90±0.42	17.27±0.22	17.60±0.20	18.30±0.17	17.73±0.63	17.80±0.06
MCHC (g/dL)	27.14±0.28	26.53±0.24	26.27±0.18	26.50±0.20	27.87±007	26.37±0.55	26.97±0.07
PLT (×10^3/μL)	248.40±28.56	248.33±31.48	210.33±9.20	285.50±0.50	249.33±12.67	234.00±14.29	289.33±6.33
WBC (per mm^3)	1.48±0.42	3.97±0.81	4.60±0.87	6.20±0.60	8.20±1.54*▲	5.97±3.18	5.07±0.81
Neutrophils (%)	19.60±3.66	16.67±4.98	22.33±4.98	31.50±10.50	19.33±4.84	26.00±1.15	19.33±1.67
Lymphocytes (%)	75.00±4.04	75.33±5.17	72.00±3.79	62.50±11.50	77.67±3.84	72.33±1.67	79.67±1.67
Eosinophils (%)	1.20±0.20	0.67±0.33	0.00±0.00	1.50±0.50	1.00±0.58	0.00±0.00	0.33±0.33
Basophils (%)	1.40±0.40	4.00±1.00	3.00±0.58	3.50±1.50	1.07±0.67	1.67±0.88	0.67±0.33
Monocytes (%)	2.80±0.97	3.33±1.86	1.67±0.67	1.00±0.00	0.33±0.33	0.00±0.00	0.00±0.00
Female							
RBC (× 10^6/μL)	7.63±0.23	8.04±0.32	8.05±0.32	7.33±0.24	7.67±0.07	7.49±0.09	7.95±0.24
Hb (g/dL)	13.98±0.18	13.90±0.06	13.83±0.18	13.10±0.30	14.00±0.06	13.40±0.21	14.10±0.42
Hct (%)	51.10±1.53	53.97±1.77	53.07±2.00	48.67±1.58	57.80±0.53	57.37±4.33	54.87±0.18
MCV (fL)	66.92±0.51	67.10±0.46	65.97±0.12	66.43±0.03	65.63±0.58	67.16±1.08	65.30±0.15
MCH (pg)	18.52±0.22	17.76±0.37	17.70±0.06	17.90±0.20	18.23±0.03	17.90±0.12	17.73±0.09
MCHC (g/dL)	27.70±0.43	26.50±0.40	26.80±0.00	26.90±0.35	27.17±0.23	26.80±0.15	27.20±0.06
PLT (×10^3/μL)	268.20±23.79	252.33±5.84	260.33±26.29	275.67±21.67	263.00±23.86	304.33±18.46	329.33±30.80
WBC (per mm^3)	3.32±0.65	1.57±0.22	2.00±0.55	2.00±0.79	3.13±0.67	2.30±0.35	2.60±0.70
Neutrophils (%)	19.80±1.16	16.67±2.73	15.67±2.85	10.00±4.73	15.33±1.76	16.00±2.65	17.00±4.04
Lymphocytes (%)	72.40±2.73	78.00±3.05	78.33±3.38	83.33±3.67	80.33±2.91	81.33±2.73	80.33±3.67
Eosinophils (%)	2.40±1.17	1.33±0.33	2.67±1.20	1.33±0.33	1.67±0.67	0.67±0.67	0.67±0.67
Basophils (%)	2.00±0.32	3.00±0.00	2.33±0.67	4.33±1.86	1.67±0.67	2.00±0.58	1.00±0.58
Monocytes (%)	3.40±1.21	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	0.00±0.00	1.00±0.58

Statistically significant ($p<0.05$) compared to the normal control group (vehicle I); * and compared to respective vehicle control groups (BPHE and BPEE against respective male and female rats of vehicle I, BPBE against respective male and female rats of vehicle II and BPAE against respective male and female rats of vehicle III); ▲. BPHE: *Barleria prionitis* hexane extract, BPEE: *Barleria prionitis* ethyl acetate extract, BPBE: *Barleria prionitis* butanol extract, BPAE: *Barleria prionitis* aqueous extract, the vehicle I: distilled water, vehicle II: corn oil, vehicle III: polyvinylpyrrolidone (3% v/v), RBC: red cell count, Hb; Hemoglobin concentration, PCV: packed cell volume, MCV; mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, PLT; platelet count, WBC; white cell count.

Table 2: Effect of selected *Barleria prionitis* extracts on biochemical parameters.

Parameters	Vehicle I	Vehicle II	Vehicle III	BPHE	BPEE	BPBE	BPAE
Male							
Creatinine (µmol/L)	75.67±7.45	53.04±1.53	58.34±4.05	68.36±4.13	79.56±2.65	70.13±4.85	52.15±3.54
BUN (mmol/L)	8.73±0.69	7.44±1.44	7.02±0.90	6.01±2.26	6.60±1.44	7.56±1.29	7.26±0.43
Total protein (g/L)	7.61±0.46	6.26±0.90	8.22±0.20	6.67±0.18	6.39±0.21	6.58±0.11	7.74±0.84
AST (U/L)	112.10±21.76	50.65±2.35	83.80±4.76	69.07±9.42	81.47±5.56	78.97±8.93	103.23±10.99
ALT (U/L)	54.13±3.51	36.66±5.86	39.77±4.28	35.89±4.46	55.09±19.62	36.47±2.24	49.08±7.90
ALP (U/L)	351.07±1.91	262.53±31.82	248.40±22.88	386.27±37.05▲	358.40±10.75	376.80±11.50▲	391.80±12.95
ɣ-GT (U/L)	6.37±1.11	8.69±1.53	2.90±0.33	6.56±0.70	5.79±1.86	6.56±2.70	5.40±0.70
Fasting serum glucose (mmol/L)	4.89±0.36	4.65±0.33	5.71±0.49	4.49±0.15	4.48±0.42	3.77±0.24	6.51±0.81
Total cholesterol (mmol/L)	66.46±2.35	58.47±5.80	61.38±4.94	74.90±3.67	78.57±3.21	75.91±10.80	90.23±3.49
Triglycerides (mmol/L)	1.36±0.17	0.82±0.06	0.80±0.10	0.91±0.07	0.97±0.07	1.06±0.10	0.93±0.05
HDL-C (mmol/L)	1.60±0.08	1.53±0.53	1.28±0.11	1.44±0.38	1.00±0.11	1.27±0.16	1.31±0.03
Female							
Creatinine (µmol/L)	65.42±3.57	55.99±4.85	54.36±1.33	71.89±6.46	91.35±16.69	67.48±4.85	59.82±2.06
BUN (mmol/L)	7.52±0.66	5.36±0.82	6.96±0.57	7.08±0.77	7.26±0.26	8.63±0.33	7.68±0.92
Total protein (g/L)	7.46±0.26	8.56±0.54	8.05±0.72	6.42±0.81	7.20±0.44	7.45±0.06	7.21±0.13
AST (U/L)	124.56±21.32	84.60±7.23	79.17±16.30	91.40±29.56	78.93±7.30	73.35±0.55	85.77±7.53
ALT (U/L)	51.45±4.43	38.99±1.78	34.92±2.93	40.55±6.60	39.29±10.76	41.03±0.29	29.39±1.46
ALP (U/L)	226.08±24.82	171.87±9.76	154.67±6.03	254.87±29.64	214.77±20.29	260.10±12.47	260.37±32.01
ɣ-GT (U/L)	6.80±0.96	3.28±1.35	7.53±0.58	5.60±2.55	5.40±1.02	5.21±0.88	6.37±0.00
Fasting serum glucose (mmol/L)	4.96±0.54	4.10±0.26	4.24±0.19	4.19±0.54	3.57±0.23	3.76±0.12	4.66±0.26
Total cholesterol (mmol/L)	58.78±4.87	48.15±11.26	51.85±8.72	56.10±4.91	62.97±2.32	75.12±3.95	62.13±2.68
Triglycerides (mmol/L)	1.06±0.08	0.88±0.05	1.18±0.20	0.87±0.15	0.81±0.06	0.97±0.10	1.16±0.06
HDL-C (mmol/L)	1.29±0.09	1.25±0.08	1.10±0.38	0.77±0.27	0.96±0.00	1.77±0.12	1.24±0.06

Statistically significant ($p<0.05$) compared to the normal control group (vehicle I); * and compared to respective vehicle control groups (BPHE and BPEE against respective male and female rats of vehicle I, BPBE against respective male and female rats of vehicle II and BPAE against respective male and female rats of vehicle III); ▲. BPHE: *Barleria prionitis* hexane extract, BPEE: *Barleria prionitis* ethyl acetate extract, BPBE: *Barleria prionitis* butanol extract, BPAE: *Barleria prionitis* aqueous extract, the vehicle I: distilled water, vehicle II: corn oil, vehicle III: polyvinylpyrrolidone (3% v/v), BUN: blood urea nitrogen, AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase, γ-GT: gamma-glutamyltransferase, HDL-C: high-density cholesterol.

Table 3: Effect of selected *Barleria prionitis* extracts on the relative weight of vital organs.

Parameters	Vehicle I	Vehicle II	Vehicle III	BPHE	BPEE	BPBE	BPAE
Male							
Heart	0.33±0.02	0.32±0.01	0.34±0.02	0.33±0.01	0.32±0.01	0.33±0.01	0.31±0.01
Liver	2.71±0.11	2.53±0.09	2.57±0.06	2.90±0.05	2.59±0.04	2.79±0.06	2.85±0.10
Small intestine	1.47±0.05	1.31±0.15	1.62±0.03	1.41±0.12	1.56±0.28	1.33±0.06	1.60±0.18
Lungs	0.49±0.07	0.45±0.03	0.47±0.02	0.53±0.02	0.46±0.02	0.49±0.02	0.47±0.01
Spleen	0.26±0.03	0.21±0.01	0.24±0.01	0.24±0.15	0.22±0.01	0.24±0.02	0.23±0.00
Kidneys	0.62±0.03	0.55±0.02	0.53±0.02	0.59±0.01	0.54±0.01	0.57±0.02	0.56±0.00
Female							
Heart	0.33±0.01	0.36±0.01	0.35±0.01	0.34±0.02	0.35±0.01	0.33±0.01	0.36±0.01
Liver	2.81±0.07	2.59±0.06	2.59±0.09	2.86±0.22	2.55±0.02	1.86±0.77	2.82±0.13
Small intestine	2.07±0.09	1.91±0.33	2.00±0.21	1.81±0.05	1.70±0.17	1.56±0.07	1.83±0.01
Lungs	0.57±0.02	0.53±0.03	0.56±0.02	0.52±0.04	0.51±0.02	0.50±0.01	0.55±0.05
Spleen	0.27±0.01	0.25±0.01	0.26±0.01	0.30±0.03	0.26±0.01	0.26±0.01	0.27±0.02
Kidneys	0.57±0.01	0.56±0.01	0.55±0.02	0.50±0.08	0.54±0.00	0.55±0.02	0.60±0.04

Statistically significant ($p<0.05$) compared to normal control group (vehicle I); * and compared to respective vehicle control groups (BPHE and BPEE against respective male and female rats of vehicle I, BPBE against respective male and female rats of vehicle II and BPAE against respective male and female rats of vehicle III); ▲. No significant difference was observed in test group rats compared to the respective vehicle I ($p>0.05$) BPHE: *Barleria prionitis* hexane extract, BPEE: *Barleria prionitis* ethyl acetate extract, BPBE: *Barleria prionitis* butanol extract, BPAE: *Barleria prionitis* aqueous extract, the vehicle I: distilled water, vehicle II: corn oil, vehicle III: polyvinylpyrrolidone (3% v/v).

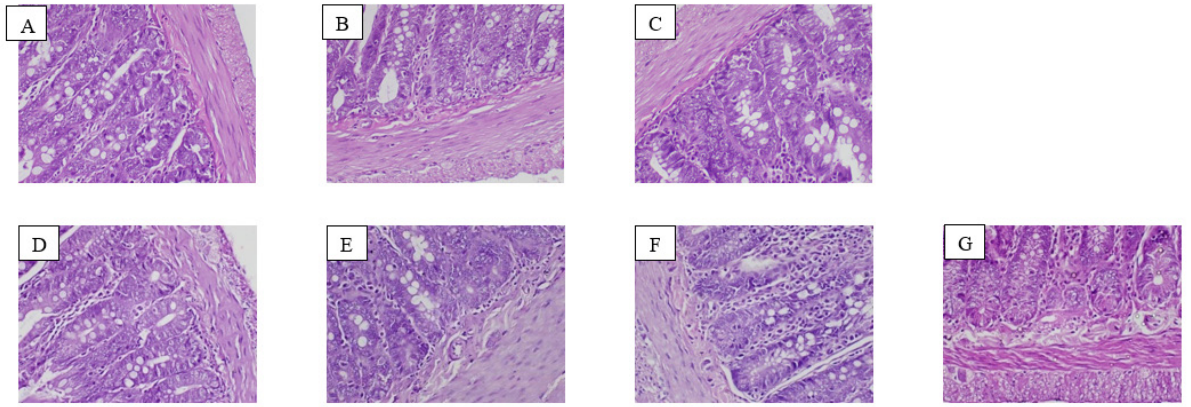

Figure 2(c): Histopathological examination of small intestine tissues (×400). A; vehicle I (distilled water), B; vehicle II (corn oil), C; vehicle III (polyvinylpyrrolidone (3% v/v), D; experimental rats administered with *Barleria prionitis* hexane extract, E: experimental rats administered with *Barleria prionitis* ethyl acetate extract, F: experimental rats administered with *Barleria prionitis* butanol extract, G: experimental rats administered with *Barleria prionitis* aqueous extract.

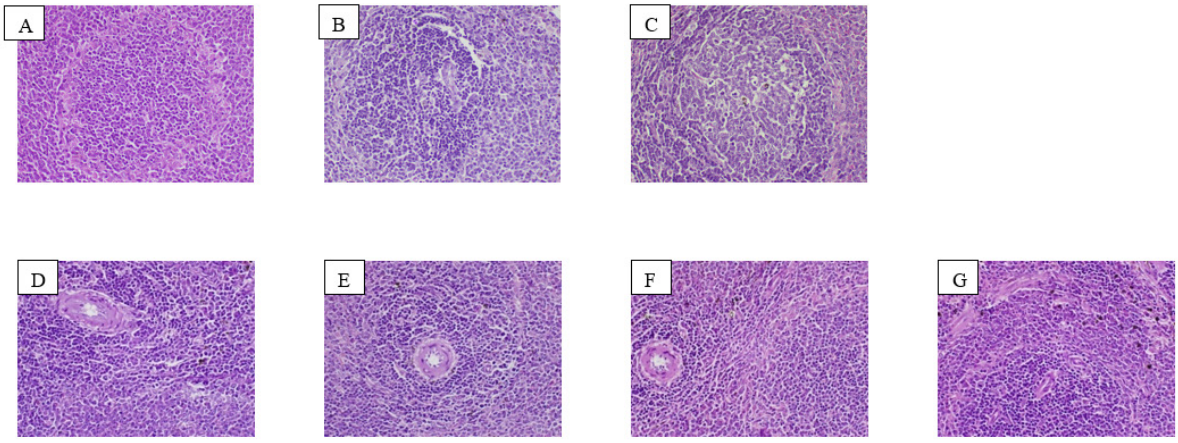

Figure 2(d): Histopathological examination of spleen tissues (×400). A; vehicle I (distilled water), B; vehicle II (corn oil), C; vehicle III (polyvinylpyrrolidone (3% v/v), D; experimental rats administered with *Barleria prionitis* hexane extract, E: experimental rats administered with *Barleria prionitis* ethyl acetate extract, F: experimental rats administered with *Barleria prionitis* butanol extract, G: experimental rats administered with *Barleria prionitis* aqueous extract.

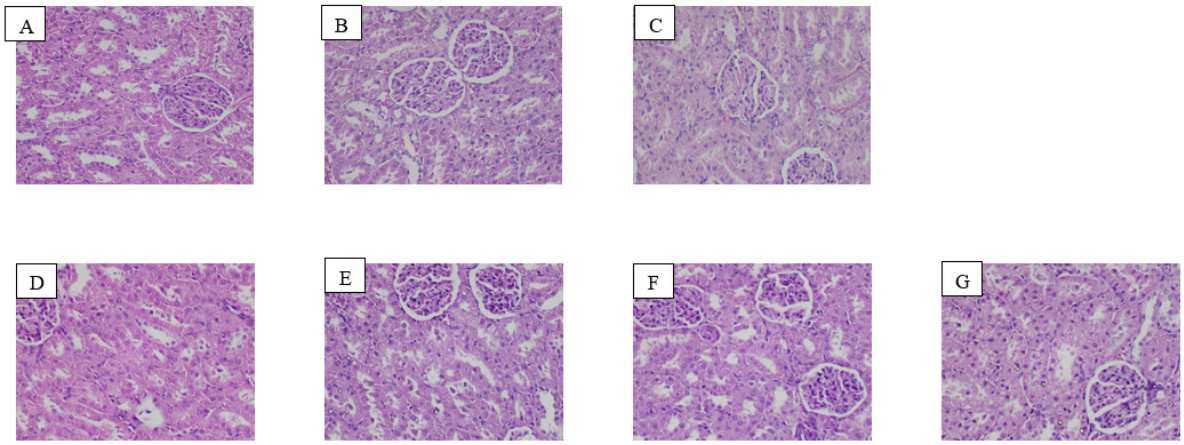

Figure 2(e): Histopathological examination of kidney tissues (×400). A; vehicle I (distilled water), B; vehicle II (corn oil), C; vehicle III (polyvinylpyrrolidone (3% v/v), D; experimental rats administered with *Barleria prionitis* hexane extract, E: experimental rats administered with *Barleria prionitis* ethyl acetate extract, F: experimental rats administered with *Barleria prionitis* butanol extract, G: experimental rats administered with *Barleria prionitis* aqueous extract.

DISCUSSION

Investigation of the acute toxicity effects of BPHE, BPEE, BPBE, and BPAE following the OECD guidelines revealed that selected extracts have no acute toxic effects *in vivo* (OECD, 2001). Wistar rats treated with the selected plant extracts at the 25, 80, 70, and 120 mg/kg doses did not develop either mortality or clinical signs of toxicity immediately following administration of the plant extracts as well as during the post-treatment period of 14 days. Therefore, selected *B. prionitis* extracts at the selected therapeutic doses were used in further investigations on sub-acute toxic effects *in vivo*.

A significant gain or loss of body weight over a short period often indicates potential toxic effects in the course of new drug administration (Jayesh et al., 2017). Interestingly, daily administration of the BPHE, BPEE, BPBE, and BPAE over 28 days showed no significant body weight gain or loss in experimental rats of treatment groups compared to either vehicle control I or respective vehicle control groups, suggesting that the selected extracts have no substantial effect on the growth of experimental animals ($p>0.05$). These findings are further supported by the findings on food consumption and water intake, which revealed the normal health status of experimental rats after the administration of plant extracts.

Deviation of hematological and biochemical parameters from the respective physiological homeostatic levels of the species often indicates a potential pathological condition in both humans and animals (Vigneshwar et al., 2021). In this context, the assessment of those hematological and biochemical parameters in animal models provides useful information on potential responses of the live animal body to different pathologies and therapeutics (Vigneshwar et al., 2021). Consequently, the selected full blood count parameters, renal, and liver function parameters, serum glucose, and lipid profile parameters were evaluated in the present study, to investigate the potential toxicity of *B. prionitis*.

The results revealed a significant increase in white cell counts in male Wistar albino rats from the BPEE treated group, suggesting potential strengthening of the immune system of experimental rats. The increase in white cell count could be attributed to the bioactive phytoconstituents present in the plant extract with the ability to boost the immune system through the increased population of defensive white cells. However, no significant elevation in white blood cell counts was observed in female Wistar albino rats from the respective treatment groups. The observed variation in white cell counts between male and female rats might be due to the sex differences in the development of the immune surveillance system and immune competence (Banerjee et al., 2012). Furthermore, an increase in total red cell count, hemoglobin concentration, and packed cell volume could be observed with most treatment groups among both male and female rats compared to the respective vehicle controls, suggesting a potential increase in the erythropoiesis after administration of *B. prionitis* extract. This is further supported by the present findings on the mean values of all selected red cell parameters that exceeded the reference ranges suggested by Kampfmann et al. (2012). The observed reduction in monocyte counts among both male and female rats of plant-treated groups implied potential anti-inflammatory properties of *B. prionitis,* in agreement with the previously published reports (Kampfmann et al*.,* 2012). The comparative reduction in monocyte counts observed in treatment groups compared to the reference ranges denoted by Vigneshwar et al. (2021) further corroborated a reduction in monocyte counts in plant-treated rats. However, the values of red cell and white cell counts, hemoglobin, MCH, and percentages of differential leukocyte counts except for basophils in the treatment groups were in line with the findings of Jacob et al. (2018), while most of them showed a variation of results compared to Vigneshwar et al. (2021) and Kampfmann et al*.* (2012). These variations observed in the hematological parameters in the present study compared to previous studies could be attributed to differences in animal age, environmental conditions, sampling sites, and different analytical protocols used (Vigneshwar et al*.* 2021).

Regarding the biochemical parameters assessed in the present study, a significant change was observed only for the ALP values of the BPHE and BPBE-treated male rats, compared to the respective vehicle controls and vehicle control I. The ALP values of the particular groups were higher than the reference values mentioned by Giknis and Clifford (2008) as well. However, no significant changes were observed in respective female rats compared to the vehicle control I or the respective control groups.

A reduction in the activity of both AST and ALT was noted in the present study in all the groups of experimental rats treated with plant extracts except BPEE, suggesting the potential hepatoprotective effects of *B. prionitis*. Although the AST values of the rats treated with BPEE were within the range, the ALT values showed comparatively high results compared to the reference intervals stated by Giknis and Clifford (2008) and Vigneshwar et al. (2021). Although these findings suggest potential liver injury by BPEE, comparable findings observed with serum total protein and the relative organ weight of the respective group exclude potential liver toxicity by BPEE. Furthermore, reductions in fasting serum glucose concentrations could be observed in rats treated with *B. prionitis* extracts compared to the vehicle control I, and the values were less than the reference values suggested by both Giknis and Clifford (2008) and Vigneshwar, et al. (2021). These findings suggest a potential hypoglycemic effect of selected extracts of *B. prionitis,* further corroborating the findings of Dheer and Bhatnagar (2010).

Vital organs such as the heart, liver, spleen, kidney, and lung often indicate features of toxicity induced by metabolic reactions (Konaté et al., 2012). However, none of the experimental groups treated with *B. prionitis* showed a significant increase or reduction in organ weights compared to vehicle control groups, excluding the potential toxicity of *B. prionitis*. Furthermore, none of the experimental groups showed pathological alterations in the selected vital organs, during the evaluation of histopathology in H and E stained sections. No features of cell injury, necrosis, fibrosis, inflammatory cell infiltrates, congestion, or hemorrhage were observed in the histology sections of the experimental rats of all groups. These findings exclude the potentially toxic effects of the plant *in vivo,* negating the conclusions made by Sawarkar et al. (2016) and Honmane et al. (2021).

CONCLUSION

The present study was conducted to evaluate the long-term toxic effects of hexane, ethyl acetate, butanol, and aqueous extracts of *B. prionitis,* which is a widely used medicinal plant in traditional practice. The findings on acute and 28-day repeated dose oral toxicity assessments of selected plant extracts resulted in neither mortality nor treatment-related hematological, biochemical, and histopathological changes in experimental rats. The significant changes observed in red cell parameters and white cell counts compared to the untreated control group suggest a potential increase in erythropoiesis and immunity in experimental rats treated with *B. prionitis* extracts. Further, the findings on total cholesterol and glucose concentrations and the activity of AST and ALT suggest potential changes in lipid and carbohydrate metabolism and liver functions following the treatments. However, the absence of statistically significant changes compared to the respective control groups and the lack of those changes in both male and female rats exclude potential toxic effects from *B. prionitis.* The findings on the relative weight of organs and histopathology further corroborated the safe use of the selected extracts at the therapeutic dose in therapeutic applications.

ACKNOWLEDGEMENTS

The grants from the National Science Foundation, Sri Lanka (RG/2016/HS -03) and the UGC block grant, University of Ruhuna (RU/PG- R/16/14) are acknowledged for the financial assistance provided.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no competing interests.

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