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RESEARCH

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Toxicity assessment of *Phlogacanthus thyrsiflorus,* a traditionally used anthelmintic plant of India

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Abstract

Background The leaves of *Phlogacanthus thyrsiflorus* are used as an anthelmintic remedy by the tribes of upper Assam. This study evaluates its toxic effects in laboratory bred mice and rats. Mice were orally dosed for 5 days, whereas rats were dosed for 28 days and variations in behaviour, feeding habits and blood parameters were recorded. The vital organs were processed for histopathology to observe any alternations from normal architecture.

Results No mortality or adverse toxic effects were manifested in this assessment. Evaluated parameters, namely feeding behaviour, body weights and relative organ weights, appeared to be similar to the control animals. Also, the haematological and serum biochemical parameters and histopathological studies revealed normal results.

Conclusions The study indicates that *P. thyrsiflorus* may not be capable of causing toxic effects in mice and rats, and hence, its traditional use as an anthelmintic could be continued. However, other studies to further validate this may be carried out.

Keywords Anthelmintic, *Phlogacanthus thyrsiflorus*, Toxicity, Traditional medicine

Background

The common use of traditional medicines in several communities is now gaining global attention [1]. However, these traditional medicines continue to be used rampantly without scientific validation of their potential toxic effects [2]. Medicinal plants contain numerous phytochemicals and metals which may be toxic to their users [3]. Hence, no matter how efficacious medicinal plants are, they must undergo toxicity assessment before they are available for public use [1].

Phlogacanthus thyrsiflorus is used by the tribes of upper Assam in India to treat helminthiasis. Its leaves have been reported to be used in the treatment of allergy [4], gout, rheumatism [5] and fever [6]. It is also known to possess good antioxidant activity [7]. Compounds such as ß-sitosterol, lupeol and betulin were the first bioactive compounds isolated from its leaf extract [8]. An earlier study had also revealed the presence of flavonoids, saponins, tannins, phenols, steroids and terpenoids in its flowers [9]. In spite of its numerous medicinal uses, there are surprisingly no reports of its safety profile.

Toxicity assessment of medicinal plants is a routine test performed by researchers to deem it suitable for human use. Toxicity tests of medicinal plants, namely *Sesbania sesban*, *Cyperus compressus* and *Asparagus racemosus*, were performed by Soren and Yadav [2]. The study reported that these plants are capable of producing toxic effects in its users [2]. Likewise, Wetchakul et al. [10] also evaluated some medicinal plants used in Thai tradition for their toxic effects. Such routine studies assist

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in establishing the safety profile of the medicinal plant and also in the determination of safe but effective dose, thereby eliminating future health complications caused by its uncontrolled usage. This study reports the toxicity profile of the methanolic leaf extract of *P. thyrsiflorus* in animal models.

Methods

Study material and animals

Fresh leaves from natural habitats were harvested, and herbarium was made. It was identified by a taxonomist and the dried leaves were extracted in methanol using a Soxhlet apparatus. The obtained crude extract was used to perform this assessment. Acute oral toxicity study was performed on female Swiss albino mice about 6–8 weeks of age and about 25–30 g. Likewise, sub-chronic oral toxicity study was performed in albino rats (Wistar strain) of both sexes, about 8 weeks of age and about 180 − 200 g. Animals were acclimatised for 15 days in the animal room at 22 ± 3 °C on a light/dark cycle of 12 h. They were housed singly in polycarbonate cages and were given sufficient food and water. Experimental procedures were carried out in accordance with the guidelines of the Institutional Ethical Committee (Animal Model) and ARRIVE.

Acute oral toxicity study

Acute oral toxicity study was executed in accordance with the guideline of Organization for Economic Cooperation and Development (OECD) [11]. A limit dose of 2000 mg/kg b.w. of plant extract was fed to 5 female Swiss albino mice. Animals were fasted for 3 h from food only before dosing and again for another 1 h after dosing. Plant extracts were dissolved using a few drops of 1% dimethyl sulphoxide (DMSO). Animals were dosed individually and observed for adverse clinical signs (tremors, convulsions, salivation, diarrhoea, lethargy, coma, gait, sleep and posture) or mortality during the first 30 min and then periodically during the first 24 h. If the first mouse survived, four additional mice were dosed at 48-h interval and kept under observation for 14 days. The LD50 was concluded to be above 2000 mg/kg if 3 or more animals survived.

Sub-chronic oral toxicity study

Sub-chronic oral toxicity study was executed employing 3 extract doses in accordance with the dosage recommended by traditional practitioners. Accordingly, 200 (lower dose), 400 (recommended dose) and 800 (higher dose) mg/kg body weight (b.w.) were used in accordance with the OECD Repeated Dose 28-day Oral Toxicity Study in Rodents guidelines [12]. Wistar rats of both sexes were divided into 8 groups ($n = 10$, 5/sex). Group I (control) received only the vehicle (water), whereas groups II (200 mg/kg b.w.), III (400 mg/kg b.w.) and IV (800 mg/kg b.w.) were administered the plant extracts. Group V (positive control) was fed with a single dose of 50 mg/kg b.w. of acetaminophen (ACP), and groups VI, VII and VIII served as the satellite controls for the control, the highest extract concentration group and ACP group, respectively. All animals were dosed once daily for 28 days and the 3 satellite groups were kept for a 2-week observation period to monitor/detect any delayed occurrence or persistence of, or recovery from toxic effects. A few drops of 1% DMSO were used to dissolve the extracts and ACP, and the same amount was also fed to the control group. During this study, animals were observed once daily for any signs of toxicity or mortality. Animals were killed by giving anaesthesia: groups I–V at the end of 28 days and groups VI–VIII (satellite groups) at the end of 42 days. Prior to this, animals were prepared by overnight fasting (but allowed free access to water) using a pre-anaesthetic, atropine (0.02 mg/kg; s/c). Following this, animals were given an anaesthetic drug, barbiturate i/p and blood samples were collected through cardiac puncture, for haematological and serum biochemical analysis.

Food and water consumption

On the last day of each week, body weights and food and water consumed by animals were measured and recorded [13–16]. The recorded results of treated animals were compared with control values.

Haematological and Serum biochemical analysis

Blood samples of animals were collected by cardiac puncture, and about 2 ml was transferred each into EDTA and non-EDTA tubes. Blood samples from EDTA tubes were analysed by a semi-automatic cell counter (Alfa Basic, Swelab, Germany) for haematological parameters, viz. red blood cell (RBC), white blood cell (WBC) and platelet counts, differential WBC count (neutrophil, lymphocyte, eosinophil and monocyte), packed cell volume (PCV), mean corpuscular volume (MCV) and haemoglobin (Hb) concentration. Likewise, blood from non-EDTA tubes was analysed using a semi-automatic biochemistry analyser (Synergy BIO 1904C, Euro Diagnostic Systems Pvt. Ltd., India) for serum biochemical parameters, viz. serum glutamic oxaloacetic transaminase (SGOT), serum glutamic–pyruvic transaminase (SGPT), alkaline phosphatase (Alk), total bilirubin (TBil), albumin (Alb), creatinine, urea, uric acid, sodium (Na^+) and potassium (K^+).

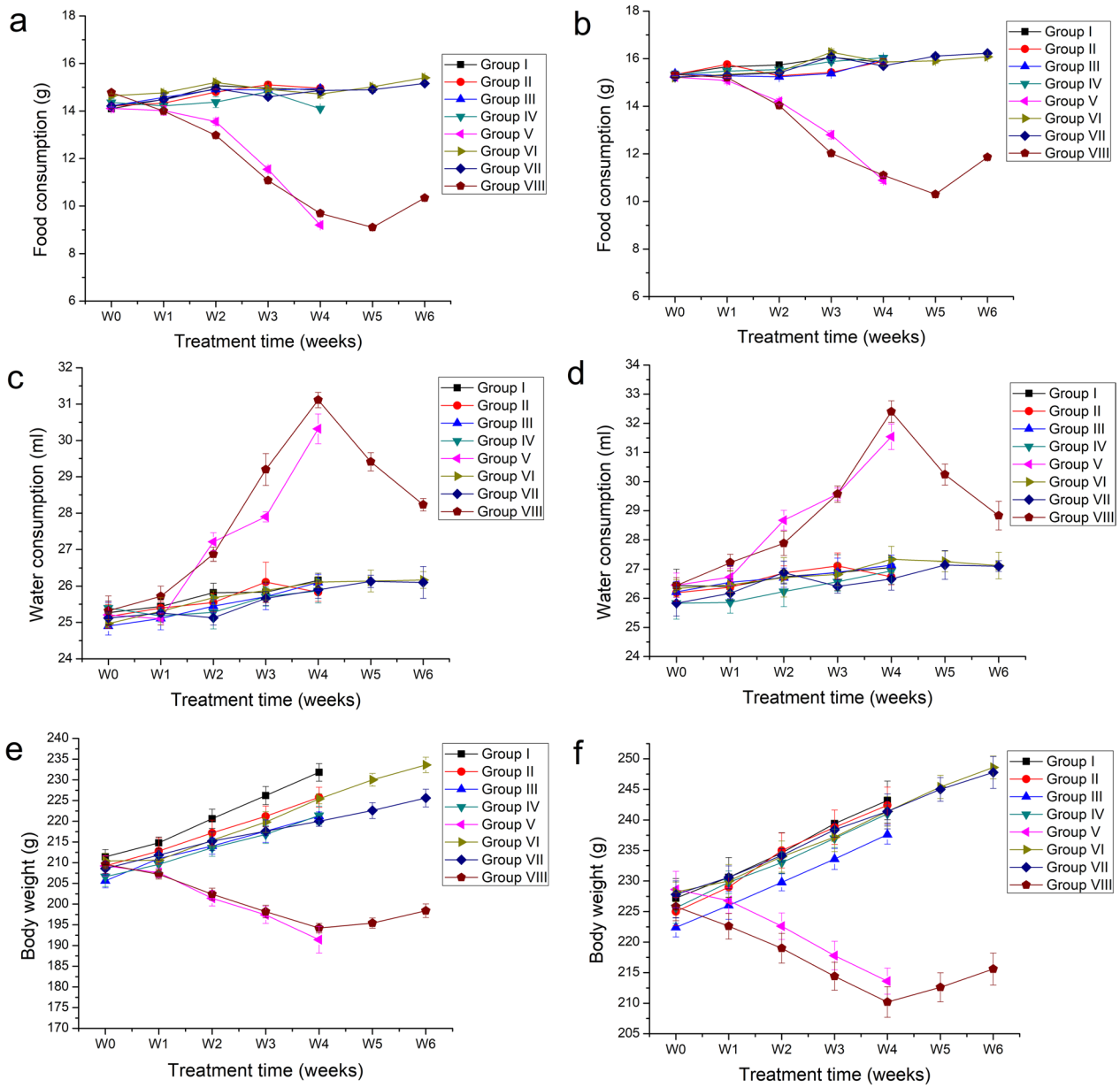

Fig. 1 Effect of *P. thyrsiflorus* leaf extract on food consumption of **a** female rats and **b** male rats; water consumption of **c** female rats and **d** male rats; body weight of **e** female rats and **f** male rats

Gross necropsy and relative organ weights (ROW)

After collection of blood samples, cervical dislocation was performed on the and a gross necropsy was done where the external body surface, orifices, thoracic and abdominal cavities and their contents were examined. Later, the liver, heart, spleen, kidney, brain, adrenal glands, testes and ovaries were excised, adherent tissues trimmed off and their weights were recorded. The relative organ weight (ROW) was calculated using the given formula [17].

$$\mathrm{ROW} = \frac{\text{absolute organ weight}(\mathrm{g})}{\text{Body weight of animal on kill day}(\mathrm{g})} \mathrm{X100}$$

Histopathology

After calculation of ROW, the liver, kidney, spleen and heart were fixed in Bouin's fixative for histopathological examinations. The paraffin-embedded tissue samples were cut in 5–6-µm-thick sections, stained with

Table 1 Effects of sub-chronic oral administration of *P. thyrsiflorus* leaf extract (PLE) and acetaminophen (ACP) on relative organ weights (ROW) of female and male Wistar rats ($n = 5$/sex)

	G-I Control	G-II PLE 200 mg/kg	G-III PLE 400 mg/kg	G-IV PLE 800 mg/kg	G-V ACP 50 mg/kg	Satellite groups		
						G-VI	G-VII	G-VIII
						Control	PLE 800 mg/kg	ACP 50 mg/kg
Female								
Heart	0.63 ± 0.04	0.58 ± 0.01	0.60 ± 0.01	0.67 ± 0.01	0.60 ± 0.02	0.60 ± 0.02	0.67 ± 0.02	0.61 ± 0.02
Liver	5.13 ± 0.18	4.90 ± 0.16[d]	3.86 ± 0.07[d]	5.12 ± 0.17[d]	7.12 ± 0.11[a]	4.80 ± 0.15	4.41 ± 0.15[g]	5.92 ± 0.11[f]
Brain	0.84 ± 0.02	0.86 ± 0.02	0.81 ± 0.03	0.82 ± 0.02	0.81 ± 0.02	0.83 ± 0.02	0.83 ± 0.06	0.86 ± 0.02
Spleen	0.41 ± 0.05	0.39 ± 0.01[d]	0.38 ± 0.01[d]	0.39 ± 0.03[d]	0.45 ± 0.02[b]	0.40 ± 0.02	0.38 ± 0.01	0.41 ± 0.01
Adrenal	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
Kidney (left)	0.81 ± 0.01	0.82 ± 0.02[e]	0.81 ± 0.02[d]	0.82 ± 0.01[e]	0.84 ± 0.01[a]	0.82 ± 0.01	0.81 ± 0.01	0.81 ± 0.02
Kidney (right)	0.84 ± 0.02	0.83 ± 0.01[d]	0.83 ± 0.02[d]	0.84 ± 0.01[e]	0.86 ± 0.01[c]	0.83 ± 0.01	0.82 ± 0.01	0.83 ± 0.01
Ovary	0.05 ± 0.00	0.05 ± 0.00	0.06 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00
Male								
Heart	0.63 ± 0.01	0.58 ± 0.01	0.63 ± 0.00	0.59 ± 0.01	0.61 ± 0.03	0.61 ± 0.02	0.61 ± 0.01	0.60 ± 0.03
Liver	7.00 ± 0.12	7.10 ± 0.29[d]	7.12 ± 0.13[d]	6.99 ± 0.14[d]	8.01 ± 0.11[a]	6.16 ± 0.19	6.22 ± 0.13[g]	6.58 ± 0.11[f]
Brain	1.01 ± 0.06	1.00 ± 0.01	0.99 ± 0.02	0.99 ± 0.01	1.00 ± 0.03	0.97 ± 0.02	0.98 ± 0.03	0.97 ± 0.02
Spleen	0.40 ± 0.03	0.39 ± 0.02	0.37 ± 0.01	0.38 ± 0.02	0.39 ± 0.01	0.41 ± 0.01	0.39 ± 0.01	0.40 ± 0.02
Adrenal	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
Kidney (left)	0.83 ± 0.01	0.82 ± 0.00	0.82 ± 0.03	0.83 ± 0.01	0.83 ± 0.01	0.82 ± 0.02	0.83 ± 0.02	0.83 ± 0.02
Kidney (right)	0.85 ± 0.02	0.84 ± 0.00	0.84 ± 0.02	0.85 ± 0.03	0.83 ± 0.03	0.84 ± 0.02	0.85 ± 0.02	0.85 ± 0.01
Testis (left)	0.72 ± 0.02	0.73 ± 0.02	0.72 ± 0.02	0.72 ± 0.02	0.73 ± 0.02	0.73 ± 0.03	0.72 ± 0.02	0.72 ± 0.02
Testis (right)	0.76 ± 0.03	0.76 ± 0.02	0.77 ± 0.03	0.76 ± 0.03	0.77 ± 0.03	0.77 ± 0.03	0.76 ± 0.02	0.76 ± 0.03

Data are expressed as mean ± S.E.M.; Treatment: G-II to G-V = 4 weeks and G-VII to G-VIII = 6 weeks; W0: week before treatment; W1 – W6: Weeks post-treatment

[a] $p < 0.001$, [b] $p < 0.01$, [c] $p < 0.05$ as compared to control value; [d] $p < 0.001$, [e] $p < 0.05$ as compared to ACP values; [f] $p < 0.001$ as compared to satellite control values; [g] $p < 0.001$ as compared to satellite ACP values, one way ANOVA

haematoxylin and eosin and then examined under a light microscope (Leica DFC425 C).

Statistical analysis

The results obtained are represented as mean ± standard error of mean (SEM). The results were analysed by ANOVA followed by Bonferroni's multiple comparison test. Results were considered to be significantly different at p value < 0.05. Statistical calculations were performed using GraphPad Prism (version 4.5).

Results

Acute oral toxicity study

A single dose of 2000 mg/kg b.w. did not cause any toxic effects or mortality in mice. All the dosed animals appeared healthy and normal in their behaviour, breathing, posture and feeding habits during the study and thereafter. Also, on administration of a higher dose of 5000 mg/kg b.w., no mortality was observed and all the animals were found to be healthy. Hence, it can be assumed that the oral LD50 of the extract is greater than 5000 mg/kg b.w. in mice.

Effects on food consumption

The highest extract-treated (800 mg/kg b.w.) female rats showed only a slight decrease (1.81%) in food consumption, but no significant changes were noticed in their male counterparts. However, animals in control groups showed an increase in food consumption by 5.24% (female) and 3.22% (male) from week 0 to 4. Similarly, in the satellite control groups, food consumption increased by 4.93% (females) and 5.47% (males) from week 0 to 6. The extract-treated satellite groups also showed a 6.26% (females) and 6.16% (males) increase in food intake. Notably, ACP-treated animals showed a significant decrease in food consumption by 34.80% and 28.45% in female and male rats, respectively, whereas the satellite ACP groups showed an increase by 30.04% (female) and 22.68% (male) (Fig. 1a, b).

Table 2 Effects of sub-chronic oral administration of *P. thyrsiflorus* leaf extract (PLE) and acetaminophen (ACP) on some haematological parameters ($n = 5$/sex)

	G-I Control	G-II PLE 200 mg/kg	G-III PLE 400 mg/kg	G-IV PLE 800 mg/kg	G-V ACP 50 mg/kg	Satellite groups		
						G-VI	G-VII	G-VIII
						Control	PLE 800 mg/kg	ACP 50 mg/kg
Female								
RBC (/cmm)	7.42 ± 0.05	7.17 ± 0.01	7.52 ± 0.08^{e}	8.42 ± 0.04^{d}	6.25 ± 0.53^{b}	7.67 ± 0.02	7.37 ± 0.02	6.93 ± 0.01
WBC (/cmm)	7600 ± 401	7780 ± 250^{d}	7500 ± 216^{d}	7690 ± 488^{d}	6302 ± 223^{a}	7500 ± 134	7638 ± 238^{h}	6980 ± 302^{g}
Neutr (%)	16.87 ± 0.27	16.67 ± 0.25^{d}	16.60 ± 0.23^{d}	16.43 ± 0.19^{d}	11.11 ± 0.93^{a}	16.76 ± 0.21	16.38 ± 0.22^{h}	13.62 ± 0.22^{g}
Lym (%)	70.6 ± 2.37	73.2 ± 1.3^{d}	70.18 ± 2.01^{d}	72.12 ± 1.90^{d}	54.01 ± 0.71^{a}	71.51 ± 0.77	73.71 ± 1.33^{h}	63.74 ± 1.05^{g}
Eos (%)	4.20 ± 0.58	4.00 ± 0.54	3.40 ± 0.48^{f}	4.12 ± 0.04	3.65 ± 0.35	3.94 ± 0.11	4.80 ± 0.03	3.79 ± 0.44
Mncs (%)	3.40 ± 0.25	3.60 ± 0.21	3.60 ± 0.21	3.30 ± 0.34	3.60 ± 0.24	3.40 ± 0.35	3.80 ± 0.16	3.60 ± 0.23
PCV (%)	37.73 ± 0.19	37.50 ± 0.06	38.11 ± 0.13	37.57 ± 0.16	34.47 ± 0.21^{c}	38.23 ± 0.06	37.84 ± 0.36	35.51 ± 0.32
HGB (gm/dL)	14.18 ± 0.04	13.40 ± 0.11^{d}	13.34 ± 0.14^{d}	13.60 ± 0.10^{d}	9.09 ± 0.57^{a}	13.42 ± 0.02	14.16 ± 0.04^{h}	10.42 ± 0.02^{g}
MCV (/cuμ)	51.11 ± 2.02	51.34 ± 0.03^{d}	50.09 ± 1.50^{d}	54.75 ± 0.33^{d}	63.99 ± 1.13^{a}	56.41 ± 0.30	54.53 ± 0.12^{i}	59.84 ± 0.09
PLT (× 10^2/cmm)	8370 ± 4870	9884 ± 6870^{d}	8754 ± 3360	9309 ± 5910^{f}	7136 ± 4860	8260 ± 2091	8572 ± 4120	7848 ± 6440
Male								
RBC (/cmm)	8.30 ± 0.02	8.32 ± 0.12^{b}	8.36 ± 0.11^{b}	8.18 ± 0.05	7.10 ± 0.07^{a}	8.13 ± 0.07	8.25 ± 0.18	8.12 ± 0.00
WBC (/cmm)	9147 ± 276	9073 ± 167	8944 ± 268	9289 ± 435	7160 ± 218	8849 ± 715	9205 ± 490	7860 ± 548
Neutr (%)	16.63 ± 0.24	16.89 ± 0.16	15.97 ± 0.29	17.29 ± 0.22	13.38 ± 0.64	16.91 ± 0.25	17.15 ± 0.36	15.36 ± 0.03
Lym (%)	73.40 ± 0.16	74.31 ± 1.06^{b}	71.60 ± 0.92^{b}	72.40 ± 0.16^{b}	63.29 ± 2.15^{a}	72.80 ± 1.15	70.60 ± 0.92	68.40 ± 1.12
Eos (%)	2.39 ± 0.15	3.10 ± 0.22	2.60 ± 0.40	3.16 ± 0.40	2.60 ± 0.50	2.20 ± 0.73	2.40 ± 0.83	3.00 ± 0.40
Mncs (%)	1.20 ± 0.66	1.23 ± 0.58	1.71 ± 0.48	1.40 ± 0.50	1.60 ± 0.24	1.80 ± 0.24	1.20 ± 0.37	1.40 ± 0.24
PCV (%)	41.18 ± 0.44	42.02 ± 0.33^{c}	41.00 ± 0.39	42.23 ± 0.26^{c}	39.00 ± 0.57	41.19 ± 0.64	40.70 ± 0.16	41.37 ± 0.15
HGB (gm/dL)	13.56 ± 0.02	13.90 ± 0.27^{b}	13.92 ± 0.18^{b}	14.14 ± 0.04^{b}	9.10 ± 0.11^{a}	14.11 ± 0.00	13.98 ± 0.28^{j}	12.80 ± 0.12^{g}
MCV (/cuμ)	50.58 ± 1.17	51.55 ± 0.12^{b}	52.53 ± 0.96^{b}	50.41 ± 0.08^{b}	61.00 ± 0.57^{a}	53.40 ± 0.12	51.21 ± 0.03^{j}	59.65 ± 0.02^{g}
PLT (× 10^2/cmm)	8404 ± 4800	9170 ± 5622^{d}	8312 ± 4081	8512 ± 2270	7042 ± 3728	9100 ± 3531	8716 ± 5367	8200 ± 5295

Data are expressed as mean ± S.E.M.; Treatment: G-II to G-V = 4 weeks and G-VII to G-VIII = 6 weeks

Female: $^{a}p < 0.001$, $^{b}p < 0.01$, $^{c}p < 0.05$ as compared to control value; $^{d}p < 0.001$, $^{e}p < 0.01$, $^{f}p < 0.05$ as compared to ACP values; $^{g}p < 0.001$ as compared to satellite control values; $^{h}p < 0.001$, $^{i}p < 0.05$ as compared to satellite ACP values, one way ANOVA

Male: $^{a}p < 0.001$ as compared to control value; $^{b}p < 0.001$, $^{c}p < 0.01$, $^{d}p < 0.05$ as compared to ACP values; $^{g}p < 0.001$ as compared to satellite control values; $^{j}p < 0.001$ as compared to satellite ACP values, one way ANOVA

Effects on water consumption

All the experimental animals showed an increase in water consumption during the experimental period. However, the highest dose-treated female animals showed a significant decrease ($p < 0.001$) in water consumption at week 2 compared to the control. From week 3 to 6, no significant decrease in water consumption was noticed. Notably among male rats, no significant differences were observed and they showed a pattern similar to the control groups. However, animals treated with ACP showed an increase in water consumption by 8.28% (female) and 16.85% (male) from week 0 to 4. Likewise, the ACP-treated satellite groups showed a 10.30% (female) and 8.29% (male) increase in water consumption (Fig. 1c, d).

Effects on body weight

Administration of *P. thyrsiflorus* leaf extract did not cause any noticeable changes in body weights. All extract-treated groups were found to maintain a similar body weight gain pattern as seen in the control animals. Also, the extract-treated satellite groups did not show any notable changes in body weight gain till week 6. In contrast, a decrease of 8.50% and 6.56% body weights was observed in ACP-treated female and male animals, respectively. Further, an increase in body weight was not observed after the withdrawal of ACP in the satellite group for two weeks, rather their body weights decreased by 5.34% in female and 4.51% in male rats from week 0 to 6 (Fig. 1e, f).

Table 3 Effects of sub-chronic oral administration of *P. thyrsiflorus* leaf extract (PLE) and acetaminophen (ACP) on some biochemical parameters of female Wistar rats ($n = 5$)

	G-I Control	G-II PLE 200 mg/kg	G-III PLE 400 mg/kg	G-IV PLE 800 mg/kg	G-V ACP 50 mg/kg	Satellite groups		
						G-VI Control	G-VII PLE 800 mg/kg	G-VIII ACP 50 mg/kg
Female								
SGOT (U/L)	135.82 ± 0.66	136.80 ± 0.41^{c}	136.84 ± 0.95^{c}	137.48 ± 0.30^{c}	260.44 ± 0.11^{a}	136.61 ± 0.83	136.75 ± 0.88^{e}	156.61 ± 0.71^{d}
SGPT (U/L)	70.59 ± 0.81	70.16 ± 0.43^{c}	70.80 ± 0.83^{c}	71.43 ± 0.53^{c}	120.72 ± 0.08^{a}	67.93 ± 0.00	71.19 ± 0.73^{e}	90.94 ± 0.94^{d}
ALP (U/L)	74.43 ± 2.30	76.72 ± 1.80^{dc}	71.33 ± 3.19^{c}	76.28 ± 2.70^{c}	247.53 ± 1.34^{a}	87.13 ± 2.76	81.04 ± 3.73^{de}	179.94 ± 3.02^{d}
TBI (mg/dl)	0.62 ± 0.02	0.53 ± 0.04^{ac}	0.63 ± 0.01^{c}	0.61 ± 0.00^{c}	0.81 ± 0.00^{a}	0.59 ± 0.00	0.62 ± 0.00^{e}	0.69 ± 0.11^{d}
ALB (gm/dl)	3.16 ± 0.08	3.48 ± 0.27^{c}	3.85 ± 0.24^{c}	3.98 ± 0.06	5.50 ± 0.16^{b}	3.93 ± 0.16	3.77 ± 0.18	3.60 ± 0.10
Crt (mg/dl)	0.67 ± 0.00	0.66 ± 0.00^{c}	0.63 ± 0.01^{c}	0.64 ± 0.01^{c}	0.87 ± 0.00^{a}	0.63 ± 0.00	0.65 ± 0.01	0.68 ± 0.01
Urea (mg/dl)	19.26 ± 0.37	19.26 ± 0.37^{c}	19.22 ± 0.39^{c}	19.31 ± 0.43^{c}	26.78 ± 0.53^{a}	19.35 ± 0.97	18.98 ± 0.15	19.70 ± 0.33
U/A (mg/dl)	2.5 ± 0.11	2.5 ± 0.05	2.66 ± 0.05	2.71 ± 0.02	3.40 ± 0.00	2.79 ± 0.03	2.76 ± 0.02	2.40 ± 0.10
Na^{+} (mEq/L)	139.20 ± 0.58	138.12 ± 1.03	138.00 ± 0.83	139.73 ± 0.34	140.88 ± 0.01	141.35 ± 1.80	142.25 ± 0.24	138.97 ± 1.18
K^{+} (mEq/L)	3.59 ± 0.05	3.63 ± 0.05	3.64 ± 0.05	3.70 ± 0.05	3.65 ± 0.05	3.64 ± 0.06	7.63 ± 0.11	3.77 ± 0.06
Male								
SGOT (U/L)	151.60 ± 0.52	152.53 ± 0.98^{b}	153.28 ± 1.4^{b}	150.67 ± 0.10^{b}	372.68 ± 0.03^{a}	152.89 ± 0.04	152.00 ± 0.00^{f}	202.08 ± 0.07^{d}
SGPT (U/L)	76.58 ± 0.99	77.91 ± 1.03^{b}	77.21 ± 1.13^{b}	78.52 ± 0.01^{b}	121.60 ± 0.07^{a}	77.31 ± 0.95	79.52 ± 0.01	77.19 ± 0.74
ALP (U/L)	94.04 ± 3.3	95.43 ± 3.9^{b}	91.97 ± 5.58^{b}	89.14 ± 4.13^{b}	290.71 ± 4.40^{a}	95.45 ± 3.5	97.90 ± 2.7^{f}	171.25 ± 1.11^{d}
TBI (mg/dl)	0.67 ± 0.01	0.68 ± 0.00^{b}	0.67 ± 0.00^{b}	0.66 ± 0.00^{b}	0.93 ± 0.01^{a}	0.61 ± 0.00	0.63 ± 0.01^{f}	0.74 ± 0.01^{d}
ALB (gm/dl)	4.28 ± 0.01	4.27 ± 0.03	4.24 ± 0.01	4.32 ± 0.02	4.06 ± 0.07	4.40 ± 0.1	4.41 ± 0.02	4.40 ± 0.01
Crt (mg/dl)	0.65 ± 0.00	0.60 ± 0.01^{c}	0.66 ± 0.00	0.62 ± 0.00	0.69 ± 0.000	0.60 ± 0.00	0.62 ± 0.00	0.68 ± 0.02^{e}
Urea (mg/dl)	25.67 ± 0.66	26.14 ± 0.93^{b}	27.15 ± 0.25^{b}	27.58 ± 0.27^{b}	38.40 ± 0.91^{a}	26.87 ± 0.41	26.87 ± 0.41	26.73 ± 0.42
U/A (mg/dl)	2.60 ± 0.12	2.60 ± 0.12^{b}	2.65 ± 0.11^{b}	2.70 ± 0.06^{b}	4.22 ± 0.00^{a}	2.83 ± 0.04	2.79 ± 0.50	2.80 ± 0.03
Na^{+} (mEq/L)	140.30 ± 0.67	139.78 ± 0.69	140.46 ± 0.71	140.39 ± 1.09	141. ± 0.83	140.81 ± 1.13	139.98 ± 0.41	141.37 ± 0.81
K^{+} (mEq/L)	3.69 ± 0.03	3.73 ± 0.05	3.76 ± 0.03	3.76 ± 0.03	3.78 ± 0.04	3.72 ± 0.05	3.77 ± 0.03	3.84 ± 0.00

Data are expressed as mean ± S.E.M.; Treatment: G-II to G-V = 4 weeks and G-VII to G-VIII = 6 weeks

Female: $^{a}p < 0.001$, $^{b}p < 0.01$ as compared to control values; $^{c}p < 0.001$ as compared to ACP values; $^{d}p < 0.001$ as compared to satellite control values; $^{e}p < 0.001$ as compared to satellite ACP values, one way ANOVA

Male: $^{a}p < 0.001$ as compared to control value; $^{b}p < 0.001$, $^{c}p < 0.01$ as compared to ACP values; $^{d}p < 0.001$, $^{e}p < 0.01$ as compared to satellite control values; $^{f}p < 0.001$ as compared to satellite ACP values, one way ANOVA

Effects on relative organ weight (ROW) to body weight

None of the animals in the extract-treated groups, including the control, satellite control as well as extract-treated satellite group showed any noticeable changes in their ROWs. On the other hand, the ACP-treated and the satellite ACP animals showed significant increase in ROWs of liver, spleen and kidney (Table 1).

Effects on haematological parameters

Administration of *P. thyrsiflorus* leaf extract did not show any significant deviations in the studied haematological parameters of all the animals (Table 2). However, the ACP-treated animals revealed a decrease in RBC, WBC, neutrophils, lymphocytes, platelet count and haemoglobin concentration and an increase in MCV. In the ACP-treated satellite groups, significant alterations in MCV, WBC, neutrophils and lymphocyte count, and haemoglobin concentration were observed.

Effects on biochemical parameters

Administration of *P. thyrsiflorus* leaf extract did not show any deviations in the studied biochemical parameters except a significant decline ($p < 0.001$) in the total bilirubin of female rats treated with 200 mg/kg b.w. of the extract (Table 3). The extract-treated satellite group did not show any alterations. In contrast, the ACP-treated animals displayed a significant increase in SGOT, SGPT, ALP, urea, creatinine and total bilirubin which was also observed in the satellite ACP-treated animals.

Histopathological studies

Histology of the liver from control group displayed conserved central vein, intact Kupffer cells and hepatocytes (Fig. 2a). Similar normal features were also observed in the liver sections of animals administered the highest

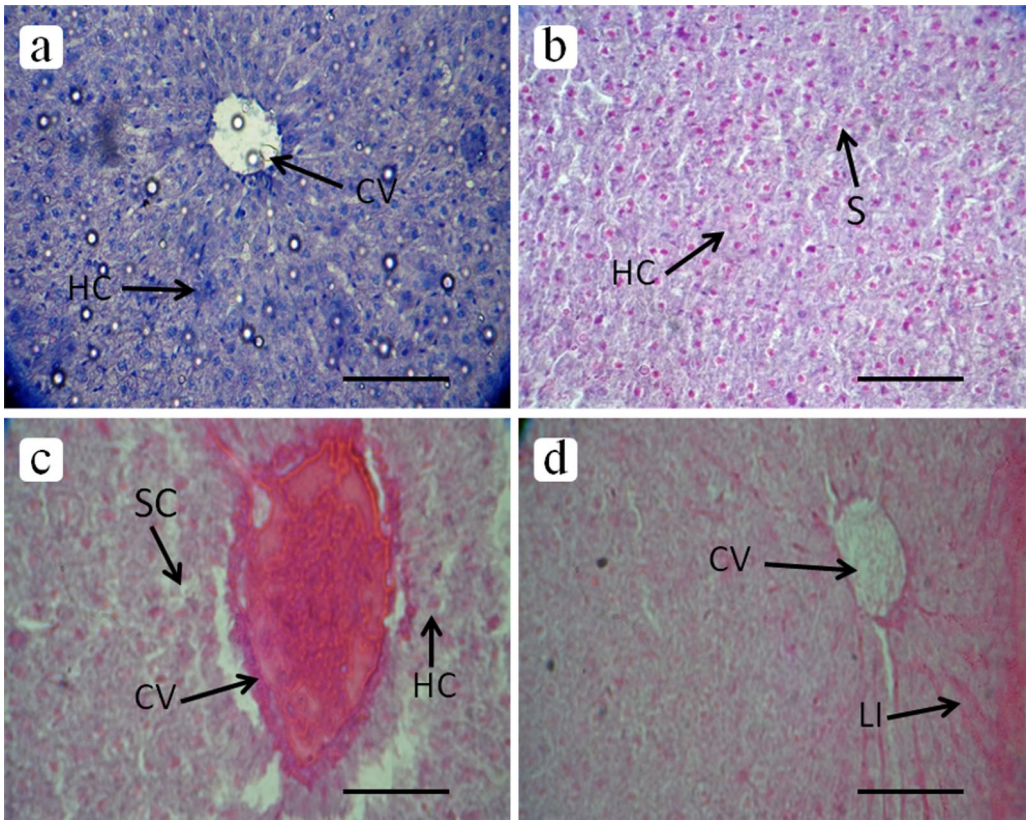

Fig. 2 **a** Photomicrographs of the liver section (magnification: 40x, bar- 100 µm) from control rat, showing clearly conserved central vein (CV), Kupffer cells (KC), hepatocytes (HC). **b** Liver section from rat treated with 800 mg/kg dose of *P. thyrsiflorus* leaf extract showing similar conserved features as control, such as HC and sinusoids (S). **c** Liver section from rat treated with 50 mg/kg of acetaminophen (ACP), showing dilation of CV, sinusoidal congestions (SC), distorted HC. **d** Liver section from ACP-treated satellite rat, showing slight dilation of CV, leucocytic infiltrations (LI)

dose (Fig. 2b). On the other hand, animals treated with ACP revealed dilation of central vein which indicates a backflow in the circulation and congestion in the sinuses (Fig. 2c). In the ACP-treated satellite group, a reduction in dilation of central vein was observed, but leucocytic congestions persisted even after two weeks of acetaminophen withdrawal (Fig. 2d).

With regard to histological features of the kidney, the glomeruli, renal tubules and capsular spaces appeared normal in the control group (Fig. 3a). Likewise, kidney sections from highest dose-treated animals did not show any alterations (Fig. 3b). In contrast, kidney sections of animals administered ACP showed significant alterations in the form of capsular space dilation of glomeruli, multiple focal tubulo-nephritis, vacuole formation around the capsule and distorted tubules (Fig. 3c). However, a slight recovering pattern was observed in ACP-treated satellite groups of animals in terms of reduction in capsular space, but leucocytic infiltrations in distal tubules appeared to be present even after the withdrawal of ACP for two weeks (Fig. 3d).

The spleen histology of rats from control (Fig. 4a) as well as extract-treated group (Fig. 4b) showed normal architecture with a well-conserved red and white pulp area with normal central artery. However, the spleen of animals treated with ACP showed some distortions of follicular cells in the pulp areas (Fig. 4c). No marked alterations were observed in the ACP-treated satellite groups (Fig. 4d).

Further, histology of the heart did not reveal any marked alterations in all the groups. All animals showed normal architecture in the cardiac muscles, connective tissues and myosin filament (Fig. 5a, b, c, d).

Discussion

Assessment of the toxicity profile of medicinal plants determines its global widespread usage. There are several similar studies on medicinal plants such as *Musanga*

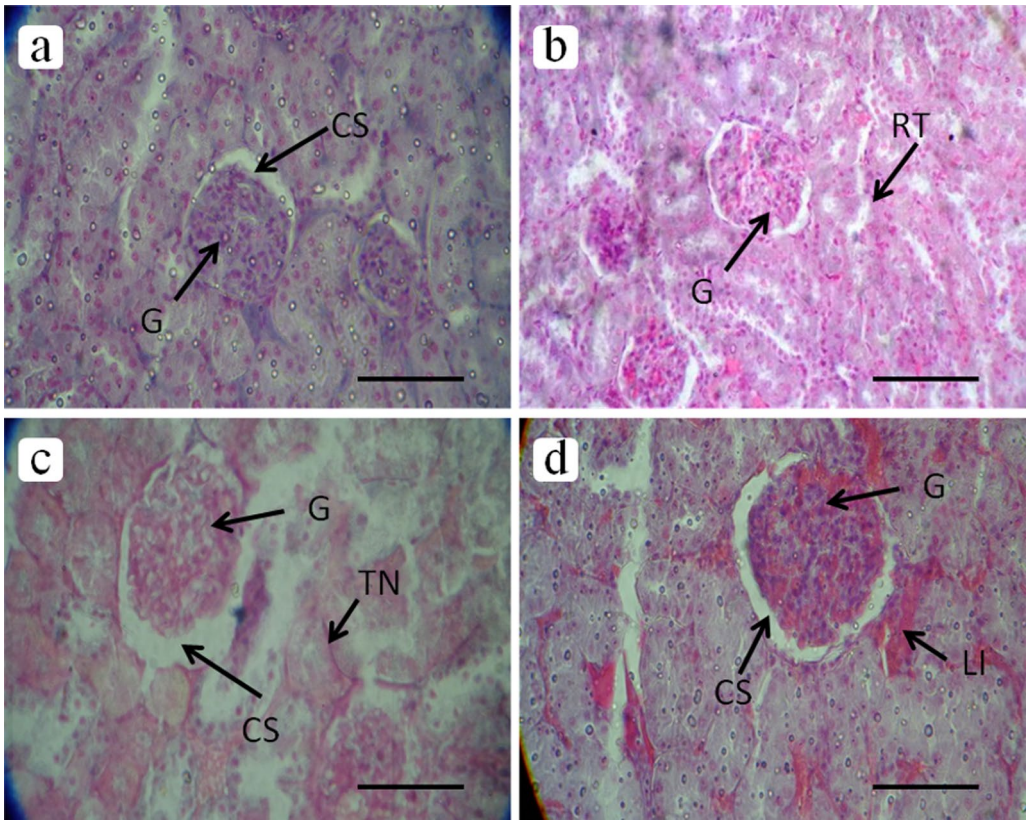

Fig. 3 **a** Photomicrographs of the kidney section (magnification: 40x, bar- 100 µm) from control rat, showing clearly conserved glomeruli (G), renal tubules (RT) and normal capsular space. **b** Kidney section from rat treated with 800 mg/kg dose of *P. thyrsiflorus* leaf extract showing conserved features of G and RTs. **c** Kidney section from rat treated with 50 mg/kg of acetaminophen (ACP), showing dilation of capsular space (CS), distorted RTs and multiple focal tubulo-nephritis (TN). **d** Kidney section from ACP-treated satellite rat, showing slight reduction in dilation of CS, leucocytic infiltrations in tubules

cecropioides from West Africa, studied by Adeneye et al. [15], *Glinus lotoides* studied by Demma et al. [14] and many more where the plant extracts have been considered to be non-toxic up to the tested dose of 2000 mg/kg, as observed in this study.

Similar to this study, a few researchers have reported the toxicological effects of medicinal plants where doses have been selected on the basis of traditionally prescribed amount, such as in the study by Mishra et al. [19] and Gogoi et al. [20]. While assessing toxicological effects of a therapeutic product/herbal drugs, it is important to determine the food and water consumption, since proper nutrient intake is essential to maintain a proper physiological status [21]. Many workers have assessed these parameters in their toxicity studies. Kumarnsit et al. in their study on chronic toxicity of *Mitragyna speciosa* (alkaloid extract) in rats noticed reduction in food consumption of animals which was attributed to the anorectic action of its extract [13]. However, the toxicity evaluation of *M. speciosa* (methanolic extract) in rodents by Harizal et al. did not observe any anomalies in the food and water consumption [22] which is similar to the results obtained in this study.

Although lowering of body weights directly indicates the toxic effect of drugs [23], the decrease in body weight noticed in this assessment could be due to decreased appetite and as such lower calorie intake, as also observed for certain plant constituents, such as P57 from *Hoodia gordonii* [24], saponin from Korean red ginseng [25] and galegine from *Verbesina encelioides* [26]. Also, organ weights comparison between treated and control groups has been used to validate the toxic effect of any test agent [27]. The changes in their weights are often associated with treatment-related effects. However, in this study, no

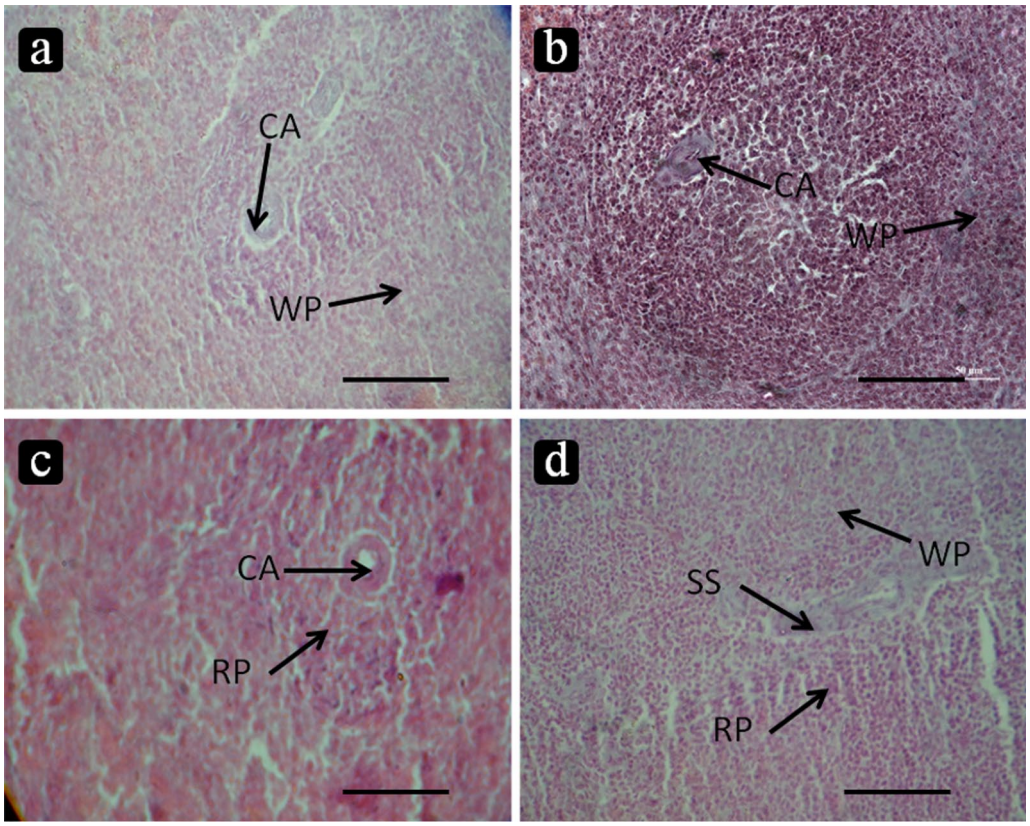

Fig. 4 **a** Photomicrographs of the spleen section (magnification: 40x, bar- 100 µm) from control rat, showing white pulp area (WP) central artery (CA), all clearly conserved. **b** Spleen section from rat treated with 800 mg/kg dose of *P. thyrsiflorus* leaf extract showing WP with a CA, all structures found to be conserved. **c** Spleen section from rat treated with 50 mg/kg of acetaminophen (ACP), showing slightly dilated CA, distorted spleen follicular cells in pulp areas. **d** Spleen section from ACP-treated satellite rat, showing normal architectures in WP, RP areas, Splenic septae, all are well conserved

significant deviations in body weights or relative organ weights were noticed.

Assessment of the hematopoietic system and biochemical parameters is a vital procedure in determining the safety profile of any extract or drug. The blood system is sensitive to toxic substances and it serves as an indicator of toxicity [28]. Any alteration is indicative of toxicity caused due to administration of drug or plant crude extract. This study did not portray any deviations in these parameters after extract administration.

Several workers have carried out histopathology studies of vital organs to study the changes caused in them after the administration of drugs or plant extracts [2, 29, 30]. Ekasari et al. carried out histopathological studies of vital organs to validate the toxic potentials of *Cassia spectabilis* on mice. They observed that its administration was incapable of causing any deformities in the architecture of the vital organs as observed in this assessment [31].

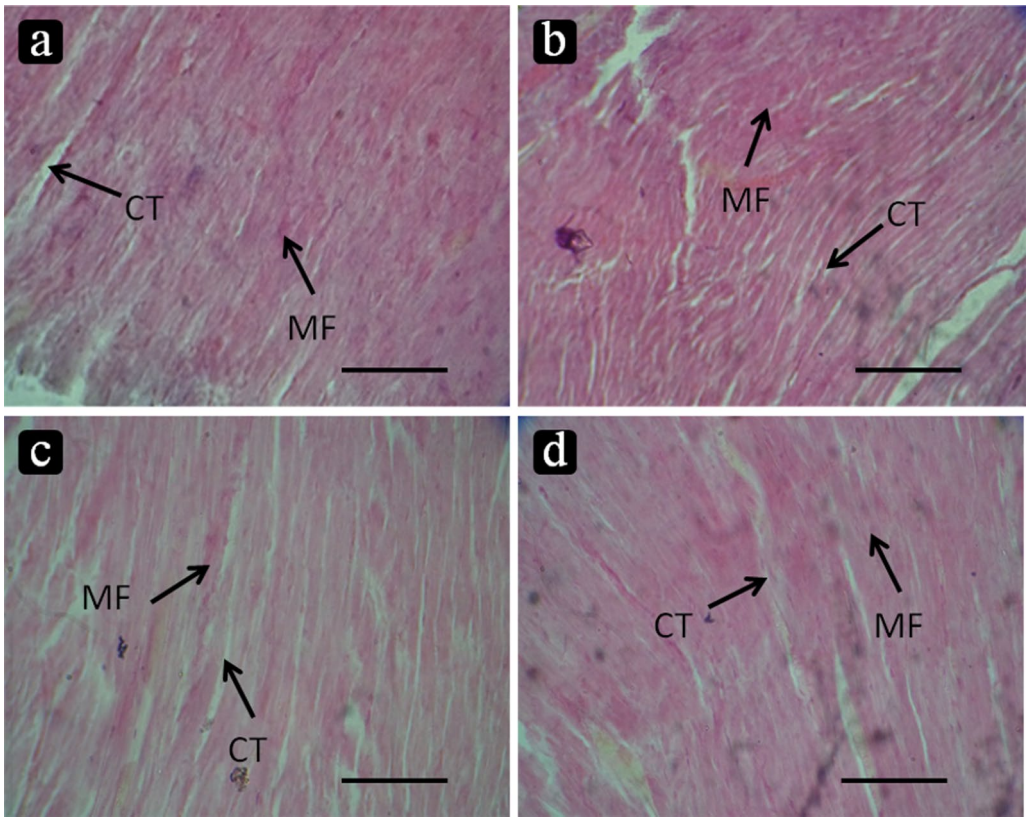

Fig. 5 **a** Photomicrographs of the heart section (magnification: 40x, bar- 100 µm) from control rat, showing conserved connective tissue (CT), myosin filament (MF). **b** Heart section from rat treated with 800 mg/kg dose of *P. thyrsiflorus* leaf extract showing normal features of CT, MF. **c, d** Heart section from rat treated with 50 mg/kg of acetaminophen (ACP) and ACP-treated satellite rat showing normal features of MF, CT, cardiac muscles (CM)

Conclusions

The findings indicate that the long-term usage of *P. thyrsiflorus* may not cause any toxic effects in its users. It can be concluded that its traditional use as a medicine by the tribes of upper Assam can be continued and that it is safe.

Abbreviations

ACP	Acetaminophen
ANOVA	Analysis of variance
ARRIVE	Animal Research: Reporting of In Vivo Experiments
ROW	Relative organ weight

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Author contributions
This study was conceptualised and supervised by AKY. KD carried out the laboratory experiments. KD, AKY and ADS analysed and interpreted the data. KD and ADS wrote the first draft. The final draft was read and approved by all the authors.

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Availability of data and materials
All generated date and material have been mentioned in this article.

Declarations

Ethics approval and consent to participate
Experimental animals used were bred and procured from the animal room of North-Eastern Hill University (NEHU). Experiments on animals were approved by the Institutional Animal Ethics Committee (Animal Models) of NEHU, Shillong (Vide, Member Secretary, IEC, NEHU, dated September 1, 2014). Experiments on animal experiments comply with the ARRIVE guidelines.

Consent for publication
Not applicable.

Competing interests
The authors declare that there are no competing interests.

Plant authentication
Plant material was identified by a taxonomist from the Department of Botany, NEHU, Shillong. Plant material was assigned accession number (Voucher number: NEHU-11920). Research involving plant was approved by the Research Committee of NEHU.

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