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Safety Evaluation of a Traditional Medicinal Plant, *Flemingia vestita* Benth and Hooker in Swiss Albino Mice: An Acute and Sub-acute Toxicity Study

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Abstract

Acute and sub-acute oral toxicity assessment of *Flemingia vestita* root-peel extract was performed in Swiss albino mice as per OECD guidelines 425 and 407. In acute toxicity test, mice were administered extract doses of 175, 550, and 1760 mg/kg body weight (b.w.), and finally, 2000 mg/kg b.w., limit dose. The treated animals were observed for adverse signs or mortality for 14 days. In the sub-acute toxicity study, mice were divided into seven groups (n = 5). Animals in group one served as control, while group five received acetaminophen to act as positive control. Groups two, three and four of animals were given 75, 150 and 300 mg/kg doses of extract for 28 days. Animals in groups six and seven served as the satellite groups for 300 mg/kg and acetaminophen-treated groups for another two weeks to monitor any delayed effects or reversal from adverse effects. The study was approved by the Member-Secretary and the Chairman, Institutional Ethics Committee (Animal Models) of North-Eastern Hill University, Shillong vide letter number: IEC/MS/Misc./08/dated September 26, 2019. Body weight, relative organ weight, haematological and biochemical parameters, and histopathology of the liver, kidney, intestine and spleen of animals were studied. No adverse effects or mortality of animals was observed at 2000 mg/kg b.w., limit dose. The LD_{50} of the extract was estimated to be greater than 2000 mg/kg. In a sub-acute toxicity study, a 300 mg/kg dose showed a noticeable decrease in food, water consumption, and body weight. Likewise, haematological observations revealed an increase in leukocyte count, and biochemical parameters showed an increase in aspartate aminotransferase in 300 mg/kg extract dose. In histopathological studies, mildly disrupted hepatocytes were observed in liver sections of high-dose treated mice. The findings suggest that *F. vestita* root-peel extract is safe for consumption but may cause mild toxicity at a high dose of 300 mg extract/kg b.w.

Keywords: Acute Toxicity, *Flemingia vestita,* Medicinal Plant, Sub-Acute Toxicity

1. Introduction

Traditional medicines occupy a crucial place in healthcare systems, with over 80% of the world's population in developing countries depend upon natural therapeutics for their primary healthcare needs[1]. Due to their usefulness in treating ailments, there has been a resurgence of interest in traditional medicines in recent years[2,3]. While traditional medicines are widely considered to be of low adverse risk in comparison to synthetic drugs, they are also not completely unrestricted from being potentially toxic[4,5]. In this connection, some reports reveal that the direct consumption of crude extracts of medicinal plants can be nephrotoxic, hepatotoxic, or may have haemolytic

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effects, and in extreme cases, the extracts may even cause deaths of consumers[6]. Therefore, if consumed without proper rationale, medicinal plants, even with established pharmacological activities, can cause harmful effects, thus make it important to assess their potential toxicity.

Flemingia vestita Benth and Hooker (Fabaceae), popularly called as *Sohphlang* by the natives in Meghalaya, is a perennial herb which grows up to the height of about 60 cm (Figure 1 (a)). It is considered as edible tuber crop in Meghalaya[7] (Figure 1 (c)). Besides India, this plant is also found in the Sichuan and Yunnan provinces of China and Nepal, and has also been reported from Laos, the Philippines and Vietnam[8,9]. The tuberous roots of *F. vestita* contain high amounts of proteins, calcium, and carbohydrates and are also rich in iron and phosphorous, thus making it a high nutritional value crop[5]. In Meghalaya, the unpeeled root tubers of this plant are regarded as a traditional remedy for intestinal helminth infections as well[10] (Figure 1 (b)). Several research studies reported that this plant possesses anthelmintic[10-12], antimicrobial[13,14], estrogenic[15], and antioxidant activities[7], to name a few. Several useful phytocompounds, such as isoflavonoids, genistein, formononetin, pseudobaptigenin, and daidzein have also been isolated from tubers of *F. vestita*[16]. Despite wide-spread use and many important bioactivities of this plant, the toxicity profiling of *F. vestita* is still lacking. Therefore, the present study intends to assess the acute and sub-acute toxicity of tuberous root-peel extract of *F. vestita* in Swiss albino mice.

2. Materials and Methods

2.1 Experimental Animals

Swiss albino mice of either sex of about 25-30 g were used in the study. The mice were bred in the Animal House of North-Eastern Hill University, Shillong. The selected animals were kept in separate cages at 22 ± 30 °C on a light/dark cycle of 12 h, housed in polypropylene cages and were given free access to standard rodent feed pellets (Pranav Agro Industries Ltd., Delhi) and distilled water ad libitum. All the experimental protocols were approved by the Member-Secretary and the Chairman, Institutional Ethics Committee (Animal Models) of North-Eastern Hill University, Shillong, vide letter number: IEC/MS/Misc./08/dated September 26, 2019.

2.2 Preparation of Plant Extract

Tubers of *F. vestita* were collected from a village, Nongspung in Mawphlang, East Khasi Hills, Meghalaya,



Figure 1. **(a)** *Flemingia vestita,* growing in the field. **(b)** Unpeeled tubers of plant. **(c)** Freshly-peeled tubers of *F. vestita* sold in Shillong market as *Sohphlang*. **(d)** Dried tuber peels of plant.

in November 2019. The plant material was identified at Botanical Survey India, Eastern Regional Centre, Shillong (Accession no. 99463). The tubers were thoroughly washed, peeled, and shade-dried (Figure 1 (d)). The dried peels were ground to fine powder using Bajaj mixer grinder and extracted in methanol (≥99.0%). The crude extract was cooled to room temperature and then stored at 4°C. The yield of extract was 40.84 g from 350 g of fresh tuber peels.

2.3 Acute Toxicity Study

This study used the protocols of the Organization for Economic Cooperation and Development (OECD, 425) revised up-and-down guidelines[17]. A starting dose of 175 mg extract/kg body weight (b.w.) was selected, and a single dose progression with each animal being dosed one at a time at 48 hours (h) intervals was done. Only one mouse was used for each test dose. When the first animal survived, dose for next animal was increased by a factor of 3.2 and was continued till 1760 mg/kg b.w. Since all the animals survived in the consecutive dose progression, a limit dose of 2000 mg/kg b.w., with five animals was used. Each animal was dosed with 2000 mg extract/kg b.w., in a sequence at 48 h interval. Animals were observed carefully every 30 min for first 2 h for signs of any adverse effects, periodically during first 24 h, and then daily for 14 days for any signs of toxicity or mortality. Phosphate Buffer Saline (PBS, pH: 7.4) was used as a vehicle in administering the test substances.

2.4 Sub-Acute Toxicity Study

It was performed as per the OECD guidelines 407[18]. Animals were divided into seven groups (G) (n = 5 mice/group). Animals in Group One (G-I) served as control, while, Groups Two (G-II), Three (G-III) and Four (G-IV) of animals were given 75, 150 and 300 mg/kg b.w., of extract. The animals of Group Five (G-V) were included as positive control and received 50 mg/kg b.w., of Acetaminophen (APAP). Group Six (G-VI) and Seven (G-VII) of animals served as satellite groups for the 300 mg/kg and APAP-treated groups, respectively. The animals in G-I to G-VII were dosed and observed daily for 28 days. The satellite groups (Groups VI and VII) were kept on observation for extra 14 days to detect any delayed adverse effects or recovery from adverse effects. At the end of the study, animals were sacrificed for relative organ weights, haematological, biochemical and histopathological studies.

2.4.1 Body Weight, Food and Water Consumption

The body weights of animals were taken at the beginning of the experiments. This practice was continued every week until the sacrifice of animals at the end of the study. Water and food intake of animals were also recorded weekly until necropsy[19], and their changes in treated groups were compared with a control group.

2.4.2 Relative Organ Weight (ROW)

The liver, kidneys, heart, and spleen were excised and weighed. The relative organ weight to body weight ratio was calculated according to Abdullah *et al*[19].

2.4.3 Haematological Assessment

The blood samples of animals were collected in vials with EDTA to measure the Haemoglobin (Hb), Packed Cell Volume (PCV), polymorphs, lymphocytes, eosinophils, platelets, Red Blood Corpuscles (RBC), total leukocytes, monocytes, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC), using a BC-2800 VET Auto Hematology Analyzer.

2.4.4 Biochemical Assessment

The blood samples of animals were collected in non-EDTA vials and were centrifuged at 3000 rpm at 4°C for 10 min. The plasma obtained was assessed for - urea, uric acid, bilirubin, sodium and potassium ions, Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP) and albumin with the aid of Biochemical-RT-9600, Rayto Analyzer (Rayto Life and Analytical Sciences).

2.4.5 Histopathological Assessment

The liver, kidney, intestine, and spleen from control, the highest dose of the extract (300 mg/kg b.w.) and APAP-treated groups were excised, washed in saline, and preserved in formalin for histopathological studies using the standard techniques[20].

2.4.6 Statistical Analysis

The results are presented as mean ± Standard Error of Mean (S.E.M.). The data were analysed by One-Way Analysis of Variance (ANOVA) followed by Tukey's test. The statistical analysis was carried out using Origin Pro 9. The *p*-value < 0.05 was considered statistically significant.

3. Results

3.1 Acute Toxicity

At the limit dose of 2000 mg/kg b.w., all the experimental animals were observed as healthy and normal in their behaviour, and no mortality was observed during the observation period. The oral LD_{50} of the extract was estimated to be greater than 2000 mg/kg b.w., in mice.

3.2 Sub-Acute Toxicity

3.2.1 Effects on Food Consumption

Figure 2 shows the effects of extract treatment on the food consumption of animals. According to the findings, the consumption of food was significantly reduced in the group treated with 300 mg extract/kg b.w., during the third and fourth weeks compared to the control group (*p<0.05). However, no such significant reduction in food consumption was observed in the groups treated with 75 mg or 150 mg extract/kg b.w.

When compared to the control group, the positive control group, which was given a 50 mg/kg APAP dose, exhibited a significant decrease in food consumption by weeks III and IV (*p<0.05). During the final two weeks (weeks V and VI), there were only slight variations in food intake among the satellite groups.

3.2.2 Effects on Water Consumption

Figure 3 shows the effects of the treatment on water consumption. The study showed that the low and medium-dose-treated groups, which received extract at doses of 75 mg/kg and 150 mg/kg b.w, respectively, as well as the control group, showed no notable changes in their water consumption patterns. However, during weeks III and IV of the study, the high-dose-treated group (300 mg/kg) displayed a significant decline in their water intake (*p<0.05), in contrast to the control group. Furthermore, the APAP-treated group exhibited a significant reduction (*p<0.05) in their water consumption during the same weeks.

It is worth noting that in both the satellite groups, the water consumption varied slightly during the final two weeks.

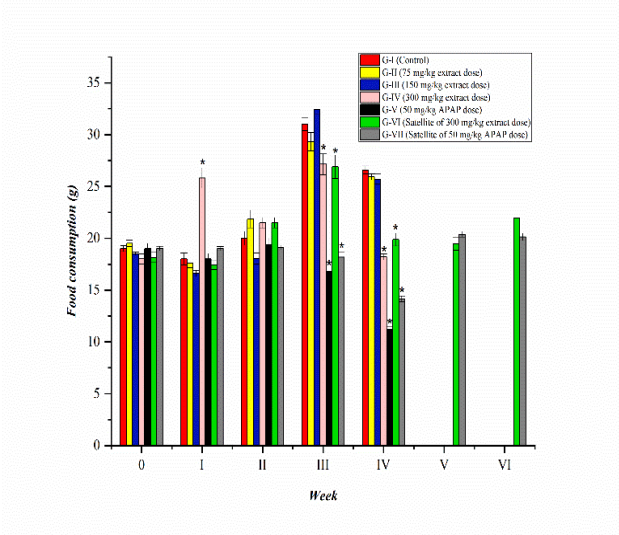


Figure 2. Effects of root-peel extract of *Flemingia vestita* on food consumption of Swiss albino mice. *p<0.05 as compared to control group; one-way ANOVA, followed by Tukey's test.

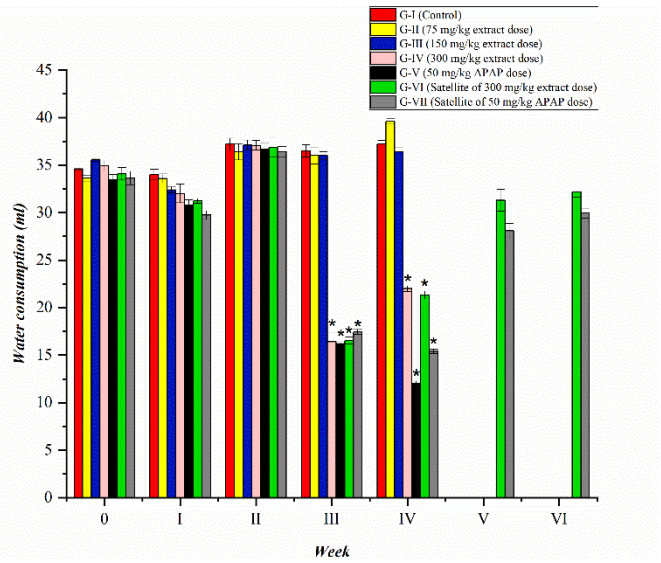


Figure 3. Effects of root-peel extract of *Flemingia vestita* on water consumption of Swiss albino mice. *p<0.05 as compared to control group; one-way ANOVA, followed by Tukey's test.

3.2.3 Effects on Body Weight

Figure 4 illustrates the effect of the extract administration on the body weight of mice. The results showed that all treated groups displayed varying levels of changes in body weight throughout the experiment. Notably, the mice that received the highest dose of the extract (300 mg/kg b.w.) showed a significant drop ($p<0.05$) in body weight in week IV. However, once the extract dosage was stopped, a reversal in the pattern was observed in the satellite group that had received 300 mg extract/kg b.w. Throughout the first four weeks, experimental animals in the control group and groups treated with extract at 75 mg/kg and 150 mg/kg dosages exhibited only slight differences in body weight and were found to be insignificant.

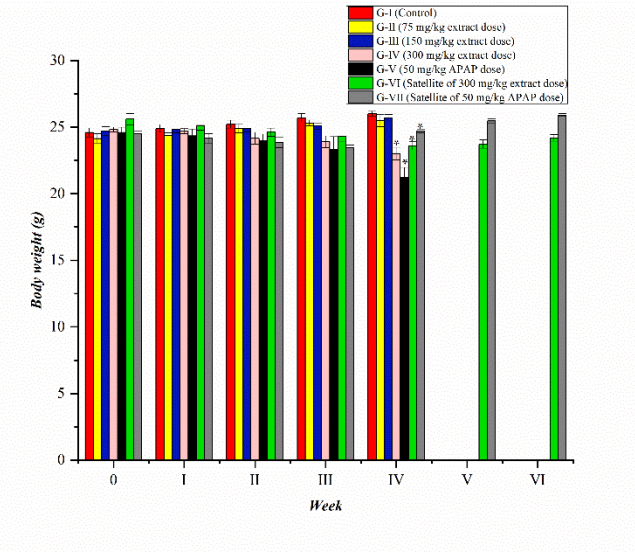


Figure 4. Effects of root-peel extract of *Flemingia vestita* on body weights of Swiss albino mice. *$p<0.05$ as compared to control group; one-way ANOVA, followed by Tukey's test.

Further, at the end of the observation period, animals that had received APAP displayed a considerable loss in body weight. Despite this, APAP-treated satellite groups exhibited a recovery in body weight by the end of week VI.

Table 1 presents the results of the study on the effect of the extract on the weight of the principal organs in relation to the body weight. The relative weights of the liver, heart, spleen and kidneys of the different groups were taken into consideration. Interestingly, the results showed that there was no significant difference in the relative weights of these organs between the extract-treated groups and the control group.

However, the group treated with APAP (acetaminophen) showed a different pattern. Herein, the weights of the liver and kidneys increased, while the weight of the spleen decreased significantly ($p<0.05$).

The haematological analysis of the mice treated with different doses of extract, including APAP is represented in Table 2. It was found that there were no significant differences in any of the parameters between the extract-treated groups of mice (Groups II, III), except for the elevation in the total leucocyte count, polymorphs, and eosinophils in the high-dosage treatment group (Group IV). Notably, the high-dose treated satellite group (Group VI) showed no significant changes in any of the parameters, except for a small decrease in the total leucocyte count after the treatment was withdrawn for two weeks.

In contrast, the haematological parameters of mice treated with 50 mg APAP/kg b.w., exhibited substantial changes ($p<0.05$). Specifically, there was a decrease in

Table 1. Relative Organ Weights (ROW) of Swiss albino mice treated with *Flemingia vestita* root-peel extract for 28 days

						Satellite	
Organs	**G-I**	**G-II**	**G-III**	**G-IV**	**G-V**	**G-VI**	**G-VII**
	(Control)	**(75 mg/kg)**	**(150 mg/kg)**	**(300 mg/kg)**	**(APAP)**	**(300 mg/kg)**	**(APAP)**
Liver	4.82±0.05	4.78±0.06	4.72±0.04	4.05±0.14	7.16±0.14*	4.52±0.20	4.90±0.06
Heart	0.59±0.01	0.58±0.01	0.59±0.01	0.63±0.01	0.63±0.01	0.62±0.01	0.59±0.01
Spleen	2.94±0.64	2.64±0.57	3.25±0.11	2.33±0.13	1.37±0.88*	2.56±0.08	1.13±0.65*
Left Kidney	0.73±0.01	0.74±0.03	0.72±0.01	0.78±0.01	0.82±0.02*	0.75±0.01	0.71±0.01
Right Kidney	0.75±0.01	0.76±0.03	0.73±0.01	0.79±0.01	0.84±0.02*	0.76±0.01	0.74±0.01

Data are expressed as mean ± S.E.M. for each group (G-I to G-VII). Treatment: G-II to G-V = 4 weeks and G-VI and G-VII = 6 weeks; n = 5 mice/group; *$p<0.05$ as compared to control group; one-way ANOVA, followed by Tukey's test.

Table 2. Haematological parameters of Swiss albino mice treated with *Flemingia vestita* root-peel extract for 28 days

						Satellite	
	G-I	G-II	G-III	G-IV	G-V	G-VI	G-VII
Parameters	**(Control)**	**(75 mg/kg)**	**(150 mg/kg)**	**(300 mg/kg)**	**(APAP)**	**(300 mg/kg)**	**(APAP)**
Hb (gm/dL)	12.28±0.12	12.30±0.15	13.02±0.06	10.46±0.17	9.42±0.14*	12.01±0.11	12.07±0.08
Total Leukocyte Count (10^3/cmm)	2.80±0.07	3.40±0.12	3.80±0.09	10.00±0.13*	6.00±0.06*	4.90±0.22*	4.20±0.25*
PCV (%)	32.88±0.58	32.40±0.63	33.02±0.72	35.56±0.10	30.54±0.10	32.00±0.52	31.90±0.36
Polymorph (%)	7.80±0.37	9.00±0.44	8.80±0.37	11.60±0.50*	10.40±0.50*	9.20±0.86	7.80±0.37
Lymphocytes (%)	65.60±1.80	62.20±0.80	63.80±1.39	70.80±1.24	85.40±0.97*	68.00±0.83	68.60±0.97
Eosinophils (%)	1.80±0.37	1.80±0.20	2.60±0.24	7.60±0.50*	2.00±0.31	2.40±1.14	1.60±0.24
Monocytes (%)	1.80±0.20	1.60±0.24	2.60±0.24	1.80±0.20	1.80±0.20	1.80±0.37	1.60±0.24
Platelets (10^5/cmm)	4.50±0.01	4.60±0.14	4.60±0.11	5.20±0.03	3.80±0.01	4.30±0.10	4.20±0.07
RBCs (10^6/cmm)	6.37±0.09	6.28±0.07	6.54±0.05	6.98±0.04	5.16±0.02*	6.40±0.13	6.12±0.05
MCV (/cuμ)	51.60±0.46	50.70±0.17	50.78±0.16	51.6±0.17	59.46±0.19*	52.42±0.35	52.74±0.54
MCH (pg)	16.60±0.12	15.24±0.07	16.20±0.07	16.56±0.09	17.25±0.08	16.64±0.18	16.43±0.12
MCHC (%)	31.22±0.13	30.35±0.37	31.24±0.32	31.70±0.28	30.88±31	30.54±0.33	30.82±0.27

Data are expressed as mean ± S.E.M for each group (G-I to G-VII). Treatment: G-II to G-V = 4 weeks and G-VI and G-VII = 6 weeks; n = 5 mice/group; *p<0.05 as compared to control group; one-way ANOVA, followed by Tukey's test.

Table 3. Biochemical parameters of Swiss albino mice treated with *Flemingia vestita* root-peel extract for 28 days

						Satellite	
Parameters	G-I	G-II	G-III	G-IV	G-V	G-VI	G-VII
	(Control)	**(75 mg/kg)**	**(150 mg/kg)**	**(300 mg/kg)**	**(APAP)**	**(300 mg/kg)**	**(APAP)**
Urea (mg/dL)	38.60±1.77	39.80±0.66	38.40±0.50	38.80±0.86	39.00±0.44	38.00±0.94	38.40±0.50
Uric acid (mg/dL)	4.33±0.03	4.16±0.06	4.20±0.04	4.22±0.04	2.79±0.01*	4.24±0.05	3.26±0.10
Na^+ (mEq/L)	141.00±0.70	139.80±1.46	139.60±0.67	139.20±1.15	148.60±1.50	142.00±0.70	143.80±0.90
K^+(mEq/L)	4.40±0.10	4.32±0.08	4.30±0.09	4.22±0.07	5.93±0.31*	4.26±0.06	4.24±0.09
Bilirubin (mg/dL)	0.72±0.003	0.72±0.01	0.71±0.01	0.73±0.01	0.75±0.013*	0.71±0.01	0.73±0.01
AST (U/L)	111.80±3.52	115.68±2.74	113.44±3.59	126.70±2.93*	191.52±1.72*	120.80±3.16	118.20±2.83
ALT (U/L)	60.80±1.31	63.50±0.63	62.68±1.23	62.08±0.71	149.24±2.77*	63.20±0.80	86.00±1.73*
AST/ALT ratio	1.83	1.82	1.80	2.04	1.30	1.91	1.37
ALP (U/L)	137.40±36.18	139.60±30.60	137.00±25.19	145.40±19.60	167.80±2.77*	139.00±5.56	139.40±7.18
Albumin (mg/dL)	3.22±0.13	3.18±0.09	3.08±0.24	1.80±0.11*	1.29±0.17*	3.12±0.09	3.04±0.08

Data are expressed as mean ± S.E.M. Treatment: G-II to G-V = 4 weeks and G-VI and G-VII = 6 weeks; n = 5 mice/group; *p<0.05 as compared to control group; one-way ANOVA, followed by Tukey's test.

haemoglobin and red blood cells and an increase in total leucocyte count, polymorphs, lymphocytes, and mean corpuscular volume. Remarkably, during the final two weeks of the study, none of the haematological parameters in the APAP-treated satellite groups changed significantly (Group VII).

The biochemical analysis of the mice treated with different doses of extract, including those given APAP, the positive control, is represented in Table 3. The result showed that 75 mg, 150 mg extract/kg b.w., treated animals did not reveal any changes in the biochemical parameters. However, the group that was treated with an extract dose of 300 mg/kg b.w., experienced an increase in their AST levels. In addition to this, the albumin levels in the same group also dropped. On the other hand, animals that were given 50 mg APAP/kg b.w., displayed significant changes in practically all of the examined biochemical markers, including uric acid, potassium, bilirubin, ALT, AST, ALP and albumin.

After dosage termination, the extract-treated satellite group did not display any significant changes to the tested biochemical indicators. Furthermore, there was even a decrease in AST level following two weeks of treatment withdrawal. Even after the treatment was stopped for two weeks, the satellite group of APAP-treated mice showed the persistence of high levels of ALT levels.

When it comes to AST: ALT ratio, a value higher than 1 was observed in all the groups including control, with the high dose treated group exhibiting the maximum value.

3.3 Histopathology

In the control group, the liver showed a normal histological structure with a clear separation of hepatocytes by sinusoids (Figure 5(a)). Similarly, the kidney sections of control animals exhibited kidney tubules and intact glomerulus surrounded by the lining of the Bowman's capsule (Figure 6(d)). The intestine also showed distinct mucosa and sub-mucosa layers, with uniformly arranged and intact villi (Figure 7(g)). The spleen of control animals displayed a normal architecture in both the white and red pulp areas (Figure 8(j)).

However, in the APAP-treated group, the liver sections displayed nuclear breakage or fragmentation, deranged hepatic plates, degenerated hepatocytes, and leukocytic infiltration in the central vein (Figure 5(c)). In the high-dose (300 mg/kg) treated group, only mildly disrupted hepatocytes were observed (Figure 5(b)).

The kidney sections of APAP-treated mice showed severe destruction of the tubules, damaged lining of the Bowman's capsule, and ruptured glomerulus (Figure 6(f)). In contrast, the 300 mg extract-treated mice showed a glomerulus surrounded by the Bowman's capsule with a distinct Bowman's space and renal tubules (Figure 6(e)).

The intestine of APAP-treated mice exhibited distortions in the villi (Figure 7(i)). However, such damages were not visible in the intestinal sections of high-dose treated mice (Figure 7(h)). The spleen sections of the APAP-treated group also displayed distortions in the red pulp region, while the 300 mg-treated mice showed normal architecture of both the red and pulp region (Figure 8(k) and (l), respectively).

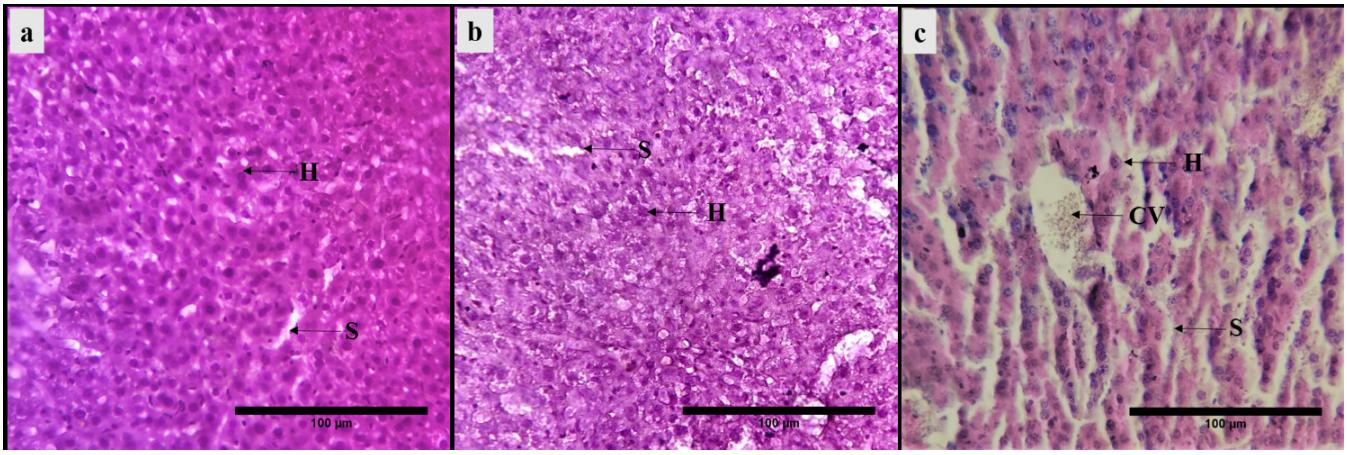


Figure 5. Histological photomicrographs of liver sections of mice. **(a)** Control. **(b)** Extract (300 mg/kg). **(c)** APAP (50 mg/kg). H – Hepatocytes, S – Sinusoids, CV – Central Vein. Scale: 100 µm.

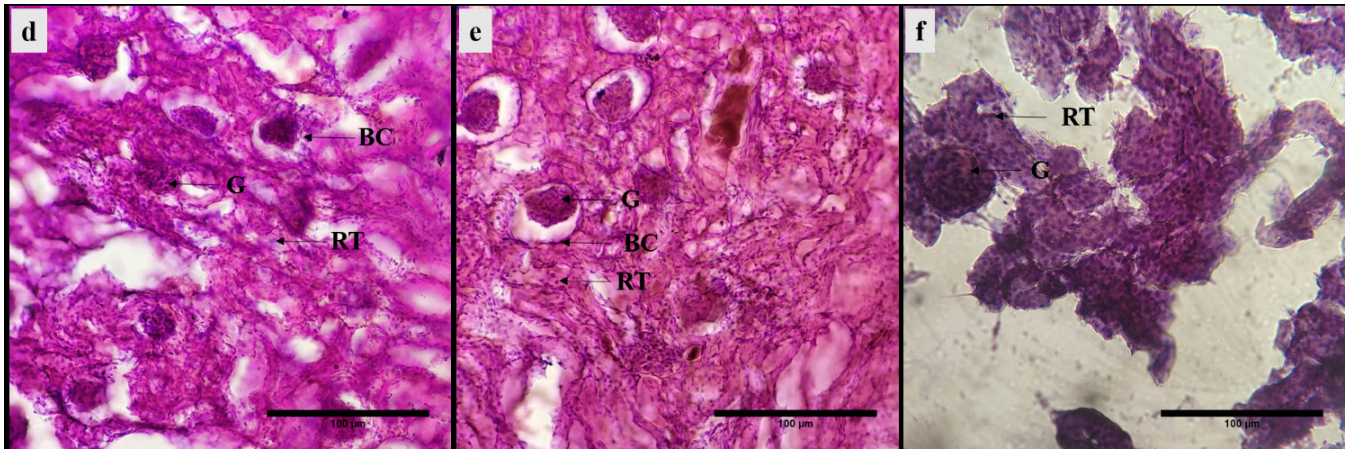


Figure 6. Histological photomicrographs of sections of kidney. **(d)** Control. **(e)** Extract (300 mg/kg). **(f)** APAP (50 mg/kg). BC – Bowman's Capsule, G – Glomerulus, RT – Renal Tubules. Scale: 100 µm.

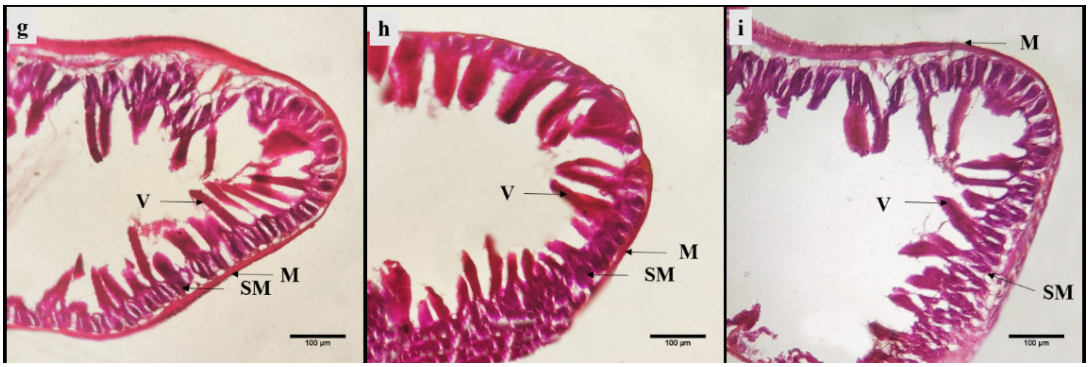


Figure 7. Histological photomicrographs of sections of intestine. **(g)** Control. **(h)** Extract (300 mg/kg). **(i)** APAP (50 mg/kg) M – Mucosa, SM – Sub-Mucosa, V – Villi. Scale: 100 µm.

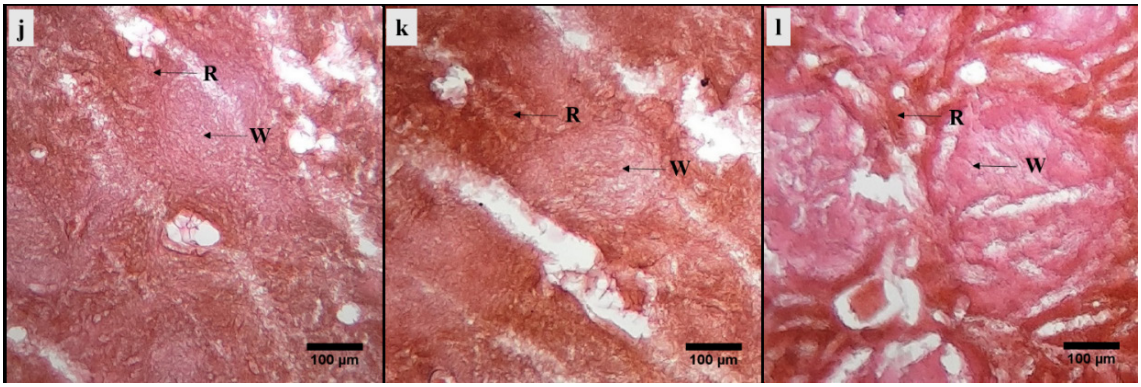


Figure 8. Histological photomicrographs of sections of spleen. **(j)** Control. **(k)** Extract (300 mg/kg), **(l)** APAP (50 mg/kg). R – Red Pulp, W – White Pulp. Scale: 100 µm.

4. Discussion

Several medicinal plants have been used as therapeutic agents in the traditional system of medicines since ancient times. These plants contain a huge diversity of bioactive compounds which also contribute as a template for the discovery of new drugs[21]. Besides efficacy investigations, the preclinical toxicity assessment of traditional medicines is considered vital in the course of drug development. Some previous preclinical toxicity studies have demonstrated that medicinal plants can also be toxic[22-25]. Thus, it is necessary to investigate the potential toxicity of medicinal plants that are used in traditional medicines to ensure their safety for human use.

Although some pharmacological activities of *F. vestita* have previously been studied, no researcher has yet attempted to study the toxicity of tuber peels of this plant. Therefore, the goal of the present study was to examine the acute and sub-acute toxicity of the tuber-peel extract of this plant in Swiss albino mice.

In the acute toxicity test, at a limit dose of 2000 mg/kg, the mice did not show any adverse effects or mortality, and therefore the oral LD50 value of extract was estimated to be greater than 2000 mg/kg. The absence of acute toxic effects can be considered important, however, the data obtained from acute toxicity studies are considered of limited value in clinical applications. The acute toxicity data only offers a general perspective of the test substance and does not necessarily reveal the full potential toxic profile of natural products. Therefore, in the present study a sub-acute toxicity study, consisting of repeated dosing of animals was conducted over 28 days.

During the toxicological tests, the animals' physiological status is significantly influenced by the consumption of food and water[26]. In the present study, the highest extract-treated group (300 mg/kg) resulted in a decrease in food and water consumption from week zero to week four. Notably, APAP-treated animals also showed a considerable reduction in food and water intake. But the satellite groups of animals showed a rise in food and water consumption as soon as the extract treatment was stopped, indicating a pattern of recovery from food and water-related adverse effects.

The reduced food intake is related to other toxicity criteria, including body weight, which is a crucial indicator of the aggravation of unfavourable effects brought out by exposure to potentially harmful substances[27,28]. Although none of the experimental animals showed mortality during the dosage period, significant suppression in weight gain was observed in the 300 mg/kg extract-treated group, as compared to the control. Even though a decline in body weight has been used as an indicator in determining the adverse consequences of drugs and chemicals[29,30], in the present study, the decrease in body weight could be due to an effect of decreased appetite, thereby resulting in lower food intake by the animals. The positive control group also showed a decrease in food intake and subsequently, also a decrease in body weight at the end of the treatment period.

The differences in relative organ weights were found to be statistically insignificant in the extract-treated groups in comparison to the control. However, a general increase in liver and kidney weights and a decrease in spleen weight were observed in the APAP-treated group. The findings suggest that APAP had an impact on the liver, potentially leading to hypertrophy or hyperplasia, as evidenced by the increase in liver weight[31]. The kidneys in this group also exhibited a significant increase in weight, which may be a result of prolonged exposure to APAP or the organ's continuous effort to eliminate it from the body. According to a report by Kluwe, an increase in relative kidney weight can be an indicator of nephrotoxicity[32]. Therefore, the observed increase in kidney weight could signify a potential nephrotoxic effect. Conversely, a decrease in spleen weight may be related to the decrease in body weight of the mice in the same group.

The haematopoietic system is considered to be very sensitive to chemical toxicity, hence assessment of haematological parameters is considered critical in toxicity studies of plant extracts[33]. This study showed a significant increase in the number of leukocytes, specifically eosinophils and polymorphs in group treated with 300 mg extract/kg b.w. The increase in leukocyte count can be correlated with an increase in production of antibodies that aid in the survival and recovery of mice exposed to the extract[34]. Importantly, the leukocyte count of the respective satellite group did not drop to normal levels despite the withdrawal of extract treatment for two weeks. This elevation in leukocyte count is known as leukocytosis and may arise due to some underlying conditions such as an inflammation, infection, or an injury to tissue[35]. The fact that the leukocyte count remained high even after treatment cessation indicates that the extract may possibly have a long-lasting impact on the leukocytes of the animals.

The biochemical serum analysis is considered important in toxicological evaluation of plant extracts/drugs[36]. In particular, the enzymes like ALT, AST, and ALP are considered critical to assess the general health of liver[37]. These enzymes typically show higher levels in the event of liver damage. AST is known to occur in the liver, cardiac muscle, skeletal muscle, kidneys, brain, pancreas, lungs, etc. However, due to its high concentration in liver tissue, ALT is considered more specific to liver than AST. In this study, the highest extract-treated group showed an elevated level of AST ($p<0.05$), but the difference in ALT level was not statistically significant. The AST/ALT ratio (De Ritis Ratio) is recognised as a marker of liver function[38], and if this ratio is large, liver-related issues are likely to occur. In this study, a dose-dependent AST/ALT ratio, which was greater than 1, was seen in all the extract-tested animals, indicating a state of possible hepatic fibrosis[39]. APAP treatment also showed a considerable increase in the levels of liver enzymes.

In the histopathological examination, the 300 mg/kg extract treated animals did not reveal any evidence of adverse effects in the selected organs in mice except mild disruptions of a few hepatocytes, which was not significantly different from that of control. However, APAP-treated group showed slight modifications in tissues of liver, including a moderate necrosis and mild inflammation, which can be correlated to the increase in liver weight and level of liver enzymes. This group also exhibited distortion in the red pulp area of spleen, which was evident from the decrease in ROW.

This is the first report on the toxicity profile of *F. vestita* tuber peels. However, genistein, which is one of the active principles of the tuber peels of this plant has been scrutinised for its toxicity[40,41], wherein the high dose of genistein was noted to have toxicological effects in experimental animals. It is important to argue here that traditional medicine practitioners prescribe the crude extract of this plant to their clients, which is a complex entity of several naturally occurring substances in it, rather than a single pure compound of synthetic origin. Also, it has also been claimed that the active principles, whether they are synthetic or natural, may be more harmful than the whole plant extract or its crude form[42], and there is a possibility that additional components in the crude extract of medicinal plant can regulate its overall safety[43]. It is in this context that it was considered necessary to assess the toxicity of *F. vestita* tuber peels, which are in particular consumed in the traditional medicine practise, rather than the pure compound of plant tubers, i.e., genistein.

5. Conclusion

This study suggests that the oral LD_{50} value of tuber peel extract of *F. vestita* is greater than 2000 mg/kg in mice, hence it may be considered safe in acute toxicity test. However, in sub-acute toxicity study, the high dose (300 mg/kg) of extract show a marked decrease in food and water consumption and body weight of animals. It also reveals an increase in leukocyte count, besides an increase in AST levels. Taken together, these observations suggest that *F. vestita* tuber peels are safe for consumption but may cause mild toxicity at high dose of 300 mg/kg in sub-acute toxicity studies in mice.

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7. References

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