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Sub-acute toxicity study of aqueous extract of *Ficus racemosa* Linn leaves with evaluation of haematological, biochemical and histopathological parameters on rat

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Sub-acute toxicity study of *Ficus racemosa* Linn aqueous leaf extract was carried out in this study, a herb which has wide application for treatment of various ailments including hyperglycaemia, gastritis, asthma, dysentery, menorrhagia, glandular enlargement and sore throat. In this study, the first group of rats served as the control group while the other two groups were given *F. racemosa* L. leaf aqueous extract (FRLAQE) at doses of 100 and 200 mg kg-[1] bodyweight, respectively via daily oral gavages for 28 days. Animals of all groups were sacrificed on 29[th] day of experimentation. For various haematological and biochemical parameters blood samples were collected to determine haemoglobin, WBC count, RBC count, PCV, ESR, bleeding time, clotting time, blood glucose, serum creatinine, urea, total bilirubin and direct bilirubin. Gross and histological examination of kidney, liver, heart, spleen and brain sections was done by veterinary pathologist. Statistics: ANOVA followed by Tuckey's HSD procedure were used to analyse the data. Haematological and biochemical study did not show any toxic effects following treatment. Pathologically, the extract was found to be non-toxic as no gross and histopathological abnormalities were observed.

Keywords: Aqueous extract, Biochemical analysis, *Ficus racemosa* linn, Haematological analysis, Histopathology, Sub-acute toxicity

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Being the "treasure house" of many plant species, North Eastern (N. E) region of India became the hot spot of the world containing 50,000 endemic plant species that contributes 20% world flora. Assam a part of North East India is a rich source of 2,000-3,000 species of the medicinal plants that are the basis of traditional healing practices since years[1] (Nath, 1975-76). Exploration of these indigenous medicinal plants leads to the development of new biomolecules from time to time which otherwise ethnic practices of use of plants by the tribal community of different states of North East India. Assam is the inhabitant of many tribes that uses plants for different health problems. Though some of their traditional healing practices are very useful to mankind but because of lack of scientific validation their remedies are completely based on trial and error mostly depending on traditional cultures, beliefs, and superstition[2]. *Ficus racemosa* Linn. (Moraceae) commonly known as "Dimoru" in Assam is large-sized, lactiferous, deciduous tree with a height of 15-18 m, devoid of prominent aerial roots[3]. *Ficus* species are used by the local tribes of Assam for the treatment of various ailments. Mishing community is one of the major tribal communities of Assam use stem of Tajik (*F. racemosa* L) in bone fracture where the traditional healers use latex of stem coated on the bamboo strips and tie over the fractured part[4]. Another tribe of Assam known as Sonowal Kachari is a plain Scheduled Tribe of Assam, inhabits in Dibrugarh, Lakhimpur, Dhemaji, Tinsukia, Jorhat and Golaghat districts of Upper Assam uses *Ficus*

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glomerata in the treatment of mumps. The people of this community crush young shoots of *Calotropis gigantea*, *Drymaria cordata* and *Ficus glomerata* together and extracted juice is useon the infected portion[5]. Again, the tribal people settled in the fringe villages of Hollongapar Gibbon Sanctuary, Jorhat, Assam uses *F. racemosa* L. (Dimoru) for the treatment of hearing loss, blood disease and headache[6]. Bioactive constituents like β-sitosterol, glauanol acetate in *F. racemosa* L., has been found to be largely responsible for the therapeutic potentials as a boon for ailments of human.

Although there is no such reporting's of traditional use of *F. racemosa* L. in the treatment of hyperglycemia by tribal people of Assam but its hypoglycemic property has been proved by many researchers time to time. The efficacy of aqueous extract of *F. racemosa* L. leaves (FRLAQE) in diabetic neuropathic situations using *in vitro* (cell line), *in vivo* (Wistar rat), and *in silico* study with protein (PDB ID: 5BTR) revealed FRLAQE at a standardized low dose and high dose improves neurite growth in *in vitro* N2A cell line, counteracting action on reactive oxygen species (ROS) generation resulting in improving diabetic neuropathy in *in vivo* DN models.

Despite these numerous pharmacological potentials of *F. racemosa* L. leaves, its subacute toxicity study is not carried out so far. However, acute and sub-acute toxicity study of *F. racemosa* L. bark has been investigated Panwar *et al.*[7] and found liver damage in sub-acute toxicity study but it is reversible. Histopathological damage to kidney was not marked but there was significant change in serum creatinine and urea. Therefore, the proposed study was undertaken toinvestigate the toxicity profile of FRLAQE in rat with a clear justification whether the extract can be applicable and safe as human medicine in future since the extract got a good efficacy against different ailments so far.

Materials and Methods

Animals

About 250-300 g of either sex Wister rats were procured from the Small Animal House Facility of College of Veterinary Science, Assam Agricultural University, Khanapara campus, Assam and they were fed with standard pelleted laboratory diet and clean drinking water. For acclimatization, the rats were provided an environment with temperature of 22-25°, humidity of 40%-70% and equal 12 h light/dark cycle for 7 days.

Plant material

Fresh leaves of *F. racemosa* L. were collected from North-Guwahati, Assam during Dec, 2020 and authenticated (Accession Number 5305) by a taxonomist, Dr. Iswar Chandra Barua, Principal Scientist, Department of Agronomy, Assam Agricultural University, Jorhat, Assam.

Extraction of the plant material

After washing the plant materials with water (3 times) sterilization was done with 70% alcohol. It was then dried at room temperature to avoid chemical changes and frequently observed forfungal contamination. Pulverized the plant materials into powder form with the help of a mill and passed them through a sieve. One hundred (100) g of powdered leaves were weighed and soaked in 1000 mL conical flask in which 400 mL of distilled water was added. The mixture was kept in a heating mantle and heated till the volume becomes one third. The extract was filtered using Whatman No. 1 filter paper and kept overnight in the deep freeze, following day, which was concentrated in a lyophilizer (HETO, Power Dry, LL 3000). The final product obtained was semi solid in nature and kept in a refrigerator for further use and yield percentage was calculated.

Experimental design

According to OECD TG407 (OECD, 2001b), 3 groups were made with 10 animals (5 male and 5 female) in each group. Two doses of FRLAQE @ 100 and 200 mg/kg orally daily for 28 days was administered to group 1 and 2 of rats and an equal volume of vehicle was administered to the control group. The animals were observed daily for the abnormal behaviour, mortality and body weight. Again, to authenticate the physical symptoms of toxicity blood was collected at weekly interval for further analysis of haematological and biochemical parameters. Animals were sacrificed on day 29 by administering ether and immediately after death of the animals, heart blood, 5 mL each was collected for haematological as well as blood biochemistry and enzyme study. The internal organs were collected, weighed and for histopathological study, 10% buffered formaldehyde solution was used for preservation of internal organs.

Study parameters

Observational parameters

The animals were observed on each day. The parameters of observation such as mortality if any,

Table 1 — Effects of FRLAQE on average feed and water intake

Treatment	Sex	Average food intake (g/day)	Average water intake (mL/day)
Control	Male	19.23±1.26	26.35±1.26
	Female	14.23±2.55	18.22±1.56
FRLAQE 100 mg/kg	Male	20.13±2.32	19.36±1.26
	Female	15.26±1.02	16.22±1.26
FRLAQE 200 mg/kg	Male	19.26±2.95	21.26±1.88
	Female	15.96±2.22	17.54±1.96

Values are expressed as mean ± standard error; n = 10 (5 females or 5 males). No significant difference between FRLAQE treatment groups and control group.

Table 2 — Effects of FRLAQE on body weight

Group	Body weight (g)				
	Week 0	Week 1	Week 2	Week 3	Week 4
Male					
Control	175.25±8.58	194.56±5.89	215.55±2.66	228.22±6.55	248.26±4.55
FRLAQE 100 mg/kg	180.22±4.56	198.25±4.96	209.88±1.45	221.22±7.59	244.22±4.53
FRLAQE 200 mg/kg	179.26±3.59	192.18±4.22	218.22±1.59	231.22±5.55	252.45±6.59
Female					
Control	180.29±4.89	190.15±8.89	199.25±4.25	222.15±5.25	249.56±2.59
FRLAQE 100 mg/kg	182.56±5.59	195.56±4.95	212.01±6.12	239.26±6.25	246.24±5.26
FRLAQE 200 mg/kg	192.78±7.89	202±5.22	215.26±8.59	235.26±4.56	244.58±2.66

Values are expressed as mean ± standard error; n = 10 (5 females and 5 males). No significant difference between FRLAQE treatment groups and control group.

motor activity, tremors, convulsions, posture, spasticity, opisthotonicity, ataxia, righthing reflex, sensation, piloerection, ptosis, lacrimation, exopthalmos, salivation, diarrhoea, writhing, skin colour, respiratory rate, daily food intake. Weight of animals was recorded every 7th day.

Biochemical parameters

Blood was collected from retro orbital plexus of rats in tubes containing Ethylenediamine Tetraacetic Acid (EDTA). Haematological and biochemical parameters were estimated by standard methods[8-10]. The plasma was subsequently analysed using fully automated multimode reader (MULTISKAN, Thermo Scientific) for the concentration of blood urea, creatinine, total bilirubin, direct bilirubin and serum glucose.

Haematological parameters

Automated haematology analyser (MELET SCHLOESING Laboratories, FLS257) was used for the complete blood count analyses[9-12].

Histopathological examination

Tissue sections were prepared as per standard method and interpreted.

Statistical analysis

Data were expressed in mean± SEM. Data were analysed by analysis of variance (ANOVA) followed by "Tukey's HSD" procedure. The statistical program used was SPSS. The significance level was $p<0.05$.

Results

A continuous dosing of *F. racemosa* L. leaf aqueous extract was done for 28 days under this experimentation. The animals did not show any unwanted behaviour and mortality was nil in the extract treated groups. The effect of *F. racemosa* L. leaf aqueous extracts on average feed and water intake is displayed in Table 1. No significant difference has been observed with the control group.

Again, the effect of FRLAQE on body weight is expressed in Table 2. The results revealed no significant difference between FRLAQE treatment groups and control group ($p<0.05$).

Effects of FRLAQE extracts on organ weight are shown in Table 3. The results showed no significant difference among FRLAQE treated groups and control group ($p<0.05$).

The status of bone marrow activity and intravascular effects were monitored by haematological

Table 3 — Effects of FRLAQE on organ weight of rats

		Control		FRLAQE 100 mg/kg		FRLAQE 200 mg/kg	
Organ name	Sex	Average organ weight (g)	Relative organ weight (%)	Average organ weight (g)	Relative organ weight (%)	Average organ weight (g)	Relative organ weight (%)
Heart	M	0.87±0.05	0.35	0.85±0.08	0.34	0.80±0.11	0.32
	F	0.79±0.07	0.31	0.73±0.02	0.26	0.71±0.06	0.25
Liver	M	9.28±0.24	3.74	8.99±0.43	3.56	8.87±0.31	3.51
	F	6.55±0.37	2.62	6.40±0.33	2.60	6.36±0.33	2.60
Lung	M	1.30±0.12	0.52	1.17±0.06	0.47	1.25±0.19	0.49
	F	1.21±0.15	0.48	1.24±0.13	0.51	1.29±0.14	0.52
Kidney	M	2.11±0.09	0.85	2.16±0.10	0.87	1.92±0.36	0.78
	F	1.37±0.10	0.54	1.21±0.04	0.49	1.19±0.15	0.47
Spleen	M	0.61±0.02	0.24	0.63±0.03	0.25	0.62±0.01	0.24
Brain	F	0.45±0.01	0.18	0.45±0.02	0.18	0.41±0.03	0.16
	M	1.85±0.05	0.75	1.94±0.04	0.79	1.88±0.05	0.78
	F	1.24±0.02	0.50	1.26±0.03	0.51	1.23±0.05	0.43

Table 4 — Effect of FRLAQE on haematological values of rats during sub-acute toxicity study

Group	Body weight						
	WBC ($X10^3$/μL)	RBC ($X10^6$/μL)	PCV (%)	ESR (mm/h)	Hb (g/dL)	Bleeding time (sec)	Clotting time (min)
		Male					
Control	14.91±1.51	6.69±0.75	36.79±3.11	2.2±0.1	15.56±1.16	32.76±2.11	4.16±0.02
FRLAQE 100 mg/kg	17.64±1.53*	6.95±1.48	35.24±3.57	2.1±0.1	13.54±0.58*	32.40±2.37	4.24±0.84
FRLAQE 200 mg/kg	13.57±3.02	6.89±0.54	35.74±4.20	2.2±0.1	15.66±0.48	32.18±0.18	4.94±0.28
		Female					
Control	14.53±1.53	7.02±0.68	35.69±3.29	2.2±0.1	13.97±0.41	32.50±1.49	4.35±0.26
FRLAQE 100 mg/kg	13.59±1.48	8.30±0.94*	34.60±4.05	2.2±0.1	13.08±0.14	33.17±1.11	4.12±0.20
FRLAQE 200 mg/kg	14.25±1.98	6.22±0.95	34.84±1.91	2.1±0.1	13.77±0.47	32.51±1.45	4.06±0.19

Values are expressed as mean ± standard error; n = 10 (5 females and 5 males). FRLAQE treatment groups and control group *p=0.05 significance difference.

examination as summarized in Table 4. During haematological examination, WBC count (17.64±1.53) was increased significantly ($p<0.05$) in male FRLAQE treated group (100 mg/kg b. wt.) as compared to control (14.91±1.51) which was not significantly different in females in extracts treated groups. Likewise, there was significant ($p<0.05$) increase in RBC count (8.30±0.94) in the females treated with FRLAQE (100 mg/kg b. wt.) in comparison to the control group (7.02±0.68) but the value appeared to be lower (6.22±0.95) in females treated with FRLAQE (200 mg/kg b. wt.) than the control group. Again, haemoglobin concentration (mean value) is significantly lower (14.54±0.58) ($p<0.05$) in males FRLAQE treated group (100 mg/kg b. wt.) on comparing to control group (15.56±1.16). Again, the bleeding time value appeared to be more (33.17±1.11) but not significant in female FRLAQE treated group (100 mg/kg b. wt.) than the control (32.50±1.49). The results of haematological assay provide a conclusion that FRLAQE is safe in sub-acute toxicity study in rats since the values fall within a normal range, suggesting further use in treating various ailments.

The results of biochemical assay of the extract treated as well as control animals are shown in Table 5. In blood chemistry, serum glucose level (99.90±2.10) and (98.76±1.20) was decreased significantly ($p<0.05$) in male FRLAQE treated groups (100 and 200 mg/kg b. wt.) when compared

Table 5 — Effect of FRLAQE on blood chemistry value of rats during sub-acute toxicity study

Group	Glucose (mg/dL)	BUN (mg/dL)	Total Bilirubin (mg/dL)	Direct Bilirubin (mg/dL)	Creatinine (mg/dL)
		Male			
Control	104.91±1.51	30.20±0.75	0.24±0.03	0.06±0.01	0.39±0.06
FRLAQE 100 mg/kg	99.90±2.10*	30.78±1.48	0.22±0.02*	0.04±0.03*	0.40±0.04
FRLAQE 200 mg/kg	98.76±1.20*	31.22±0.54	0.20±0.06 *	0.05±0.03	0.40±0.03
		Female			
Control	93.90±1.90	27.02±0.68	0.18±0.03	0.05±0.02	0.34±0.02
FRLAQE 100 mg/kg	92.24±1.10	28.30±0.94	0.20±0.05*	0.06±0.04	0.34±0.04
FRLAQE 200 mg/kg	90.60±1.34*	27.22±0.95	0.019±0.02	0.05±0.01	0.35±0.03

Values are expressed as mean ± standard error; n = 10 (5 females and 5 males). FRLAQE treatment groups and control group *p=0.05 significance difference.

with control (104.91±1.51). Likewise, there was significant ($p<0.05$) decrease in serum glucose level (90.60±1.34) in female FRLAQE@ 100 mg/kg b. wt. group than the control group (93.90±1.90), but the value appeared to be in a normal range. Again, total bilirubin (mean value) appeared to be lower (0.22±0.02 and 0.20±0.06) significantly ($p<0.05$) in male FRLAQE treated group (100 and 200 mg/kg b. wt.) than the control group (0.24±0.03) alsototal bilirubin (mean value) appeared to be lower (0.20±0.05) significantly ($p<0.05$) in female FRLAQE@ 100 mg/kg b. wt.as compared to control group (0.18±0.03). The direct bilirubin (mean value) decreased (0.04±0.03) significantly ($p<0.05$) in male FRLAQE@ 100 mg/kg b. wt.when compared to control group (0.06±0.01). From the biochemical assay of the extract treated animals, it has been observed that the results are considered as normal for this species of animal depicting no abnormalities in the use of FRLAQE further.

Histopathological examination of the internal organs revealed no abnormalities in extract treated animals. The results are displayed in (Fig. 1-5). Liver shows normal architecture as well as normal advent of radiating hepatocytes and portal triad. Fatty changes are almost nil suggesting no pathological significance of the extract. Again, there is normal appearance of renal parenchyma, tubules and glomeruli with clear bowman's space implies no signs of renal toxicity on the extract treated animals. Histopathological examination of heart, spleen and brain of extract treated animals showed normal architecture of these organs as well as normal cellular detailing which reveals the safe nature of FRLAQE.

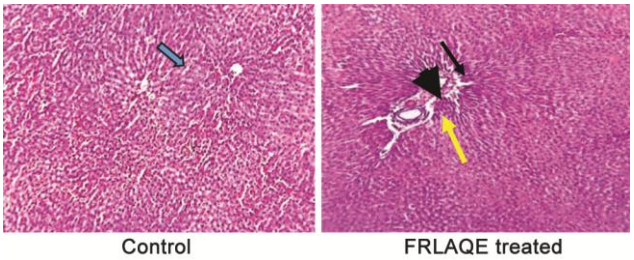

Fig. 1 — Showing normal hepatic architecture of liver with normal appearance of radiating hepatocytes and portal triad (blue arrow – central vein, black arrow – portal vein, yellow arrow - bile duct, arrow head- hepatic artery). (FRLAQE treated H & E, × 10X; Control H & E, 4X)

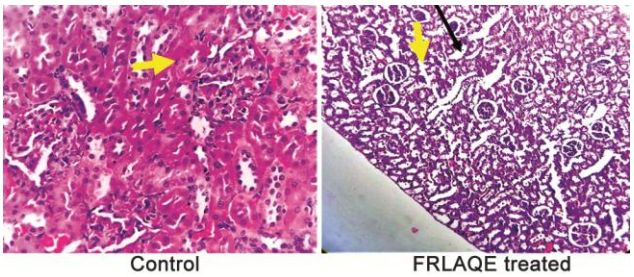

Fig. 2 — Showing normal appearance of renal parenchyma, tubules (black arrow) and glomeruli (yellow arrow) with clear bowman's space. (FRLAQE treated H & E, × 10X; Control H & E, 40X)

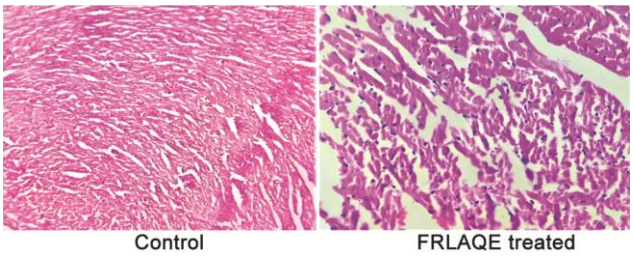

Fig. 3 — Showing normal myocardial striations with normal myocytes. H & E, × 10X

Discussion

People in the ancient era were solely dependent on traditional beliefs and thoughts for curing any kind of illness and the same is continuing generations after generations. However, due to urbanization, some of the valuable traditional medicines as well as curing

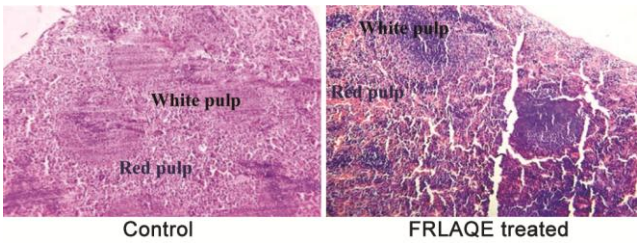

Fig. 4 — Showing normal splenic architecture with normal white and red pulp. H & E, × 10X

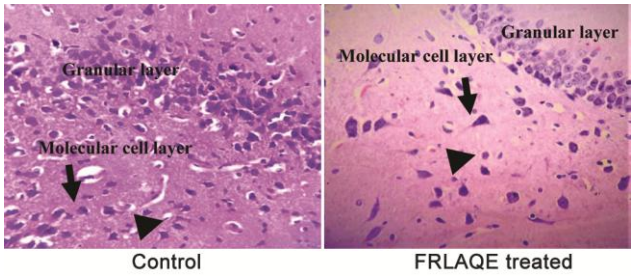

Fig. 5 — Showing normal cerebral architecture, granular and molecular cell layer (arrow –pyramidal neurons, arrowhead – glial cell). H & E, × 40X

methods get lost and replaced with the synthetic medicine without realizing the harmful side effects and resistance. Though, it cannot be acceptable that traditional practices are completely safe but before ignoring one must be sure enough for their toxicity profile since the nature lies all the secrets for ecological balance. Therefore, exploration of this traditional medicine for their pharmacological as well as toxicological property is important.

F. racemosa (cluster fig) is traditionally used in many health issues considering some as dysentery, hyperglycaemia, biliary disorders, jaundice and inflammatory conditions[13]. Some of the literature reported its toxic character for prolonged use[7,14]. Since its pharmacological evidence has been investigated in diabetic neuropathy which itself is a chronic disease, so it becomes essential to investigate its toxicity profile for its prolonged use.

Blood being the medium of carrying absorbed nutrients, chemicals, drugs as well as toxic compounds to the entire body. Therefore, haematological evaluation plays an important role in determining the pharmacological and toxicological consequences of any substances that enters in the body. The components of blood mainly red blood cell, white blood cells, haemoglobin and platelets are very sensitive to the effects of the toxic compounds that manifest the physiological and pathological status of an individual[15]. There were increase in WBC count, RBC count and bleeding time in FRLAQE treated male rats on comparing the control animals but all values lay within the normal range for this species so increase these haematological values is not indicative for toxicity of haematopoiesis and leucopoiesis in experimental animals.

Almost all the drugs, chemicals and xenobiotics are excreted through kidney after metabolism so biochemical parameters associated with liver and kidney plays an important role to justify its proper functioning and healthy condition. Some of the reports suggested the toxicity of liver and kidney associated with the use of phyto-therapeutic compounds which otherwise pocesses good pharmacological efficacy[16]. Serum biochemical parameters related to kidney functions *viz*., creatinine and BUN demonstrated no significant differences with respect to FRLAQE treated animals, although serum glucose, total bilirubin, and direct bilirubin in FRLAQE treated animals were found to be lower but all values lay within the normal range and hence, the results are considered as normal for this animal species.

From our investigation, it has been observed that FRLAQE is totally safe without any abnormalities of haematological and biochemical parameters. One of the important studies proved that *F. racemosa* leaves extract on experimental animals was safe even in chronic use. Body weight, feed consumption and behaviour of the animals were found normal and likewise haematological and biochemical evaluation resulted no appearance of abnormalities of the animals[17]. This fact is also added with normal appearance of organs, organs weight and normal architecture of organs during histopathological examination[17]. In another study, the ethanolic extract of *F. racemosa* leaves showed hepatoprotective effect by the levels of SGOT, SGPT, and serum bilirubinin CCl4- induced hepatotoxicity in rats[18]. Again, the results of the repeated-dose 28-day sub-chronic toxicity of the herbal mixture (1:1) of *F. racemosa* and *Azadirachta indica* showed no evidence of toxicities with regards to the behavioural pattern and body weight analysis. Variations in the haematological and biochemical parameters were seen but the values occupied the normal range[19]. From the above experiment it is proved that FRLAQE does not possess hepatic as well as renal toxicity in rats. In support of the safety profile of FRLAQE, the

biochemical and haematological parameters revealed no abnormal value when compared with the control group. Moreover, no abnormality has been observed on histopathological examination of various internal organs too. Therefore, the results obtained suggest that *F. racemosa* L. leaf aqueous extract is fairly nontoxic.

Conclusion

In summary, it is concluded from the study that *F. racemosa* L. leaf aqueous extract was found to be nontoxic when oral subacute toxicities in rats were performed. Chronic toxicity study needs to be done before concludes safe use of *F. racemosa* L. leaf aqueous extract for prolong use.

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Conflict of Interests

The authors declared no conflict of interest.

Author Contributions

Conceptualization: CCB, Data curation: ICB, Investigation: LB, AK, MH, Methodology: CCB, AK, Formal analysis: BD, ANP, Writing-original draft: SH, Writing-review and editing: CCB, SH, Supervision: CCB.

Ethics Statement

The experimental protocol was approved by IAEC committee of the University (770/03/ac/CPCSEA/ FVSc, AAU/IAEC/06/22) and its ethical rules were followed throughout the experimental procedures.

Data Availability

The authors confirm that all the data supporting the findings of this study are available within the article.

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