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In vivo Evaluation of Analgesic, Antidiarrheal, Hypoglycemic, and Anti-Inflammatory Activities of *Sarcolobus carinatus* Methanolic Extract in Swiss Albino Mice

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Abstract

In this study, *Sarcolobus carinatus*, also known as 'Bawali-lota', is subjected to several *in vivo* biological activity assays, such as central and peripheral analgesic, antidiarrheal, hypoglycemic, and anti-inflammatory activity. The study was carried out on Swiss Albino mice models using pure methanolic extract of *S. carinatus* at three different doses (200 mg/kg, 400 mg/kg, and 600 mg/kg of body weight). Central and peripheral analgesics showed a dose-response relationship with moderate activity, where the highest activity for peripheral activity was (69.32 ± 0.85) % inhibition of writhing. The sample also showed a dose-response relationship for anti-diarrheal and anti-inflammatory activity with (65.75 ± 2.92) % reduction of diarrhea and (32.31± 1.52) % reduction of paw edema for 600 mg/kg body weight of dose in comparison to (75.34 ± 0.5) % and (49.75 ± 0.5) % reduction by the standard, respectively. For hypoglycemic activity, the sample did not show a dose-response relationship and the activity was low to moderate level. All the results were statistically significant with $P < 0.05$. The findings prove that the *S. carinatus* plant is enriched with phytochemicals with therapeutic potential.

Key words: *Sarcolobus carinatus*, analgesic, anti-diarrheal, anti-inflammatory, hypoglycemic.

Introduction

Natural products that are derived from plants are still an essential source of pharmacologically active substances. However, thorough in vivo studies are necessary to evaluate efficacy, bioavailability, and safety within complicated biological systems to convert early in vitro results into clinically meaningful outcomes. Many acute and chronic disorders are linked to complicated biological reactions like inflammation and pain, which are frequently treated with synthetic medications that may have unfavorable side effects with extended usage (Wehling, 2014). Similar to this, diarrhea and hyperglycemia are major worldwide health issues, especially in developing nations where access to safe and efficient treatments may be restricted (Chen *et al*., 2023; "Diabetes," n.d.). The need to find plant-based substitutes with better safety profiles and multi-targeted therapeutic benefits has grown as a result of these difficulties.

Sarcolobus, a plant genus of the Apocynaceae family, currently comprises approximately 22 recognized species. This genus is native to Andaman Islands, Bismarck Archipelago, Cambodia, Borneo, Caroline Islands, China Southeast, Fiji, Jawa, Lesser Sunda Islands, India, Malaya, Maluku, Nicobar Islands, Myanmar, New Caledonia, New Guinea, Philippines, Queensland, Solomon Islands, Laos, Sulawesi, Northern Territory, Sumatera, Thailand, Vietnam, Bangladesh, Vanuatu and Wallis-Futuna Islands ("Sarcolobus R.Br. | Plants of the World Online | Kew Science," n.d.). Many plants in the genus have long been used in traditional medicine to treat a variety of

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illnesses, although there is still little scientific evidence supporting their pharmacological properties (Wangensteen *et al.*, 2006), and *Sarcolobus carinatus*, the subject of this study, is such a plant. Its roots and leaves are used medicinally. Its root is used for stomachache, gonorrhea and constipation. Leaves are used to treat cuts, insect bites, ulcers, swellings, sores, and to improve healing after childbirth (Wim Giesen *et al*., 2007.). Its tender and fleshy fruit is being eaten by locals, and its roots and follicles are used as fish poison during fishing (Wim Giesen *et al*., 2007.). However, very few scientific studies have been conducted on this species.

Using well-established animal models, the current study aims to explore the in vivo pharmacological activity of *S. carinatus*, with particular attention to its anti-inflammatory, central and peripheral analgesic, antidiarrheal and hypoglycemic properties. The purpose of this endeavor is to investigate the plant's potential as a source of new medicinal compounds and to offer scientific evidence in favor of the plant's traditional use.

Materials and Methods

Collection and preparation of the plant sample. Jahangirnagar University's Department of Botany taxonomically identified the fresh plant (whole) sample of *S. carinatus* that was obtained in April 2024 from Sundarbans, Bangladesh (Voucher specimen number: JUH 10392). The freshly harvested plants (whole) were washed, dried for a few days at room temperature, and then oven-dried at approximately 40 °C. After being dried, the sample was ground into a powder and stored in an airtight container. For additional extractions, powdered specimen was utilized.

Extraction. The cold extraction procedure, which required soaking the sample in methanol in a container with a flat bottom and stirring it often, was used to extract approximately 500 g of the specimen powder in methanol. Following a cotton filter, the sample's methanolic solution was further filtered using Whatman filter paper. By evaporating the mixture in a rotary vacuum evaporator at around 40°C, the crude extract was obtained. *In vivo* bioactivity was assessed using crude extract of methanol at three distinct body weight doses: 200 mg/kg, 400 mg/kg, and 600 mg/kg.

Medicines and reagents. Every chemical and reagent utilized in the experiments was of analytical grade. Square Pharmaceuticals Ltd. provided standard medications for bioactivity tests, such as loperamide and glibenclamide. Gonoshasthya Pharmaceuticals, Essential Drugs Company, and Incepta Pharmaceuticals Ltd. provided morphine, diclofenac-sodium, and thiopental-sodium, respectively. Castor oil and a glucose sample were purchased from the local grocery store.

Experimental animals. The international center for diarrheal diseases and research, Bangladesh (ICDDR, B), Dhaka, provided swiss albino mice weighing between 28 and 30 g of either sex that were 4-5 weeks old. They were housed in polypropylene cages and fed water and ICDDR,B manufactured rodent chow throughout a 12 h light/dark cycle at a fixed normal temperature ((24 ± 2)ºC; relative humidity ranged from 60-70%). During the use of animal models in bioactivity tests, ethical guidelines were strictly followed. Twenty Swiss-Albino mice were used as test subjects. They were split up into five groups, each with four mice. Three different dosages of crude methanolic extract were administered orally (200 mg/kg, 400 mg/kg, and 600 mg/kg of body weight) to three groups, which were denoted as MeSF-200 (Methanol Soluble Fraction at 200 mg/kg of body weight), MeSF-400 (Methanol soluble fraction at 400 mg/kg of body weight) and MeSF-600 (Methanol soluble fraction at 600 mg/kg of body weight), respectively. The remaining two groups of mice acted as the control and standard groups.

*Central analgesic activity***.** The heat-induced tail flicking assay was utilized to assess the central analgesic efficacy of *S. carinatus* (Moniruzzaman and Imam, 2014). Normal saline was fed to the control group (CTL), and the methanolic extract at different dosages was fed to the test groups. On the

other hand, the standard group (STD) received morphine subcutaneously at a dose of 2 mg/kg of body weight. Each test animal's tail was submerged in 55°C hot water, and the amount of time it took for the tail to remove itself from the water was noted at intervals of 30, 60 and 90 min following the delivery of standard and sample doses. The percentage of elongation in tail flicking reactions with time was calculated using the following formula:

$$\% \text{ time elongation} = \frac{T_t - T_c}{T_c} \times 100$$

Where, T_t = Average duration of test samples tail flicking

T_c = Average duration of the control group's tail flicking

Peripheral analgesic activity. The writhing test caused by acetic acid was used to assess the test sample's capacity to produce analgesia in pain stimulation through the peripheral nervous system (Schachtel *et al.*, 2010). For the assessment, three different dosages of test samples were administered orally to three test groups. As a control, 1% of Tween-80, prepared in normal saline, was administered orally at a dose of 0.1 ml/10 g of body weight. Diclofenac-Na was administered intraperitoneally as standard at a dose of 50 mg/kg of body weight. Each test animal received an intraperitoneal injection of 1% acetic acid after 30 min. Each mouse's abdominal constrictions were counted starting five minutes after injection and continuing for ten minutes. The following formula was used to determine the percentage of inhibition in writhing:

$$\% \text{ inhibition} = \frac{\text{Control's writhing response} - \text{Samples' writhing response}}{\text{Control's writhing response}} \times 100$$

Anti-diarrheal activity. The crude extract's anti-diarrheal properties were assessed using the already established castor oil-induced diarrhea method with slight adjustments (Shoba and Thomas, 2001). The assay's positive control was loperamide hydrochloride (50 mg/kg of body weight). Three test groups of mice were given a suspension of crude extract of methanol of the sample in saline water at three different doses. The control group was given a normal saline solution. Every mouse was placed in a different box with a floor liner (blotting paper). Each mouse received 1 milliliter of extremely pure analytical-grade castor oil to cause diarrhea after 30 min. Over the course of four hrs, the quantity of fecal stools produced by each mouse was noted. The following formula was used to determine the percentage decrease in diarrheal feces as compared to the control:

$$\% \text{ inhibition} = \frac{f_c - f_t}{f_c} \times 100$$

Where, f_c = Total number of fecal droppings in the control group

f_t = Total number of fecal droppings in the test group

Hypoglycemic activity. A modified version of the oral glucose tolerance test was used to assess the test sample's ability to lower the mice's blood glucose levels (Bogdanet e*t al.*, 2020). In this experiment, the glucose level in the blood of each mouse in all five groups after fasting them for 12 hrs, was recorded before administration of glucose. After that, 10% glucose solution was administered orally to each group at a dose of 2 g/kg of body weight. The rise in blood glucose levels of the standard (Glibenclamide, at a dose of 10 mg/kg of body weight), control (1% Tween-80 solution in saline), and treatment groups (200, 400 and 600 mg/kg of body weight) were monitored using a portable glucometer (Quick Check Blood Glucose Monitoring Kit) from tail-vein blood samples collected at 0 (baseline), 30, 60 and 120 min.

Anti-inflammatory activity. A slightly modified version of the right hind paw edema method was carried out to measure the anti-inflammatory activity of the sample (Barung *et al.*, 2021). At the start of the experiment (0 hrs), the mice were orally administered the test samples, standard (0.5 ml diclofenac solution (2 mg/ml) in 9.5 ml normal saline) and control materials (1% Tween-80 solution in saline) using a feeding needle. After 30 min, the right hind paw volume was measured by using a Vernier caliper and right after that, 0.1 ml freshly taken hen's egg white

was injected in the right hind paws of the mice by subcutaneous injection. After administration of egg white, the right hind paw diameter of the mice was measured at 30, 60, and 120 min time intervals. The percentage of inhibition of edema was then calculated using the following equation:

$$\% \text{ inhibition of edema } = \frac{E_c - E_t}{E_c} \times 100$$

Where, E_c = Average edema in control

E_t = Average edema in test sample

Statistical analysis. Mean ± SEM was used to present all measured and calculated data. Statistical analysis was performed using one-way or two-way ANOVA, as appropriate, followed by Tukey's multiple-comparison test. A value of $P < 0.05$ was considered statistically significant.

Results and Discussion

Central analgesic activity. To evaluate the central analgesic activity, the amount of time each mouse kept its tail in the hot water was recorded at 0, 30, 60, and 90 min after treatment. The percentages of elongation time of tail withdrawal were then calculated from the obtained data, which are illustrated in figure 1:

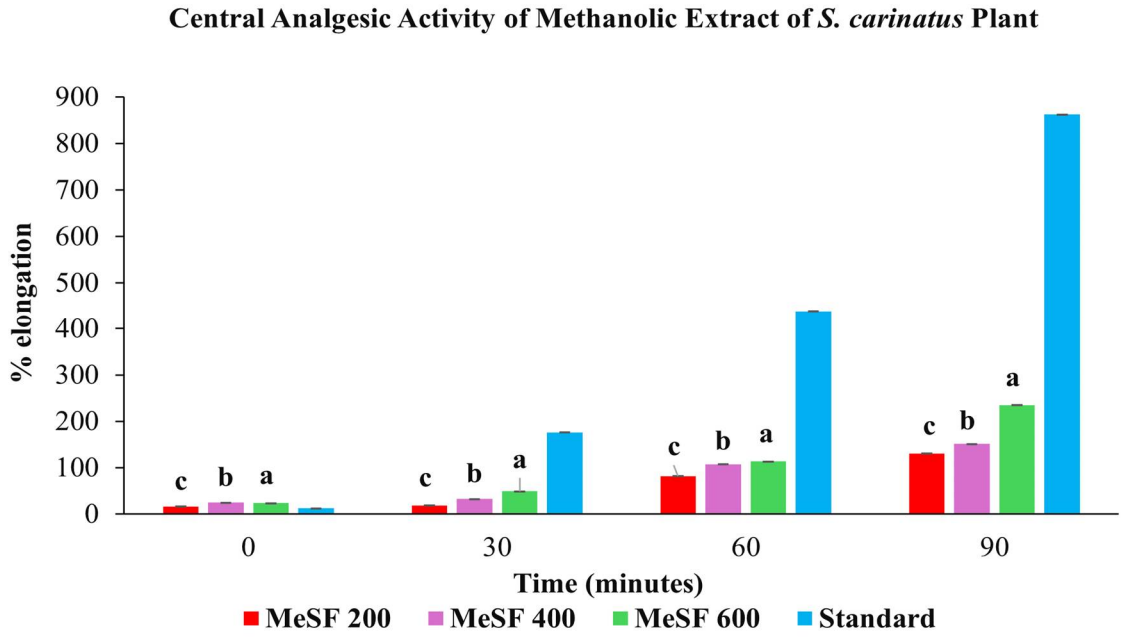

Figure 1. The percentage of time elongation in the tail immersion assay of methanolic extract of *S. carinatus* plant. The data are illustrated as mean ± SEM, and each letter (a–c) denotes significant differences among groups.

The results displayed that *S. carinatus* has a moderate level of activity with relatively higher efficacy at higher doses and longer post-treatment intervals. After 30 min of administration, the sample showed a low level of activity, but after longer time intervals (60 and 90 min), it showed a moderate level of activity. *S. carinatus* showed the highest level of activity after 90 min at 600 mg/kg of body weight dose which was (234.73 ± 0.097) %. The statistical analysis data specified that the results are statistically significant ($P < 0.05$).

Although the values for the extract were considerably lower than those of the standard, the results suggest the presence of active phytochemicals in the plant extract of *S. carinatus*, which are capable of acting as a pain reliever.

Peripheral analgesic activity. In the test of pain inhibition effect, three different doses of crude methanolic extract were administered to mice. The reduction of writhing (induced by a 1% acetic acid solution) by the doses is shown in figure 2.

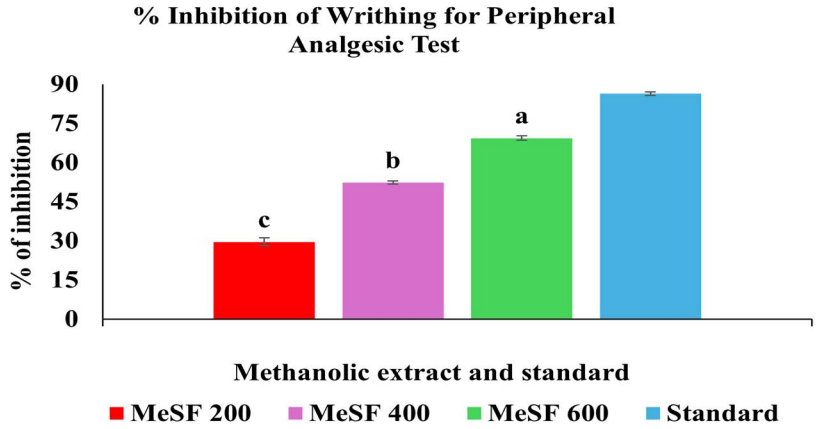

Figure 2. % inhibition of writhing responses of various crude methanolic extract doses in comparison to the standard. The data are illustrated as mean ± SEM, and each letter (a-c) denotes significant differences among groups.

The extract exhibited moderate peripheral analgesic activity, demonstrating a dose-dependent increase in inhibition of writhing, although its effect remained lower than that of the standard drug. The highest inhibition was (69.32 ± 0.85) %, achieved by the 600 mg/kg of body weight dose, which suggests the dose-dependent increase in analgesic property. Statistical analysis confirmed these results were significant ($P < 0.05$).

The ethnomedicinal use of the leaves in painful inflammatory conditions such as cuts, insect bites, ulcers, swellings, and sores (Wim Giesen *et al*., n.d.) supports the observed peripheral analgesic activity of the plant extract. This suggests that the extract may act through peripheral mechanisms, possibly by inhibiting inflammatory mediators involved in pain generation (Gupta *et al*., 2015).

Anti-diarrheal activity. Figure 3 illustrates how the sample crude extract at various doses reduced the number of diarrheal stools in mouse models when compared to the standard (loperamide):

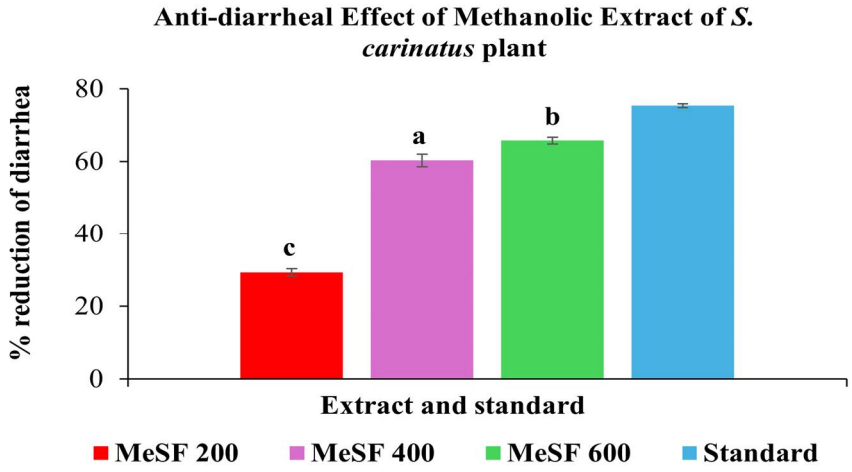

Figure 3. Effects of various doses of methanolic extract of *S. carinatus* on diarrhea of mice triggered by castor oil. The data are illustrated as mean ± SEM, and each letter (a–c) denotes significant differences among groups.

Methanolic extract of *S. carinatus* plant exhibited a moderate dose response increase in the percentage of reduction of diarrhea. The MeSF 600 dose showed the highest reduction in diarrheal stool, among the doses with a (65.75 ± 0.92) % reduction of diarrhea. Statistical analysis confirmed these results were significant ($P < 0.05$).

The plant is traditionally reported to be used for the treatment of constipation (Wim Giesen *et al.*, n.d.). However, in the current study, the extract demonstrated significant antidiarrheal activity, showing a dose-dependent reduction in diarrheal feces. This apparent inconsistency may be explained by the complex phytochemical composition of the plant, where different bioactive constituents may exert bidirectional modulatory effects on gastrointestinal motility and secretion (Chomentowski *et al*., 2025). Although the present findings indicate significant pharmacological activity of the extract, the exact bioactive constituents responsible for the effect remain to be confirmed. Therefore, the observed activities may be associated with the presence of secondary metabolites reported in medicinal plants, but further phytochemical screening, quantitative estimation, and compound isolation are required to establish a direct relationship.

Hypoglycemic activity. The effect of methanolic extract of *S. carinatus* plant at three different doses on lowering the glucose level in blood was observed to evaluate its hypoglycemic activity, and the result is illustrated in figure 4.

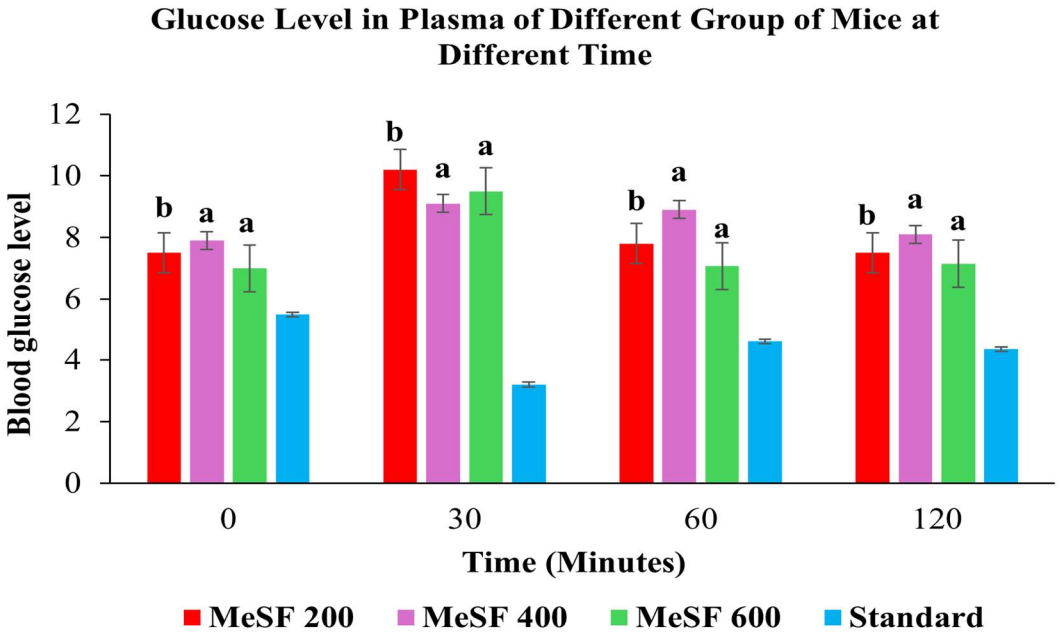

Figure 4. Hypoglycemic activity of various doses of methanolic extract of *S. carinatus* plant in comparison to the standard. The data are illustrated as mean ± SEM, and each letter (a and b) denotes significant differences among groups.

The extract exhibited no clear dose-response relationship in hypoglycemic activity assay. Dose MeSF 600 showed the highest decrease in glucose level after 120 minutes, which was (7.15 ± 0.760) mmol/L. Overall, the extract showed mild to moderate level of hypoglycemic activity. The calculated P-value indicated that the results are statistically significant ($P < 0.05$).

The reduction of blood glucose may be linked to the presence of bioactive secondary phytochemicals,

which are reported to exert glucose-lowering activity through multiple mechanisms, including stimulation of insulin release, enhancement of glucose uptake, or inhibition of carbohydrate-digesting enzymes (Velmurugan, 2025). Further studies are required to relate the activity with specific phytochemicals.

Anti-inflammatory activity. The methanolic extract of the plant *S. carinatus* was subjected to anti-inflammatory test induced by egg white. The obtained results are demonstrated in figure 5.

At the early phase (30 min), the extract exhibited slightly higher inhibition of edema compared to the standard drug. However, in the later phase (60-120 min), the standard drug showed markedly higher inhibition. Overall, the extract demonstrated moderate anti-inflammatory activity. Calculation of the P values showed that the results were statistically significant ($P < 0.05$). The result demonstrated the potential anti-inflammatory activity of *S. carinatus*, which further supports its traditional use in inflammation (Wim Giesen *et al.*, n.d.).

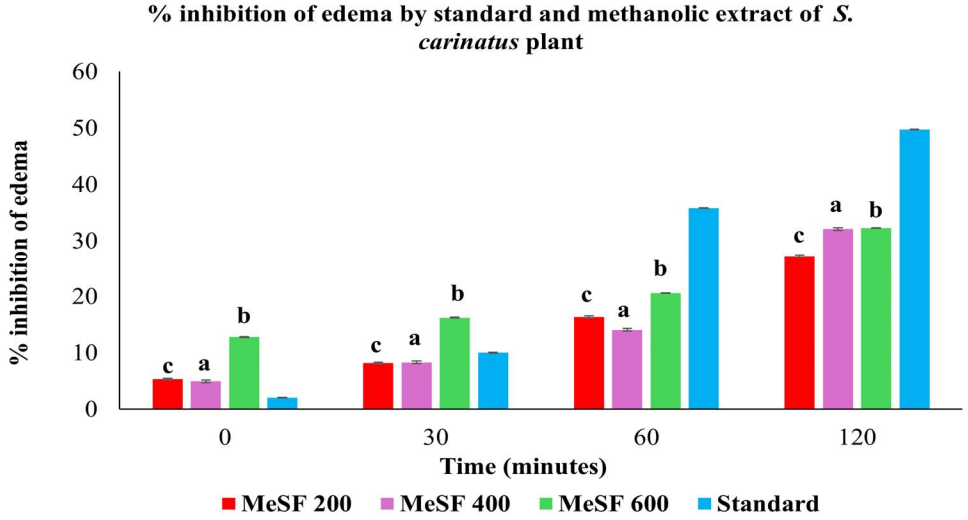

Figure 5. Anti-inflammatory activity of three different doses of methanolic extract of *S. carinatus* in comparison to the standard. The data are illustrated as mean ± SEM, and each letter (a–c) denotes significant differences among groups.

Conclusion

In vivo pharmacological evaluations of *S. carinatus* underscored the therapeutic value of the plant. The plant extract exhibited dose-dependent central and peripheral analgesic activities, reinforcing its potential role in pain management. Significant antidiarrheal effects were also observed, suggesting gastrointestinal protective efficacy. Moreover, the extract showed hypoglycemic activity, highlighting its potential role in glucose regulation. The plant also displayed moderate anti-inflammatory activity in a dose-responsive fashion. The findings are encouraging enough to warrant more thorough research into the active chemical substances causing these therapeutic effects.

Conflict of interest

The authors declare that there's no conflict of interest.

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Data availability

The raw data used to support the findings of this study are available from the corresponding author upon request.

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