Assessment of Antidiarrheal and Superoxide Scavenging Activities of Ethanolic Frond Extract from *Stenochlaena palustris* Bedd.

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Received: 18 January 2021 / Revised: 19 February 2021 / Accepted: 4 March 2021
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Abstract: The present study aimed to evaluate the antidiarrheal and superoxide scavenging activities of ethanolic extract of *Stenochlaena palustris*, an edible fern from *Blechnaceae* family. The antidiarrheal activity was determined against castor oil induced diarrhea model, gastrointestinal transit and enteropooling tests in Swiss albino mice. The test groups received various doses (100, 200, and 400 mg/kg, body weight) of the extract, whereas positive control received loperamide (3 mg/kg, b.w.) and negative control was supplied with 1% tween-80 in distilled water (10 mL/kg, b.w.). At three test doses (100, 200 and 400 mg/kg), the extract showed significant (*p* < 0.001) and dose dependent antidiarrheal activity in all models. The delay and reduction in the onset, frequency, and weight of diarrheic feces as well as inhibition of the gastrointestinal transit and intraluminal fluid accumulation were observed in all models and highly comparable to the standard loperamide. The extract also exhibited high inhibitory ability to scavenge superoxide anion (O$_2^-$). The highest percentage inhibition of superoxide anion was found to be 84.32 ± 0.15% at the concentration 800 µg/mL.

Keywords: Antidiarrheal activity, Castor oil, Enteropooling assay, Gastrointestinal motility, *Stenochlaena palustris*, Superoxide anion

1. INTRODUCTION

Pteridophytes (ferns and their allies) are expected to contain more phytochemicals than other plants and are being used as eminent sources of food and medicine for the alleviation and maintenance of human illness and health [1,2]. However, among 13000 species, less number of species has been investigated for biological activities in pharmaceuticals and modern therapies [1,2]. *Stenochlaena palustris* Bedd. (Family: *Blechnaceae*) commonly known as Deki Lota (Bangladesh), Kalakai or Midin (Indonesia, Malaysia and India), is an edible epiphytic fern that plays an important ethnobotanical role (Asia) and found mainly in South-East Asian rainforests and Australia [3-5]. Traditionally, the leaves of this adaptable fern are used for fever, remedy for swellings, diarrhea, ulcers, skin disease, stomachache, abortifacient, and contraceptive [4-7]. Especially, in Malaysia, when cooked with boiling water, this fern is a common household remedy in treating diarrhea [4,5]. Phytochemical studies have shown that *S. palustris* have a lot of potential as herbal medicine because of its high content of antioxidants, polyphenols, terpenoids as well as glycosides [5-7]. For the biological activity, previous researches have reported this fern as a potent antibacterial, antifungal, anti diabetic, and antioxidant agent [5-9]. However, there is no pharmacological study available in literature to confirm the antidiarrheal activity of *S. palustris*.

Diarrhea, a gastrointestinal disorder, occurs when lining of the intestine is unable to absorb fluid, or it actively secretes fluid and is characterized by passing of loose or watery bowel movements three or more times in a day [10,11]. According to the WHO report, diarrhea is a major public health problem especially second leading cause for child malnutrition and mortality in developing countries [12]. Every year, nearly about 1.7 billion cases of childhood diarrheal disease and kills around 525,000 children less than five year of age [12]. Loperamide and raccadotril are the standard opioid-like antimotility drugs currently used for the treatment of diarrhea but are often linked with adverse effects and contraindications limiting their uses [11,13,14]. Bronchospasm, vomiting, constipation, fever, abnormal heart rhythms, itching are associated with use of these drugs.
Studies on antidiarrheal activity of edible fern S. palustris

[13,14]. The use of loperamide in children under two years of age is contraindicated as fatal paralytic ileus associated with abdominal distention has been observed [14]. Inclination towards folk medicine for searching out new leads for effective alternative therapy toward diseases is a common approach. Therefore, in this study, Ethanolic frond extract of S. palustris was employed for the pharmacological investigation to ascertain the antidiarrheal activity on castor oil induced diarrhea model in mice. Furthermore, superoxide radical scavenging activity of S. palustris was also evaluated in this present study.

2. MATERIALS AND METHODS

2.1. Plant collection, identification and extract preparation

Adequate amount of fresh fronds or leaves of S. palustris were collected from Sundarban, Sathkhira, Bangladesh in the month of September 2015. The plant was recognized and authenticated by taxonomists at Bangladesh National Herbarium, Mirpur, Dhaka, where a voucher specimen has been deposited (Accession no.: DACB-36544) for future reference.

The collected fronds after washing were shed dried at room temperature for a period of 7 days and were grinded by capacitor start motor (Wuhu Motor Factory, Wuhu, China) into fine powder. The powdered fronds were extracted by using ethanol as solvent with the help of a Soxhlet apparatus. The extract obtained was concentrated under reduced pressure in a rotary vacuum evaporator EYELA N1000 (Tokyo, Japan) and their percent yields were determined.

2.2. Chemicals, drugs and test animal

Disodium phosphate (Na₂HPO₄), phenazine methosulfate (PMS), reduced nicotinamide adenine dinucleotide (NADH), ascorbic acid, and analytical grade extracting solvent ethanol, were obtained from Sigma-Aldrich (St. Louis, MO, USA). Nitroblue tetrazolium (NBT) and tween-80 were purchased from Thermo Fisher (Waltham, MA, USA) and Loba Chemie Pvt. Ltd. (Mumbai, India), respectively. Castor oil (Qualikems Fine Chemicals Pvt. Ltd., New Delhi, India) and charcoal meal (SD Fine-Chem Ltd., Mumbai, India) were procured from local pharmacy in Bangladesh. Standard antidiarrheal drug, loperamide was the product of Beximco Pharmaceuticals Ltd. (Dhaka, Bangladesh).

Healthy Swiss albino mice of either sex aged 4-6 weeks (weighing 20-30 g) were used for this study. The mice were purchased from the Animal Research Branch of the International Centre for Diarrheal Disease and Research, Bangladesh (ICDDR, B). The mice were kept for 7 days prior to experimentation under standard environmental condition (25 ± 1°C; 50-56% relative humidity with 12:12 h light/dark cycle) in the animal house of the Department of Pharmacy, Khulna University, Bangladesh for adaptation after their purchase. During this time, the animals were supplied with standard pellet diet and tap water ad libitum. Experiments on animals were performed according to both internationally accepted [15] and ethical guidelines of ICCDR, B.

2.3. Acute toxicity study

Standard Lorke’s method was employed for an acute toxicity test of ethanolic frond extract of S. palustris in mice [16]. This method has two phases comprising of a total twelve test animals. In phase 1, nine mice were taken and divided into three groups where extract at 10, 100 and 1000 mg/kg doses were administered. After that, animals were placed under observation for 24 h to monitor their behavior as well their mortality. Phase 2 includes three test animals distributed into three groups of one animal each and were administered higher doses (1600, 2900 and 5000 mg/kg) of extract, and then observed for 24 h for their behavioral change as well as mortality. LD₅₀ is calculated as the geometric mean of the highest non-lethal dose and the least toxic dose. More observations and weighing were carried on for 14 days and the experiment was then terminated.

2.4. Grouping and dosing

In all three models (castor oil induced) used in this study, experimental animals were randomly selected and screened based on their susceptibility to these models, and were divided into five groups (control, positive and three test groups) consisting of six mice in each group. The first group was assigned as control group and was supplied with vehicle (1% tween-80 in distilled water) at a volume of 10 mL/kg, body weight while positive control group received standard antimotility drug loperamide (3 mg/kg) in all models. The test groups (3, 4 and 5) received different doses (100, 200 and 400 mg/kg, respectively) of Ethanolic extract of S. palustris. All groups received their respective doses orally with the help of feeding needle. Several dose levels in this study were determined and selected based on the result of acute toxicity study by Lorke’s method [16]. LD₅₀ value of the extract was found greater than 5 g/kg. 1/8th of middle acute toxicity dose (Phase 2, 1600 mg/kg) was used to determine the middle dose and one half and 2 times the middle dose was selected for the lowest and highest doses, respectively. The absence of mortality and signs of over toxicity up to 4 times of highest effective dose (400 mg/kg) to acute middle acute toxicity dose (1600 mg/kg) suggested that ethanolic frond extract has a wider safety margin.
2.5. Castor oil induced diarrhea
The method previously described by Shoba and Thomas was adapted for this study with minor modification [17]. Swiss albino mice of either sex were fasted for 18 h with free access to water and treated as described previously in grouping and dosing section. After one hour of respective doses or treatments, diarrhea was induced orally by the administration of 0.5 mL castor oil to each mouse and placed separately in transparent polyvinyl cage where the floor was lined with blotting paper for the ease of counting feces and was changed every hour for the total observation period of 4 h. The time of onset of diarrhea, total number of diarrheic faeces and the total weight of diarrheic faeces by each mouse were recorded and compared with the control group. The percentage of inhibition of defecation was calculated for each group by following formula which corresponds to antidiarrheal activity.

\[
\%\text{Inhibition} = \frac{\text{Average number of DFC} - \text{Average number of DFT}}{\text{Average number of DFC}} \times 100
\]

Where, DFC = Diarrheic faeces in the control group and DFT = Diarrheic faeces in the test group.

2.6. Castor oil induced gastrointestinal motility
The effect of \textit{S. palustris} extract on gastrointestinal motility using activated charcoal suspension was evaluated according to the method described by Yasmeen \textit{et al.} and Umer \textit{et al.} with slight modification [18,19]. Mice were fasted for 18 h and group and treated as described under grouping and dosing section. After one hour of test and vehicle compound administration, 0.5 mL castor oil was administered orally with the help of feeding needle. Again, after one hour of administration of castor oil, each mouse received 1 mL of charcoal meal (3% charcoal suspension) as peristaltic marker by oral route. The animals were then sacrificed by cervical dislocation after an hour of castor oil administration. After that, abdomen of each mouse was opened carefully and the small intestine was removed, and tied at the pyloric end and the ileocecocal junction. The weight of filled intestine was then measured and the intestinal contents were collected by milking into a graduated tube to measure their volume. Each empty intestine was again reweighed and the difference between the full and the empty intestines was calculated. Lastly, the percentage inhibitions of the volume and weight of intestinal contents were determined according to the given formula.

\[
\%\text{Inhibition}(V) = \frac{\text{MVICC} - \text{MVICT}}{\text{MVICC}} \times 100
\]

\[
\%\text{Inhibition}(W) = \frac{\text{MWICC} - \text{MWICT}}{\text{MWICC}} \times 100
\]

Where, MVICC = Mean volume of the intestinal content of the control group and MVICT = Mean volume of the intestinal content of the test group.

2.7. Castor oil induced enteropooling test
The inhibition of intraluminal fluid accumulation (enteropooling) in small intestine by ethanolic extract of \textit{S. palustris} was determined based on the method described by Robert \textit{et al.} and Maxwell \textit{et al.}, [20,21]. Mice of either sex were deprived of both food and water for 18 h and grouped and dosed as described previously. Each test animal received 0.5 mL of castor oil orally one hour after the administration of test and control treatment. All mice under investigation were sacrificed by cervical dislocation after an hour of castor oil administration. After that, abdomen of each mouse was opened carefully and the small intestine was removed, and tied at the pyloric end and the ileocecocal junction. The weight of filled intestine was then measured and the intestinal contents were collected by milking into a graduated tube to measure their volume. Each empty intestine was again reweighed and the difference between the full and the empty intestines was calculated. Lastly, the percentage inhibitions of the volume and weight of intestinal contents were determined according to the given formula.

2.8. Superoxide radical scavenging activity
Superoxide anion scavenging activity based on the reduction of nitroblue tetrazolium (NBT) was measured using standard method followed by slight modification [22]. Superoxide radicals were generated through non-enzymatic phenazine methosulfate (PMS)/reduced nicotinamide adenine dinucleotide (NADH) system that reduces NBT into a purple-colored formazan. The capacity of \textit{S. palustris} extracts to inhibit formazan formation upon photochemical reduction of NBT corresponds to the superoxide radical scavenging activity. In this test, the reaction mixture (3 mL phosphate buffer, pH 7.4) contained 1 mL NBT (150 μM), NADH (468 μM) solutions for superoxide radical generation, and different ethanolic concentrations of \textit{S. palustris} (6.25-800 μg/mL). Then, reaction was accelerated by adding
1mL of 60 µM PMS solution and total reaction mixture was incubated at 25°C for 5 minutes. The absorbance was taken at 560 nm against an appropriate blank solution. L-ascorbic acid was used as the positive control. The decrease in absorbance (NBT reduction) at 560 nm with the frond extract and the reference compound ascorbic indicates the abilities to quench superoxide radicals in the reaction mixture. The percentage of superoxide radical scavenging was calculated according to the following equation: \( \left( \frac{A_o - A_1}{A_o} \right) \times 100 \) where \( A_o \) is the absorbance of the blank and \( A_1 \) is the absorbance of the sample extract and standard.

### 2.9. Statistical analysis

The obtained experimental data exhibited normal distribution and were expressed as mean ± standard error of mean (SEM) (n=6) of responses. Statistical significance differences within and between the groups was determined by using One-way Analysis of Variance (ANOVA) followed by Tukey’s post hoc test using Statistical Package for the Social Sciences (SPSS) version 20.0. A value of \( p < 0.05 \) was considered statistically significant in the present investigation.

### 3. RESULTS AND DISCUSSION

The frond of *S. palustris* yielded 17.6% extract of dried fern material. During acute toxicity test, no changes in the behavior pattern and no signs of toxicity or death were observed over a period of 24 h for the ethanolic extract of *S. palustris*. Even at the highest dose (5 g/kg), extract was found devoid of mortality of any test animals depicting that the LD\(_{50}\) of the extract was greater than 5 g/kg. Also, the test animals showed no significant variation in weight and appetite during the observation period of 14 days.

Ethanolic extract of *S. palustris* at the discrete doses (100, 200 and 400 mg/kg) were evaluated for their antidiarrheal potential against castor oil induced diarrhea, gastrointestinal transit, and enteropooling (intraluminal fluid accumulation) model in Swiss albino mice and shown in Tables 1-3. In castor oil induced diarrheal test, *S. palustris* extract, in a dose dependent manner, demonstrated significant (\( p < 0.001 \)) antidiarrheal effect in the mice by prolonging and reducing the onset, frequency, and weight of diarrheal feces. As shown in Table 1, the extract at all doses (100, 200, and 400 mg/kg) showed marked delay in onset of diarrhea (113.25 ± 6.77, 144.50 ± 8.56, and 172.92 ± 12.40 min) as compared to control (42.50 ± 4.61 min). Similarly, the number of diarrheic (wet) feces significantly (\( p < 0.001 \)) inhibited (1.83 ± 0.40) as compared with the control (9.17 ± 0.75). Loperamide 3 mg/kg (positive control) had also shown highly significant delay and reduction (\( p < 0.001 \)) of the onset (184.42 ± 6.17) and frequency of diarrhea (1.33 ± 0.33). The highest percentage of inhibition of defecation was observed at a dose 400 mg/kg (80.04%) of ethanolic frond extract which is favorably comparable with percentage inhibition of standard drug loperamide (85.50%) and showing significant antidiarrheal effect of fern extract. In addition, there was also a significant (\( p < 0.001 \)) dose dependent reduction in the average weight of wet fecal outputs with 3 mg/kg and 400 mg/kg of the standard

### Table 1. Effect of ethanolic extract of *S. palustris* fronds on castor oil induced diarrheal model in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>Onset of diarrhea (min)</th>
<th>Total number of diarrheic (wet) feces</th>
<th>Weight of diarrheic (wet) feces (g)</th>
<th>% Inhibition of defecation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1% tween 80 in DW)</td>
<td>10 mL/kg</td>
<td>42.50 ± 4.61*</td>
<td>9.17 ± 0.75*</td>
<td>0.81 ± 0.06*</td>
<td>-</td>
</tr>
<tr>
<td>Loperamide</td>
<td>3 mg/kg</td>
<td>184.42 ± 6.17*</td>
<td>1.33 ± 0.33*</td>
<td>0.10 ± 0.03*</td>
<td>85.50</td>
</tr>
<tr>
<td><em>S. palustris</em></td>
<td>100 mg/kg</td>
<td>113.25 ± 6.77*</td>
<td>4.00 ± 0.37*</td>
<td>0.27 ± 0.04*</td>
<td>56.38</td>
</tr>
<tr>
<td></td>
<td>200 mg/kg</td>
<td>144.50 ± 8.56*</td>
<td>2.50 ± 0.62*</td>
<td>0.20 ± 0.04*</td>
<td>72.74</td>
</tr>
<tr>
<td></td>
<td>400 mg/kg</td>
<td>172.92 ± 12.40*</td>
<td>1.83 ± 0.40*</td>
<td>0.13 ± 0.05*</td>
<td>80.04</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, SEM = Standard error for mean (n=6), *Significant difference (\( p<0.001 \)) in comparison to control analyzed using one-way ANOVA followed by Tukey’s post hoc test, *Compared with standard loperamide
and extract displaying the highest effect (0.10 ± 0.03 and 0.13 ± 0.05 g) relative to the control group. Epithelial cells lining of the intestinal lumen of tremendous absorptive capacity serve as selective permeable barriers and involved in bidirectional transport of nutrients, electrolytes and fluid from the intestinal lumen [23]. Irritation in the intestinal mucosa results in the induction of inflammatory mediators like prostaglandin, nitric oxide, histamine, tachykinins etc. which damage the mucosal architecture and compromise the epithelial barrier [23]. This increases the permeability of the mucosal cells marked by increased volume of the intestinal content by inhibiting absorption and enhancing the secretion of fluid and electrolytes thereby stimulating peristaltic activity and diarrhea. Herein, castor oil produce diarrhea through the release of its active component ricinoleic acid by a hypersecretory response [24]. Ricinoleic acid through irritation and inflammation of the gastrointestinal mucosa triggers the release of prostaglandins that results in the stimulation of intestinal motility and electrolyte secretion [20,25]. Successful inhibition of the castor oil induced diarrhea in comparison with standard loperamide by ethanolic extract of S. palustris might be due to its antimotility and antisecretory effect [26]. The presence of tannins and flavonoids in the fern extract are responsible for reducing or inhibiting peristaltic movement, intestinal motility and hydroelectrolytic secretion that evoked the antidiarrheal activity [5,26-29].

The effect of S. palustris extract on charcoal meal intestinal transit model in mice is shown in Table 2. The impact of test extract on gut motility was evaluated in this method. The administration of extract (100, 200, and 400 mg/kg) significantly (p < 0.001) decreased the propulsion of charcoal meal in gastrointestinal (GI) tract of mice as compared with the control (p < 0.001) in a dose dependent manner. The distance traveled by the charcoal meal (peristaltic marker) for the control was 44.17 ± 2.12 cm with peristaltic index or motility of 82.18 ± 3.56%. On the other hand, significant inhibition of gastrointestinal transit was observed in case of three test doses as charcoal marker travelled lesser distance (25.67 ± 2.50, 18.05 ± 1.75, and 13.67 ± 2.36 cm) in small intestine with reduced peristaltic index of 46.74 ± 4.59, 32.52 ± 3.28, and 26.37 ± 3.31%. Loperamide (3 mg/kg) also showed a significant reduction (20.61 ± 2.45 cm, p < 0.001) in the distance traveled by the charcoal meal and resulted lowest percentage of motility index (20.61 ± 2.45%) as compared with the control group (Table 2). Also, the percentage inhibition of charcoal meal by the highest dose of this extract (400 mg/kg) (67.91%) showed highly comparable antimotility effects to that of the standard (74.92%). Antidiarrheal agents act by reducing the increased GI peristalsis to control diarrhea [30]. The above finding suggests that antidiarrheal effect of the extract is due to its ability to influence the

<table>
<thead>
<tr>
<th>Treatment (n=6)</th>
<th>Dose</th>
<th>Length of small intestine (cm)</th>
<th>Distance travelled by peristaltic marker (cm)</th>
<th>Peristaltic index (%)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1% tween 80 in DW)</td>
<td>10 mL/kg</td>
<td>53.75 ± 1.63</td>
<td>44.17 ± 2.12*</td>
<td>82.18 ± 3.56*</td>
<td>-</td>
</tr>
<tr>
<td>Loperamide</td>
<td>3 mg/kg</td>
<td>54.59 ± 1.52</td>
<td>11.25 ± 1.68*</td>
<td>20.61 ± 2.45*</td>
<td>74.92</td>
</tr>
<tr>
<td>S. palustris</td>
<td>100 mg/kg</td>
<td>54.92 ± 1.16</td>
<td>25.67 ± 2.50*</td>
<td>46.74 ± 4.59*</td>
<td>43.12</td>
</tr>
<tr>
<td></td>
<td>200 mg/kg</td>
<td>55.50 ± 1.86</td>
<td>18.05 ± 1.75*</td>
<td>32.52 ± 3.28*</td>
<td>60.42</td>
</tr>
<tr>
<td></td>
<td>400 mg/kg</td>
<td>51.83 ± 2.01</td>
<td>13.67 ± 2.36*</td>
<td>26.37 ± 3.31*</td>
<td>67.91</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, SEM = Standard error for mean (n=6), *Significant difference (p<0.001) in comparison to control analyzed using one-way ANOVA followed by Tukey’s post hoc test, **Compared with standard loperamide
peristaltic movement by increasing intestinal absorption of water and electrolytes in GI tract and thus delaying gastrointestinal transit in mice [5-7,31].

The inhibition of intraluminal fluid accumulation produced after oral administration of castor oil was evaluated in castor oil induced enteropooling model and results are presented in Table 3. In this model, the ethanolic extract of S. palustris extract significantly (p < 0.001) reduced the volume of intestinal fluid and weight of the intestinal contents in a dose dependent manner at all test doses as compared with the control (p < 0.001). Among three test doses, the highest percentage inhibition of volume and weight of intestinal contents was shown by the extract at the dose of 400 mg/kg and found to be 76.92 and 73.79%, respectively. The maximum level of intestinal fluid reduction was seen in the standard drug (3 mg/kg) (82.05 and 79.61%) which is close and highly comparable to the highest dose of test extract. The volume and weight of intestinal contents of the control were 0.78 ± 0.07 and 1.03 ± 0.06 (Table 3), respectively and were reduced gradually after the administration of standard and test doses. Ricinoleic acid, active component of castor oil, activates the nitric oxide (NO) pathway and induces NO dependent GI secretions along with prostaglandin synthesis [32]. The presence phytochemical constituents such as terpenoids and flavonoids in the extract are responsible for the inhibition of nitric oxide pathway which halts intestinal secretion and produce antisecretory effect [5,7,33-35]. Fig. 3 shows overall antidiarrheal effect of S. palustris extract on three castor oil induced diarrheal model in mice.

Superoxide anion (O$_2^•$) radical acts as precursor of the more reactive oxygen species (hydrogen peroxide, hydroxyl radical, and singlet oxygen) and has the detrimental effect on the cellular components in a biological system contributing to the tissue damage and various diseases [36,37]. It also initiates lipid oxidation by generating singlet oxygen [36]. No extensive results on superoxide scavenging activity from the ethanolic frond extract of S. palustris have been reported. Table 4 shows the superoxide anion (O$_2^•$) radical activity of the ethanolic frond extract of S. palustris measured on the basis of reduction of nitroblue tetrazolium (NBT) at various concentrations (6.25-800 µg/mL) using ascorbic acid as standard. Extract demonstrated high ability to scavenge superoxide anion in a concentration

<table>
<thead>
<tr>
<th>Treatment (n=6)</th>
<th>Dose</th>
<th>Volume of intestinal contents (mL)</th>
<th>% Inhibition</th>
<th>Weight of intestinal contents (g)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1% tween 80 in DW)</td>
<td>10 mL/kg</td>
<td>0.78 ± 0.07$^a$</td>
<td>-</td>
<td>1.03 ± 0.06$^a$</td>
<td>-</td>
</tr>
<tr>
<td>Loperamide</td>
<td>3 mg/kg</td>
<td>0.14 ± 0.03$^*$</td>
<td>82.05</td>
<td>0.21 ± 0.03$^*$</td>
<td>79.61</td>
</tr>
<tr>
<td>S. palustris</td>
<td>100 mg/kg</td>
<td>0.37 ± 0.05$^*$</td>
<td>52.56</td>
<td>0.51 ± 0.08$^*$</td>
<td>50.49</td>
</tr>
<tr>
<td></td>
<td>200 mg/kg</td>
<td>0.25 ± 0.05$^*$</td>
<td>67.95</td>
<td>0.34 ± 0.04$^*$</td>
<td>66.99</td>
</tr>
<tr>
<td></td>
<td>400 mg/kg</td>
<td>0.18 ± 0.03$^*$</td>
<td>76.92</td>
<td>0.27 ± 0.06$^*$</td>
<td>73.79</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, SEM = Standard error for mean (n=6), *Significant difference (p<0.001) in comparison to control analyzed using one-way ANOVA followed by Tukey’s post hoc test, $^a$Compared with standard loperamide.

Fig. 3. Effect of ethanolic frond extract of S. palustris on the inhibition of diarrhea using three castor oil induced diarrheal models in mice.

Table 4. Superoxide anion radical scavenging activity of ethanolic frond extract of S. palustris and ascorbic acid

<table>
<thead>
<tr>
<th>S. palustris extract concentration (µg/mL)</th>
<th>Superoxide anion (O$_2^•$) scavenging (% inhibition)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.25</td>
<td>5.92 ± 0.17</td>
</tr>
<tr>
<td>12.5</td>
<td>12.65 ± 0.18</td>
</tr>
<tr>
<td>25</td>
<td>20.26 ± 0.13</td>
</tr>
<tr>
<td>50</td>
<td>35.27 ± 0.13</td>
</tr>
<tr>
<td>100</td>
<td>47.13 ± 0.20</td>
</tr>
<tr>
<td>200</td>
<td>58.43 ± 0.18</td>
</tr>
<tr>
<td>400</td>
<td>70.85 ± 0.14</td>
</tr>
<tr>
<td>800</td>
<td>84.32 ± 0.15</td>
</tr>
</tbody>
</table>

Standard ascorbic acid (800 µg/mL) | 91.59 ± 0.12 |
dependent manner. At the concentration 800 µg/mL, extract showed the highest percentage inhibition of superoxide anion (84.32 ± 0.15%) which is highly comparable to standard ascorbic acid (91.59 ± 0.12%). Higher inhibitory effects of the extract on superoxide anion formation corresponds the fern to be a promising antioxidant agent.

4. CONCLUSION

Overall results of the present study revealed that ethanolic extract of *S. palustris* fern possessed significant antidiarrheal activity on evaluation in animal models using Swiss albino mice. The extract demonstrated a significant dose dependent delay in the onset of diarrhea, reduced the frequency of wet feces as well as antimotility and antisecretory effects at all three test doses evaluated experimentally. The findings of the study provide a scientific support to confirm the traditional claim of antidiarrheal activity of the fern.

REFERENCES


