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Study of Analgesic, Neuropharmacological and Anti-diarrheal Activities of Ethanol Extract of *Solanum sisymbriifolium* Fruits

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ABSTRACT

Background: The aim of the present study was to investigate the possible analgesic, neuropharmacological and anti-diarrheal activities of the ethanol extract of fruits of *Solanum sisymbriifolium* Lam. (Family: Solanaceae) in Swiss Albino mice. **Methods:** Analgesic activity of the fruits extract was conducted using acetic acid induced writhing inhibition test. The neuropharmacological activities were evaluated by hole cross, hole board and elevated plus maze tests and the anti-diarrheal activity was carried out by castor oil induced diarrhea inhibition method. **Results:** The ethanol extract of fruits of *Solanum sisymbriifolium* demonstrated a statistically significant ($p<0.001$) analgesic effect at both the dose of 200 and 400 mg kg^{-1} body weight, inhibiting pain by 80.03 and 86.64%, respectively in a dose dependent manner. The extract showed anxiolytic behavior of mice in the elevated plus maze test at the dose 400 mg kg^{-1} body weight and also significantly displayed a dose dependent suppression of locomotor activity and exploratory behavior in mice that was carried out by hole cross and hole board test. The extract showed highly significant ($p<0.001$) anti-diarrheal activity evidenced by decrease in mean number of stool and total weight of fecal output in a dose dependent manner. **Conclusion:** The present findings suggest that the plant widely available in Bangladesh could be a prominent source of medicinally important natural compounds.

Key words: *Solanum sisymbriifolium*, analgesic, anxiolytic, sedative, anti-diarrheal

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INTRODUCTION

Solanum sisymbriifolium is a member of a large plant genus *Solanum*, which contains at least 1,500 species and are commonly known as Sticky Nightshade. Among the species, *Solanum sisymbriifolium* (Family: Solanaceae) is a spiny species and has very prickly, sticky leaves; white flowers and bright red slightly sticky fruits[1]. It is a perennial erect, rhizomatous herb and grows in agricultural, disturbed and urban areas. They are found in native South America as well as most of its non-native range and are thus currently distributed throughout the world[1]. It is popular in Bangladesh as Kanta Begun, Kantikari (Bengali)[2].

The leaves of *S. sisymbriifolium* are traditionally being used as febrifuge and diuretic, while its roots are used as diuretic, analgesic, hysteria, contraceptive, antisyphilitic and hepatoprotective. The fruits and flowers are used as analgesic and in the synthesis of corticosteroids and oral contraceptives[3].

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S. sisymbriifolium is medicinally important as it is a rich source of variety of phytoconstituents. A new neolignan, designated as sisymbrifolin and carpesterol were isolated from the berries of this shrub[1] are well known to date. The chemical constituents previously reported to be found in roots of *S. sisymbriifolium* were isonuatigenin-3-O-β-solatriose[4] and cuscohygrine, solacaproine[3]. Presence of phytochemical constituents such as alkaloids, flavonoids, steroids and tannins[5] were also found from the whole plant.

Extract from the plant has been reported to exert hypotensive effects in rats[4]. Literature survey reveals that nuatigenosido, isolated as one of the prospective active compounds from the roots, was shown to lower blood pressure and augment the contractile force in the right atrium of animal models[6]. Investigation on the dried fruit was found to possess potent anticonvulsant and CNS depressant activities in rodents[7]. In addition to these, the methanol extract of the whole plant showed dose dependent antinociceptive activity in mice[5].

From the existing information it is evident that the plant possesses some important pharmacological activities. However, the fruits of the plant are not

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explored significantly for its analgesic, neuropharmacological and anti-diarrheal activities; thus pharmacological studies have been incorporated which consisted of performing *in vivo* procedure to identify the pharmacological activities of the ethanol extract of the fruits of this plant.

MATERIALS AND METHODS

Plant collection and identification: The fruits of *S. sisymbriifolium* were collected in the month of August, 2011 from Aftabnagar, Dhaka, Bangladesh. Collected plant part was identified by a taxonomist from Bangladesh National Herbarium, Mirpur, Dhaka, where a voucher specimen under the Accession Number DACB 35894 has been conserved for future reference.

Preparation and extraction of plant material: The dried, coarsely powdered material (584 gm) was extracted by cold maceration process over 72 h with ethanol (1L) at room temperature. The extract was concentrated with a rotary evaporator (IKA, Germany) at low temperature of 45°C and reduced pressure to get the dried crude ethanolic extract.

Experimental animals: All the pharmacological experiments were conducted using 20 young Swiss Albino mice, weighing 20-25 g and were purchased from Animal Research Branch of the International Centre for Diarrheal Disease and Research, Bangladesh (ICDDR, B). The animals were grouped and housed separately in cages for adaptation under standard laboratory conditions (relative humidity 55-65%, room temperature 25.0±2.0°C and 12 hours light dark cycle) and were fed with standard (ICDDR, B formulated) diets and had free access to tap water. The animals were allowed to acclimatize to laboratory conditions 24 hours before the start of the experiment. The research protocols were in accordance with the principles and guidelines adopted by the Animal Experimentation Ethics Committee (AEEC) of East West University. All the experiments were performed on an isolated and noiseless condition.

Analgesic activity: The analgesic activity of the fruits extract was evaluated using acetic acid induced writhing inhibition test in mice[8]. The writhes were induced by intraperitoneal injection of 0.7% (v/v) acetic acid to the experimental animals to create pain sensation. Mice were fasted overnight with water *ad libitum* and randomly divided into four groups of each case containing five in number. The extract solutions of dose 200 mg kg^{-1} and 400 mg kg^{-1} body weight were administered to the test groups. The control group received 1% (v/v) tween-80 (Merck, Germany) in normal saline (Beximco Infusions Ltd., Bangladesh) at a dose of 0.5 mL $mice^{-1}$ and the positive control group received diclofenac sodium (Square Pharmaceuticals Ltd., Bangladesh) at a dose of 10 mg kg^{-1}. After 30 min of the oral administration, 0.7% acetic acid was induced by intraperitoneal injection to each of the mice.

The number of painful muscular contractions referred to as 'writhing' was counted over a period of 20 minutes just 5 min after the administration of acetic acid. Mice did not always perform full writhing. The incomplete writhing was taken as a half-writhing. Accordingly two half-writhings were considered as one full writhing[9]. Analgesic activity was expressed as writhing inhibition (%) and was calculated for each animal by using the following formula:

$$\text{Writhing inhibition (\%)} = \frac{Wc - Ws}{Wc} \times 100$$

where, W_c is the mean number of writhing of control and W_s is the mean number of writhing of the test sample.

Neuropharmacological activities: The neuropharmacological activities of the ethanol extract of *S. sisymbriifolium* fruits were conducted using hole cross, hole board and elevated plus maze tests. During every experiment four groups of mice were taken each consisting five in number. The groups that received particular treatment:

Group 1: Control (1% (v/v) tween-80 in normal saline, 0.5 mL $mice^{-1}$)
Group 2: Positive control (diazepam, Square Pharmaceuticals Ltd., Bangladesh, 1 mg kg^{-1} b.wt.)
Group 3: Test sample 1 (ethanol extract at the dose of 200 mg kg^{-1} b.wt.)
Group 4: Test sample 2 (ethanol extract at the dose of 400 mg kg^{-1} b.wt.)

Hole cross test: The method described by Subhan *et al.*[10] was adopted for screening sedative activity in mice. A wooden box on which the experiment was carried out was partitioned in the middle having a size of 30×20×14 cm. A hole of 3 cm diameter was made at a height of 7.5 cm in the center of the box. Each mouse was placed in the box to observe the spontaneous movements from one chamber to another over a period of 3 min after the oral administration. The observation was conducted for 0, 30, 60, 120 min.

Hole board test: The head dip test method described by Somani *et al.*[11] was adopted to see the emotional behavior of the mice. The apparatus was used, using a white printed wooden board (40×40×25 cm) with 16

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equidistant holes (diameter 3 cm), elevated from the ground of 25 cm from which mice could peep through the holes. Each of the mice was placed at the center of the board and moved freely in the box. A head dip up to eye into holes was used to indicate exploratory behavior. The latency until the first head dipped and the number of counts for head dipped by each of the treated animal were observed for a period of 5 minutes after 30 minutes of feeding.

Elevated plus maze test: The anxiolytic activity was adopted using the elevated plus maze test[12]. Briefly, the apparatus consisted of two arms crossed with two enclosed arms (16×5×12cm) and two open arms (16×5cm). The arms were connected together with a central square from the floor. The entire maze is elevated to a height of 40 cm above the floor. The whole set up of the apparatus were placed in a dimly illuminated room. 30 minutes after the feeding of individual, mice were placed at the centre of the maze facing towards one of the open arms. All tests were recorded using a digital video camera for a period of 5 minutes. From the video, number of parameters noted, are as follows:

- Number of entries in the open, closed arms and centre
- Number and duration of rearing
- Number and duration of grooming
- Number and duration of stretch attend postures

Anti-diarrheal activity: Castor oil induced diarrheal inhibition model was used to evaluate possible anti-diarrheal activity of the extract following the method described by Shoba and Thomas[13]. The experimental mice were fasted for 24 h before the test with free access to water. The mice were then randomly selected and divided into four groups, each groups containing 5 mice. Of the experimental groups, group-1, control, received 0.5 mL of 1% (v/v) tween-80 in normal saline solution orally. Group 2 or positive control received 0.5 mL of the anti-motility drug Loperamide (Square Pharmaceuticals Ltd., Bangladesh) at the dose of 2 mg kg^{-1} body weight as oral suspension. The test groups were administered orally with 0.5 mL of ethanolic extract solution of *S. sisymbriifolium* at the dose of 200 and 400 mg kg^{-1} body weight. Mice were fed with the samples 30 min prior to the oral administration of 0.2 ml of castor oil (BDH Chemicals Ltd., UK). Each mouse was then placed in separate beaker having fresh adsorbent filter paper beneath it. The presence of diarrhea was examined for three hours after the administration of castor oil. During an observation period, number of parameters that were to check was: (a) onset of dry and wet stool (b) number of wet stool (c) weight of dry and wet stool and (d) total weight of fecal output.

Statistical analysis: Data were presented as Mean±SEM. SPSS for WINDOWS™ (version 12.0) was applied for the analysis and statistically analyzed by one-way ANOVA followed by Dunnett t-test (2-sided). $p<0.05$ was taken to be the level of significance, $p<0.001$ was taken to be the level of highly significance.

RESULTS

Analgesic activity: In the acetic acid induced writhing inhibition test, ethanol extract of *S. sisymbriifolium* fruits demonstrated a statistically highly significant ($p<0.001$) analgesic effect at both the dose of 200 and 400 mg kg^{-1} b.wt., inhibiting pain by 80.03 and 86.64%, respectively in a dose dependent manner when compared to the control (Table 1).

Hole cross test: The number of hole crossed from one chamber to another by mice of the control group was similar from 0 to 120 min. In the hole cross test, ethanol extract of *S. sisymbriifolium* fruits at both doses of 200 and 400 mg kg^{-1} b.wt. showed a decrease in locomotion in the test animals from second observation period (30 min) and was sustained up to 5th observation period (120 min) as evident by the reduction in number of hole crossed by the treated mice compared to the control group (Table 2). The result was comparable to the control group and was statistically significant ($p<0.05$) for both the doses at 30 min and for 400 mg kg^{-1} b.wt. at 120 min observation (Table 2). CNS was depressed till the completion of observation period. The positive control, diazepam also showed a significant ($p<0.05$) decrease in locomotion activity in the test animal (Table 2).

Hole board test: At the dose 200 mg kg^{-1} b.wt., ethanol extract of *S. sisymbriifolium* fruits has significantly ($p<0.001$) increased the number of head dip responses (52.5±1.16) when compared to the control group which was statistically highly significant ($p<0.001$) (Table 3). But the dose of 400 mg kg^{-1} b.wt. has decreased the activity (35.6±0.51) significantly ($p<0.001$) when compared with control. At both doses of 200 and

Table 1: Effect of ethanol extract of *S. sisymbriifolium* fruits (SSF) and controls on acetic acid induced writhing in mice

Group	Dose (p.o.)	No. of writhing	Inhibition (%)
Control (1% tween-80 in saline)	0.5 mL	78.7±0.22	-
Positive control (Diclofenac sodium)	10 mg kg^{-1} b. wt.	1.5±0.16**	98.09
SSF 200	200 mg kg^{-1} b.wt.	15.7±0.41**	80.03
SSF 400	400 mg kg^{-1} b.wt.	10.5±0.39**	86.64

Values are Mean±SEM (n = 5), **$p<0.001$ Dunnett t-test as compared to negative control

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Table 2: Effect of ethanol extract of *S. sisymbriifolium* fruits (SSF) on number of movement in hole cross test

		No. of movements				
Groups	Dose (p.o.)	0 min	30 min	60 min	90 min	120 min
Control (1% tween-80 in saline)	0.5 mL	10.0±0.71	8.6±0.60*	7.2±0.58	7.0±0.55	6.2±0.37
Positive control (Diazepam)	1 mg kg^{-1} b.wt.	7.8±0.74	6.2±0.80*	5.8±0.74	4.2±1.16*	3.6±0.51*
SSF 200	200 mg kg^{-1} b.wt.	12.4±0.12	11.8±0.58*	8.0±0.84	6.0±0.70	4.8±0.20
SSF 400	400 mg kg^{-1} b.w.t.	12.4±0.74	11.6±0.50*	6.2±0.66	7.6±0.81	3.8±0.49*

Values are Mean±SEM (n = 5), *p<0.05 compared to control

Table 3: Effect of ethanol extract of *S. sisymbriifolium* (SSF) fruits and controls in hole board test in mice

Treatment	Dose (p.o.)	No. of head pocking	Latency until the firstentry (sec)
Control (1% tween-80 in saline)	0.5 mL	44.8±1.24	14.8±0.37
Positive control (Diazepam)	1 mg kg^{-1}	29.6±0.93**	02.0±0.45**
SSF 200	200 mg kg^{-1}	52.5±1.16**	06.0±0.55**
SSF 400	400 mg kg^{-1}	35.6±0.51**	05.2±0.37 **

Values are Mean±SEM (n = 5), **p<0.001 compared to control

Table 4: Effect of ethanol extract of *S. sisymbriifolium* fruits (SSF) and controls on elevated plus maze test in mice

	Time spent(sec)		No. of entry					
Treatment (dose, p.o.)	Open arm	Close arm	Open arm	Center	Close arm	No. of stretch attend postures	No. of grooming	No. of rearing
Control (0.5 mL)	7.6±3.20	215±19.76	1.2±0.49	4.2±0.2	9.4±1.91	9.2±1.42	3.8±0.58	14.2±1.66
Positive control (1 mg kg^{-1})	6.8±2.82	260±12.96	1.6±0.81	4.6±0.75	12.0±3.03	8.6±2.29	6.2±0.80	9.4±3.37
SSF 200 (200 mg kg^{-1})	16±6.78	236.4±17.36	1.0±0.55	2.4±1.03	7.2±0.73	5.8±0.66	4.2±0.8	10.4±1.96
SSF 400 (400 mg kg^{-1})	11.2±4.32	236.8±14.73	1.4±0.75	7.6±1.29*	11.2±1.32	14.2±0.49	3.8±1.2	14.6±3.17

Values are Mean±SEM (n = 5)Control: 1% tween-80 in saline, Positive control: Diazepam

Table 5: Effect of ethanol extract of *S. sisymbriifolium* fruits (SSF) and controls on castor oil induced diarrhea in mice

Treatment	Dose (p.o.)	Total latent period (min)	No. of stool	Total weight of fecal output
Control	0.5 mg $mice^{-1}$	42.8±1.16	14.4±1.75	0.935±0.02
Positive control	2 mg kg^{-1}	95.2±1.71**	08.6±0.75*	0.731±0.03**
SSF 200	200 mg kg^{-1}	42.6±1.29	06.2±0.37**	0.396±0.3**
SSF 400	400 mg kg^{-1}	14.2±0.86**	09.2±0.86*	0.557±0.03**

Values are Mean±SEM (n = 5), *p<0.05, **p<0.001 compared to control, Positive control: Loperamide

400 mg kg^{-1} b.wt. showed a highly significant decrease (p<0.001) in the latency period (6.0±0.55 and 5.2±0.37), respectively (Table 3). The positive control, diazepam also showed highly significant (p<0.001) decrease in both head dip responses (29.6±0.93) and latency period (2.0±0.45) compare to control group (Table 3).

Elevated plus maze test: In elevated plus maze test, the extract at the dose 200 mg kg^{-1} decreased the number of entry in the open arm while at the dose 400 mg kg^{-1} increased the number of entry in the open arm compared to control which were statistically not significant (Table 4).

Anti-diarrheal activity: In castor oil induced diarrhea inhibition test, *S. sisymbriifolium* fruits extract caused small reduction in the latent period at the dose 200 mg kg^{-1} and the result was non-significant when compared with the control (Table 5). Whereas highly significant (p<0.001) decrease in latent period (i.e hastened the onset of diarrheal episode) (14.2±0.86) at the dose of 400 mg kg^{-1} of body weight was observed compared with the control group (42.8±1.16). At dose 200 mg kg^{-1}, the extract showed a marked significant reduction in the total number of stool compared with control (p<0.001) and at higher dose there was a significant (p<0.05) reduction in the total number of stool when compared with the control group (Table 5). At both doses of 200 and 400mg kg^{-1} b.wt., there was a reduction in total weight of fecal output which was found to be statistically highly significant (p<0.001) (Table 5).

DISCUSSION

Analgesic activity: The acetic acid induced writhing inhibition test has long been used as a screening tool for the assessment of analgesic properties of plant extracts and natural products and the method was found effective to evaluate peripherally active analgesics. In the analgesic activity performed, the writhing inhibition increased as the dose of extract was increased. The similar result was also reported by the other researchers with extract of the whole plant[5]. The agents reducing the number of writhing will render analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition[8]. The significant pain reduction of the plant extract at both the doses might be due to the presence of analgesic principles such as flavanoids, alkaloids, tannins and steroids[14,15,16] acting with the prostaglandin pathways.

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Hole cross test: Gamma-amino-butyric acid (GABA) is the major inhibitory neurotransmitter in the CNS. Different anxiolytic, sedative hypnotic drugs elucidate their action through $GABA_A$ receptor. The sedative effect recorded here may be related to an interaction with benzodiazepines related compounds that binds to receptors in the CNS. Therefore it is possible that extract of *S. sisymbriifolium* fruits may act by potentiating GABAergic inhibition in the CNS or may be due to the activation of GABA receptor by the extracts. Earlier investigation on phytoconstituents and plants suggests that many flavonoids and steroids were found to be the ligands for $GABA_A$ receptors in the CNS which led to the assumption that they can act like benzodiazepine-like molecules[17]. So, it is probable that phytoconstituents present in this extract is responsible for its CNS activity. The decrease number of hole crosses by diazepam treated mice compare to control may be due to the dose (1 mg kg^{-1}) used in the test that can produce sedation in mice as reported by Takeda *et al.*[18].

Hole board test: The hole board experiment is a measure of exploratory behavior in animals and is an accepted parameter for evaluating anxiety conditions in animals. In this test, the test compounds showed dose dependent decrease in the number of nose poking. The extract produced a significant decrease in the nose poking and latency time period at higher dose levels and was more pronounced when compared to a control. This indicates a decrease in the curiosity or exploratory behavior of test animals and also provides evidence in favor of a CNS depressant action[19,20]. This observation also indicates the anxiogenic activity of the extract at higher dose (400 mg kg^{-1}). The presence of secondary metabolites like alkaloids, flavanoids, tannins, solasodine individually or in combination would account for the observed pharmacological effects of this plant in this study. However, the increase number of head dipping behavior at dose 200 mg kg^{-1} indicates the anxiolytic activity of the extract[18]. The decrease number of head dipping behavior in mice treated with diazepam may be due to the sedative effect of diazepam at the experimental dose (1 mg kg^{-1}).

Elevated plus maze test: The test is used to evaluate psychomotor performance and emotional aspects of mice. The present work has been done to see the anxiolytic activity by the fruits extract of *S. sisymbriifolium* as assessed by elevated plus maze test. Results obtained on the test after treatment with the extract at dose 400 mg kg^{-1} b.wt. revealed anxiolytic activity, since increases in open arm entry parameters are the most representative indices of anxiolytic activity[21]. The decrease in open arm entry at dose 200 mg kg^{-1} b.wt. indicates the anxiogenic activity of the extract as described by Takeda *et al.*[18].

Anti-diarrheal activity: Anti-diarrheal activity of the ethanolic fruit extract of *S. sisymbriifolium* was tested using the model of castor oil induced diarrhea inhibition in mice. Castor oil, used to induce diarrhea in mice, mixes with bile and pancreatic enzymes and liberates recinoleic acid from the triglycerides upon oral administration. However, it is well evident that castor oil produces diarrhea due to its most active component recinoleic acid which causes irritation and inflammation of the intestinal mucosa, leading to the release of prostaglandins that results in the stimulation of motility and secretion. The recinoleic acid thus liberated readily forms recinoleate salts which stimulates the intestinal epithelial cell's adenyl cyclase[22] or release prostaglandin[23]. Since the ethanolic extract of *S. sisymbriifolium* successfully inhibited the castor oil induced diarrhea, extract might have exerted its anti-diarrheal action via anti-secretory mechanism which was also the evident from the decreased number of stool as well as the decrease in total weight of fecal output. Again, flavonoids present in the plant extract are reported to inhibit release of autacoids and prostaglandins, thereby inhibit motility and secretion induced by castor oil[24]. Previous studies showed that antidysentric and antidiarrheal properties of medicinal plants were mostly due to tannins, alkaloids, flavonoids, saponins, triterpenes, sterols[25,26]. On the basis of the result of castor oil induced diarrhea, it can be concluded that the ethanolic fruit extract of *S. sisymbriifolium* might possess anti-diarrheal activity.

CONCLUSION

The study has shown that the ethanolic fruit extract of *S. sisymbriifolium* possess significant analgesic, CNS and anti-diarrheal activities in laboratory animals at the doses investigated. Further experiments and detailed phytochemical analysis can be carried out to determine the phytoconstituents responsible for mechanism of actions involved.

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