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Pharmacological Activities of *Senna obtusifolia* Linn.: A Medicinal Plant of Bangladesh

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Abstract

The crude methanol extract of seed of *Senna obtusifolia* Linn. has been investigated for anxiolytic, anti-atherothrombosis, membrane stabilizing and alpha-amylase inhibitory activities. The anxiolytic activity was examined in mice by using the hole cross and open field test (OFT). The anti-atherothrombosis activity was evaluated and compared with that of standard streptokinase. The membrane stabilizing activity was tested by using hypotonic solution- and heat-induced hemolysis of human erythrocyte. The plant extract was also assessed for anti-diabetic activity through *in vitro* α-amylase inhibitory potential. The α-amylase inhibitory activity of *S. obtusifolia* was measured using the starch-iodine method. The crude extract of *S. obtusifolia* showed moderate anxiolytic activity. In the *in-vitro* anti-atherothrombosis test, the extract exhibited mild activity as compared to the standard, streptokinase (81.53%). In membrane stabilizing activity test, the plant extract at 1.0 mg/ml inhibited the heat-induced hemolysis of RBCs by 56.37% whereas the standard acetyl salicylic acid (ASA) demonstrated 71.36% inhibition of hemolysis. Our results revealed that the extract produced dose-dependent prevention of digestion of carbohydrates by inhibiting α-amylase. These findings demonstrated that *S. obtusifolia* may be a good candidate for further analysis because of its effective pharmacological properties.

Key words: *Senna obtusifolia*; anxiolytic; anti-atherothrombosis; membrane stabilizing; alpha amylase.

Introduction

Medicinal plants have immensely contributed to the development of human health and welfare. Plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines (Farombi, 2003). They are used in modern medicines where they occupy a very significant place as raw materials for important drugs (Audu *et al.,* 2007). Natural constituents can be derived from any part of plant's bark, leaves, flowers, roots, fruits, seeds etc. This means that any part of the plant may contain active components. This has attracted a great deal of research interest in natural product chemistry.

Senna obtusifolia Linn. (commonly known as 'Sicklepod') belonging to the family Fabaceae grows as a weed during rainy season throughout Bangladesh, India and other tropical regions of the world like America, Asia and Africa. It grows on well-drained fertile soil. The green leaves of the plant are fermented to produce a high-protein food product called "kawal" which is eaten by many people in Sudan as a substitute of meat. Its leaves, seeds, and root are also used in folk medicine, primarily in Asia. It is believed to possess laxative effect, as well as to be beneficial for eyes (Dirar, 1984).

The plant has been traditionally used for the treatment of dizziness, dysentery and eye inflammation etc. (Guo *et al.,* 1998). The leaves are used locally as a remedy for gout, sciatica, joints pain, stomach-ache and head-ache (Zafar 1994). The leaf decoction is used as febrifuge and for treatment of gingivitis, urinary tract infections, diarrhea, fever and cough (Doughari *et al.,* 2008). Chemical review of the plant revealed the presence of different phytoconstituents like anthraquinones, phytosterols, triterpenoids and flavonoids (Sob *et al*., 2008; Sudi *et al.,* 2011).

As part of our ongoing efforts to study medicinal plants of Bangladesh (Khair *et al.,* 2014; Chakma *et al.,* 2013; Kuddus *et al.,* 2011), we evaluated the anxiolytic, anti-atherothrombosis, membrane stabilizing and alpha-amylase inhibitory activities of *S. obtusifolia* as well as to justify the rationale behind for its folk uses.

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Materials and Methods

Collection of plant materials: Seeds of *S. obtusifolia* were collected from Chittagong Pahartoli, Bangladesh in June 2012 and were identified at the Forest Research Institute, Chittagong, Bangladesh where a voucher specimen has been maintained for future reference.

Drying and grinding: After collection, the seeds were washed with running tap water. These clean seeds were dried at a temperature not exceeding 40 ^{o}C. The dry materials were ground to a coarse powder with the help of a grinder and kept in an airtight container. The container was then stored in a cool and dark place until extraction was commenced.

Hot extraction by soxhlet extractor: Exactly 120gm of powdered seed was extracted with 750 ml of methanol (99.98%) with a Soxhlet apparatus (Quickfit, England). The extract was concentrated with a rotary evaporator (Heidolph, Germany) under reduced temperature and pressure to provide a gummy residue (yield 15.87%).

Chemicals: All chemicals and solvents used in this study were of analytical grade and purchased from Merck, Germany. Standard drugs such as diazepam, acetyl salicylic acid and acarbose were obtained from Square Pharmaceuticals Ltd as gift samples.

Experimental animals: For the experiment *Swiss albino* mice of either sex, 6-7 weeks of age, weighing between 25-30 g, were collected from the Animal Resources Branch of the International Centre for Diarrheal Disease and Research, Bangladesh (ICDDR,B). The mice were maintained under standard environmental conditions of temperature: (27.0 ± 1.0 °C), relative humidity: 55-65% and 12 h light/12 hr dark cycle and had free access to ICDDR,B formulated diet and water *ad libitum*. Appropriate measures were taken to minimize the pain or discomfort of animals and the mice were acclimatized to laboratory condition for one week prior to experiments. All protocols for animal experiment followed by the institutional animal ethical committee's animal handling procedure to minimize the pain and reduce discomforts (Zimmermann, 1983).

Test for anxiolytic activity

Treatment schedule: The anxiolytic activity of *S. obtusifolia* was examined by using the hole board test and open field test (OFT). The animals were divided into four groups, with each group consisting of seven mice. First group received normal saline, second group received diazepam (1 mg/kg b.w., p.o.), while the third and fourth groups received plant extract at 200 and 400 mg/kg, b.w. respectively.

Hole cross test: The hole cross is a white painted wooden board (30 cm × 20 cm × 14 cm) with 16 holes (each of diameter 3 cm) evenly distributed on the base of box. The number of passages of a mouse through the hole from one chamber to the other was counted for a period of 5 min at 30 min after oral administration of both doses of the test drug (Kishore *et al.,* 2012).

Open field test: The open field test is used to observe general motor activity, exploratory behavior and measures of anxiety. The open field area was made of plain wood and consisted of a square area (45 cm × 45 cm × 20 cm). The floor had a square sheet of wood (45 cm × 45 cm) with the surface divided into sixteen small squares. Mice were divided into four groups of 7 mice and treated similarly as described in the hole cross test. About 30 min after treatment, mice of both the control and treated groups were placed individually at the center of the open field and behavioral activities were recorded for 5 min. Subsequently, hand operated counters and stopwatches were used to score the following behavioral parameters for a period of 5 min: (1) the number of entries and time spent in the centre, (2) periphery and corners of the field, (3) the number of crossings (number of square floor units entered) as a measure of distance traveled, (4) rearing (number of times the animal stood on hind legs) and (5) assisted rearing (forepaws touching the walls of the apparatus) (Kishore *et al.,* 2012).

Test for anti-atherothrombosis activity: The anti-atherothrombosis activity of the crude extract was evaluated by using streptokinase as standard (Prasad *et al.,* 2006). For this study, 4 ml venous blood was drawn from healthy volunteers and distributed in three (for extract, reference standard and for negative control) pre-weighed sterile micro-centrifuge tubes (0.5 ml/tube). The tubes were incubated at 37 °C for 45 min. After clot formation, serum was completely removed without disturbing the clot and each tube was weighed again to determine the weight of clot (clot weight = weight of clot containing tube – weight of tube alone). Then, 100 µl of methanol extract at a dose of 5 µg/µl, 100 µl of streptokinase and 100 µl of methanol were separately added to the pre-marked tubes

containing the clot. The tubes were then incubated at 37 °C for 90 min and observed for clot lysis. Afterwards, the fluid released was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis. The experiment was repeated for three times in different days with fresh blood samples collected from 10 healthy volunteers (male and female) having no history of contraceptives and anticoagulants.

Test for membrane stabilizing activity: For this experiment, three clean centrifuge tubes were taken for positive control (acetyl salicylic acid), three for negative control (99.8% methanol) and six for crude methanol extract. One milliliter of 10% RBC suspension was added to each tube. Then 1.0 ml methanol and 1.0 ml acetyl salicylic acid (0.1 mg) were added to the negative control and positive control tubes, respectively. On the other hand, for the test group, 1.0 ml of methanol extract (1000 mg/kg) was added. The pH (7.4 ± 0.2) of the reaction mixtures was adjusted by phosphate buffer. The tubes were incubated in water bath and after cooling, these were centrifuged at 2500 rpm for 5 min. After filtration the absorbance of the supernatants was measured at 556 nm. The total inhibition of hemolysis was then calculated by determining the % inhibition of hemolysis (Shinde *et al.,* 1999).

Test for alpha-amylase inhibitory activity: The α-amylase inhibitory activity of *S. obtusifolia* was measured using the starch-iodine method (Komaki*et et al.,* 2003). Twenty microliter of α-amylase solution (0.030 mg/ml) was mixed with 1.3 ml of Tris-HCl buffer (0.01 M containing 0.006 M NaCl, pH 6.8) and the crude extract (80, 160, 320 µl). After incubation at 37 ºC for 20 min, 100 µl of the starch solution (0.1%) was added, and the mixture re-incubated for 20 min, after which 2 ml of 0.01% acidic iodine solution was added. The absorbance of the sample was measured at 565 nm and percentage inhibition was calculated as % Inhibition of enzyme activity = (A-C) × 100/ (B-C) where, A = absorbance of the sample, B = absorbance of blank (no extract), and C = absorbance of control (no starch).

Statistical analysis: Results are expressed as the mean ± SEM (SEM = Standard Error of Mean). Statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dunnett's multiple comparisons. The results obtained were compared with the vehicle control group; p=0.05 was considered as statistically significant.

Results and Discussion

The present study was designed to evaluate anxiolytic, anti-atherothrombosis, membrane stabilizing and α-amylase inhibitory activities of the methanol extract of seed of *S. obtusifolia*. The results are summarized in the Tables 1-3.

Table 1. Anxiolytic activity of crude methanol extract of *S. obtusifolia* in mice by hole cross and open-field test.

Treatment	Dose (mg/kg)	Hole cross test		Open field test	
		Number of hole crossing	% Inhibition of hole crossed	Number of square crossed	% Inhibition of square crossed
Saline	1 ml	25.67 ± 1.08	0.0	251.67 ± 2.94	0.0
Diazepam	1	3.33 ± 0.71	87.01	39.67 ± 2.54	84.24
SOME	200	5.0 ± 0.41	80.51	66.67 ± 1.42	73.51
SOME	400	13.0 ± 0.41	49.36	181.31 ± 1.78	28.07

All values are mean ± SEM (n=7); SEM= Standard Error of Mean; SOME = S. obtusifolia methanol extrac.t

During the evaluation of anxiolytic activity by hole cross test, the number of line crossings was found to increase significantly in case of diazepam treated animals as compared to control animals. The plant extracts at the 200 and 400 mg/kg b.w. (p.o) dose showed significant increase in the number of line crossing as compared to control animals as shown in Table-1.In the open field test, administration of plant extract in mice showed significant increase in the number of squares crossed during 5 min intervals of test as compared with the control as show in Table-1.The adrenergic and dopaminergic system have been shown to play a role in anxiety. Benzodiazepine have been extensively, used for the last 40 years to treat several forms of anxiety, but due to their unwanted side effects,

alternative treatment strategies were sought with favorable side effect profiles. Medicinal plants are a good source to find new remedies for these disorders. Mechanism of anxiolytic action of plants may be by interaction with some of the natural endogenous mediators in the body as reported by various workers (Contarino *et al.,* 1999).

Table 2. Anti-atherothrombosis and membrane stabilization activities of crude methanol extract of *S. obtusifolia/*

Test groups	Anti-atherothrombosis activity	Membrane stabilizing activity
	% Clot lysis	Total inhibition of hemolysis
Control	2.87 ± 0.94	00.00 ± 0.018
Streptokinase (positive control)	81.53 ± 3.7	ND
Positive control (ASA, 0.1mg/ml)	ND	71.36 ± 0.021*
SOME (1.0 mg/ml)	ND	56.37 ± 0.0212*
SOME (0.5mg/ml)	12.26 ± 2.4	38.63 ± 0.01414*

*Values are mean ± SEM,*p= 0.05; ND = Not determined; SEM= Standard Error of Mean; SOME = S. obtusifolia methanol extract*

Table 3. Inhibition (%) of amylase activities of crude methanol extract of *S. obtusifolia.*

Test group	Total inhibition of α-amylase activity
Control (DW)	0
Positive control (50 µg/ml)	79.80 ± 0.00141*
SOME (400 µg/ml)	57.90 ± 0.003536*
SOME (200 µg/ml)	40.35 ± 0.005401*
SOME (100 µg/ml)	22.81 ± 0.004083*
SOME (50 µg/ml)	16.67 ± 0.002042
SOME (25 µg/ml)	4.38 ± 0.009356

*DW = Distilled water, Values are mean ± SEM; *p= 0.05; SEM= Standard Error of Mean; SOME = S. obtusifolia methanol extract*

Effect of most of the anxiolytic agent is to enhance the response to GABA, by facilitating the opening of GABA-activated chloride channels. Thus, the present study showed that *S. obtusifolia* extract demonstrated anxiolytic activity in mice as evident by open field and hole cross models. The effects were dose dependent.

In the anti-atherothrombosis activity test, streptokinase, a positive control (30,000 IU) showed 81.53% clot lysis. On the other hand, clots when treated with 100 µl methanol (negative control) showed only negligible lysis (2.87%). In the same time, by treating clots with 500 µl (µg/ ml) of the extract, 12.56% clot lysis was obtained. Statistical representation of the effective clot lysis percentage has been shown in Table 2.

The extract at 500 and 1000 µg/ml inhibited the heat-induced hemolysis of RBCs by 38.63, 56.37%, respectively whereas standard acetyl salicylic acid showed 71.36% inhibition of hemolysis (Table 2). The stabilization of cell membrane for crude methanol extract was found to be moderate. Although the precise mechanism of this membrane stabilization is yet to be elucidated, it is thought that the plant might inhibit the release of lysosomal content of neutrophils at the site of inflammation. The red blood cell stability test is based on the result that a number of non-steroidal anti-inflammatory agents inhibit heat-induced rupture of erythrocytes, most probably by stabilizing the membrane of the cell. The erythrocyte membrane may be considered as a model of the lysosomal membrane. Agents that can prevent the rupture of the latter, and thereby prevent damage to the tissue caused by the release of the hydrolytic enzymes contained within the lysosome may be expected to improve some symptoms of inflammation. It has been demonstrated that certain herbal preparations were capable of stabilizing the red blood cell membrane and this may be indicative of their ability to exert anti-inflammatory activity (Oyedapo and Famurewa 1999).

During the alpha-amylase inhibitory activity test, the extract of *S. obtusifolia* displayed concentration dependent inhibitory effect on the starch breakdown *in vitro* as shown in Table 3. In the present study, the crude extract showed 57.90%, 40.35%, 22.81%, 16.67% and 4.38% inhibition of α-amylase enzyme activity at 400, 200, 100, 50 and 25 µg/ml, respectively whereas the standard acarbose (50 µg/ml) produced 79.80% inhibition. The ability of *S. obtusifolia* to inhibit and hypotonic and thermal enzyme activity was found to be statistically significant (p=0.05). The anti-diabetic activity of medicinal plants could be evaluated using several methods; *in vitro* α-amylase inhibitory assay is one of such techniques. The extract of *S. obtusifolia* inhibited α-amylase but its activity was significantly less than that of positive control acarbose (p=0.05). Alpha-amylase is an enzyme responsible for breaking down of α-1,4-glycosidic bonds in starch. Therefore, the enzyme increases the availability of glucose in the blood. *S. obtusifolia* extract could be useful in post-prandial hyperglycemia by reducing the hydrolysis of carbohydrates. The observed

activity may be due to the presence of chemical constituents such as phenolic compounds (tannins and flavonoids) and terpenoids in the extract (Ebi and Ofoefule 2000; Kim *et al*., 2006). Phenolics have been reported to inhibit α-amylase activities. They also have anti-hyperglycemic activity and inhibit the development of diabetes (Hanamura *et al*., 2006; Zunino *et al.,* 2007).

Conclusion

These primary findings suggest the presence of bioactive secondary metabolites in this plant extract that are responsible for anxiolytic, anti-atherothrombosis, membrane stabilizing and alpha-amylase inhibitory activities. This warrants for systematic chemical investigation of *S. obtusifolia* to isolate the active molecules.

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