Stamford Journal of Pharmaceutical Sciences

S. J. Pharm. Sci. 1(1&2): 63-68

Anti-diarrhoeal and CNS Depressant Activity of Methanolic Extract of *Saccharum spontaneum* Linn.

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Received- 25 October, 2008 Accepted for Publication-5 December, 2008

ABSTRACT

Preliminary phytochemical screening of the methanolic extract of the whole plant of *Saccharum spontaneum* Linn. (Family- *Gramineae*) revealed the presence of alkaloids, flavonoids, reducing sugar, tannins and saponins. The antidiarrhoeal activity of the extract (200 and 400 mg/kg) was assessed on experimental animal and a dose dependent decrease in the total number of faecal dropping was obsereved in castor oil induced diarrhoea in mice. The plant extract was also assessed for effect on the central nervous system (CNS) using a number of neuropharmacological experimental models in mice. The extract produced a dose-dependent reduction of the onset and duration of pentobarbitone-induced hypnosis, reduction of locomotor and exploratory activities in the open field and hole cross tests. These results suggest that the extract possesses antidiarrhoeal and CNS depressant activity.

Key Words: *Saccharum spontaneum*, Castor oil, Loperamide, Pentobarbitone.

INTRODUCTION

Over the years, medicinal plants have been found useful in the treatment and management of various health problems. About 80% of the world population relies on the use of traditional medicine, which is predominantly based on plant material (WHO, 1993). Scientific studies available on a good number of medicinal plants indicate that promising phytochemicals can be developed for many health problems (Gupta, 1994). Herbal drugs have gained importance in recent years because of their efficacy and cost effectiveness. These drugs are invariably single plant extracts of fractions thereof or mixtures of fractions/extracts from different plants, which have been carefully standardized for their safety and efficacy (Subramoniam and Pushpangadan, 1999). *Saccharum spontaneum* Linn. (Family- Gramineae) locally known as Kaich is a tall erect reed-like perennial grass with plume like inflorescence, grows in marshes in Chittagong and other areas. Leaves and stalks contain lignin, carbohydrates, proteins and amino acids (Ghani, 2003). Roots and root-stocks contain starch and polyphenolic compounds. Aerial parts possess laxative and aphrodisiac properties, and are useful in burning sensations, strangury, phthisis, vesical calculi, blood diseases, biliousness and haemorrhagic diathesis (Chopra et al., 1992). Roots are used as galactagogue and diuretic (Ghani, 2003). The present study was undertaken to investigate antidiarrhoeal and CNS depressant activity of the methanolic extract of *Saccharum spontaneum* in the experimental animal model.

EXPERIMENTALS

Plant Material

The plant *S. spontaneum* was collected from Dhaka in the month of May 2007. A voucher specimen for this collection has been maintained in Bangladesh National Herbarium (DACB 32882), Dhaka, Bangladesh.

Extraction

The powdered plant sample (500 g) was soaked in 1.5 L of methanol for 16 days and then filtered through a cotton plug followed by Whitman filter paper number 1. The extract was concentrated with a rotary evaporator and it afforded 15 g of the methanol extract.

Phytochemical Screening

A preliminary phytochemical screening of all extracts carried out using the standard method of Trease and Evans (1989).

Animals

Swiss albino mice of either sex (120 - 140 g) were obtained from the Animal house of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). The animals were housed under standard laboratory conditions (relative humidity 55 - 65%, r.t. 23.0 ± 2.0^{o}C and 12 h light: dark cycle). The animals were fed with standard diet and water *ad libitum*. The University Animal Research Ethical Committee approved the experimental protocol.

Effect of Extract on Castor Oil-Induced Diarrhoea

The method described by Uddin et al. (2005) and Awouters et al. (1978) was adopted to study the effect of the *S. spontaneum* extract on castor oil-induced diarrhoea. Mice were weighed and grouped into 4 groups (n = 5). Group 1 received distilled water, group 2 and 3 were administered 200 and 400 mg/kg extract orally while group 4 received loperamide (5 mg/kg) orally. Each animal was then given 0.5 mL of castor oil orally after 30 min of treatment and placed in transparent cages to observe for consistency of faecal matter and frequency of defecation for 3 h. Faeces were collected with an absorbent sheet of paper placed beneath the transparent cages (Mukherjee et al., 1998). The wet faeces were read at the end of the experiment by lifting up the upper part of the cage containing the sheet of paper and animals. The percent (%) inhibition of defecation was measured using the following formula.

% Inhibition of defecation = [(A - B) / A] x 100

A = Mean number of defecation caused by castor oil

B = Mean number of defecation caused by drug or extract

Pentobarbitone Induced Sleeping Time Test

The animals were randomly divided into four groups consisting of five mice each. The test groups received of *S. spontaneum* extracts at the doses of 200 and 400 mg/kg while positive control was treated with diazepam (1 mg/kg i.p.) and control with vehicle (1% Tween 80 in water). Thirty minutes later, pentobarbitone (40 mg/kg, i.p., Sigma Chemicals, USA) was administered to each mouse to induce sleep. The animals were observed for the latent period (time between pentobarbitone administration to loss of righting reflex) and duration of sleep (time between the loss and recovery of righting reflex) (Williamson et al., 1996).

Open Field Test

This experiment was carried out as described by Gupta et al. (1971). The animals were divided into control and test groups. The test groups received *S. spontaneum* methanolic extracts at the doses of 200 and 400 mg/kg body weight orally whereas control group received vehicle (1% Tween 80 in water). The floor of an open field of half square meter was divided into a series of squares each alternatively colored black and white. The apparatus had 40 cm height a wall. The number of squares visited by the animals was counted for 3 min, on 0, 30, 60 and 90 min during the study period.

Hole Cross Test

The method described by Takagi et al. (1971) was adopted for this study. A steel partition was fixed in the middle of a cage 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 cage. The number of passages of a mouse through the hole from one chamber to other was counted for a period of 3 min on 0, 30, 60and 90 min after the oral treatment with *S. spontaneum* methanolic extracts at the doses of 200 and 400 mg/kg.

Statistical Analysis

Data obtained from pharmacological experiments are expressed as mean±SEM. Difference between the control and the treatments in these experiments were tested for significance using one-way analysis of variance (ANOVA), followed by Dunnet's *t*-test for multiple comparisons using SPSS software.

RESULTS

Prelimenary phytochemical studies revealed the presence of alkaloids, flavonoids, reducing sugar, tannins and saponins.

Effect of Extract on Castor Oil-Induced Diarrhoea

In the castor oil-induced diarrhoea experiment, the mice group that did not receive the plant extract showed typical diarrhoeal signs and symptoms such as watery and frequent defecation. The methanolic extract of *S. spontaneum* produced a notable antidiarrhoeal effect in mice (Table-1).

Table 1: Effect of the methanolic extract of *S. spontaneum* on castor oil-induced diarrhoea in mice.

Group	Traeatment	Latent time (min)	Number of faeces at first hour		Number of watery faeces at second hour	Number of watery faeces at third hour	Number of watery faeces at fourth hour
			Hard stool	Watery stool			
Control	Castor oil (10 ml/kg)	12.90±0.43	8.20 ± 0.48	3.0 ±0.32	11.4 ± 0 .67	13.6 ± 0.40	11.4±0.45
Standard	Castor oil + loperamide (5 mg/kg)	39.4±1.06 ***	3.20 ±0.37 ***	1.2 ± 0.20 ** (%inhibition 60.71%)	5.2 ± 0.37 *** (54.38%)	5.4±0.40 *** (60.29%)	4.0±0.79 * (64.9%)
Test-1	Castor oil + crude extract (200 mg/kg)	27.44±1.07* **	6.0 ± 0.32 **	1.8 ± 0.20 * (%inhibition 30.36%)	3.6 ± 0.24 *** (68.42%)	2.8 ± 0.37*** (79.41%)	4.2±1.02* (63.15%)
Test-2	Castor oil + crude extract (400 mg/kg)	35.20±0.63* **	4.6 ± 0.50 ***	1.0 ± 0.32 *** (% inhibition 50.0%)	2.2 ± 0.03 *** (80.70%)	2.0 ± 0.32*** (85.29%)	3.4±1.68* (70.17%)

Values are expressed as mean±SEM (n=5); One-way ANOVA; ***p< 0.001, **p<0.01 and *p<0.05 compared to control.

Both doses of the extract significantly decreased ($p < 0.001$) the total number of wet faeces produced by administration of castor oil (2.8 ± 0.37 at the dose of 200 mg/kg and 2.0 ± 0.32 at the dose of 400 mg/kg) as compared to the castor oil-treated control group (13.6 ± 0.40) at third hour of observation. The percentage of inhibition of castor oil-induced diarrhoea in the extract-treated mice was 79.41and 85.29%, respectively, at the doses of 200 and 400 mg/kg. The effect of the extract was found to be better to that of the standard drug, loperamide (3 mg/kg), which produced an inhibition of 60.29% (Table-1).

Effect of Extract on Pentobarbitone Induced Sleeping Time

In the Pentobarbitone induced hypnosis test, the extract at the doses of 200 and 400 mg/kg significantly induced the sleep at an earlier stage and also prolonged the duration of sleeping time in test animals as compared to control (Table 2).

Table 2: Effect of extract Pentobarbitone induced Sleeping Time

Group	Treatment	Route of administration	Onset of sleep (min)	Duration of sleep (min)
Control	1% aqueous tween 80	p.o.	15 ± 0.39	45.04 ± 0.89
Positive control	Diazepam 1mg/kg	i.p.	10.34 ± 0.22*	76.68 ± 0.84*
Test Group I	Crude extract 200mg/kg	p.o.	7.3 ± 0.25*	66.42 ± 1.02*
Test group II	Crude extract 400mg/kg	p.o.	6.38 ± 0.26*	98.06 ± 1.08*

Values are expressed as mean±SEM (n=5); One-way ANOVA; *p<0.001 compared to control

Effect of Extract on Open Field Test in Mice.

In the open field test, the extracts showed a noticeable decrease in locomotion in the test animals from the second observation period at both dose levels (200 and 400 mg/kg body weight). The results were dose dependent and statistically significant (Table-3).

Table 3: Effect of extract on open field test in mice.

Group	Treatment	Route of administration	Number of Movements			
			0 min	30 min	60 min	90 min
Control	1% aqueous tween 80	p.o.	115.4 ±1.5	105.2 ±0.8	93.4 ±0.51	89.0 ±0.71
Test group I	Crude extract 200mg/kg	p.o.	110.6 ±1.36	61.6 ±2.09*	24.4±0.81*	24.6 ±1.12*
Test group II	Crude extract 400mg/kg	p.o.	101.2±1.46*	14.6 ±0.75*	4.4 ±0.24*	0.60 ±0.24*

Values are expressed as mean±SEM (n=5); One-way ANOVA; *p<0. 01 compared to control

Effect of Extract on Hole Cross Test in Mice.

In the hole cross test, the extracts showed a decrease in locomotion in the test animals from the second observation period at both dose levels (200 and 400 mg/kg body weight). The results were dose dependent and statistically significant (Table-4).

Table 4: Effect of extract on Hole cross test in mice.

Group	Treatment	Route of administration	Number of movements			
			0 min	30 min	60 min	90 min
Control	1% aqueous tween 80	p.o.	10.2± 0.37	11.75±0.48	11.0 ±0.32	9.4±0.4
Test group I	Crude extract 200mg/kg	p.o.	9.6 ±0.51	6.2 ±0.37*	4.0 ±0.32*	2.4±0.24*
Test group II	Crude extract 400mg/kg	p.o.	9.2 ±0.37	1.6 ±0.24*	1.2±0.2*	2.0±0.32*

Values are expressed as mean±SEM (n=5); One-way ANOVA; *p<0. 001 compared to control

DISCUSSION

Castor oil induced diarrhoea model is widely used for the evaluation of antidiarrhoel property of drugs. Ricinoleic acid, the active metabolite of ricinoleic acid which is present in castor oil is responsible for the diarrhoea inducing property of castor oil (Gaginella and Philips, 1975). It stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa. Its action also stimulates the release of endogenous prostaglandin which stimulates motility and secretion (Pierce et al., 1971, Galvez et al., 1993). In this study, the methanolic extract of *S. spontaneum* displayed a significant and dose-dependent antidiarrhoeal activity against castor oil-induced diarrhoea in mice. The results were found to be better to that of the standard drug loperamide (3 mg/kg) with regard to the severity of diarrhea. Loperamide is widely used in the management of diarrhoeal disorders effectively antagonizes diarrhoea induced by castor oil (Niemegeers et al., 1974). The antidiarrhoeal property of the methanolic extract of *S. spontaneum* found in the present study could be owing to the presence of tannins, alkaloids, saponins, flavonoids in this plant. Previous studies showed that antidysenteric and antidiarrhoeal properties of medicinal plants were mostly due to tannins, alkaloids, saponins, flavonoids, sterol and triterpenes (Galvez et al., 1991, 1993; Longanga et al., 2000). The result obtained in this study infers the antidiarrhoeal property of the methanolic extract of *S. spontaneum*. To evaluate the effect of methanolic extract *of S. spontaneum* on the CNS in mice, a number of methods namely pentobarbitone-induced hypnosis, open field, hole cross, were adopted. In the pentobarbitone-induced hypnosis test, both extracts, at the doses of 200 and 400 mg/kg body

weight, dose dependently induced sleep at a rapid stage as compared to control, and increased the duration of sleep (Table-2). Pentobarbitone when given at appropriate dose induces sedation or hypnosis in animals by potentiating the GABA mediated postsynaptic inhibition through an allosteric modification of GABA receptors (Collier et al., 1968). Substances that have CNS depressant activity either decrease the time for onset of sleep or prolong the duration of sleep or both. The results obtained in this test, indicate that these extracts might have depressant action on the CNS. The extract significantly decreased the locomotor activity as shown by the results of the open field and hole cross tests (Table-3 and Table-4). The methanolic extract of *S. spontaneum* at 400 mg/kg dose showed more prominent depressant activity than the 200 mg/kg dose.

CONCLUSION

The results of the present study indicates the anti-diarrhoeal and CNS depressant activity of *S. spontaneum* which deserves further studies to establish its therapeutic value as well as its mechanism of action.

ACKNOWLEDGEMENT

We are really grateful to Prof. Abdul Ghani for his valuable cooperation and help.

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