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Antinociceptive Activity of Methanol Extract of *Solanum sisymbriifolium* Lamk.

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Abstract: The antinociceptive activity of the methanolic extract of the whole plant of *Solanum sisymbriifolium* Lamk. was investigated using acetic acid-induced writhing model in swiss albino mice. At the doses of 125, 250 and 500 mg kg⁻¹ body weight, the extract showed 44.19, 59.53 and 73.02% writhing inhibition, respectively in test animals which were comparable to that of standard drug aspirin (85.12%) and the results were statistically significant (p<0.001). At the above doses, the extract exhibited significant and dose dependant antinociceptive activity in acetic acid-induced writhing model in mice. Phytochemical investigation of the extract indicated the presence of alkaloid, flavonoids, steroid and tannin.

Key words: *Solanum sisymbriifolium*, antinociceptive activity, acetic acid-induced writhing, phytochemical test

INTRODUCTION

Solanaceae is a large plant family of 72 genera and about 1500 species, of which over 1225 species belong to the genus *Solanum*. A large number of plants this family are traditionally used in different therapeutic purposes including in the treatment of pain and inflammation^[1,2]. Some species of this genus has been reported to possess antinociceptive activity^[3,4]. Previously Ibarrola^[5] presumed that the flowers of *Solanum sisymbriifolium* may possess antinociceptive activity. The plant is reported to contain hypotensive agents^[6]. But still the plant is devoid of proper pharmacological evaluation. As part of our continuing search for plants possessing antinociceptive activity^[7,8], we now report on the antinociceptive activity of the whole plant (root, stem and leaves) of *Solanum sisymbriifolium* Lamk.

MATERIALS AND METHODS

Animals: Swiss albino mice of either sex (20-25 g) obtained from the Animal Resources Branch of the International Center for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) were used. The animals were housed under standard environmental condition and fed with standard diet (ICDDR, B formulated) and water *ad libitum*.

Plant material and extraction: The whole plant (root, stem and leaves) of *S. sisymbriifolium* was collected from

Khulna in the month of June 2003 and was identified by the experts of Bangladesh National Herbarium, Dhaka, Bangladesh. The dried plant parts were pulverized into coarse powder with the help of a suitable grinder (Capacitor start motor, Wuhu Motors factory, China). Cold extraction by methanol yielded approximately 6% extract of the dried plant parts.

Phytochemical tests: The following tests were carried out according to the methods described by Harborne^[9].

Test for alkaloids: Dragendorff's reagent was used to assess the presence of alkaloids in the extract.

Test for flavonoids: Cyanidin test was used to determine the presence of flavonoids. Methanolic solutions of the plant extracts were used. In the presence of HCl and metallic magnesium, flavonoids present in the extract was reduced to anthocyanins which was determined by their absorbance at 510 nm.

Table 1: Contents of alkaloids, flavonoids, steroids and tannins in the *S. sisymbriifolium* plant extracts

Types of compounds	Methanol extract of <i>Solanum sisymbriifolium</i>
Alkaloids	++
Flavonoids	+++
Steroids	++
Tannin	++++

++ = Low level, +++ = Moderate level, ++++ = High level,

Table 2: Effect of the methanolic extract of *solanum sisymbriifolium* on acetic acid-induced writhing in mice

Treatment	Dose ^a (mg kg ⁻¹ , p.o.)	Writhings ^b	Inhibition (%)
Control (1% Tween 80, 10 mL kg ⁻¹ , p.o.)	-	35.83±2.33	-
Aspirin	100	5.33±0.80*	85.12
<i>S. sisymbriifolium</i> extract	125	20.00±1.41*	44.19
	250	14.50±0.88*	59.53
	500	9.67±1.23*	73.02
One-way ANOVA	f-value	67.50	
	df	4, 25	
	p-value	< 0.001	

^aAdministered 45 min before 0.7% acetic acid administration (mL kg⁻¹, i.p.), ^bCounted for 15 min, starting 5 min after acetic acid administration. Values are mean ±SEM, *p<0.001 vs. control, one way ANOVA followed by Dunnett's Multiple Comparison Test

Test for steroids: Libermann-Burchard reagent was used to assess the presence of steroids in the extract.

Test for tannins: Liquid extract (1 mg mL⁻¹) was mixed with the methylene blue solution (7.0 x 10⁻⁵ M) followed by the determination of residual methylene blue by its absorbance at 668 nm.

Antinociceptive activity study using acetic acid induced writhing assay: The antinociceptive activity was studied using acetic acid-induced writhing model in mice^[10]. The animals were divided into control, positive control and test groups containing six mice in each group. The animals of test groups were fed with the methanol extract of the whole plant of *S. sisymbriifolium* at the doses of 125, 250 and 500 mg kg⁻¹ body weight, positive control group with reference drug (aspirin) at the dose of 100 mg kg⁻¹ and control group with vehicle (1% Tween 80 in water) at a dose of 10 mL kg⁻¹ body weight orally 45 min before intraperitoneal administration of 0.7% acetic acid. After a 5 min interval for proper absorption of acetic acid, the mice were observed for specific contraction of body referred as writhing for 15 min.

Statistical analysis: All the data were analyzed by Prism 4 for windows (Graphpad software) using one-way ANOVA followed by Dunnett's Multiple Comparison Test. p<0.05 was considered significant.

RESULTS AND DISCUSSION

Phytochemical study of the extract showed the positive response of alkaloid test, flavonoids test, steroid test and tannin test (Table 1). At the doses of 125, 250 and 500 mg kg⁻¹ body weight, the extract produced 44.19, 59.53 and 73.02% writhing inhibition in test animals (Table 2). The results were statistically significant (p<0.001) and were comparable to that of the standard drug aspirin, which showed 85.12% writhing inhibition at the dose of 100 mg kg⁻¹ body weight. The intensities of writhing inhibition of the extract increased with the increase of dose (Table 2).

Acetic acid-induced writhing model represents pain sensation by triggering localized inflammatory response. Acetic acid, which is used to induce writhing, causes algia by liberation of endogenous substances, which then excite the pain nerve endings^[11]. Increased levels of PGE₂ and PGF_{2α} in the peritoneal fluid have been reported to be responsible for pain sensation caused by intraperitoneal administration of acetic acid^[12]. On the basis of the result of acetic acid induced writhing test, it could be concluded that the methanolic extract of *S. sisymbriifolium* possess antinociceptive activity and the mode of action might involve a peripheral mechanism of pain inhibition.

Further pharmacological investigation of bioactivity guided phytochemical studies are required to find out the actual active constituents responsible for antinociceptive action of the extract of *S. sisymbriifolium*.

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