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ANALGESIC, ANTI-INFLAMMATORY AND ANXIOLYTIC ACTIVITIES OF *SARCOCHLAMYS PULCHERRIMA* GAUDICH

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ABSTRACT

The aim of the current study was to evaluate analgesic, anti-inflammatory and anxiolytic activities of methanol extract of the leaves of *Sarcochlamys pulcherrima* Gaudich (SPME). In analgesic activity test, the crude extract revealed 54.35% protection at 200 mg/kg b.w. as compared to that 69.67% exhibited by standard diclofenac sodium. In the anti-inflammatory test, the crude extracts at the dose of 400 µg/ml showed 37.03 % inhibition of protein denaturation whereas standard acetyl salicylic acid (ASA) exhibited 76.75% inhibition. The anxiolytic activity was examined in mice by using the hole board test and open field test (OFT). The results demonstrated that the plant extract significantly increased the number of line crossing as compared to control in hole cross tests. In open field test, the extract showed significant increase in the number of square crossed. The efficacy of the extract (200-400 mg/kg b.w.) was compared with standard anxiolytic drug diazepam (1 mg/kg b.w.). It can be concluded that the methanol extract of *S. pulcherrima* has moderate analgesic, anti-inflammatory and antipyretic activities in various animal models and this strongly supports the ethno-pharmacological uses of this plant as analgesic, anti-inflammatory and anxiolytic agent. The role of the various classes of secondary metabolites such as alkaloids, flavonoids and triterpenoids present in the plant extract needs to be evaluated in future studies.

INTRODUCTION

Though considerable progress has been achieved in medical science during the last few decades, management of chronic pain still remains a challenge for medical community. All the currently available analgesic drugs such as NSAIDs have same adverse effects.¹ As a result, more people are turning to herbal medicines as the alternative treatment of pain. Plants are an important source of new chemical substances with potential therapeutic uses. Chronic inflammatory diseases is still one of the world's major health problems.² Inflammation is the response of living tissues to injury. It involves a complex array of enzyme activation, mediator release, extravasations of fluid, cell migration, tissue breakdown and repair.³ Inflammation has become the focus of global scientific research because of its implication in virtually all human and animal diseases. The conventional drugs used to ameliorate this phenomenon are either too expensive or toxic and are not commonly available to the rural folks that make up a large population.^{4,5}

Sarcochlamys pulcherrima Gaudich (Bengali name- Brihati, karabi; Family- Urticaceae) is an evergreen shrub or small tree growing from 2-6 m tall. In Bangladesh, this plant is widely distributed in Forests of Chittagong, Chittagong Hill Tracts, Sylhet and Mymensingh. As part of our continuing studies of the medicinal plants of Bangladesh^{6,7}, the present study has been designed to evaluate the analgesic, anti-inflammatory and anxiolytic activities of crude extracts of *Sarcochlamys pulcherrima* leaf to find out the logical evidence for the folk uses of this plant.

MATERIALS AND METHOD

Collection of plant materials

The leaves of *S. pulcherrima* were collected from Chittagong Hill Tracts, Bangladesh in June 2013 and were identified at Forest Research Institute; Chittagong, Bangladesh, where a voucher specimen has been maintained.

Drying and grinding

After collection, the leaves were separated from dust and then washed with running tap water. These leaves were dried in the shade and followed by hot air oven at temperature not exceeding 50 °C. The dried materials were ground into a coarse powder with the help of a grinder and stored in airtight container and in a cool and dark place until extraction was commenced.

Hot extraction by Soxhlet extractor

Exactly 140 gm of powder was extracted with 700 ml of methanol (99.98%) in 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 16.70%).

Chemicals

All the chemicals and solvents used in this study were of analytical grade and purchased from Merck, Germany. Standard drugs such as acetyl salicylic acid, diclofenac sodium, and diazepam were obtained from Square Pharmaceuticals Ltd.

Animal

Young Swiss albino mice of either sex, 6-7 weeks of age, weighing between 20-25 gm, were collected from the Animal Research Branch of the Bangladesh Council of Scientific and Industrial Research (BCSIR), Chittagong, Bangladesh. The animals were maintained under standard environmental conditions, temperature: (27.0 ± 1.0 °C), relative humidity: 55-65% and 12 h light/12 h dark cycle and had free access to feed and water *ad libitum*. The mice were acclimatized to laboratory condition for one week prior to experiments. All protocols for animal experiment were approved by the institutional animal ethical committee.⁸

Analgesic activity

The analgesic activity of crude extract was evaluated using formalin-induced writhing method in mice.⁹ Experimental animals (Swiss albino mice) were randomly selected and divided into three groups denoted as group-I, group-II, and group-III consisting of 7 mice in each group. Each group received a particular treatment *i.e.* control, standard and the two doses of the extract. Each mouse was weighed properly and the doses of the test samples and control materials were adjusted accordingly. Test samples (About 200 and 400 mg/kg b.w. of the plant extract), control and diclofenac sodium were given orally by means of a feeding needle. An interval of thirty min was given to ensure proper absorption of the administered substances. Then the writhing inducing chemical, formalin solution (5%) was administered intraperitoneally to each of the animals of a group. After an interval of 10 min, which was given for absorption of formalin, number of squirms (writhing) was counted for 5 min.

Anti-inflammatory activity

To determine the anti-inflammatory activity of the methanol extract of *S. pulcherrima*, 9 clean centrifuge tubes (three for standard acetyl salicylic acid, three for negative control methanol and three for crude extract) were used. 1.0 ml of 5% egg albumin solution was kept into all test tubes. Then 1 ml of acetyl salicylic acid (0.1 mg), 1 ml of methanol and 1ml of SPME (500 µg/ml) added to the positive and negative and test groups marked test tubes, respectively. The pH (5.6±0.2) of all the reaction mixtures was adjusted by 1N HCl. These were heated, cooled and after filtration, the absorbance was measured spectrophotometrically at 660 nm.¹⁰

Anxiolytic activity

Treatment Schedule

The anxiolytic activity was examined by using the hole board test and open field test (OFT). The animals were divided in to four groups, with each group consisting of three mice. First group receives normal saline, second group received diazepam (1 mg/kg b.w.), third and fourth groups received plant extract (200 and 400 mg/kg b.w.).

Hole Cross Test

The hole board 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 test groups received crude extract at the dose of 200 and 400 mg/kg b.w. orally whereas the control group received saline and positive control received diazepam (2 mg/kg-i.p.). 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.¹¹

Open field test

The open field test is one of the tests 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 3 mice and treated similarly as described in hole cross test. About 30 min after treatment, mice of both the control and treated groups were placed individually in the center of the open field and behavioral activities were videotaped 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).¹¹

STATISTICAL ANALYSIS

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 group. p values <0.05 were considered to be statistically significant compared with the control.

RESULTS

Analgesic activity

The pain killer dose 200 mg/kg b.w. was found to be significantly active in comparison to the standard, diclofenac sodium. Total writhing were 16 at 200 mg/kg b.w. while the standard paracetamol produced 11. SPME exhibited 54.35% protection compared to 69.67% as exhibited by standard diclofenac sodium (Table 1).

Anti-inflammatory activity

In the present study for *in-vitro* anti-inflammatory test, the crude methanol extract at 500 $\mu\text{g/ml}$ showed 37.03% inhibition of protein denaturation whereas standard acetyl salicylic acid (ASA) exhibited 76.75% (Table 2). The ability of SPME was found to be moderate in inhibiting heat-induced protein denaturation.

Anxiolytic activity

Hole cross test

The number of hole crossings were increased 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-3.

Open field test

There was significant anxiolytic activity observed with diazepam, plant extracts, SPME when compared to control. 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 control as show in table-3.

DISCUSSION

In analgesic activity test, the formalin-induced pain as an experimental model of analgesia is useful for elucidating mechanism of pain since it measures the response to a long-lasting nociceptive stimulus and, therefore, resembles clinical pain. Subcutaneous injection of dilute formalin into mice hind-paw produces biphasic nociceptive response namely: the first transient phase is caused by the direct effect of formalin on sensory C-fibers, and the second prolonged phase is associated with the development of the injury induced spinal sensitization, responsible for facilitated pain processing, a central sensitization of the dorsal horn neuron occurs during inflammatory pain.¹² Drugs that act centrally, such as the narcotics, inhibit both phases of formalin-induced pain, while peripherally acting drugs such as aspirin only inhibit the late phases.¹³ Results of the present study showed that the crude extract of *S. pulcherrima* inhibit both the early and late phases of formalin-induced pain, thus suggesting its central and peripheral anti-nociceptive actions.

Anxiety disorders are due to involvement of GABAergic, serotonergic, involvement. The adrenergic and dopaminergic system have also been shown to play a role in anxiety. BZA 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. Despite the wide spread traditional use of *S. pulcherrima* for treating various disorders there are no reports of scientific evaluation of its anxiolytic activity. The present work demonstrates that the *S. pulcherrima* extract had anxiolytic activity in mice by Open field and Hole cross models.¹⁴

Table-1: Analgesic activity of SPME 200 mg/kg in formalin induced test.

Animal group	Total Writhing	% Writhing	% Protection	T-test (value of p)
Control	29.67	100	0	-
Diclofenac sodium (25 mg/kg)	9.0	30.33	69.67	5.31 (<0.01)
SPME (200 mg/kg)	16.0	46.07	54.35	3.22 (<0.003)

Here, n = Number of animals= 07

Table-2: *In-vitro* anti-inflammatory activity of SPME and controls.

Test groups	Total inhibition of protein denaturation
Control	00.00±0.0141
Standard ASA (100 µg/ml)	76.75±0.0008 ^a
SPME (500 µg/ml)	37.03±0.0004 ^b

^ap<0.0005, ^bp<0.00001. ASA = Acetyl Salicylic Acid

Table 3: Anxiolytic effect of SPME in mice by hole cross test and open field test.

Treatment	Dose (mg/kg)	Hole cross test		Open field test	
		Number of hole crossing	% Inhibition of hole cross	Number of square crossed	% Inhibition of Square cross
Saline	1 ml	10.33±1.08	0.0	88.33±2.94	0.0
Diazepam	1	3.00±0.71	70.96	36.00±2.54	59.24
SPME	200	5.67±0.41	45.16	53.00±1.42	40.00
SPME	400	4.67±0.41	54.84	44.67±1.78	49.43

All values are mean ±SEM (n=7);

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