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Research Article

ANTINOCICEPTIVE ACTIVITY OF THE ETHANOLIC EXTRACT OF *JASMINUM SCANDENS* VAHL.

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ABSTRACT
In hot plate test and acetic acid induced writhing test methods the ethanolic extract of *Jasminum scandens* Vahl. (JS) produced significant antinociceptive effect in mice. A sub-effective dose of JS extract also potentiated the analgesic activity of sub-effective doses of both morphine and aspirin. While trying to explore the mechanism of antinociceptive action of JS, it was observed that naloxone did not antagonize this effect in albino mice in hot plate test. This indicates that an opioid mechanism may not be responsible for the central analgesic activity of JS and therefore some other mediators might be involved for its central analgesic activity. In comparison to both of the standard drugs morphine and aspirin, more than ten times doses of JS extract were required to produce significant antinociceptive activity. The observations suggest that JS possesses both central and peripheral analgesic activity. It may be useful in relieving both the visceral and integumental pain.
KEY WORDS: Analgesic, Antinociceptive, *Jasminum scandens* Vahl.

INTRODUCTION

Jasminum scandens vahl. (Family: Oleaceae) is a scandent shrub with drooping branches. The leaves are opposite, 4-9 cm long, ovate to lanceolate, acuminate, entire and base rounded. The Cymes of the plant are subcapitate, pubescent, dense and often on short axillary branches. The Flowers are white, fragrant and fruits are about 8 mm long and ellipsoid. In Bangladesh the plant is often found in the forests of Dhaka, Sylhet, Chittagong and Chittagong Hill Tracts. The Chakma people named it as 'Muichchaludi' and the Murong community as 'Kao Rong'. In Khagrachari of Chittagong Hill Tracts different parts of the plant are used traditionally for treatment of various ailments. The roots are useful in the treatment of ringworm and leaves are used in eye infection[1]. In Narail (Bangladesh) the leaves of it are traditionally used as poultice in muscle pain.

MATERIALS AND METHODS

Animals

Adult Swiss Albino mice (18–25 g) of either sex were procured from International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) and were housed in the animal house of Pharmacy Discipline, Khulna University, Bangladesh, with 12:12 h light:dark cycles. Standard pellets obtained from ICDDR,B were used as a basal diet during the experimental period. Animal studies were conducted following a protocol suggested by the scientist of Pharmacology Laboratory, Bangladesh Council of Scientific and Industrial Research (BCSIR), Chittagong.

Plant materials

The aerial parts of the plant were collected in July from Narail, Bangladesh. The taxonomical identification of the plant was done by the taxonomist and botanist of the Forest Research Institute Herbarium (FRIH), Chittagong, Bangladesh. The specimen preserved in the Pharmacognosy laboratory of the Department of Pharmacy, Southern University Bangladesh as a reference.

Preparation of extract

The dried plant material was made into fine powder (800 g). The powder was extracted by both maceration and hot percolation methods[2]. At first the powder material was macerated for 24 h in 70% v/v ethanol. Then it was subjected to percolation by using 70% v/v ethanol as solvent. The both extracts were filtered and the filtrates were combined and the plant residue was brought to dryness using rotary evaporator under 1 atmospheric pressure at 40°C. Yield value was calculated by using the equation: % yield = $(W_e/W_p) \times 100$; where, W_e = Weight of dried extract and W_p = Weight of dried powder material. Percent yield was found to be 19.5%.

Chemicals and Drugs

Solvent

Ethanol (≥99.5%), reagent grade for analysis were used as solvent in hot percolation of the plant material.

Test sample

The ethanolic stem extract of *Jasminum scandens* (JS) in the form of suspension was fed orally in a volume of 10 ml/kg/wt, in doses of 100, 250, and 500 mg/kg of body weight in both the experimental models. Where as, to study the potentiation of analgesic activity of standard drugs, it was used in the dose of 5 mg/kg/wt, p.o. For naloxone antagonism, JS in the dose (500 mg/kg, p.o.), which produced maximum analgesic action was used. Dose selection of test drug was based on previously studied by preliminary trial in our laboratory.

Acetic acid

Glacial Acetic acid 100% extra pure was used for induction of pain in writhing test on mice and diluted with distilled water before administration.

Morphine sulphate

Morphine sulphate was dissolved in distilled water and used in the doses of 40 mg/kg, s.c as standard drug and 4 mg/kg, s.c to study its potentiation effect by JS extract.

Aspirin (acetylsalicylic acid)

Acetylsalicylic acid was used as a 2% aqueous suspension in gum acacia in the doses of 50 mg/kg orally as standard drug and 25 mg/kg, orally to study its potentiation by JS.

Naloxone hydrochloride

It was used in the dose of 1 mg/kg, s.c.

Experimental Design

Two methods – hot plate test and acetic acid induced writhing test methods were employed to evaluate the analgesic activity.

For antinociceptive activity

The animals were divided into five groups in both the experimental models with each group consisting of five animals[2]. Group I received distilled water and served as control. Groups II, III and IV were administered three graded doses of JS extract (100, 250 and 500 mg/kg, p.o) in hot plate as well as in acetic acid induced writhing method. Where as, group V received morphine sulphate in hot plate test and aspirin in acetic acid induced writhing method as standard drugs for comparison respectively (Table 1 and 4).

For naloxone antagonism

The animals were divided into 6 groups of five animals each[2]. Pretreatment drugs were given 30 minutes before inducing pain by hot plate. Groups I, II, III and IV received distilled water, morphine sulphate (40 mg/kg, sc), JS (500 mg/kg, p.o) and naloxone HCl (1 mg/kg, sc) respectively. In group V, naloxone HCl (1 mg/kg, sc) and l (40 mg/kg, sc) were given and group VI was treated with naloxone HCl (1 mg/kg, sc) and JS in the dose of (500 mg/kg, p.o). In group V and VI naloxone HCl was given 30 minutes prior to morphine sulphate or test drug (Table 3).

Potentiation of antinociceptive activity of morphine sulphate and aspirin by JS extract

The animals were divided into four groups to investigate potentiation of antinociceptive activity of morphine sulphate as well as aspirin by JS extract, with each group consisting of five animals[2]. Group–I received distilled water and served as control. Group–II received sub-effective doses of morphine sulphate (4 mg/kg, s.c) in hot plate test and aspirin (25 mg/kg, p.o) in acetic acid induced writhing method. Group–Ill received JS extract 5 mg/kg, p.o, and Group–IV was given combination of morphine sulphate and JS extract in hot plate test and similarly aspirin and JS extract in above doses in acetic acid induced writhing model (Table 2 and 5).

Hot plate test

Aluminum hot plate was used to screen the antinociceptive activity of JS[3]. The mice were subjected to a preliminary screening and those showing variation of more than 2 second between two reaction times at 15 minutes interval or more than three seconds from group mean were discarded. The mice were kept on hot plate and the time when animal withdraws the paws (reaction time) was noted. The increase in reaction time in drug treated group in comparison to control indicates the antinociceptive activity. Thirty seconds was taken as cut off point for recording hot plate response as exposure more than thirty seconds caused tissue damage. The reaction time was noted before and 30, 60, 90 and 120 minutes after drug administration. A statistical evaluation of the results of analgesic activity is given in table 1, 2 & 3.

Acetic acid-induced writhing test

The writhing was induced by administration of 1 ml/100 g of 0.6% acetic acid, i.p. It consisted of a wave of constriction and elongation of abdominal musculature followed by extension of hind limbs. The animals were pretreated with drugs 45 minutes before induction of writhing. The animals were observed for onset and number of writhing within a span of 20 minutes. The animals showing a positive response within the period of 20 minutes on preliminary screening were included in the study. The abolition or inhibition of writhing response in number and frequency were considered to be the criteria for analgesic activity[3]. A statistical evaluation of the results of analgesic activity is given in table 4, 5 & 6.

Study of interaction of (JS) extract and naloxone HCl

Study of interaction of (JS) extract and naloxone HCl was performed following the methods described by Gupta and Tandon[2]. This experiment was done to explore the possible mechanism of central analgesic activity of JS.

Statistical analysis

The results of the analgesic test were subjected to Student's *t*-test for comparison of test groups with the control group. Values with $P < 0.5$ were considered significant.

RESULTS

Hot plate method

Oral administration of JS extract showed a significant analgesic activity (P<0.05, 0.01, 0.001) in a dose dependant manner. The analgesia began at 30 minutes, remained for 2 hrs and the peak effect was noted at 1 hr in comparison to control. The maximum analgesic response was observed in 500 mg/kg dose. Morphine (40 mg/kg, s.c.) showed significant (P<0.001) antinociception. In comparison to morphine, more than ten times dose of JS extract was required to produce comparable significant antinociceptive activity (Table 1).

Potentiation of morphine analgesia by JS

The sub-effective doses of JS (5 mg/kg, po) and morphine HCl (4 mg/kg, sc) did not show any analgesic effect. However, their co-administration produced a statistically significant (P<0.01) analgesia with a peak activity at 90 minutes (Table 2).

Interaction of JS and naloxone

Pretreatment with naloxone (1 mg/kg, s.c.) reversed the morphine analgesia significantly (P<0.5, <0.0005, <0.0001,) but failed to do so for the JS extract 500 mg/kg, p.o (Table 3).

Acetic acid induced writhing method

In the dose of 500 mg/kg, JS delayed the onset (P<0.05) and decreased the number of writhing in 20 minutes (P<0.0001). In the doses of 100 and 250 mg/kg, though delayed the onset of writhing, it was not statistically significant, however, it decreased the number of writhing significantly (P<0.0001, and P<0.0001) respectively. The maximum effect was seen in the dose of 500 mg/kg, p.o. Aspirin (50 mg/kg, p.o) showed significant decrease in number of writhing as well as induction time in comparison to control (Table 4).

Potentiation of aspirin analgesia by CB

Aspirin (25 mg/kg, p.o) and JS extract (5 mg/kg, p.o) did not exhibit analgesic activity whereas simultaneous administration of both the drugs in above doses showed a significant (P<0.0001) increase in onset of as well as decrease in the number of writhing which indicates potentiation of aspirin analgesia by JS (Table 5).

DISCUSSION

In both the experimental models - hot plate test and acetic acid induced writhing test methods the ethanolic extract of *Jasminum scandens* produced a significant antinociceptive effect in mice. A sub-effective dose of JS extract also potentiated the analgesic activity of sub-effective doses of both morphine and aspirin. These findings therefore are indicative that, it can lower the requirement of morphine and aspirin. Regular dosages of morphine sulfate can cause drop in blood pressure and other common side effects of the drug includes sedation, mental clouding, lethargy, constipation, nausea, vomiting, respiratory distress and blurred vision[4] and the plant parts may avoid dose related untoward effects of these drugs if used as an adjuvant therapy reducing the dose of morphine.

Aspirin also produce some side effects at regular dosages as well. The analgesic doses are associated with nausea, vomiting, epigastric distress, increased occult blood loss in stool, gastric mucosal damage and peptic ulcer. Antiinfalmmatory doses can produce salicylism which requires dose titration to one which is just below that prodcuing these symptoms[4].The plant can be used to reduce the side effects of aspirin as an adjuvant.

In this study, while trying to explore the mechanism of antinociceptive action of JS, it was observed that naloxone did not antagonize this effect in albino mice in hot plate test. Since naloxone is opioid receptors antagonist[5]. This indicates that an opioid mechanism may not be responsible for the central analgesic activity of JS and therefore some other mediators might be involved for its central analgesic activity, which still remain to be elucidated. In comparison to both of the standard drugs morphine and aspirin, more than ten times doses of JS extract were required to produce significant antinociceptive activity.

CONCLUSION

The above observations suggest that *Jasminum scandens* vahl. possesses both central and peripheral analgesic activity. It may be useful in relieving both the visceral and integumental pain. The central antinociception action appears not to be mediated by opioid receptor mechanism. The potentiation of analgesic action of morphine and aspirin by JS is indicative of its possible use as an adjuvant therapy.

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Table 1: Antinociceptive effect of *Jasminum scandens* extract in mine (hot plate method)

Group no. (n = 5)	Drug, dose and route	Reaction time (sec) (mean ± SEM)				
		Time after drug administration				
		0 min	30 min	60 min	90 min	120 min
I	D.W. (10 ml/kg, p.o.)	7.3±0.33	7.2±0.21	7.5±0.22	7.6±0.33	7.3±0.21
II	JSE (100 mg/kg, p.o.)	7.0±0.25^{I}	9.6±1.33^{F}	11.1±1.45^{G}	11.6±1.56^{H}	9.8±1.47^{I}
III	JSE (250 mg/kg, p.o.)	7.1±0.40^{I}	12.3±1.63^{D}	18.6±2.42^{B}	18.5±2.18^{A}	16.0±1.77^{B}
IV	JSE (500 mg/kg, p.o.)	7.1±0.54^{I}	13.6±1.89^{D}	21.5±0.88^{A}	19.6±0.40^{A}	19.0±1.56^{A}
V	Morphine (40 mg/kg, s.c.)	6.8±0.21^{H}	32.0±0.20^{A}	30.0±0.20^{A}	28.0±0.42^{A}	27.0±0.60^{A}

$^{A}P<0.0001$, $^{B}P<0.0005$, $^{C}P<0.001$, $^{D}P<0.005$, $^{E}P<0.01$, $^{F}P<0.05$, $^{G}P<0.025$, $^{H}P<0.1$,$^{I}P<0.5$

Table 2: Potentiation of morphine analgesia in mice by *Jasminum scandens* extract (hot plate method)

Group no. (n = 5)	Drug, dose and route	Reaction time (sec) (mean ± SEM)				
		Time after drug administration				
		0 min	30 min	60 min	90 min	120 min
I	D.W. (10 ml/kg, p.o.)	7.3±0.33	7.2±0.21	7.5±0.22	7.6±0.33	7.3±0.21
II	Morphine (4 mg/kg, s.c)	7.2±0.25^{I}	9.3±0.21^{A}	9.1±0.36^{C}	9.3±0.33^{E}	8.5±0.34^{F}
III	JSE (5 mg/kg, p.o.)	7.1±0.42^{I}	7.8±0.21^{F}	8.0±0.36^{G}	7.3±0.34^{I}	7.2±0.34^{I}
IV	Morphine (4 mg/kg, s.c) & JSE (5 mg/kg, p.o.)	7.2±0.33^{I}	15.8±1.5^{A}	17.3±1.5^{A}	16.6±1.5^{A}	10.6±0.36^{A}

$^{A}P<0.0001$, $^{B}P<0.0005$, $^{C}P<0.001$, $^{D}P<0.005$, $^{E}P<0.01$, $^{F}P<0.05$, $^{G}P<0.025$, $^{H}P<0.1$, $^{I}P<0.5$

Table 3: Interaction of *Jasminum scandens* extract and naloxone in mice (hot plate method)

Group no. (n = 5)	Drug, dose and route	Reaction time (sec) (mean ± SEM)				
		Time after drug administration				
		0 min	30 min	60 min	90 min	120 min
I	D.W. (10 ml/kg, p.o.)	7.3±0.33	7.2±0.21	7.5±0.22	7.6±0.33	7.3±0.21
II	Morphine (40 mg/kg, s.c)	6.8±0.21^{H}	32.3±0.21^{A}	30.2±0.21^{A}	28.5±0.42^{A}	27.6±0.60^{A}
III	JSE (500 mg/kg, p.o.)	7.1±0.54^{I}	13.6±1.89^{D}	21.5±0.88^{A}	19.6±0.40^{A}	19.0±1.56^{A}
IV	Naloxone (1 mg/kg, s.c)	7.16±0.44^{I}	6.83±0.47^{I}	6.66±0.42^{D}	6.83±0.30^{H}	6.83±0.56^{I}
V	Naloxone (1 mg/kg, s.c) + Morphine (40 mg/kg, s.c)	7.6±0.21^{I}	11.2±0.21^{A}	10.5±0.21^{A}	9.7±0.38^{B}	7.9±0.21^{I}
VI	Naloxone (1 mg/kg, s.c) + CBE (500 mg/kg, p.o.)	7.1±0.70^{I}	17.3±1.75^{A}	18.8±0.42^{A}	17.6±0.91^{A}	14.6±1.45^{A}

^{A}P<0.0001, ^{B}P<0.0005, ^{C}P<0.001, ^{D}P<0.005, ^{E}P<0.01, ^{F}P<0.05, ^{G}P<0.025, ^{H}P<0.1, ^{I}P<0.5

Table 4: Antinociceptive effect of *Jasminum scandens* extract in mice (acetic acid method)

Group no. (n = 5)	Drug, dose and route	Writhing response (mean ± SEM)	
		Onset of writhing (minutes)	Number of writhing (in 20 minutes)
I	D.W. (10 ml/kg, p.o.)	4.5±0.22	38.3±1.37^{A}
II	JSE (100 mg/kg, p.o)	4.6±0.33^{I}	25.3±2.10^{A}
III	JSE (250 mg/kg, p.o.)	4.9±0.45^{G}	16.6±0.79^{A}
IV	JSE (500 mg/kg, p.o)	5.1±0.78^{G}	12.5±0.51^{A}
V	Aspirin (50 mg/kg, p.o)	8.3±1.00^{D}	7.6±0.27^{A}

^{A}P<0.0001, ^{B}P<0.0005, ^{C}P<0.001, ^{D}P<0.005, ^{E}P<0.01, ^{F}P<0.05, ^{G}P<0.025, ^{H}P<0.1, ^{I}P<0.5

Table 5: Potentiation of analgesic action of aspirin in mice by *Jasminum scandens* extract (acetic acid writhing method)

Group no. (n = 5)	Drug, dose and route	Writhing response (mean ± SEM)	
		Onset of writhing (minutes)	Number of writhing (in 20 minutes)
I	D.W. (10 ml/kg, p.o.)	4.5±0.22	38.3±1.37
II	Aspirin (25 mg/kg, p.o)	4.8±0.15^{G}	31.3±1.33^{D}
III	JSE (5 mg/kg, p.o.)	4.6±0.23^{I}	33.5±1.23^{C}
IV	Aspirin (25 mg/kg, p.o) & JSE (5 mg/kg, p.o)	6.1±0.37^{A}	21.3±2.41^{A}

^{A}P<0.0001, ^{B}P<0.0005, ^{C}P<0.001, ^{D}P<0.005, ^{E}P<0.01, ^{F}P<0.05, ^{G}P<0.025, ^{H}P<0.1, ^{I}P<0.5

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