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Evaluation of antinociceptive activity of methanolic extract of leaves of *Stephania japonica* Linn

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ABSTRACT

Ethnapharmacological relevance: Stephania japonica is a common plant, widely distributed in all over Bangladesh. Traditionally, this plant is considered as one of the important ingredients in treatment of a variety of ailments including inflammation, pain, rheumatism, cancer, bone fracture, fever etc. However, the scientific reports regarding the antinociceptive effect of this plant are very limited. This study evaluated the antinociceptive effect of methanolic extract of *S. japonica* (MESJ) leaves.

Materials and methods: The antinociceptive effect of MESJ was investigated using both heat- and chemical-induced nociceptive models such as hot plate, tail immersion, acetic acid-induced writhing, formalin and glutamate tests at the doses of 50, 100 and 200 mg/kg. Morphine (5 mg/kg) and diclofenac sodium (10 mg/kg) were used as reference drugs in thermal and chemical models, respectively. Moreover, naloxone (2 mg/kg) was used in the thermal models to justify the possible role of the opioid receptors.

Results: MESJ produced a significant and dose-dependent increase in the hot plate and tail immersion latencies which were reversed by the treatment with naloxone, suggests the possible involvement of opioid receptors in this activity. Moreover, MESJ inhibited acetic acid-induced writhing, formalin and glutamate-induced lickings in a dose-dependent manner. In parallel, the reference drugs also produced desired antinociceptive effects in this study.

Conclusion: These results strongly support the antinociceptive activity of the leaves of *Stephania japonica* and rationalize the traditional use of the leaves in treatment of different painful conditions.

1. Introduction

The plant *Stephania japonica* Linn. belongs to the family Menisperaceae, a slender wiry climber or twining shrub (Senthamarai et al., 2012), is widely used in the traditional medicine of Bangladesh in treatment of a wide range of diseases and disorders including inflammation, cancer, asthma, fever, sleep disturbance, edema, and bone fracture (Kirtikar and Basu, 1981; Jahan et al., 2010). Especially its leaves, which are extensively used to treat different kinds of painful conditions, more specifically, the crushed leaves for body pain (Seraj et al., 2013; Jahan et al., 2010) and warmed leaves for rheumatism (Rahman et al., 2007). In 1982, Matsui and his group have isolated two hasubanan type alkaloid oxostephamiersine (284 mg) and 16-oxoprometaphanine (238.5 mg) and one bisbenzylisoquinoline type alkaloid stebisimine (192.5 mg) from the methanolic extract *S. japonica* leaves (Matsui et al., 1982). Recently, more eight hasubanan alkaloids have been detected in the aerial parts showing affinity to the human δ-and μ-opioid receptors. The IC_{50} values were found ranging from 0.7 to 46 μM (Carroll et al., 2010). Moreover, both ethanolic and methanolic extracts from the leaves have also been found to exhibit potent antioxidant activity *in vitro* and inhibit acetic acid-induced writhing in mice (Rahman et al., 2011; Uddin et al., 2014).

However, these limited number of scientific reports revealing its action against different painful conditions influenced us to design the present study. Here we tried to investigate the central antinociceptive effect of MESJ using two heat-induced nociceptive models such as hot plate and tail immersion tests. Although it is well established that the centrally acting analgesics can also be effective in peripheral nociceptions, we applied MESJ in several chemical-induced models like acetic acid-induced writhing, formalin- and glutamate-induced licking tests in mice to evaluate the peripheral action of this plant leaves. In addition, we also tried to understand the possible molecular mechanism(s) underlying by

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antagonizing the effect in heat-induced models with naloxone.

2. Materials and methods

2.1. Plant material and extraction

The leaves of *S. japonica* were collected from Comilla, Bangladesh, in March 2013. The collected samples were then identified with a voucher number of DACB: 42,020, by Busra Khan, Principle Scientific Officer, Bangladesh National Herbarium. 250 g powdered leaves were then macerated with methanol and 14.76 g (Yield 5.90%) extract was obtained from this process. Extraction details are explained in Supplementary materials.

2.2. Phytochemical screening

MESJ was qualitatively analyzed to detect the phytochemicals according to Ghani (2003) as described in Supplementary materials.

2.3. Drugs and chemicals

The following drugs and chemicals were used in this study: morphine sulphate (Gonoshasthaya Pharmaceuticals Ltd., Savar, Bangladesh), naloxone (Hameln Pharmaceuticals GmbH, Hameln, Germany), diclofenac sodium (Square Pharmaceuticals Ltd., Dhaka, Bangladesh), methanol, 99% dimethylsulfoxide (DMSO), L-glutamic acid, acetic acid, and formalin (Merck, Darmstadt, Germany).

2.4. Animals

Male Swiss Albino mice of 20–25 g body weight were used in this study. Animal handling details are described in Supplementary materials. All experimental animals were treated following the Ethical Principles and Guidelines for Scientific Experiments on Animals (1995) formulated by The Swiss Academy of Medical Sciences and the Swiss Academy of Sciences. All experimental protocols conducted in this study were approved by the Institutional Ethics Committee of Stamford University Bangladesh (SUB/IAEC/14.05).

2.5. Drugs and treatments

Morphine sulphate (5 mg/kg) was employed in hot plate and tail immersion tests and diclofenac sodium (10 mg/kg) was used in writhing and licking tests. These drugs were administered intraperitoneally (i.p.) 15 min before the induction of nociception. In both chemical- and heat-induced pain models, DMSO (vehicle, 0.1 ml/mouse) and MESJ (50, 100, and 200 mg/kg) were administered orally 30 min prior the nociceptive stimuli.

2.6. Acute toxicity test

This test was performed according to a previously established method (Moniruzzaman et al., 2015) as described in Supplementary materials.

2.7. Antinociceptive activity analyses

The antinociceptive activity of MESJ was evaluated using hot plate, tail immersion, acetic acid-induced writhing, formalin, and glutamate tests according to the standard protocols as described in the Supplementary materials.

2.8. Involvement of opioid receptors

The possible involvement of opioid receptors was investigated according to the method described in Supplementary materials.

2.9. Statistical analysis

The results are expressed as Mean ± SEM. The statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's and Bonferroni's post hoc tests using SPSS software.

3. Results and discussion

The present study evaluated the antinociceptive effect of methanolic extract of *S. japonica* (MESJ) leaves in different models of acute nociception. The results obtained from this study demonstrated that MESJ can modulate the nociceptive responses induced by heat as well as acetic acid, formalin, and glutamate. It has also been found that MESJ did not produce any allergic reaction or mortality of the animals within 72 h of observation period. This implies that the extract is not toxic with the experimental doses tested up to 3000 mg/kg. Moreover, some classes of the secondary metabolites such as alkaloids, tannins, glycosides, flavonoids, saponins and carbohydrates were detected in the phytochemical screening of MESJ. Furthermore, the Liebermann's test for alkaloids revealed that either atropine, hyoscyamine, hyoscine, or/and ephedrine is/are present in MESJ (Suppl Table 1). There are several reports demonstrated that atropine, hyoscyamine, and hyoscine possess antinociceptive property, where ephedrine has been reported to enhance opioid-mediated antinociception (Ghelardini et al., 1990; Tekol et al., 1994; del Valle-Laisequilla et al., 2012). Taken together, it is possible that the above-mentioned groups of phytochemicals in MESJ might contribute at least in a part in the observed antinociceptive activities.

The hot plate and tail immersions tests are the well established methods in pain research act through supraspinal and spinal pathway, respectively (Arslan and Bektas, 2010). In these tests, the oral administration of MESJ at 50, 100 and 200 mg/kg doses caused a dose-dependent increase in the reaction latencies of the animals. The effect is significant ($p < 0.01$) with the doses of 100 and 200 mg/kg 60 min after the extract administration. The results also revealed that naloxone was able to significantly ($p < 0.05$) reverse the observed antinociceptive activity, suggesting the probable role of the opioid mechanisms in MESJ-mediated action (Tables 1 and Suppl Table 2). These findings were partially supported by the work published by Carroll et al. (2010). Therefore, it is clear that MESJ possesses central antinociceptive activity, which could be mediated through the spinal and supraspinal mechanisms involving opioid receptors.

The acetic acid is well known to induce peritoneal resident cells causing lipid peroxidation and liberation of several endogenous mediators, which induce noxious stimuli are characterized as writhing (Koster et al., 1959). The results of the present study revealed that pretreatment with MESJ as well as diclofenac sodium were able to inhibit number of abdominal writhes, significantly ($p < 0.01$) in a dose-dependent manner (Table 2). The percentage of writhing inhibition produced by MESJ was calculated as 35.59%, 55.08% and 74.79% for 50, 100 and 200 mg/kg doses, respectively. This outcome also can be supported by Rahman et al. (2011) who demonstrated the antioxidant and writhing inhibitory effects of MESJ. Moreover, the antioxidants are generally known to relieve pain through the prevention of lipid peroxidation and prostaglandin synthesis (Islam et al., 2014). Therefore, our observations and previous report suggest that MESJ is endowed with writhing