Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Original article doi: 10.12980/jclm.3.2015j5-60

Antidepressant, anxiolytic and anti-nociceptive activities of ethanol extract of *Steudnera colocasiifolia* K. Koch leaves in mice model

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ARTICLE INFO

Article history:
Received 19 May 2015
Received in revised form 3 Jul, 2nd revised form 24 Jul, 3rd revised form 6 Sep 2015
Accepted 18 Oct 2015
Available online 9 Nov 2015

Keywords:
Steudnera colocasiifolia K. Koch
Antinociceptive
Antidepressant
Anxiolytic
Hole board

ABSTRACT

Objective: To estimate the antidepressant, anxiolytic and antinociceptive activities of ethanol extract of *Steudnera colocasiifolia* K. Koch (*S. colocasiifolia*) leaves.
Methods: Swiss albino mice treated with 1% Tween solution, standard drugs and ethanol extract of *S. colocasiifolia,* respectively, were subjected to the neurological and antinociceptive investigations. The tail suspension test and forced swimming test were used for testing antidepressant activity, where the parameter is the measurement of immobility time. Anxiolytic activity was evaluated by hole board model. Anti-nociceptive potential of the extract was also screened for centrally acting analgesic activity by using formalin induced licking response model and acetic acid induced writhing test was used for testing peripheral analgesic action.
Results: Ethanol extract of *S. colocasiifolia* significantly decreased the period of immobility in both tested models (tail suspension and forced swimming models) of antidepressant activity. In the hole board model, there was a dose dependant (at 100 and 200 mg/kg) and a significant increase in the number of head dipping by comparing with control (1% Tween solution) ($P < 0.05$ and $P < 0.001$). In formalin induced licking model, a significant inhibition of pain compared to standard diclofenac sodium was observed ($P < 0.05$ and $P < 0.001$). In acetic acid induced test, there was a significant reduction of writhing response and pain in mice treated with leaves extract of *S. colocasiifolia* at 200 mg/kg body weight ($P < 0.05$ and $P < 0.001$).
Conclusions: The results proofed the prospective antidepressant, anxiolytic and antinociceptive activities of ethanol extract of *S. colocasiifolia* leaves.

1. Introduction

Depression is one of the main mental health problems of people all over the world, and it is connected with many disabilities[1,2]. It is a persistent illness that changes thoughts, mood and behavior of any person and has been expected to affect up to 21% population of the earth[3]. Synthetic drugs taken as antidepressant in proper dosages are regularly connected with their anticipated reactions like powerlessness in driving abilities, dry mouth, sexual brokenness and blockage and most of patients are hesitant to take this type of treatment[4]. Therefore, natural plants may be potential sources of novel antidepressant drugs and the usage of plant extracts and their phytoconstituents may act as an improved means in the management of depression and anxiety. In many nations, many medicinal plants from natural resources, especially Chinese medicine, such as *Plantago asiatica* and *Hypercarium perforatum* were successfully used to treat depression[5,6].

Anxiety-related disorders such as patient's nervousness, obsessive-compulsive disorder and post-traumatic stress are the foremost causes of infirmity in the world[7]. Currently, the most common approved medicines for anxiety disorders are benzodiazepines. However, the medical uses of benzodiazepines are limited by their side effects such as psychomotor destruction, potentiating activity of other sedatives and reliance liability[8].

The majority of antinociceptive (analgesic) drugs for example, cyclooxygenase (COX)-2 inhibitors and opioids show a broad scope of unfavorable impacts including kidney difficulty, gastrointestinal disorders and other redundant effects. Drug regulatory authorities are forcing to attach a boxed warning on the label of some COX-2 selective inhibitors for many risks like gastrointestinal and cardiovascular risks[9]. As well, the dependence and misuse of opioids are a going up issue. Painkiller choice is determined by the type of pain, for example, conventional analgesics are low effective on the treatment of neuropathic pain[10]. Therefore, the advent of safe and successful

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Foundation Project: Supported by a research grant from Managing Committee of the Department of Pharmacy, International Islamic University Chittagong, Bangladesh (Grant No. Pharm-P&D 71/09-15/54).

analgesic drugs is still demanding for the efforts of drug developer.

Steudnera colocasiifolia K. Koch (Araceae) (*S. colocasiifolia*) is a kind of evergreen herb with short stem and green petiole. Its petiole is slender and cylindric with length ranged from 30–50 cm and the leaves of *S. colocasiifolia* are paler with sharp edge[11]. It commonly grows in dense forests, wet meadows, by streams and in seasonally moist lowland forest. It distributed in Bangladesh, India, Myanmar, Thailand and China. Locally it is used to treat injuries, cuts, snake and insect bites and skin ulcers. The whole plant extract of *S. colocasiifolia* has anti-arthritic and anti-inflammatory activities[12].

There are many species of plants that have the medical values, which are not investigated yet. The aim of this study was to examine antidepressant, anxiolytic and anti-nociceptive activities of *S. colocasiifolia* leaves extract by using different test methods on mice. There are many studies about this plant. To our knowledge, no earlier studies have been conducted experimentally to characterize the antidepressant, anxiolytic and analgesic effects of *S. colocasiifolia.*

2. Materials and methods

2.1. Plant material

The fresh leaves of *S. colocasiifolia* (voucher specimen ID. No: 1316 CTGUH) were collected from Alu Tila, Khagrachari, Chittagong, Bangladesh in September 2014. It was authenticated by Pro. Shaikh Bokhtear Uddin, Department of Botany, University of Chittagong, Bangladesh.

2.2. Preparation of extract

The leaves were dehydrated for 10 days under shadow and on ground. The grounded leaves (250 mg) were saturated in a sufficient amount of ethanol for one week at room temperature with irregular shaking and stirring. Then the entire mixture was filtered and the obtained filtrate was conducted to concentrate for a sticky mass by using a rotary evaporator. The sticky mass was reserved at room temperature under a ceiling fan for drying the extract (about 7%). The leaves extract was prepared for the screening of antidepressant, anxiolytic and anti-nociceptive activities.

2.3. Experimental animals

Swiss albino mice at 25–30 g weight were collected from animal lab, Jahangir Nagar University, Savar, Bangladesh. The mice were fed with standard lab nourishment and refined water *ad libitum* and kept in room having legitimate ventilation in natural day-night cycle. All the experiments were conducted in an isolated and noiseless condition. The study protocol was approved by the P&D Committee, Department of Pharmacy, International Islamic University Chittagong, Bangladesh. The mice were acclimatized to laboratory conditions for 7 days prior to experimentation.

2.4. Chemicals and equipments

In this paper, imipramine hydrochloride and diazepam (Square Pharmaceutical, Bangladesh), diclofenac sodium (Beximco Pharmaceuticals Ltd., Dhaka, Bangladesh), formaldehyde (MERCK, Mumbai, India), acetic acid (MERCK, Mumbai, India), normal saline solution (0.9% NaCl) and Tween 80 (BDH Chemicals, UK) were used. All other reagents were of analytical grade.

2.5. Preparation of test doses

The extracts were suspended in 1% Tween 80 solution. Various concentrations were prepared from a stock solution (40 mg/mL). The solutions were freshly prepared to administer orally.

2.6. Acute toxicity study

For acute toxicity study, twenty Swiss albino female mice were used. According to the method of Walum *et al*., selected mice were divided into four groups with five mice each[13]. Different doses (1 g/kg, 2 g/kg, 3 g/kg and 4 g/kg) of ethanol extract of *S. colocasiifolia* leaves (EESC) were administered by oral feeding gavage. Then the mice were observed for common toxicity signs.

2.7. Antidepressant activity

2.7.1. Tail suspension test (TST)

TST generally used as behavioral model for evaluating antidepressant activity in mice, was established by Steru *et al*.[14]. Mice were moved from their housing colony to the laboratory in their own cages and then they were allowed to adapt to the laboratory conditions for 1–2 h. The Group I was treated as control (1% Tween 80, 10 mL/kg body weight, *p.o.*), Group II received the standard drug (imipramine, 10 mg/kg body weight, *p.o.*) and mice in Group III and IV received EESC at 100 and 200 mg/kg body weight, respectively, by oral administration. Each mouse was individually suspended to the rim of a table at a hight of 50 cm above the floor, by using adhesive tape placed on approximately 1 cm from the tip of the tail. Every mouse during the test was both acoustically and visually isolated from other mice. The whole period of immobility was recorded manually for 6 min by a stop watch. Mice were considered to be immobile when they didn't show any body movement, hung passively and totally motionless. The test was conducted in a room with weak light and each mouse was used only once in the test. The observer recording the immobility of mice, was blind to the drug managements which was given to the animals under test[15,16].

2.7.2. Forced swim test (FST)

FST first designed by Porsolt *et al*.[17] is frequently used as behavioral model for screening antidepressant-like activity in rodents. According to this method, mice were independently forced to swim in open glass compartment (25 cm × 15 cm × 25 cm) containing fresh water to a height of 15 cm and maintained at (26 ± 1) ˚C. At such height of water, mice were not able to hold up themselves by touching the base or side walls of the compartment with their hind-paws or tail. Water in the compartment was changed after subjecting every mouse to forced swimming test because "used water" had been shown to change the activities. Each mouse showed vigorous movement during initial 2 min period of the test. The duration of immobility was manually recorded throughout the next 4 min of the total 6 min testing time.

It was considered to be immobile when mouse stopped struggling and remained suspended motionless in water, making only those actions necessary to keep their head above water. Then mice were dried and returned to their housing conditions[15,16].

2.8. Anxiolytic activity

2.8.1. Hole board test

The hole board equipment consisted of a wood box (40 cm × 40 cm × 25 cm) with 16 holes (each with 3 cm in diameter) evenly dispersed on the base of box. The apparatus was elevated to the height of 25 cm. Mice (n = 6) were treated with EESC (100 and 200 mg/kg, *p.o.*), diazepam (1 mg/kg, *i.p.*) and 1% Tween solution, respectively, for 30 min before they were placed in the hole board apparatus and the numbers of head dipping during a 5 min period were recorded[18].

2.9. Anti-nociceptive activity

2.9.1. Acetic acid induced writhing test

Mice were separated into four groups of either sex containing five mice of each group. For acetic acid induced writhing test, 0.6% (v/v) acetic acid solution (10 mL/kg body weight) was injected intraperitoneally to mice and the number of mice writhing and stretching was counted over 20 min[19,20]. Group I was treated as control group receiving 1% Tween solution 10 mL/kg. Group II was treated with diclofenac sodium at 10 mg/kg as a standard, and Group III and IV were treated with EESC at 100 and 200 mg/kg, respectively, by oral administration 30 min before acetic acid injection. The decreased percentage of writhing was measured by following equation:

$$\% \text{ Decrease} = \frac{W_{control} - W_{drug/extracts}}{W_{control}} \times 100$$

where, $W_{control}$ is the number of writhing with 1% Tween solution treatment and $W_{drug/extracts}$ is the number of writhing with standard or extracts solution treatment.

2.9.2. Formalin test

A total of 20 μL of 2.5% formalin in water was injected subcutaneously to a hind paw of the mice 30 min after oral administration of the diclofenac sodium (10 mg/kg), EESC at 100 and 200 mg/kg to Groups II, III and IV, respectively. Group I taken as control received 1% Tween solution with formalin (20 μL of 2.5%) during the experiment.

The time spent in licking and biting responses of the injected paw were taken as an indicator of pain response and the data were expressed as total licking time in the early phase (0–5 min) and the late phase (15–30 min) after formalin injection[21]. Decreased percentage of licking and biting at early phase and late phase was measured by previous equation.

2.10. Statistical analysis

The data were expressed as mean ± SEM. Statistical analysis was carried out with Dunnett's test by using the statistical software SPSS 16.0 (IBM corporation, NY). The obtained results were compared with the negative control (1% Tween solution) group and $P < 0.05$ and $P < 0.001$ were considered as statistical significant.

3. Results

3.1. Acute toxicity

The extract administered up to high dose (4000 mg/kg) produced no mortality in mice. The mice did not show any sign of restlessness, respiratory distress, general irritation, coma or convulsion. Therefore, this extract was considered safe.

3.2. Antidepressant activity

3.2.1. TST

In TST, mice treated with two doses of EESC (100 mg/kg and 200 mg/kg, *p.o.*) showed significant decreases in their time of immobility, which were (132.32 ± 1.83) and (111.19 ± 2.27) s, respectively, by comparing with control (205.9 ± 1.01) s ($P < 0.001$). Similarly, mice treated with imipramine (10 mg/kg), as estimated, showed a significant decline in the time of immobility [(82.05 ± 1.23) s; $P < 0.001$], which proved that this extract had an antidepressant effect on mice (Table 1).

Table 1
Effect of EESC and imipramine on tail suspension model in mice.

Treatment	Time of immobile (s)	% Decrease
Control (1% Tween)	205.9 ± 1.01	
Imipramine hydrochloride (10 mg/kg)	82.05 ± 1.23[b]	60.15
EESC (100 mg/kg)	132.32 ± 1.83[b]	35.74
EESC (200 mg/kg)	111.19 ± 2.27[b]	45.99

[b]: $P < 0.001$ compared with control; Dunnett test as compared to negative control (1% Tween); Statistical representation of immobile time of mice by EESC and standard drug for TST processed by Dennett's test by using SPSS 16.0 for Windows.

3.2.2. FST

The potential antidepressant effect of EESC after oral administration was also evaluated in the forced swimming tests. In FST, mice treated with two doses of EESC (100 mg/kg and 200 mg/kg, *p.o.*, respectively) showed reduces in their time of immobility, which was significant [(122.2 ± 2.61) s and (98.17 ± 3.41) s respectively; $P < 0.001$] as compared with the control (194.27 ± 4.81) s. Similarly, mice treated with imipramine (10 mg/kg), as anticipated, demonstrated a significant diminish in the time of immobility [(88.66 ± 2.93) s; $P < 0.001$] (Table 2).

Table 2
Effect of EESC and imipramine on forced swim model in mice.

Treatment	Time of immobile (s)	% Decrease
Control (1% Tween)	194.27 ± 4.81	
Imipramine hydrochloride (10 mg/kg)	88.66 ± 2.93[b]	54.36
EESC (100 mg/kg)	122.2 ± 2.61[b]	37.09
EESC (200 mg/kg)	98.17 ± 3.41[b]	49.47

[b]: $P < 0.001$ compared with control; Dunnett test as compared to negative control (1% Tween); Statistical representation of immobile time of mice by EESC and standard drug for swimming test processed by Dennett's test by using SPSS 16.0.

3.3. Anxiolytic activity

The number of head dipping was increased (148.77%) significantly in case of standard drug diazepam treated mice as compared to the control mice ($P < 0.001$). The EESC at both dose levels showed an increase (45.58% and 86.10%, respectively) in the number of head dipping significantly as compared to the control mice ($P < 0.05$ and $P < 0.001$). All results are shown in Table 3. This test proved that EESC has significant anxiolytic activity. Because head dipping of mice increased with all the tested treatment.

Table 3
Effect of *S. colocasiifolia* leaves extract on head dipping of mice in hole board.

Treatment	Number of head dipping	% Increase
Control (1% Tween)	26.33 ± 0.56	
Diazepam (1 mg/kg)	65.50 ± 1.20[b]	148.77
EESC (100 mg/kg)	38.33 ± 1.70[a]	45.58
EESC (200 mg/kg)	49.00 ± 2.16[b]	86.10

[a]: $P<0.05$; [b]: $P<0.001$ compared with control. Dunnett test as compared to negative control (1% Tween); Statistical representation of number of head dipping of mice by EESC and standard drug processed by Dennett's test by using SPSS16.0.

3.4. Anti-nociceptive activity

3.4.1. Acetic acid test

Treatment with the EESC at doses of 100 mg/kg and 200 mg/kg, *p.o.* significantly decreased the number of writhing after acetic acid induction in mice. Highest anti-nociceptive activity (43.82%) was found at a dose of 200 mg/kg. Diclofenac sodium (10 mg/kg) shown 69.66% defense against acetic acid induced writhing in mice (Table 4).

Table 4
Effect of *S. colocasiifolia* leaves extract on acetic acid induced writhing response in mice.

Treatment	Number of writhing	% Inhibition
Control (1% Tween)	59.33 ± 1.84	
Diclofenac-Na (10 mg/kg)	18.00 ± 0.82[b]	69.66 ± 1.38
EESC (100 mg/kg)	44.33 ± 0.62[a]	26.97 ± 1.05
EESC (200 mg/kg)	31.67 ± 0.47[b]	43.82 ± 0.79

[a]:$P < 0.05$; [b]:$P < 0.001$ compared with control; Dunnett test as compared to negative control (1% Tween); Statistical representation of number of writhing of mice by EESC and standard drug processed by Dennett's test by using SPSS 16.0.

3.4.2. Formalin test

The outcome of EESC in formalin test is presented in Table 5. At both doses, there was dose reliant reduce of paw licking time in early phase but dose of 200 mg/kg significantly abridged latency to distress in late phase compared to the late phase of the test control ($P < 0.001$). And the standard antinociceptive drug Diclofenac sodium (10 mg/kg) significantly decreased the licking activity against both phases of formalin-induced nociception.

Table 5
Antinociceptive profile of *S. colocasiifolia* leaves extract assessed by the formalin test in mice.

Treatment	Early phase (1st 5 mins)	% inhibition	Late phase (Last 15 mins)	% Inhibition
Control (1% Tween)	57.31 ± 1.06		41.74 ± 1.46	
Diclofenac-Na (10 mg/kg)	14.95 ± 0.60[b]	73.91 ± 1.05	13.26 ± 0.94[b]	68.22 ± 2.26
EESC (100 mg/kg)	39.26 ± 1.50[a]	31.50 ± 2.62	21.54 ± 2.04[b]	48.39 ± 4.89
EESC (200 mg/kg)	30.62 ± 1.09[b]	46.58 ± 1.91	16.87 ± 0.99[b]	59.58 ± 2.36

[a]: $P < 0.05$; [b]: $P < 0.001$ compared with control; Dunnett test as compared to negative control (1% Tween); Statistical representation of paw licking time of mice by EESC and standard drug processed by Dennett's test by using SPSS 16.0.

4. Discussion

Nowadays any kind of stress results in progressive deterioration of brain functions. Abnormal functioning of brain leads to imbalance of various neurotransmitters like Gamma amino butyric acid (GABA), 5-hydroxytrptamine (serotonin) and various amino acids and their metabolite in pathophysiology of depression and anxiety states. Here two main activities of central nervous system, antidepressant and anxiolytic activities of EESC were evaluated. In the present study, the antidepressant effect of *S. colocasiifolia* was appraised by FST and TST where duration of immobility was considered as screening parameter. Antidepressants decreased this parameter. Here immobility of mice treated with extract and reference drug (imipramine hydrochloride) were compared with that in negative control (1% Tween solution) group. The result showed that extract of *S. colocasiifolia* leaves at 100 mg/kg and 200 mg/kg significantly reduced the duration of immobility time, respectively, in FST and TST ($P < 0.05$ and $P < 0.001$). In this work, anxiolytic effects were determined by the hole board test. The number of head dipping in hole board test gives a sign of exploratory trend and the increase of the number of head dipping shows an indication of anxiolytic activity. In the present study, there was an increase in the number of head dipping when treated with *S. colocasiifolia* which indicated that there was an anxiolytic activity[22]. GABA is the foremost inhibitory neurotransmitter in the central nervous system[23]. In different types of anti-anxiety drugs, sedative–hypnotic drugs shown their action through GABA[24,25]. Most of the anxiolytic agents exert their action by opening of activated GABA-chloride channel[26]. The results indicated that the *S. colocasiifolia* leaves possess similar activity to that of benzodiazepines.

The study data presented here revealed that the EESC had antinociceptive activities. The abdominal constriction induced by acetic acid is a sensitive procedure to assess peripherally acting analgesics[27]. Normally, acetic acid causes pain by therapeutic endogenous materials such as prostaglandins and histamine, which excite nerve endings[28,29]. The ethanol extract at 100 and 200 mg/kg in this study both significantly inhibited nociception in mice with a percentage of 26.97% and 43.82%, respectively, by comparing with control, while dose at 200 mg/kg showed more statistically significant ($P < 0.001$) antinociceptive activity in acetic acid pain.

The EESC was then tested against other models of experimental pain. Formalin induced inflammation and neurogenic pains[30]. Injected formalin has been reported to cause an immediate and physically powerful increase in the spontaneous activity of C-fiber afferent and evoke a distinct quantifiable behavior indicative of pain demonstrated in paw licking by the animals[31-34].

In formalin test, the nociceptive reaction had two phases. The early phase (0–5 min) and late phase (15–30 min) represented the neurogenic and inflammatory pain responses, correspondingly[27]. The control group had the highest licking time and the extract reduced it remarkably in both phases dose dependently. The percentages of pain inhibition are 31.5%, 46.58% at early phase and 48.39%, 59.58% at late phase by 100 and 200 mg/kg, respectively. The standard drug diclofenac sodium (10 mg/kg) also caused significant inhibition of pain in both phases.

The activity against nociception exerted by this extract may be attributed to the presence of secondary metabolites like saponins, flavonoids, tannins and terpenes[35]. Therefore this extract inhibits different types of pain as well as narcotic pains may in part explain the mechanisms of its action.

Our present study revealed that *S. colocasiifolia* had significant analgesic and anxiolytic effects, along with prominent decrease in neuro-pharmacological activities due to its active chemical constituents. However, further studies are required to authenticate the mechanism of action behind the effects observed in our study.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors greatly thanks the Managing Committee of the Department of Pharmacy, International Islamic University Chittagong, Bangladesh, for providing all the laboratory amenities and supporting with a research grant to accomplish the study (Grant No. Pharm-P&D 71/09-15/54). The authors are also thankful to all members of GUSTO (a research group), for their kind help in the experiment.

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