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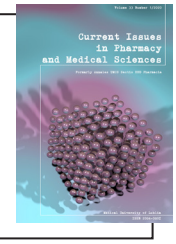

Assessment of antidepressant and sedative-hypnotic activities of methanolic crude extracts of *Stephania japonica* (Thunb.) Miers. whole plants

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ABSTRACT

In this study, methanolic crude extracts of *Stephania japonica* (Thunb.) Miers. (MESJ) whole plants were examined for possible antidepressant and sedative-hypnotic activities. Herein, the forced swimming test and tail suspension test were conducted to explore the antidepressant activity. In addition, the open field test and hole-board test were performed to evaluate the sedative-hypnotic activities. In the acute toxicity test, the MESJ ensured safety up to a dose of 2000 mg/kg, p.o. The experimental doses were 100 and 200 mg/kg p.o. In both the forced swimming test and tail suspension test, the extract significantly ($p<0.01$ and $p<0.05$) inhibited immobility time in a dose dependent manner compared to the control. These results (13.56-26.46% inhibition) indicate the mild antidepressant activity of MESJ compared to nortriptyline (60.4-64.6% inhibition). The open field test and hole-board test demonstrated the dose dependent significant ($p<0.001$, $p<0.01$ and $p<0.05$) and moderate sedative-hypnotic activities of the extract compared to diazepam. However, these activities were found to gradually decrease after 60 min in the open field test and must be considered as short-term activities, compared to diazepam. It can be claimed that the methanolic crude extract of *Stephania japonica* possesses mild antidepressant and moderate but short-term sedative-hypnotic activities.

INTRODUCTION

Depression is an alarming public health related concern in modern civilization [1]. It is predicted that by 2020, depression might be the 2nd principal reason of disease burden after heart complications [2]. Usually it may be comorbid with other chronic disease like diabetes, arthritis etc. where further deterioration of health status may occur [3-6]. Major depressive disorders may be responsible for respiratory complications, stroke, heart disorders, suicidal tendency etc. [7,8]. Neurotransmitters like norepinephrine, serotonin, γ-aminobutyric acid (GABA), dopamine, acetylcholine etc. are related to the maintenance of depression. Overactivity, deficiency in general activities or other type abnormal activities of these neurotransmitters may trigger the development of depressive disorders [9,10]. Due to unexpected adverse effects of many available pharmacotherapies and to find out better alternatives, new exploration is being carried on for successful management of depressive disorders [11].

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Health related complaints especially about insomnia and other sleep disorders are very common complaints heard by healthcare practitioners [12]. Tiredness, irritability, problems with concentration etc. are the outcomes of these conditions which make one's life very troublesome. Furthermore, the combination of nervousness, tension, indecision accompanying by physiological arousal can exaggerate sleep disturbances [13]. In this situation, inhibition of synaptic transmission is expected and usually can be done by γ-aminobutyric acid receptor type A (GABAA) agonists (e.g. benzodiazepines) [14]. Many more attempts are being taken to develop new therapeutic approaches to solve these type of health problems.

Leading researchers have been exploring traditional uses of natural plants to discover new therapeutic approaches. *Stephania japonica (*Thunb.) Miers. is a shrub with greenish yellow flowers that belongs to a large family, Menispermaceae, consisting of 65 genera & 350 species [15]. It is found in the countries of Southern Asia, Eastern Asia and Australasia [16]. Traditionally, the whole plants of *S. japonica* are used in treating skin diseases, cough, convulsions,

asthma, kidney disorders etc. [17-21]. The roots and leaves are astringent in nature and bitter in taste and are used in urinary problems, fever, dyspepsia and diarrhea [22]. The roots are also used in convulsions and stomach aches in Indonesia [23,24]. Plant preparations of *S. japonica* were also employed by the Tonchonga and Chakma tribes for urinary burning sensations in Bangladesh [25]. Many species of the genus Stephania are also found to be used in a notable number of traditional practices. *S. glabra* (Roxb.) Miers is used as antipyretic, antiasthamatic, antidysenteric and antituberculosis agent [26,27]; *S. tetrandra,* S. Moore possesses analgesic & antipyretic properties and *S. dinklagei* Diels has analgesic & sedative activities [28]. The aforementioned properties of *S. japonica* and other species of the same genus Stephania influenced the research team to explore new possible potential uses of the experimental plant.

Though herbal plants are generally considered as safe, they may contain a number of known and unknown constituents possessing toxic potential [29,30]. Hence, before treatment with a natural plant, confirmation about its toxic state is highly recommended [31].

In this study, we tried to find out whether material drawn from the whole plants of *S. japonica* had antidepressant and sedative-hypnotic activities.

MATERIALS AND METHODS

Plant collection and extraction

The whole plants of *S. japonica* were picked up from Kishoreganj, Bangladesh, in December, 2018. The samples were then identified by one of the expert taxonomists of Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh and a voucher (DACB accession number: 46793) was deposited there for further reference. Powdered dried plants (about 250 g) were macerated in sufficient amount of methanol with occasional stirring at 25±2°C for 15 days. A Buchner funnel and a cotton filter were then used to filter the solution. The solvent was completely removed by rotary evaporator at 50°C temperature and reduced pressure. Finally, the expected methanolic extract of *S. japonica* was obtained.

Animals

About 25-30g of Swiss albino male mice were picked up from Animal Resources Branch of the International Center for Diarrhoeal Disease Research, Bangladesh (icddr, b). The mice were constrained in optimum laboratory environment (relative humidity 55-60%; room temperature 25±2°C; 12 h light/dark cycle) and were provided with standard diet (icddr,b formulated) and clean water *ad libitum* in adaptation time. The animals were conformed to the standard ambience for 14 days before performing the tests. The mice were kept starved overnight prior to the tests. All experiments were carried out according to the National Institute of Health Guidelines for the care and use of laboratory animals and the European Council Directive on 24 November 1986 for Care and Use of Laboratory Animals (86/609/EEC), and approved by the Local Ethics Committee.

Chemicals

Diazepam, nortriptyline, methanol, tween-80, and normal saline (0.9% NaCl) were the principle chemicals in this experiment. Diazepam and nortriptyline were purchased from Incepta Pharmaceuticals Ltd and used as the standard in sedative-hypnotic and antidepressant activity tests, respectively.

Acute toxicity test

To determine the median lethal dose (LD_{50}) of the methanolic crude extract of *S. japonica* (MESJ), the method established by Litchfield and Wilcoxon was followed [32]. Four groups of mice, each containing five individuals, were used – three MESJ groups and a control group. Group-I was treated with normal saline, p.o. and the remaining three groups received preparations of MESJ at doses of 500, 1000, and 2000 mg/kg body weight, p.o. respectively. Subsequently, routine observation was undertaken up to seven days to report any abnormalities (abnormal weight change, hypersensitivity or death) in comparison to the control group.

Forced swimming test

In the forced swimming test, the antidepressant potential of MESJ was determined. This test was carried out by following the method reported by Porsolt *et al.* [33]. The mice were forced to swim by putting them in a cylinder (35 cm high, 20 cm diameter) made up of Plexiglas that contained around 25 cm water. There were four groups, each of which consisted of five mice – a control (CTL), a standard (STD) and two MESJ groups. The CTL and STD group were treated with only normal saline (0.1 ml/mice, p.o.) and nortriptyline (1 mg/kg, i.p.), respectively. The MESJ groups were treated with freshly prepared plant extract solutions at two different doses (100 and 200 mg/kg, p.o.), and 45 min after the administration of the test solutions, the swimming test was conducted for six min, and the duration (in second) of immobility during the last five min was noted.

Tail suspension test

Whether MESJ possessed antidepressant activity was the objective of this test. Here, the method of Steru *et al.* was the basis of the experimental procedure [34]. Like the forced swimming test, there were four groups of mice. The CTL and STD group received normal saline (0.1 ml/mice, p.o.) and nortriptyline (1 mg/kg, i.p.), respectively. The MESJ groups received two different doses of the extract (100 & 200 mg/kg, p.o.). After a period of 45 min, the experimental mice were kept hanging about 30 cm above the floor with adhesive tape positioned approximately 1-2 cm from the tip of the tail. The duration (in second) of immobility in a five min period was recorded.

Open field test

Open field test was carried out according to Gupta *et al.* so as to determine the sedative potential of MESJ [35]. An open field of 0.5 square meter was made by using a wooden apparatus that contained a series of alternatively painted (black and white) squares. The wooden apparatus

had a surrounding wall of 50 cm in height. Four groups of mice each containing five individuals were used for this test. The CTL and STD group were given normal saline (0.1 ml/mice, p.o.) and diazepam (1 mg/kg, i.p.), respectively. The MESJ groups were given two different doses of the extract solutions (100 & 200 mg/kg, p.o.). After a period of 30, 60, 90 and 120 min, the no. of squares crossed by the mice during a period of three min was noted.

Hole-board Test

The method described by Öztürk *et al.* was the basis of the hole-board test [36]. In this test, a flat platform (60 cm × 30 cm) was used. It contained 16 holes that were of equal size and evenly spaced. Four groups of mice each containing five individuals were used. The CTL and STD group received normal saline (0.1 ml/mice, p.o.) and diazepam (1 mg/kg, i.p.), respectively. The MESJ groups received two different doses of the extract (100 & 200 mg/kg, p.o.). After a period of 30 min in case of the control & crude extract and 15 min in case of the standard, the mice were placed on the perforated flat platform and the no. of head dips in a period of five min was recorded.

Statistical Analysis

The aftermaths are presented as Mean ± SEM. One-way analysis of variance (ANOVA) was used for analyzing the results. $p<0.05$ indicates the statistical significance of the findings of this study.

RESULTS

Acute toxicity test

A test of acute toxicity was done to establish the safe dose ranges of methanolic crude extract of *S. japonica* (MESJ). We found a wide safe dose range for MESJ as it presented no toxicity at all the experimental doses (500, 1000 and 2000 mg/kg, p.o.). From this experiment, we can say that the LD_{50} of MESJ is > 2000 mg/kg in case of oral administration.

Forced swimming test

The results of the forced swimming test are shown in Table 1. Decreasing immobility was the expected outcome in this test and considered as the antidepressant potential. In comparison to the control (CLT) therapy (normal saline), the standard (STD) drug (nortriptyline) reduced the immobility time period very significantly (64.60%, $p<0.001$). The methanolic crude extract of *S. japonica* (MESJ) also decreased the immobility time at both doses, which was statistically significant ($p<0.01$), but the effect was considered as mild compared to the standard treatment.

Table 1. Antidepressant activity of MESJ in forced swimming test

Group	Immobility Time (s) (Mean ± SEM)	% of Inhibition
CTL	116.4±4.18	0
STD	41.2±2.8***	64.60
$MESJ_{100}$	90±3.57**	22.34
$MESJ_{200}$	85.6±5.08**	26.46

Values are expressed as Mean ± SEM (n=5). ***p<0.001, **p<0.01, *p<0.05 indicate significant compared to the control. CLT = control (normal saline), STD = standard drug, $MESJ_{100}$ = methanolic crude extract at dose 100 mg/kg b.w., $MESJ_{200}$ = methanolic crude extract at dose 200 mg/kg b.w.

Tail suspension test

In the tail suspension test, MESJ decreased the immobility time to some extent (Table 2) at both doses. Compared to the standard drug, nortriptyline (60.40% inhibition, $p<0.001$), the experimental plant extract showed mild activity. This findings of MESJ were in a dose dependent fashion compared to that of the control group.

Table 2. Antidepressant activity of MESJ in the tail suspension test

Group	Immobility Time (s) (Mean ± SEM)	% of Inhibition
CTL	129.8±5.85	0
STD	51.4±5.64***	60.40
$MESJ_{100}$	112.2±4.92	13.56
$MESJ_{200}$	106±4.34*	18.34

Values are expressed as Mean ± SEM (n=5). ***p<0.001, **p<0.01, *p<0.05 indicate significant compared to the control. CLT = control (normal saline), STD = standard drug, $MESJ_{100}$ = methanolic crude extract at dose 100 mg/kg b.w., $MESJ_{200}$ = methanolic crude extract at dose 200 mg/kg b.w.

Open field test

The outcomes of the open field test are shown in Table 3. The number of squares crossed by the experimental animal is supposed to be decreased if the test sample possesses sedative-hypnotic potential. Compared to the control treatment, the highest activity of the standard drug (diazepam) was found at 60 min (71.97% inhibition) and the other outcomes were very close to this up to 120 min. Like the standard drug, the total number of squares crossed by the MESJ-treated animals decreased in the time of 60 min, albeit the number of squares crossed was greater than that crossed by the STD group. Hence, MESJ can be considered as having moderate sedative-hypnotic potential compared to the standard treatment. In a dose dependent manner, the activity of $MESJ_{200}$ was higher than that of $MESJ_{100}$. In both cases, the effectiveness gradually decreased as we see in the table indicating that MESJ possesses short duration of sedative-hypnotic activity.

Table 3. Sedative-hypnotic activity of MESJ according to the open field test

Group	30 min		60 min		90 min		120 min	
	Square Crossed (Mean ± SEM)	% of Inhibition	Square Crossed (Mean ± SEM)	% of Inhibition	Square Crossed (Mean ± SEM)	% of Inhibition	Square Crossed (Mean ± SEM)	% of Inhibition
CTL	75.6±3.09	0	74.2±3.64	0	69.8±2.85	0	64.2±3.12	0
STD	43.2±3.40***	42.86	20.8±2.08***	71.97	24.8±2.33***	64.47	22.8±1.07***	64.49
$MESJ_{100}$	59.4±3.11**	21.43	51.6±4.39**	30.46	53.6±6.79	23.21	53.8±1.93*	16.20
$MESJ_{200}$	56.0±4.10**	25.93	44.0±3.86***	40.70	50.2±5.19*	28.08	56.4±4.95	12.15

Values are expressed as Mean ± SEM (n=5). ***p<0.001, **p<0.01, *p<0.05 indicate significant compared to the control. CLT = control (normal saline), STD = standard drug, $MESJ_{100}$ = methanolic crude extract at dose 100 mg/kg b.w., $MESJ_{200}$ = methanolic crude extract at dose 200 mg/kg b.w.

Hole-board Test

Table 4 lists the findings of hole-board test. Here, a decreasing number of head dips was attributed to the sedative-hypnotic potential of any test sample. The results of this test indicate that MESJ had moderate sedative-hypnotic activity compared to the standard treatment (diazepam). The findings of MESJ were almost in a dose dependent manner compared to the control group.

Table 4. Sedative-hypnotic activity of MESJ according to the hole-board test

Group	Head Dips (Mean ± SEM)	% of Inhibition
CTL	64.4±4.57	0
STD	20.2±2.03***	68.63
$MESJ_{100}$	44.2±5.02*	31.37
$MESJ_{200}$	42.8±2.71**	33.54

Values are expressed as Mean ± SEM (n=5). ***p<0.001, **p<0.01, *p<0.05 indicate significant compared to the control. CLT = control (normal saline), STD = standard drug, $MESJ_{100}$ = methanolic crude extract at dose 100 mg/kg b.w., $MESJ_{200}$ = methanolic crude extract at dose 200 mg/kg b.w.

DISCUSSION

Usually, research work with plant parts' or whole plants' extract are carried out to justify their traditional applications and to determine whether they contain only medicinally important constituents or that which are toxic [37]. In this experiment, we tried to confirm whether *S. japonica* plants had antidepressant and sedative-hypnotic activities. First of all, the possibility of acute toxicity was determined. This test indicated us that the methanolic crude extract of *S. japonica* contained no harmful constituents capable of creating acute toxic reactions in the experimental mice. This finding ensures the safety margin of *S. japonica* plants up to a high dose (2000 mg/kg, p.o.) in the case of traditional practices.

The forced swimming test and tail suspension test were designed to assess the antidepressant potential of *S. japonica* plants. In both cases, stressful situations were developed for the experimental animal to which they were not fully used to. This unexpected and stressful situation made them depressed. As a result, a state of loss of motivation, confusion, indecisiveness, lack of energy etc. were observed in the mice. In this condition, the mice remained immobile until an antidepressant agent improved the outcome. In both of the tests, the experimental crude extract decreased the immobility time to some extent in a dose dependent manner compared to the control treatment. Here, both tests presented exhibited the mild antidepressant potential of *S. japonica* plants compared to the standard drug (nortriptyline).

Sedative-hypnotics agents exert a relaxing effect in the CNS that slows down the general functioning of human body. In the open field test and hole-board test, we tried to explore the sedative-hypnotic potential of *S. japonica* plants. Decreasing the number of squares crossed in open field test and head dips in hole-board test by the experimental mice mimicked the relaxed conditions of human body functions. In both cases, the activities were statistically significant compared to the control groups and moderate in comparison to the standard drug (diazepam). Here, the effectiveness was directly proportional to the experimental dose. But the duration of action as a sedative-hypnotic agent was found to be short in the open field test where the activity gradually decreased after 60 min compared to the standard drug.

CONCLUSION

The methanolic crude extract of *Stephania japonica* (Thunb.) Miers. possesses antidepressant & sedative-hypnotic activities at the experimental doses (100 and 200 mg/kg), while up to a dose of 2000 mg/kg, it is considered harmless to use. The antidepressant potential at the experimental doses is mild in action compared to the standard therapy (nortriptyline). Beyond the aforementioned, the whole plants of *S. japonica* possess moderate but short-term sedative-hypnotic activity at the experimental doses compared to the standard treatment (diazepam). According to the aforementioned findings, it is obvious that *Stephania japonica* is a plant of enormous medicinal importance. Further broad study of this plant might result in the development of many unexplored medicinal properties and eventually new additions to the existing pharmacotherapies.

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