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Evaluation of *In-vivo* Neuropharmacological Effect of *Sarcochlamys pulcherrima* Leaf Extract in Animal Model

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MNA, MRI, MAS and SC designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MJA, MNA, MIAC, IJE and SFT managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To evaluate *in-vivo* neuropharmacological effect of methanolic leaf extract of *S. pulcherrima* in animal model.
Place and Duration of Study: This study was conducted in the Department of Pharmacy, Faculty of Science and Engineering, International Islamic University Chittagong, during the period between September 2013 and April 2014.
Methodology: Neuropharmacological activity of crude methanolic extract of *S. pulcherrima* leaf was determined by using standard animal behavioral models. Such as hole cross test and open field test for exploratory activity, thiopental sodium induced sleeping times tests for sedative activity and elevated plus maze test for anxiolytic activity.
Results: Methanolic leaf extract of *S. pulcherrima* showed a significant dose dependent suppression of exploratory activity of swiss albino mice in hole cross and open field test

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respectively at both dose which is 200 mg/kg and 400 mg/kg (P<0.05). In the case of thiopental sodium induced hypnosis test, methanolic leaf extract exhibited significant sedative activity at 200 mg/kg and 400 mg/kg doses respectively (P<0.05). At 400 mg/kg dose, significant anxiolytic activity was demonstrated by methanolic leaf extract of *S. pulcherrima* in elevated plus maze test (P<0.05). The results of this study exhibited that methanolic extract of *S. pulcherrima* leaf contains significant exploratory, sedative as well as medium anxiolytic activity.

Keywords: S. pulcherrima; exploratory; hole cross; open field; sedative; elevated plus maze; anxiolytic.

1. INTRODUCTION

For the discovery of safe, effective and advanced medicinal agents, medicinal plants are the major source. Traditional medicine based on medicinal plants has always played a crucial role in the health care systems around the world. From the ancient times traditional medicines are used to treat various diseases. Consumption of folk medicines are increasing day by day due to its safety, efficacy, low prices and minimum side effects than chemically formulated medicine. Eighty percent people of developing countries are still dependable on traditional medicine [1]. These traditional plants contain valuable secondary metabolites, which can be used in therapeutics purposes. Over 25% of modern medicines that are commonly used worldwide contains compounds extracted from medicinal plants [2].

Anxiety and depression are the most common psychiatric disorders, already cover 20% of the adult population, suffering from these illnesses at some time during their lives [3-5]. It has grown up to be an important area of research interest in psychopharmacology during this decade [6].

Drugs acting on the central nervous system (CNS) are still the most widely used pharmacological agents [7]. Benzodiazepines are among the most prescribed and effective anti-anxiety drugs used worldwide [8]. Barbiturates and Ethanol are also frequently used. Both barbiturates and benzodiazepines show their CNS effect by interaction with postsynaptic gamma aminobutyric acid receptor ($GABA_A$ receptor) [9]. The most serious shortcoming of barbiturates as a depressant is linked to their narrow margin of safety, and only 10 times of their therapeutic dose may be lethal [10].

Moreover barbiturates can grow both psychological and physiological dependence [11,12]. Benzodiazepines are the most commonly used CNS depressant which leads to tolerance and physical dependence, for example diazepam typically produces sedation at dose of 5 to 10 mg in user of first time, but those who repeatedly use it may become tolerant to doses of several hundred milligrams [13]. Ethanol produces its depressant action by changing membrane fluidity and interaction with the GABA system [10,14]; also it has a tolerance and physical dependence activity. Statistically it has been shown; alcohol addiction in American society is 5% to 10% for men and 3% to 5% for women [15]. A natural CNS depressant with minimum or no toxicity is therefore, essential.

Sarchochlamys pulcherrima (Roxb.) Gaud. (Urticaceae) is an evergreen shrub or small tree without stinging hairs with alternate or spiral leaves and short or few lateral branches and 3 to 6 meters tall in length. In Bangladesh, the plant was collected from the hill tract area in the sub district of Banshkhali at the northern area of Chittagong, Bangladesh. The plant is also known as “Rakhali Chora” or “Morichia” by local community, “Jungalya Shak” by Chakma tribe and “Ma Cha Da” by Marma tribe. The plant is widely distributed in the forests and hill tract areas of Chittagong, Sylhet, Mymensingh and other topical areas of Indian subcontinent as Thailand, China, Indonesia and East Himalayas. Traditionally it is used as folk medicine and food by different tribes and communities of Assam in India and in neighboring countries [16]. To treat eye complications, boils disease and fever blisters, leaf of this plant is effective [17]. This plant is also useful for the treatment of diarrhea and dysentery [18]. Young shoots, leaves and fruits are eaten as vegetable [19,20]. In this modern era, life is so hectic that people feel mental pressure and stress which cause anxiety and depressive disorders. These are the most frequent psychiatric conditions. According to a statement, more than 20% of the adult population suffers from these conditions at some stage during their life [21,22]. In recent times it has become a major and vast area of research in neuropharmacology [23]. The objective of the

present study is to evaluate the neuropharmacological effect of *S. pulcherrima* leaf extract.

2. MATERIALS AND METHODS

2.1 Collection and Proper Identification of Plant

The leaves of *S. pulcherrima* were collected from hill tract area of Banshkhali, (22.04: latitude and 91.95: longitude), Chittagong district, Bangladesh. Collected leaves were identified and authenticated by Dr. Shaikh Bokhtear Uddin, Associate Professor, Department of Botany, University of Chittagong.

2.2 Preparation of Extract

The leaves were dried for a period of 2 weeks under shade at room temperature and made fine powder by grinding them in a suitable grinder. Then the powdered leaves (250 gm) were soaked in sufficient amount of methanol (1:3) for one week with proper shaking and then filtered through a cotton plug followed by Whitman filter paper number 1. The solvent was evaporated at 45ºC temperature in water bath to yield methanolic semisolid crude extract that was preserved in a refrigerator at 2-4ºC for further use. About 25 to 30 gm of extract was found with the yield value of 10-12%.

2.3 Experimental Animals

Swiss Albino mice weighing 25-30 gm of both gender were collected from International Center for Diarrheal Diseases Research, Bangladesh (ICDDRB) and housed in polypropylene cages under controlled conditions. The animals were exposed to alternative 12:12 hours light and dark cycle at an ambient temperature of 26±2ºC. Animals were allowed free access to drinking water and pellet diet. Mice were acclimatized for 7 days in the laboratory environment prior to the study.

2.4 Hole Cross Test

The hole cross test was performed according to the method, described by Takagi (1971) [24], for screening CNS depressant activity in mice. The animals were divided into three groups as control, positive control and test. The test groups received methanolic extract of *S. pulcherrima* at the doses of 200 and 400 mg/kg, p.o. separately whereas the control group received vehicle (1% Tween 80 in water) with the dose of 10 ml/kg, p.o. In the test, a wood- made cage having a size of 30 × 20 × 14 (cm) with a fixed partition in the middle of the cage having a hole of 3 cm in diameter at the lower side of partition. In the determination of *in-vivo* sedative effect the number of passage of a mouse from one chamber to another was counted for a period of 3 minutes at 0, 30, 60, 90 and 120 min. after oral administration of extract solution. Standard diazepam (1 mg/kg, i.p.) was used as positive control. The results were represented by graphical representation plotting the number passage against the respective groups of mice.

2.5 Open Field Test

The open field test was performed according to the method, described by Gupta (1971), where the animals were divided into control, positive control and test groups. The test groups received *S. pulcherrima* methanolic leaf extracts at the doses of 200 and 400 mg/kg body weight orally whereas the control group received vehicle (1% Tween 80 in water) with the dose of 10 ml/kg, p.o. The floor of an open field having a size of 16×16 square inch. was divided into 16 square areas with black and white colored areas alternatively. The apparatus had a 40 cm height wall. To determine *in-vivo* sedative effect in this test the number of square areas, passed by a mouse, were counted for a period of 3 minutes at 0, 30, 60, 90 and 120 min. after oral administration of extract solution or administration of reference standard drug. In current study diazepam (1 mg/kg, i.p.) was used as reference standard drug. The results were displayed by graphical representation by plotting the number of square areas, passed by the mice, against the respective group of mice [25].

2.6 Thiopental Sodium Induced Hypnosis Test

Thiopental sodium induced hypnosis was performed according to the method, described by Ferrini, where the animals were randomly divided into three groups as control, standard and test groups consisting of 3 mice each. The test groups received methanol extract solution of the leaves of *S. pulcherrima* at the dose of 400 mg/kg (p.o.) and 200 mg/kg (p.o.) while the standard group was treated with diazepam (1 mg/kg, i.p.) and control group with vehicle (1% Tween 80 in water) at the dose 10 ml/kg, (p.o.).

Twenty minutes later, thiopental sodium (40 mg/kg, i.p.) were administered to each mice to induce sleep. The animals were observed for about 3 hours while the latent period (time between thiopental administrations to loss of righting reflex) and duration of sleep (time between the loss and recovery of righting reflex). The results were expressed by graphical representation by plotting the time of onset of sleep versus respective group of mice the duration of sleep versus respective group of mice [26].

2.7 Elevated Plus-Maze (EPM Test)

The elevated plus maze (EPM) is a rodent model of anxiety that is used as a screening test for putative anxiolytic or anxiogenic compounds and as a general research tool in neurobiological anxiety research. The EPM apparatus consists of two open arms (5×10 cm) and two closed arms (5×10×l5 cm) radiating from a platform (5×5 cm) to form a plus sign figure. The apparatus was situated 40 cm above the floor [27]. The open arms edges were 0.5 cm in height to keep the mice from falling and the closed-arms edges were 15 cm in height. Sixty minutes after administration of the test drugs, each animal was placed at the center of the maze facing one of the enclosed arms. During the 5-min test period, the number of open and enclosed arms entries, plus the time spent in open and enclosed arms, was recorded. Entry into an arm was defined as the point when the animal places all four paws onto the arm. The procedure was conducted in a sound attenuated room; observations made from an adjacent corner [28].

2.8 Statistical Analysis

The data was expressed as mean ± standard error of mean (S.E.M.). Statistical comparisons were performed using one-way ANOVA followed by Dunnett's multiple comparison test. The values obtained were compared with the vehicle control group and were considered statistically significant when $P<0.05$.

3. RESULTS AND DISCUSSION

3.1 Hole- Cross Test

In this test, hole crossed by mice was decreased with increased time. Dose dependent decrease of motion was exhibited where maximum suppression of locomotor activity was displayed by 400 mg/kg ($P<0.05$), dose of methanolic extract of *S. pulcherrima* which was compared with standard drug diazepam.

3.2 Open Field Test

In this experiment, dose dependent decrease of motion was exhibited where maximum suppression of locomotor activity was displayed by 400 mg/kg dose of methanolic extract of *S. pulcherrima* that was comparable with standard drug diazepam. Open field test of *S. pulcherrima* treated groups (200 and 400 mg/kg body weight) exhibited significant and dose-dependent reduction of movement from its initial value at 0 to 120 min (Fig. 2). The number of squares traveled by the mice was reduced significantly at the dose level of 200 mg/kg and 400 mg/kg body weight ($P<0.05$) of methanolic extract of *S. pulcherrima* leaves.

3.3 Thiopental Sodium Induced Hypnosis (TSIH) Test

In this study, methanolic leaf extract of *S. pulcherrima* produced medium dose dependent hypnosis effect where 400 mg/kg dose exhibited quick onset of sleep and prolonged duration of sleep than that of 200 mg/kg dose. The effect of methanolic leaf extract (200 and 400 mg/kg) on the onset of sleep was comparable to that of standard diazepam. Both doses of the extract potentiated the duration of thiopental sodium induced sleeping time in test animals compared to controls (Table 1).

3.4 Elevated Plus Maze (EPM)

In the case of EPM, anxiolytic activity was observed by elevated plus maze test. Methanolic extract of *S. pulcherrima* demonstrated minimal activity in case of percent of entry into open arm at the dose of 200 mg/kg and 400 mg/kg respectively. On the contrary, methanolic leaf extract showed significant activity in case of percent of time spent in open arm at the dose of 400 mg/kg. Here, the percentage of time spent in open arm for the extract at 400 mg/kg showed more significant effect than the extract at 200 mg/kg and the percentage of time spent in open arm for the extract at 400 mg/kg was significantly adjacent to the standard drug Diazepam.

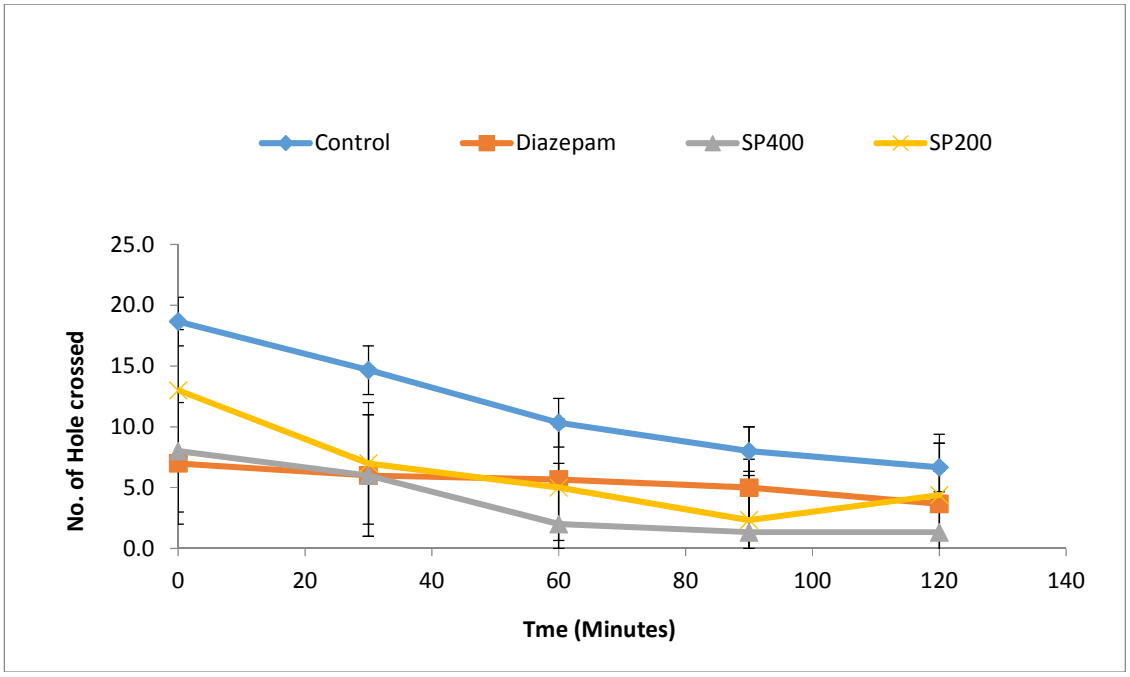

Fig. 1. Effect of methanolic extract of *S. pulcherrima* on exploratory behavior (hole cross test) in mice

Values are mean ± S.E.M., (n=3); P<0.05. Dunnett's test as compared to control

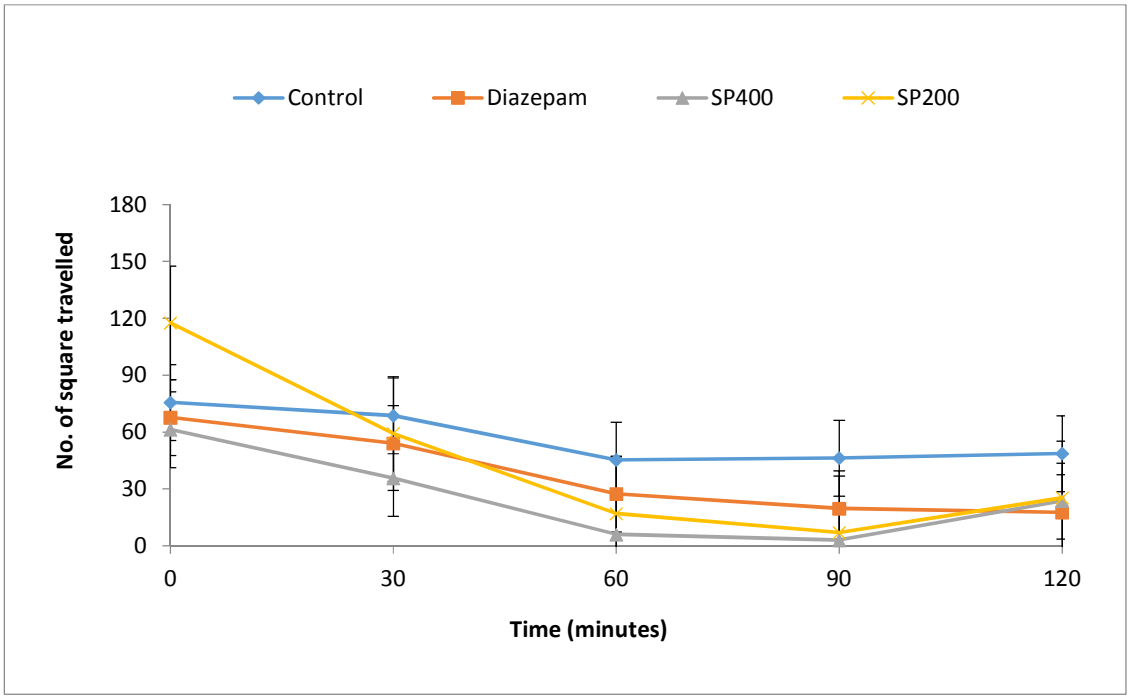

Fig. 2. Effect of methanolic extract of *S. pulcherrima* on exploratory behavior (open field test) in mice

Values are mean ± S.E.M., (n=3); P< 0.05, Dunnett's test as compared to control

Table 1. Effect of methanolic extract of *S. pulcherrima* on thiopental sodium induced sleeping time

Treatment	Dose(mg/kg)	Onset of sleep(min)	Duration of sleep(min)
Control	Vehicle	42.30±0.971	47.70±0.700
Diazepam	1	14.50±1.617**	145.00±3.215**
S. pulcherrima	200	25.67±0.370**	79.33±0.401**
S. pulcherrima	400	17.33±0.524**	98.67±0.370**

*Values are expressed as mean ± S.E.M, (n=3); **P<0.05, Dunnett's test as compared to control*

Table 2. Effect of methanolic extract of *S. pulcherrima* on EPM test during 5 min test session

Treatment	Dose (mg/kg)	% entry into open arm	% time spent in open arm
Control	Vehicle	25.00±1.52	27.33±2.33
Diazepam	1	77.14±2.86**	78.20±6.20**
S. pulcherrima	200	27.33±1.45	36.67±1.45
S. pulcherrima	400	33.67±1.85	43.33±0.88**

*Values are expressed as mean ± S.E.M., (n=3); **P<0.05, Dunnett's test as compared to control*

4. CONCLUSION

From the above study, it can be concluded that the crude methanolic extract of *S. pulcherrima* contains significant exploratory and sedative activity as well as moderate anxiolytic activity. Level of excitability of the CNS and sedation resulting from depression of the central nervous system was measured by locomotor activity in open field and hole cross tests [29]. The result indicated that methanolic leaf extract significantly decreased the locomotor activity. Standard drug diazepam increases open arm exploration are considered as anxiolytic activity [30]. In this study, we observed that the administration of two different doses of methanolic leaf extract of *S. pulcherrima* induced an anxiolytic-like effect in mice, as it increased open arm entries and the time spent in the open arms of the EPM when compared to the control animals. Significant sedative effect of methanolic leaf extract was found by the reduction in sleeping latency and increase of thiopental sodium induced sleeping time. In central nervous system (CNS), Gamma-amino-butyric acid (GABA) is a major inhibitory neurotransmitter. CNS depressant drugs mainly exert their action through GABAA receptor [31]. So methanolic leaf extract of *S. pulcherrima* may acts by hyperpolarization of the CNS through GABA receptor or benzodiazepine receptor located adjacent to the GABA receptor. All of the results were dose-dependent and statistically significant. Therefore, it can be suggested that this methanolic leaf extract of *S. pulcherrima* may fulfill the therapeutic need for the treatment of neuropharmacological disorders. However, further investigation is necessary to determine the precise phyto-constituents and mechanism of action that are responsible for the biological activities of methanolic leaf extract of *S. pulcherrima*.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All experimental procedures were given ethical clearance and approved by the P & D Committee (59/04-10), Department of Pharmacy, International Islamic University Chittagong, Bangladesh for animal experiments according to governmental guidelines.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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