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Antidepressant and Sedative-hypnotic Activities of Methanolic Extract of *Grewia asiatica* Linn. Leaves in Mice

Rasna Jebin[1], Md. Islam Molla[2], Surid Mohammad Chowdhury[1] and Md. Rajdoula Rafe[2]

[1]Department of Pharmacy, Southeast University, Dhaka-1213, Bangladesh
[2]Department of Pharmacy, Jagannath University, Dhaka-1100, Bangladesh

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Abstract

Grewia asiatica (Family-Malvaceae), known as Phalsa in Bangladesh, is also native to India, Nepal, Pakistan, Cambodia and Thailand. The plant has a long history of traditional uses. The present investigation was designed to evaluate acute toxicity test, antidepressant and sedative-hypnotic activities of the methanolic extract of the leaves of *G. asiatica*. In acute toxicity test, methanolic soluble fraction of the plant extract of *G. asiatica* showed no significant changes in the body weight between the control and treated group at the doses of 1000, 2000 and 3000 mg/kg body weight. The extract could not significantly reduce immobility time in comparison with control group and standard drug (Nortriptyline) treated group in forced swimming and tail suspension tests. Sedative-hypnotic activity was evident at the doses of 100 and 200 mg/kg body weight after observing in hole board test. Sedative-hypnotic activity of short duration of action was also evident in hole cross and open field test.

Key words: *Grewia asiatica*, sedative-hypnotic, toxicity, immobility, inhibition.

Introduction

Medicinal plants are the nature's best gift to the humankind. People have trust on these natural plants throughout the human civilization for their health related benefits (Tripathi *et al.*, 2010). The traditional uses include either the whole plants or plant parts. They contain a variety of phytochemicals that exert beneficial pharmacological effects. Along with pharmacologically important ones, they may contain harmful chemicals too. That is why, extensive studies are being carried out to screen out the phytochemicals possessing medicinal importances.

Grewia asiatica L. (Family: Malvaceae) is a plant of medicinal importances though it is cultivated mainly for its fruits (Zia-Ul-Haq *et al.*, 2013). It is commonly known as Phalsa. Its scientific name indicates its habitat. It is widely distributed in many countries of South Asia, particularly in India, Pakistan, Bangladesh, Nepal, Laos, Thailand, Cambodia etc. (Hays, 1953; Lim, 2012). Different portions of *G. asiatica* have distinct traditional uses. Fruits are thought to be helpful for liver disorders, heart diseases, indigestion, stomatitis, thirst, fevers, asthma, tuberculosis, diarrhea and sexual problems (Sharma and Sisodia, 2009; Morton, 1987; Lavekar *et al.*, 2008; Pallavi *et al.*, 2011; Mishra *et al.*, 2012). Roots have beneficial effects in urinary tract diseases and rheumatic disorders and barks are believed to cure urinary diseases and lessen burning sensation in vagina (Khan and Hanif, 2006; Sisodia and Singh, 2009). The leaves have application to skin irritation, rashes and skin eruptions (Zia-Ul-Haq *et al.*, 2012; Farnsworth *et al.*, 1985). Many of this traditional uses are yet to be established. *G. asiatica* contains a number of phytochemicals such as flavonoids, tannins, phenolic compounds, proteins, amino acids

Correspondence to: Md. Rajdoula Rafe; Phone: +8801748358585; E-mail: rafi.soyeb@gmail.com
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and vitamin C that may contribute to the establishment of its known traditional uses or new therapeutic uses (Gupta *et al.*, 2008).

Treatment with natural plants is considered almost safe. Sometimes it may create complexity in the treatment approaches, as a plant contains various type of chemicals (Nasri and Shirzad, 2013). For this reason, evaluation of safety margin and toxic state is very much needed for the preparations made from natural plants (Haq, 2004).

People of today's advanced world are highly affected by depressive disorders which account for about 4.4% of world's total diseased conditions (WHO, 2002). Abnormalities in the activity of neurotransmitters is responsible for the development of depression (Milak *et al.*, 2005). Deficiency in the general activity of certain neurotransmitters- norepinephrine, γ-aminobutyric acid (GABA), serotonin, dopamine, etc. or overactivity of some neurotransmitter systems- substance P, corticotropin-releasing factor, acetylcholine etc. may contribute to the development of major depressive disorders (Mann *et al.*, 2012). They result in a great risk of suicidal tendency, heart diseases, stroke, respiratory disorders, etc. (Angst *et al.*, 2002; Stark *et al.*, 1995). Along with the existing therapies, many more attempts are being taken to develop new approaches. A lot of recent researches exhibited that medicinal plants are used for the treatment of diseases in a specific part of the body like the nervous system and cardiovascular systems (Rafe *et al.*, 2018). This study was aimed at finding out if the leaf extracts of *Grewia asiatica* L. had any kind of neurological activities.

Materials and Methods

Collection and preparation of plant materials: The leaves of *G. asiatica* were collected from Maulovibazar, Bangladesh in March, 2016 and identified by an expert taxonomist. A voucher specimen was submitted to the national herbarium, Mirpur, Dhaka, Bangladesh (Accession number: DACB 42991). Leaves were then washed properly to remove dirty materials and shade dried for several days with occasional sun drying. These were then dried in an oven for 24 hours at considerably low temperature for better grinding. The dried leaves were grounded into coarse powder by a grinding machine.

Cold extraction of the plant materials: Powdered plant materials (leaves) having a weight of about 200 g were taken in an amber colored reagent bottle and soaked in 1.5 liter of methanol. The bottle with its contents were sealed and kept for a period of about 7 days with occasional shaking and stirring. The whole mixture was then filtered through cotton and then through Whatman No. 1 filters paper and was concentrated with a rotary evaporator under reduced pressure and temperature to afford crude extract.

Experimental animals: Both male and female Swiss albino mice weighing 25-30 g were used in pharmacological test and were housed at room temperature in a 12 hrs light/dark cycle. Food and water were available *ad libitum*. The animals were collected from Animal Resource Branch of the International Center for Diarrheal Disease Research (ICDDR, B). The animals were kept in standard laboratory condition and provided with standard diet (ICDDRB, B formulated) and clean water *ad libitum* during acclimatization period. Tests were performed only after the mice had acclimated to the above environment. All experiments were conducted between 8:00 and 13:00 every day to avoid any temporal factor. Each animal was used for only one experimental condition. All experiments were carried out in accordance with international guideline outlined in the Guide for the Care and Use of Laboratory Animals.

Drugs: Nortriptyline and diazepam were purchased from Square Pharmaceuticals Limited. Diazepam was suspended in the solvent. All the solutions were freshly made on the day of testing and administered to a final volume of 1 mg/kg body weight of mice.

Acute toxicity test: Median lethal dose (LD_{50}) values were determined by dividing the mice into four groups- one control and three test groups. Each

group contained five mice. The crude extract was administered orally to the mice of group-I, group-II and group-III at the dose of 1000, 2000, and 3000 mg/kg body weight, respectively while the group-IV received only normal saline. The test animals were observed for a period of 72 h for any unexpected results- hypersensitivity, mortality etc. (Walker *et al.*, 2008).

Forced swimming test: In this test, the swimming sessions were conducted by placing the animals in individual Plexiglas's cylinders (40 cm high, 24 cm diameter) that contained around 20 cm of water. Four different groups each containing five mice were treated orally with the control (0.1 ml/mice), nortriptyline (1mg/kg) and two different doses of the extract (100 and 200 mg/kg), respectively. After a period of 45 min, all animals were forced to swim for 6 min, and the time spent in immobility during the last 5 min of a 6 min observation period was recorded as immobile (Porsolt *et al.*, 1978).

Tail suspension test: In tail suspension test, four groups each containing five mice were studied. They were given orally the control (0.1 ml/mice), nortriptyline (1mg/kg) and two different doses of the extract (100 and 200 mg/kg), respectively. Then the mice were suspended 30 cm above the floor by adhesive tape placed approximately 1-2 cm from the tip of the tail. The duration of immobility was measured for a 5 min period. This was done after a period of 45 min of administering the test samples (Steru *et al.*, 1985).

Hole-board test: The hole board test was performed by using a flat platform of 60 cm × 30 cm that contained equal sized and evenly spaced sixteen holes. Four groups each containing five mice were studied. The mice groups were treated orally with the control (0.1 ml/mice), diazepam (1mg/kg) and two different doses of the extract (100 and 200 mg/kg), respectively. Then they were individually placed at the center of the perforated board and the number of head dips was registered during a period of 5 min. This was performed 30 min after the control & crude extract administration and 15 min after the standard drug (diazepam) administration (Öztürk *et al.*, 1996).

Open-field test: This experiment was carried out to determine sedative action of the crude extracts on CNS in mice. A wooden apparatus of 0.5 square meter open field containing an alternatively painted (black and white) series of squares was used in this test. The surrounding wall of the apparatus was 50 cm in height. Four groups each containing five mice were treated orally with the control (0.1 ml/mice), diazepam (1mg/kg) and two different doses of the extract (100 and 200 mg/kg) respectively. At 30, 60, 90 and 120 min following the administration, the number of squares passed by the animals was counted for 3 min (Gupta *et al.*, 1971).

Hole cross test: A special cage with a size of 30 × 20 × 14 cm was used in this test. The cage had a fixed divider in the middle containing a hole with a diameter of 3 cm. Four groups each containing five mice were treated orally with the control (0.1 ml/mice), diazepam (1mg/kg) and two different doses of the extract (100 and 200 mg/kg). Then they were observed whether they crossed the divider by the hole to go from one chamber to another and vice versa for 3 min. This observation was done at 30, 60, 90 and 120 min following the oral treatment (Takagi *et al.*, 1971).

Results and Discussion

Acute toxicity test: After oral administration of *G. asiatica* extract at the doses of 1000, 2000 and 3000 mg/kg, p.o. no toxicity and no significant changes in the body weight between the control and treated group were demonstrated at these doses. This result indicates that the LD_{50} was higher than 3000 mg/kg.

Antidepressant activity: As it is seen in table 1, nortriptyline significantly decreased immobility time compared to the control group in forced swimming test. Here, the doses of the methanolic extract of *G. asiatica* (MEGA) could slightly reduce immobility time in comparison with control group.

In tail suspension test, nortriptyline significantly decreased immobility time compared to the control group, where both of the doses of the MEGA could not significantly reduce immobility time in

comparison with control group (Table 1). Both of the doses showed lower inhibitory activity compared to the standard drug.

Sedative-hypnotic activity: Hole board test is well established as a means to assay potential anxiolytic and sedative effects of any agents by observing the exploratory behavior in rodents. This experiment is advantageous due to its methodological simplicity and several behavioral responses of an animal can be readily observed and quantified when exposed to an unfamiliar environment. It was found that the head-dipping behavior of the animals is directly related to their emotional state. Based on this observation, it was suggested that the expression of an anxiety state in animals might be reflected by an increase in head-dipping behavior while a decrease in the number of head dips was found to be correlated with the sedative effect. Our results revealed that the methanolic extract of *Grewia asiatica* caused a dose-dependent reduction in head-dip response in the animals (47.37 and 26.32 % head-dip inhibition for 100 and 200 mg/kg doses, respectively) suggesting that the extract possesses significant sedative activity. The observed effects in the treated groups were significantly different from that of the control group (Table 2).

Table 1. Effect of MEGA on forced swimming test and tail suspension test (n=5).

Treatment	Dose (mg/kg body weight)	Immobility time (sec) for forced swimming test (mean ± SEM)	Immobility time (sec) for tail suspension test (mean ± SEM)
Control	0.1ml/mice	141 ± 1.21	185 ± 0.71
Nortriptyline	1	90 ± 1.97	101 ± 1.52
MEGA 100	100	132 ± 0.97	165 ± 1.01
MEGA 200	200	125 ± 1.28	167 ± 0.48

Here, n=5, SEM= Standard error of mean, MEGA = Methanolic extract of *G. asiatica*.

Table 2. Effect of MEGA on hole board test (n=5).

Treatment	Dose (mg/kg)	Number of head dips (mean± SEM)	% of inhibition
Control	0.1 ml/mice	28.5 ± 4.94	0
Diazepam	1	12.5 ± 2.12	56.14
MEGA 100	100	21 ± 2.18	26.32
MEGA 200	200	15 ± 2.82	47.37

Here, n=5, SEM= Standard error of mean, MEGA = Methanolic extract of *G. asiatica.*

The sedative effects of MEGA was studied by observing spontaneous locomotors activity of mice in hole cross and open field tests. Our result for open field test demonstrated that the oral administration of MEGA in both of the doses (100 and 200 mg/kg) caused a marked reduction in number of square crossed by the mice (Table 3). This ability of MEGA to suppress the locomotors activity suggests that the extract is endowed with central nervous system depressant activity.

The sedative effects of MEGA was also recorded by spontaneous locomotors activity of mice in hole cross tests. Our result demonstrated that the oral administration of MEGA in all doses (100 and 200 mg/kg) caused a marked reduction in number of hole crossed (Table 4). The suppressive effect was found at 30 min and continued up to 120 min after administration of MEGA. This ability of MEGA to suppress the locomotor activity suggests that the extract is endowed with central nervous system depressant activity.

Table 3. Effect of MEGA on open field test (n=5).

Treatment	Dose	Number of square crossed (% of inhibition)			
		After 30 min	60 min	90 min	120 min
Control	0.1 ml/mice	67.5 ± 3.53	69.2 ± 1.06	71.5 ± 2.82	70.8 ± 3.82
Diazepam	1	19 ± 1.41 (71.87)	8.6 ± 3.11 (87.5)	7.5 ±3.01 (89.51)	5.75 ± 1.95 (91.88)
MEGA 100	100	59 ± 1.40 (12.59)	64 ± 2.83 (7.51)	35.5 ± 3.14 (50.35)	54.5 ± 3.54 (23.02)
MEGA 200	200	51.5 ± 2.12 (23.70)	50 ± 2.03 (27.74)	37.5 ± 3.51 (47.55)	47.5 ± 3.03 (32.91)

Here, n=5, SEM= Standard error of mean, MEGA = Methanolic extract of *G. asiatica.*

Table 4. Effect of MEGA on hole cross test (n=5).

Treatment	Dose (mg/kg)	Number of hole crossed (% of inhibition)			
		After 30 min	60 min	90 min	120 min
Control	0.1 ml/kg	13.5 ± 2.12	15.2 ± 2.54	12.72 ± 0.28	15 ± 3.53
Diazepam	1	5 ± 1.41 (62.96)	3.5 ± 0.89 (76.97)	2.8 ± 0.56 (77.99)	2 ± 0.17 (86.66)
MEGA 100	100	13 ± 4.24 (3.70)	8 ± 1.41 (47.37)	9 ± 1.05 (29.25)	10 ± 0.89 (33.33)
MEGA 200	200	12.5 ± 2.12 (7.41)	10 ± 2.83 (34.21)	4.5 ± 0.07 (64.62)	11.5 ± 0.78 (23.33)

Here, n=5, SEM= Standard error of mean, MEGA = Methanolic extract of *G. asiatica.*

In this study at hand, we considered acute toxicity test, antidepressant and sedative-hypnotic activity of the methanolic extract of *G. asiatica* in mice. In case of acute toxicity studied at the doses of 1000, 2000, 3000 mg/kg, it was found that this plant is safe to use as a medicinal plant after observing the result since its LD_{50} value is elevated than the dosage of 3000 mg/kg.

Two test using animal models for bioactivity investigation, the forced swimming test (FST) and tail suspension test (TST), were used for exploration of antidepressant study. The immobility exhibited by mice when subjected to unavoidable stress is considered as a reflection of a state of despair or lowered mood, which is believed to imitate depressive disorders in humans (Tobler *et al.,* 2001). In addition, the immobility time has been shown to be abated by treatment with antidepressant drugs. In both of these animal models, results pointed out that the doses of the extract could not trim down the immobility time significantly as compared to the control. Nonetheless, the activities were observed almost similar in both doses.

A dose-dependent reduction in the number of head dips in the hole-board test brought forth the fact that the methanolic extract of *G. asiatica* resulted in a dose-dependent decline in head-dip response in the animals proposing that it possesses significant sedative activity compared to the standard diazepam.

The emotional state of animals was evaluated by the utilization of the open field test. The effect of the extract in both doses significantly reduced the number of square crossed after 30 min, 60 min & 90 min but after 120 min it turns to increase its movement that is indicative of sedative activity at short term duration of action. The outcome of hole cross tests signified that the plant under study dropped the frequency as well as the bountifulness of

movements. The locomotor activity lowering effect was also evident at the doses of 100 & 200 mg/kg after 30 min, 60 min & 90 min assisted to significantly reducing the number of movement but after 120 min it turns into its original position. It also provides an apparent indication that the extract has good sedative activity with short duration of action.

Conclusion

Present study has confirmed the sedative-hypnotic effects of crude methanolic leaf extract of *Grewia asiatica* in Swiss albino mice. The results obtained in this study suggest that the methanolic leaf extract of *G. asiatica* possesses insignificant anti-depressant activity in acute animal models of depression. Assessment of the sedative-hypnotic activity was performed carefully on the basis of motor activity and exploratory behavior in hole cross, open field and hole board test. Noticeable sedative activity was found at shorter duration of action in mice models. The extract was not found to be associated with any form of toxicity in mice after investigated at the doses of 1000, 2000, 3000 mg/kg, hence human consumption of this plant might be safe and sound. Consequently, considering the potential bioactivity, extensive study regarding the plant materials can further be undertaken to delve out their unexplored efficacy and to rationalize their applications as traditional medicines.

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