International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Original Article **CODEN: IJPNL6**

In vivo antidepressant and anxiolytic activities of ethanol extract of *Trema orientalis* leaves in mice

Md. Saiful Islam Arman[1], Mohammad Shah Hafez Kabir[2*], Sumya Afroze[3], Md. Forhad Alam[4], Md. Amdadul Haque[5], Tariqul Islam[5], Abdullah Al Ragib[6], Md. Tareq Rahman[7], Khondoker Md. Akter-Uz-Zaman[1], Md. Mominur Rahman[2]

[1]Department of Pharmacy, Manarat International University, Dhaka, Bangladesh.
[2]Department of Pharmacy, International Islamic University Chittagong, Chittagong-4203, Bangladesh
[3]Department of Microbiology, University of Chittagong, Chittagong-4331, Bangladesh
[4]Department of Microbiology, Primeasia University, Dhaka-1213, Bangladesh
[5]Department of Pharmacy, Noakhali Science and Technology University, Sonapur, Noakhali-3814, Bangladesh
[6]Department of Applied Chemistry and Chemical Engineering, Noakhali Science and Technology University, Sonapur, Noakhali-3814, Bangladesh
[7]Department of Pharmacy, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh

***Corresponding author e-mail:** mohammadshahhafezkabir@yahoo.com

Received on: 28-05-2016; Revised on: 16-06-2016; Accepted on: 26-06-2016

ABSTRACT

The goal of our investigation was to determine whether the leaf extracts of *Trema orientalis* held any significant antidepressant and anxiolytic properties. Leaves of *T. orientalis* was extracted with pure ethanol (EETO). The forced swimming and tail suspension tests were used as predictive animal models of antidepressant activity, where the time of immobility was considered. Anxiolytic activity was evaluated by elevated plus mazes (EPM) and hole board model. For all *in vivo* tests, doses of 200 and 400 mg/kg body weight were used. The extract also significantly decreases the duration of immobility in both animal models of antidepressant activity, forced swimming and tail suspension tests. In EPM test, the extract significantly increased time spent in open arms compared to control and in the hole board test, they also significantly increase in the number of head pokes in comparison to control. The overall results of the study indicated significant antidepressant and anxiolytic activities of ethanol extract of *T. orientalis* leaves. So this plant deserves further investigation to isolate the active constituents responsible for these activities and to establish the mechanism of action.

Key words: *Trema orientalis*, antidepressant, anxiolytic, forced swimming test, tail suspension tests, EPM, hole board test.

INTRODUCTION

Anxiety and depression disorders are currently among the most prevalent psychiatric disorders worldwide [1, 2]. Benzodiazepines (indirect GABAA receptor agonists) and selective serotonin reuptake inhibitors (SSRis) are the drugs of choice for the treatment of anxiety [1]. SSRIs are also commonly used to treat depressive disorders [3]. However, the chronic use of benzodiazepines produces tolerance,

and a treatment disruption can induce an abrupt withdrawal syndrome [4]. On the other hand, chronic use of SSRis can produce considerable side effects [5]. Therefore, the search for new compounds with anxiolytic and antidepressant properties with less potential to produce adverse effects continues.

Bangladesh is a country with a very diverse flora and fauna, and many of its indigenous cultures have an extensive pharmacopeia of medicinal plants, knowledge that has been passed through generations. During the last few decades, there has been a stimulation of popular interest in plants used for the treatment of different illnesses including anxiety and depression disorders.

Nowadays there is little information or research on the use and management of Bangladeshi medicinal plants. A thorough scientific validation of the efficacy of medicinal plants would guarantee the reliability of their usage and also it would help to identify those plants that are ineffective and this type of survey performed in Mexico and Central America [6].

Trema orientalis is an evergreen tree which belongs to the family Ulmaceae. It has been used extensively in various ways. *T. orientalis* is the same as *Celtis orientalis* Linn., *Celtis guineensis* Schum. and Thonn., *Trema bracteolate* Hochst Blume, *Sponia orientalis* Linn. Decne, and *Trema guineensis* (Schum. and Thonn.) Ficalho. Aside its uses in paper production and in the manufacturing of poles, it has been used for medicinal purposes including the treatment of respiratory, inflammatory, and helminthic diseases. Almost every part of the plant is used as medicine in various parts of Africa [7][8]. The generic name *Trema* is derived from a Greek word which means perforation or hole and alludes to pitted seeds of the tree, whereas the specific name *orientalis* is derived from the Latin word "*orientalis*" meaning eastern. The plant has common names such as pigeon wood, hop out, charcoal tree, Indian charcoal tree, Indian nettle tree, and gunpowder tree [9]. Phytoconstituents namely (-)-ampelopsin f, (+)-catechin, (+)-syringaresinol, (-)-epicatechin, hexacosanoic acid, N-(trans-p-coumaroyl) tyramine, simiarenone and trans-4-hydroxycinnamic acid isolated from *T. orientalis* [10].

The aim of the present study to evaluate the antidepressant and anxiolytic activity of ethanol extract of *Trema orientalis* leaves.

MATERIALS AND METHODS

Plant material

Fresh leaves of *Trema orientalis* were collected from Bandarban, Chittagong, Bangladesh in the month of September 2013. It was authenticated by Dr. Shaikh Bokhtear Uddin, Professor, Department of Botany, University of Chittagong, Chittagong-4331, Bangladesh.

Preparation of Extract

The leaves were dried for a period of 10 days under shade and ground. The ground leaves (500 gm) were soaked in sufficient amount of ethanol for one week at room temperature with occasional shaking and stirring then the whole mixture was filtered and the filtrate thus obtained was concentrated using a rotary evaporator (Bibby RE200, Sterlin Ltd, UK) to get a viscous mass. The viscous mass was kept at room temperature under a ceiling fan to get a dried extract (about 5.5%). The extract prepared was for thrombolytic effect screening.

Drugs and chemicals

The chemicals used were: ethanol (Merck, Germany), Imipramine hydrochloride and Diazepam (Eskayef Banlgadesh Ltd; Tongi, Bangladesh). Dimethylsulfoxide (DMSO) was from Sigma-Aldrich.

Antidepressant activity assay

Tail suspension test (TST)

TST generally used as behavioral model for evaluating antidepressant activity in mice, was established by Steru et al [11]. Mice were moved from their housing colony to the laboratory in their own cages and then they were allowed adapt to the laboratory conditions for 1-2 h. Mice (n=6) were treated with ethanol extract of *T. orientalis* leaves (200 and 400 mg/kg), positive control group with Imipramine hydrochloride (10 mg/kg) and negative group with 1% Tween-80 10 mL/kg. Each mouse was individually suspended to the rim of a table at a height 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 anybody 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 [12].

Forced swim test (FST)

Forced swim test first designed by Porsoltis *et al*. [13] is frequently used as behavioral model for screening antidepressant-like activity in rodents. According this method, mice were independently forced to swim in open glass compartment

(25×15×25 cm) containing freshwater 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 of that "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 towel dried and returned to their housing conditions.

Anxiolytic activity assay
Elevated plus maze test
The elevated plus maze (EPM) consisted of two open arms (35×5 cm) crossed with two closed arms (35×5×20 cm). The arms were connected together with a central square of 5×5 cm. The apparatus was elevated to the height of 25 cm in a dimly illuminated room. Mice (n = 6) were treated with ethanol extract of *T. orientalis* leaves (200 and 400 mg/kg, p.o.), diazepam (1 mg/kg, i.p.) or 1% Tween-80 10 mL/kg 30 min before being placed individually in the centre of the EPM, facing a closed arm. The time spent in both the open and closed arms was recorded for 5 min [14].

Hole-board test for exploratory behavior in mice
The study was conducted using a wooden board measuring 20 cm by 40 cm with sixteen evenly spaced holes [15]. The animals were randomly grouped into four groups each containing six mice. Control group was treated with 1% Tween-80 10 mL/kg. Examined mice treated with ethanol extract of *T. orientalis* leaves at doses of 200 mg/kg and 400 mg/kg respectively; while those in positive control group received diazepam 1 mg/kg *i.p.* Thirty minutes after treatment, the mice were placed singly on the board and the number of times the mice dipped their head into the holes at the level of their eyes during a five minute trial period was counted using a tally counter.

Statistical analysis
The results were expressed as means ± standard of errors of means (SEM). The data were analyzed using One-Way analysis of variance (ANOVA) followed by Dunnett t-test to determine the level of significance. The mean difference is statistically significant at the 0.05 level. The statistical analysis was carried out using the SPSS program (Version 22.0).

RESULTS

Antidepressant activity
Tail suspension test
In this test (**Table 1**), mice treated with two doses of the ethanol extract of *T. orientalis* leaves (200 and 400 mg/kg) showed decreases in their immobility times, which was significant ($P < 0.05$ and $P < 0.001$) when compared with negative control and the ethanol extract showed antidepressant activity with lowest immobile time (120.86 ± 2.11 sec; $P < 0.001$) at 400mg/kg dose. Similarly, mice treated with Imipramine hydrochloride (10 mg/kg), as expected, showed a significant decrease in the immobility time (86.4 ± 1.35sec; $P < 0.001$).

Forced Swim test
The possible antidepressant effect of EETO after oral administration was studied in the forced swimming test. In this test (**Table 2**), mice treated with two doses of every sample (200 and 400 mg/kg) showed decreases in their immobility times, which was significant ($P < 0.05$-0.001), when compared with negative control (194.27 ± 4.81). The EETO at its dose of 400 mg/kg showed highest antidepressant activity, where it decrease immobile time with 45.97% (104.97 ± 3.32 sec; $P < 0.001$). Similarly, mice treated with Imipramine (10 mg/kg), as expected, showed a significant decrease in the immobility time (88.66 ± 2.93; $P < 0.001$).

Anxiolytic activity
Elevated plus maze test
As shown in **Table 3**, in the elevated plus-maze test diazepam significantly increased the time spent in the open arms. Meanwhile, mice treated with the extract of *T. orientalis* at both doses (200 mg/kg and 400 mg/kg) showed a trend towards increased time spent in these arms, although this did not reach significance at all treatment. EETO and METS at their 400 mg/kg dose, showed significantly ($P < 0.001$) increased the time spent in the open arms.

Hole board test
The number of head dipping was increased significantly ($P < 0.001$) in case of Diazepam treated mice as compared to the positive control mice. The EETO at both dose levels showed an increase in the number of head dipping significantly ($P < 0.05$, $P < 0.001$) as compared to the positive control mice and percentage of increase of head dipping was 63.96% and 87.63%, at 200mg/kg and 400 mg/kg respectively. All other sample treatment also increased number of head dipping, which are shown

in **Table 4**.

DISCUSSIONS

The purpose of this study was assessed the antidepressant-like effect of EETO using animal behavioral models. A major problem in the screening for new antidepressant effect is the establishment of a valid animal model able to sufficiently and accurately identified diverse depressant treatments, without making errors of omission [16]. In that case, the forced swimming and tail suspension tests have been validated as a suitable tool for predicting the antidepressant properties of drugs [17]. The characteristic behavior evaluated in these tests, termed immobility, has been considered to reflect behavioral despair similar to that seen in human depression, and it is well known that antidepressant drugs are able to reduce the immobility time in rodents [18]. It is interesting to note that the immobility shown by mice when subjected to unavoidable stress such as forced swimming test is thought to reflect a state of despair or lowered mood, which is thought to reflect depressive disorders in humans. In addition, the immobility time is reduced by treatment with antidepressant drugs [19]. There is a significant correlation between the clinical efficacy of antidepressant drugs and their potency in the FST, this was not found in any other model [20][21]. Interestingly, our data indicate that higher doses of plant extracts were more effective than smaller doses both in forced swimming and tail suspension tests. The result showed that EETO at 200 mg/kg and 400 mg/kg significantly reduced the duration of immobility time, respectively, in FST and TST ($P < 0.05$ and $P < 0.01$).

The anxiety indicators in the elevated plus-maze (the time spent in the open arms) showed up being sensitive to the agents which were thought to act via the GABAA receptor complex [22]. The therapeutic action of the benzodiazepines and other pharmacological compounds used to treat anxiety, panic, insomnia, and epilepsy is mediated by an enhancement of GABAergic neuronal inhibition through GABAA receptors [23, 24]. Moreover, it is reported that piperine modulates γ-aminobutyric acid (GABA) type A (GABAA) receptors and exhibited antidepressant activity in rodents [25, 26]. In light with these reports, our high-piperine containing methanolic extract have increased anxiolytic-like behavior and exploratory activity in Aβ (1–42)-treated rats. In elevated plus maze test, the extract significantly increased time spent in open arms compared to control and in the hole board test, they also significantly increase in the number of head pokes in comparison to control.

CONCLUSION

The results of the study indicated significant antidepressant and anxiolytic activities of ethanol extract of leaves of *T. orientalis*. The leaf extract of the plant may have potential application in the treatment of conditions like depression and anxiety. But further studies are also required to identify the phytoconstituents responsible for these bioactivities and to establish the mechanism of action of such activities.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Acknowledgements

The authors are grateful to the authority of International Islamic University Chittagong, Bangladesh, for providing the facilities to conduct this research work. The authors thank GUSTO (A research group) for the financial support. The authors are thankful to all members of GUSTO (A research group) for their kind help in the experiments.
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Table 1: Antidepressant activity of ethanol extract of leaves of *T. orientalis* on tail suspension test in mice.

Treatment	Immobile (sec)	% Decrease
Control (1% Tween-80)	207.7 ± 1.97	-
Imipramine hydrochloride (10 mg/kg)	86.4 ± 1.35[b]	58.40
EETO (200 mg/kg)	160.50 ± 2.98[a]	22.73
EETO (400 mg/kg)	120.86 ± 2.11[b]	41.81

All values are expressed as mean ± SEM (n=5). One-Way ANOVA followed by Dunnett t-test: and significance levels are [a]$P < 0.05$ and [b]$P < 0.01$.

Table 2: Antidepressant activity of ethanol extract of leaves of *T. orientalis* on swimming test in mice.

Treatment	Immobile (sec)	% Decrease
Control (1% Tween-80)	194.27 ± 4.81	-
Imipramine hydrochloride (10 mg/kg)	88.66 ± 2.93[b]	54.36
EETO (200 mg/kg)	144.50 ± 4.01[a]	25.62
EETO (400 mg/kg)	104.97 ± 3.32[b]	45.97

All values are expressed as mean ± SEM (n=5). One-Way ANOVA followed by Dunnett t-test: and significance levels are [a]$P < 0.05$ and [b]$P < 0.01$.

Table 3: Anxiolytic activity of different extracts and fractions of leaves of *T. orientalis* on EPM in mice.

Group	Time spent in closed arm	Time spent in opened arm
Control(1% Tween-80)	285.16 ± 1.63	10.15 ± 1.16
Diazepam (1mg/kg)	246.43 ± 2.21[a]	48.27 ± 1.52[b]
EETO (200 mg/kg)	262.26 ± 4.16	31.14 ± 2.38[a]
EETO (400 mg/kg)	253.67 ± 3.76[a]	41.32 ± 2.64[b]

All values are expressed as mean ± SEM (n=5). One-Way ANOVA followed by Dunnett t-test: and significance levels are [a]$P < 0.05$ and [b]$P < 0.01$.

Table 4: Anxiolytic activity of extracts and fractions of leaves of *T. orientalis* on hole board in mice.

Treatment	Number of head dipping	% Increase
Control(1% Tween-80)	28.3±0.75	-
Diazepam (1mg/kg)	66.8 ± 1.30[b]	136.04
EETO (200 mg/kg)	46.4 ± 2.38[a]	63.96
EETO (400 mg/kg)	53.1 ± 2.75[b]	87.63

All values are expressed as mean ± SEM (n=5). One-Way ANOVA followed by Dunnett t-test: and significance levels are [a]$P < 0.05$ and [b]$P < 0.01$.

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