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# **Evaluation of Neuropharmacological Activities of Methanolic Extract of** *Bacopa monnieri* L. in Mice Model

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# ABSTRACT

Now-a-days, the use of natural product has increased its popularity day by day all over the world. One of the most effective therapeutic medicinal plants is *Bacopa monnieri*. In the current study, the methanolic distillate of *B. monnieri* was designed for antidepressant in mice model. *B. monnieri* was evaluated for anti-depressant venture in the forced swimming test (FST), tail suspension test (TST) and elevated plus maze test. In force swimming test, imipramine (30 mg/kg) used as a standard drug and in TST as well as elevated plus maze test, diazepam (10 mg/kg) used as a standard and the plant extract (100 mg/kg and 200 mg/kg) was administered as test group and the control group was given deionized water. In this test, the test extracts (100 mg/kg and 200 mg/kg) compare to both control and standard group. The higher dose (200 mg/kg) represented more significant effect than dose 100 mg/kg. In comparison to the standard and control, at dose 200 mg/kg represented more significant effect at p<0.05 among three of the test.

Keywords: Bacopa monnieri, Neuropharmacological Activities, Methanolic Extract, TST, and Mice Model.

# **INTRODUCTION**

Depression stands for low behavioral & disinclination to the particular action that can have negative impact on ones thinking, motivation, perception, behavior, and other positive senses. It can either be short term or long term (De Zwart *et al.*, 2018). Depression is included in a usual and familiar risky condition for life (Uddin *et al.*, 2017). Patients suffering from depression normally show a reduction in the neurotransmitters like - serotonin, nor epinephrine and dopamine (Brown *et al.*, 1997). Synthetic drugs of neurological disorders have more side effects (such as insomnia, sedation, muscle relaxation, anxiety, weight gain etc.) than their efficacious (Barua *et al.*, 2009; Shazeed-Ul-Karim, 2019). The efficacy of natural (herbal) drugs is better than synthetic drugs as herbal drug is generally considered to be safe for human health (Zhang, 2004).

*B. monnieri* is a small, aquatic herb having several branches, light purple flowers, and tiny oblong leaves. It is commonly known as Brahmi. The plant is spread all along the warm and wet lands zones of our planet and especially in Bangladesh. Traditionally, it is

utilize as a brain tonic to alleviate anxiety or epileptic disorders in patients and to improve intellectual development (Chopra, 1958). Different medicinal plants (including *B. monnieri*) could be considered as alternative medicine source to treat neurological disorders (Galdino *et al.*, 2009).

In Bangladesh, Brahmi is used to prevent miscarriage and to treat rheumatism (Sudharani et al., 2011). It possesses a wide range of traditional medicine values as a neurological tonic to treat toothache, respiratory, cardiac and digestive disorders and purifies blood (Valko et al., 2007; Kumar et al., 2009). Brahmi also possesses analgesic. sedative. antipyretic, antiulcerogenic, anti-inflammatory, antioxidant, antimicrobial, antidiarrhoeal anti-lipid peroxidative and free radical scavenging activities (Sairam et al., 2001; Kishore and Singh, 2005; Afjalus et al., 2013). B. monnieri contains different phytochemicals such as alkaloid brahmine, nicotinine, herpestine, ß-sitosterol, D-mannitol, stigmastanol, stigmasterol and betulinic acid (Chopra et al., 1956; Sastri et al., 1959; Avanigadda and Vangalapati, 2011). The present undertaken examine research was to the neuropharmacological activities of methanolic isolate of B. monnieri in mice models.

## MATERIALS AND METHODS

**Collection of plant material -** Whole plant of *B. monnieri* was collected from the Mongla of Bagerhat district during the first week of December, 2017. The collected plant samples were recognized by Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh.

**Preparation of** *B. monnieri* Methanolic Extract (BMME) - Extraneous materials were removed from the fresh plants by washing. Then the washed plants

were dried under a shade and then powdered by grinder, weighed with analytical balance and stored properly in air tight jar. About 300 g of powder was taken in beaker and then soaked in 1200 ml methanol for 7days. The mixture was stirred at an interval of 18 hours. The solution was elapse through Whatman filter paper no.1 for three times. Next, the solvent was expelled by rotary evaporator at pressure at the temperatures below  $45^{\circ}$  C leaving a residue, deep brown in color. The residue was stored in air tight jar at  $40^{\circ}$  C.

Collection and maintenance of animals - Swissalbino mice were selected for the animal trial. The mice were aged between 4 to 5 weeks and the weighted between 20 to 25 g. The mice were calmed from the Animal Research centre of the Jahangirnagar Dhaka. University, Savar, Bangladesh. The experimental mice were kept in favorable environment (temperature: 25°C, relative humidity: 58-62% and 12 h light/12 h dark cycle) for 2 weeks so that they can get adjusted with the environment. The mice were fed rodent food (commercial formulation) and pure drinking water (Hossain et al., 2009). Here, the animal trial was permitted and accepted by the Institutional Ethical Committee.

**Preparation of sample -** The sample was prepared by dissolving *B. monnieri* Methanolic Extract (BMME) in deionized water at two doses (100 mg/kg body weight, 200 mg/kg body weight).

**Evaluation of Neuropharmacological activity** -Neuropharmacological (antidepressant) pursuit was determined by forced swimming test, TST as well as elevated plus maze test. Drugs and chemicals of neuropharmacological pursuit were representing in **Table 1**.

**Table 1:** Drugs and chemicals of neuropharmacological activity.

Drugs and Reagents	Purpose	Source
Imipramine HCI	Antidepressant drug	Sandoz (Novartis Bangladesh Ltd.)
Diazepam	Depressant drug	Sedil (Square Pharmaceuticals Ltd.)
Deionized water	Control	

**Forced swimming test (FST) -** The forced swimming test was conducted according to the protocol of Porsolt *et al.* (1977) with minor changes. All the mice were kept in a room having controlled environmental condition (temperature: 25°C; humidity: 48-54%; 12h/12h light–dark cycle). Chow and pure drinking water were provided. Mice were exposing to random division for control, standard and two treatment groups (Group-3 and Group-4). Each group was consisted of 5 mice. In standard group, imipramine HCl (30 mg/kg body weight) was administered orally.

Two treatment groups received the extract of *B. monnieri* (100, and 200 mg/kg body weight, respectively), and the control group received deionized water only. Mice were kept in an acrylic cylinder (height 46 cm and diameter 20 cm) which was loaded with water at 25°C of 16 cm depth for 2 minutes. Next to the pre-test session, the mice were exposing to the identical conditions for 4 minutes. A mouse was considered secure when it remained floating in the water, except for negligible movements (Sakakibara *et al.*, 2006).

Tail suspension test (TST) - The TST was carried out according to Steru et al. (1985) with minor modifications. All the mice were kept in a room having controlled environmental condition (temperature: 25°C; humidity: 48-54%; 12h/12h lightdark cycle). Chow and pure drinking water were provided. Mice were exposing to random division for control, standard and two treatment groups (Group-3 and Group-4). Each group was consisted of 5 mice. In standard group, Diazepam (1 mg/kg body weight) was administered orally. Two treatment groups received the extract of B. monnieri (100, and 200 mg/kg body weight, respectively) and the control group received deionized water only. Two stands, each having a clamp situated about 24 cm from the ground surface and they were kept apart at 25 cm intervals. A mouse was hung 6 cm from the tails end on a stand, and the value was recorded for 6 minutes (Sakakibara et al., 2006).

**Elevated plus maze test** - The materials utilized for the elevated plus maze test was in the configuration of a+ and composed of 2 unlocked arms ( $25 \times 5 \times 0.5$  cm) across each other and perpendicular to 2 closed arms  $(25 \times 5 \times 15 \text{ cm})$  with a centered platform  $(5 \times 5 \times 0.5 \text{ cm})$ . The expose arms had a tiny (0.5 cm) wall to reduce the number of falls, whereas the closed arms had a high (15 cm) wall to enclose the arm. The entire system was 50 cm above the floor. The apparatus was build of wood. The platform is white and the walls are hard board. The reason of such variation in materials and color is to ease the measurement of elevated plus maze. The mice were kept in the center and allowed to move through the maze freely. The behavior was noted for 5 min to ensure the chance for phenotypic detection. The unlocked and closed elevated arms caused an exploration conflict. The values of the elevated plus maze test are taken by skilled person (Komada et al., 2008).

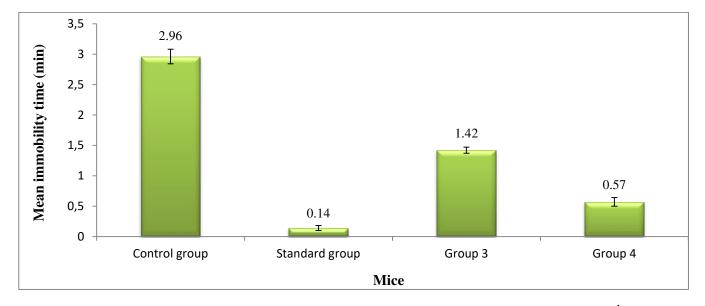
**Statistical analysis** - The data obtained from the experiment was evaluated by SPSS windows version 16.0 using one way investigate of variance (ANOVA) followed by Dennett's post-Hoc analysis. All the results obtained from the experiment were expressed as mean $\pm$  SEM and level of significance was set at p<0.05.

#### **RESULT AND DISCUSSION**

Forced swimming test - The forced swimming test (FST) was one of the better widely used animal models for determining antidepressant effect in mice. The findings were shown in (Table 2 and Fig 1). In forced swimming test, control group of mice showed immobility time of (2.96±0.12) min whereas immobility time of standard drug (imipramine HCl) group mice was (0.14±0.04) min. Test drug BMME at dose of 100 mg/kg showed immobility time of (1.42±0.05) and higher dose of BMME showed immobility time of  $(0.57\pm0.07)$ . When test drugs and standard drug collate to control group of animals, a significant difference was observed in immobility period of both the groups collate to control. Alcoholic extract of BMME at 2 doses of (100, 200 mg/kg) and standard drug imipramine HCl significantly (P<0.01) reduce in immobility time was seen as collate to the control group. In both doses of BMME (100, 200 mg/kg) contributed a greater decrease in immobility time as collate to the control group.

Table 2: The	effect of B.	monnieri	Methanolic	Extract	(BMME)	of in	forced	swimming	test;	Values	of
immobility tim	e were represe	ented in me	$an \pm SEM.$								

Mice group	Dose	Immobility time in minute (mean ± SEM)	% of inhibition
Control group (Deionized water)	10ml/kg	$2.96 \pm 0.12$	0
Standard group (Imipramine HCl)	30mg/kg	$0.14 \pm 0.04^{***}$	95.27
Group 3	BMME 100 mg/kg	$1.42 \pm 0.05^{***}$	52.03
Group 4	BMME 200 mg/kg	$0.57 \pm 0.07^{***}$	80.74



**Fig 1:** Graphical representation of effect of plant of *B. monnieri* on force swimming test on immobility time in mice. Control=Deionized water, 10 ml/mice, Imipramine HCl= 30 mg/kg, Group 3=BMME 100 mg/kg, Group 4= BMME 200 mg/kg, body weight. \* p < 0.05 collate with the control group (Dunnett's test).

**Tail suspension Test** - The TST has become one of the most widely used animal models for assessing antidepressant–like pursuit in mice. The results were summarized in (**Table 3 and Fig 2**). In TST, control group of mice showed immobility time of  $(2.95\pm0.09)$ min, whereas immobility duration of standard drug (Diazepam) group animal was  $(3.33\pm0.09)$  min. Test drug BMME at dose of 100 mg/kg showed immobility time of  $(2.29\pm0.08)$  min and higher dose (200 mg/kg)of BMME showed immobility time was  $(1.37\pm0.18)$ min.

When test drugs and standard drug collate to control group of animals, there was a notably difference in immobility duration of both the groups collate to control. Alcoholic extract of BMME at 2 doses of (100, 200 mg/kg) significantly (P<0.01) decrease in

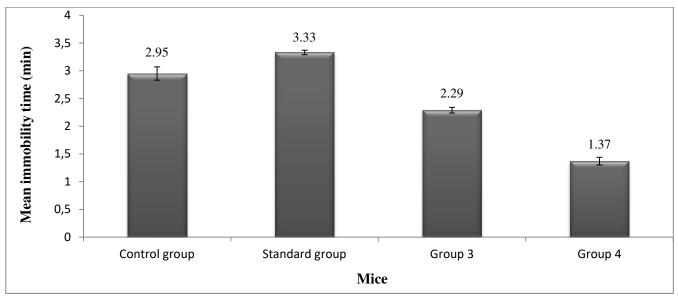
the immobility time was seen as collate to the control group. In both doses of BMME (100, 200 mg/kg) contributed a greater decrease in immobility time as collate to the control group.

**Elevated plus maze test -** In elevated plus maze test, Group 3=BMME 100 mg/kg, Group 4= BMME 200 mg/kg body weight, significantly enlarged the number of complete of mice into the unlocked arms and the time depleted in the unlocked arms of the elevated plus maze. However, the effects of treatment of mice with all doses on unlocked arm entries and time depleted in unlocked arms were near to each other test animals statistically significant. It indicated that the experiment was positive. The results were shown in **Table 4**.

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**Table 3:** The effect of *B. monnieri* Methanolic Extract (BMME) in TST; Values of immobility time are represented in mean  $\pm$  SEM.

Mice group	Dose	Immobility time in minute (mean ± SEM)	% of inhibition
Control group (Deionized water)	10 ml/kg	$2.95 \pm 0.09$	0
Standard group (Diazepam)	10 mg/kg	$3.33 \pm 0.09$	12.88
Group 3	BMME 100 mg/kg	2.29±0.08*	22.37
Group 4	BMME 200 mg/kg	$1.37 \pm 0.18$ ***	53.56

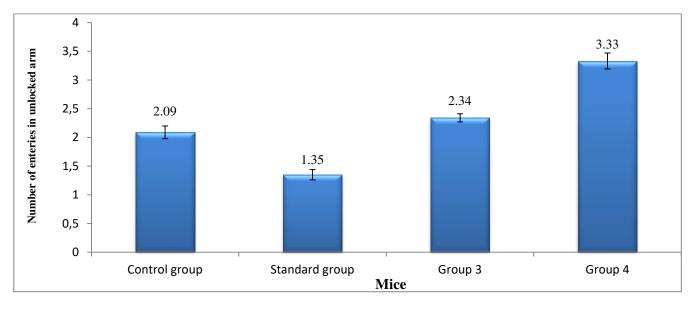


**Fig 2:** Graphical representation of effect of plant of *B. monnieri* on TST on immobility time in mice. Control=Deionized water, 10 ml/mice, Diazepam= 10 mg/kg, Group 3=BMME 100 mg/kg, Group 4=BMME 200 mg/kg, body weight. \*p < 0.05 collate with the control group (Dunnett's test).

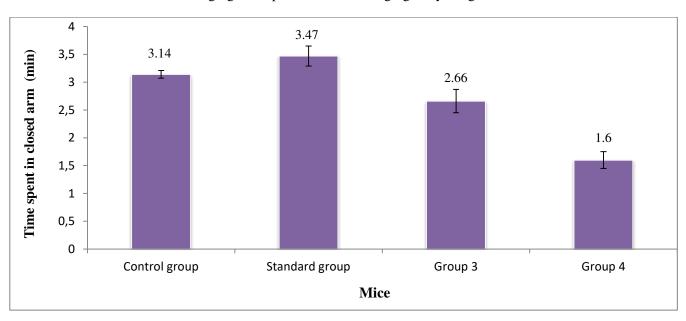
Table 4: The effect of B. monnieri Methanolic Extract (	(BMME) in elevated plus maze test.
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Mice group	Doses (mg/kg)	Time (min) spent in unlocked arm (mean±SEM)	Time (min) spent in closed arm (mean±SEM)	Number of entries in unlocked arm (n±SEM)	Number of entries in closed arm (n±SEM)
Control group (Deionized water)	10 ml/kg	$2.09 \pm 0.11$	$3.14 \pm 0.07$	8.4 ± 0.93	17.2 ± 1.77
Standard group (Diazepam)	10 mg/kg	$1.35 \pm 0.09$	$3.47 \pm 0.18$	$6.4 \pm 0.5$	24.8 ± 2.13***
Group 3	BMME 100 mg/kg	$2.34 \pm 0.07 **$	$2.66 \pm 0.21*$	15.6 ± 1.72*	$12 \pm 1.82*$
Group 4	BMME 200 mg/kg	$3.33 \pm 0.14$ ***	$1.6 \pm 0.15^{***}$	26 ± 1.94***	9.4 ± 0.51**





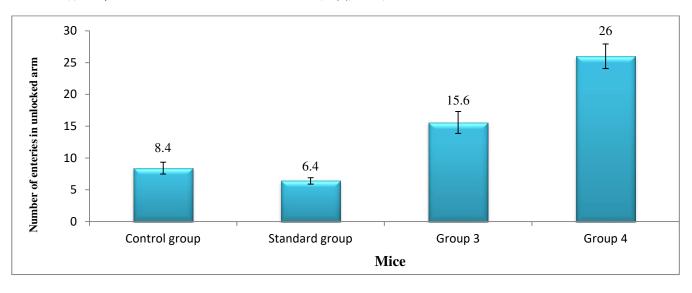
**Fig 3:** Graphical representation of effect of *B. monnieri* on elevated plus maze test of the time depleted in unlocked arms in mice. Control=Deionized water, 10 ml/mice, Diazepam=10 mg/kg, Group 3=BMME 100 mg/kg, Group 4=BMME 200 mg/kg body weight.



**Fig 4:** Graphical representation of effect of B. *monnieri* on elevated plus maze test of the time depleted in closed arms in mice. Control=Deionized water, 10 ml/mice, Diazepam=10 mg/kg, Group 3=BMME 100 mg/kg, Group 4= BMME 200 mg/kg, body weight.

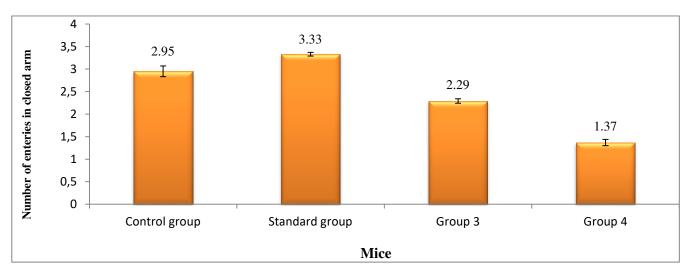
Our findings were almost similar to the findings of the following researchers. In the current experiment, the antidepressant pursuit of brahmi has been assessed in the case of albino mice in FST and TST. Stress performs vital role in depression (Ahmed *et al.*, 2009). These animals face physical stress and thereby suffering through depression. FST and TST mice models of depression that renders a quick as well as well-rounded behavior screening assays for

antidepressants. The immobility has been anticipated to display a phase of behavioral despair and inability to adjust the stress condition (Plaznik *et al.*, 1988). Antidepressant drugs reduce time required for immobility in both the case of FST and TST (Chatterjee *et al.*, 2010). Bacopasides I and II, bacopasaponin C, bacosides A and B, and the *B. monnieri* extract displayed antidepressant capacity.



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**Fig 5:** Graphical representation of effect of parts of *B. monnieri* on elevated plus maze test of the number of entries in unlocked arms in mice. Control=Deionized water, 10 ml/mice, Diazepam=10 mg/kg, Group 3=BMME 100 mg/kg, Group 4=BMME 200 mg/kg, body weight.



**Fig 6:** Graphical representation of effect of parts of *B. monnieri* on elevated plus maze test of the number of entries in closed arms in mice. Control=Deionized water, 10 ml/mice, Diazepam=10 mg/kg, Group 3=BMME 100 mg/kg, Group 4=BMME 200 mg/kg, body weight.

On the other hand, bacopaside VII did not possess any antidepressant capacity while tested on forced swimming and tail-suspension models in experimental mice (Sairam *et al.*, 2002; and Zhou *et al.*, 2007). Behavioral studies in mice had shown that *Bacopa* developed acquisition, retention, motor learning and long term extinction of newly adopted behavior (Singh and Dharwan, 1997). The results reflected the butanolethanol and methanol extracts gradually decreased the immobility times both in TST and FST in mice after oral administration for five consecutive days. In the effective doses for FST and TST, the experimental samples did not show any inhibitory impact against locomotion in mice (Shen *et al.*, 2009). Mannan *et al.* (2015) investigated a potent and dosedependent antidepressant pursuit in several mice models. Their main results of the MEBM gradually reduced the time of immobility in the TST as well as the forced swimming test (p < 0.001).

In the study of Wasnik *et al.* (2015), mice were treated with alcoholic extract of *B. monnieri* at dose of 40 mg/kg and 80 mg/kg for 7 days once daily and it was collate to the control group which were not treated

except the administration of vehicle 1 % Na CMC at the dose of 10 ml / kg. Imipramine (20 mg/kg) which was used as standard, employed daily once for 7 days to measure the antidepressant effect utilizing 2 mice models which were well established i.e. force swim test (FST) and TST. Lower dose of BME was found to be less effective in changing the period of immobility of mice. However, other doses significantly decreased the immobility time of mice in FST and TST.

# CONCLUSION

In the current research FST and TST ensured that *B. monnieri* has an effective antidepressant pursuit in the mice model. The methanol extracts and numerous fractions of *B. monnieri* were investigated for their antidepressant pursuit in the TST and forced swimming test (FST) in mice. Researchers can further investigate the presence of antioxidants in *B. monnieri* that will be helpful in pharmaceutical industries and food industries for the discovery of drugs and food products to maintain human and animal health.

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## **CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest about the publication of the article.

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