

# New insight in neuropharmacological activities of *Dioscorea alata*



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## ABSTRACT

The purpose of our present study was to evaluate the neuropharmacological activities of the methanolic extract of *Dioscorea alata* tuber using mice model. Neuropharmacological activities of this extract were determined using standard behavioral mice models like-elevated plus maze and hole board test for anxiolytic activity; open field, hole cross, tail suspension and force swimming test for exploratory activities of mice. Our result showed that *D. alata* extract possesses significant dose dependent indicative of neophilia in elevated plus maze and hole board test. The time spent in open arm was  $28 \pm 0.95$  seconds (for 250 mg/kg extract feeding group) and  $82 \pm 2.02$  seconds (for

500 mg/ kg extract feeding group); whereas the control groups spent time was  $19.0 \pm 2.17$  seconds. In addition, the crude also showed a significant dose dependent suppression of exploratory activity by decreasing serotonin level of swiss albino mice in open field, hole cross, tail suspension and force swimming test respectively at both doses ( $p < 0.001$ ). The action on inhibition of serotonin and noradrenalin may play important role in motor activity. Finally, it can be concluded that the methanolic extract of *D. alata* showed significant antidepressant and anxiolytic activities, and it might be a potential source of natural antidepressant and anxiolytic agents.

**Keywords:** *D. alata*, Elevated plus maze, Open field, Hole cross

## INTRODUCTION

The number of patients enduring the neurological disorder have been growing around the world specially in developing countries.<sup>1,2</sup> Neurodegenerative disorders like alzheimers disease, parkinsons disease, anxiety, depression are more common.<sup>3</sup> Oxidative stress plays a vital role in neurodegeneration. Free radicals are chemical species having unpaired electron responsible for functioning of oxidative stress. They are important for maintaining normal physiological function. Reactive oxygen species (ROS) includes oxygen centered radicals like  $O_2^-$ ,  $OH^-$ ; and some also non radical derivatives of oxygen species such as  $H_2O_2$ ,  $HOCl$  etc. derived from oxygen and generated by metabolic processes and some other external factors. Excess ROS causes production of lipid peroxides which results in DNA damage, protein and lipid damage leading to neuronal and cellular death.<sup>4,5</sup> Dietary antioxidant and some bodily antioxidant enzyme protect from free radical and ROS induced damage.

Medicinal plants are important candidate for developing new drugs and treating different diseases by discovering and isolating pharmacologically active lead compounds.<sup>6</sup> *Dioscorea alata* usually has known as yam is in the family of Dioscoreaceae. It

is non woody vine, twining attaining 10-15 m in length. Tubers usually single, varying in size and shape, and have succulent caudiciforms with an underground tuberous root. These tubers are edible one which have medicinal and pharmacological properties which are of significant economic importance.<sup>7</sup> Studies have shown that *D. alata* possesses anti-diarrheal and antidiabetic activities.<sup>8,9</sup> It also has antibacterial activity and antioxidant capacity.<sup>10</sup> It has function in neuroprotection against hyperhomocysteinemia-induced selective oxidative stress in brain regions of rats.<sup>11</sup> The aim of our study was to evaluate the total neuropharmacological activities of the tubers of *D. alata* in mice model.

## MATERIALS AND METHODS

### Materials

Standard Diazepam was purchased from Square Pharmaceuticals Limited, Bangladesh. All other reagents needed for this study were analytical grades which were collected from the pharmacognosy laboratory of Noakhali Science and Technology University.

### Collection of plant

Tubers of *Dioscorea alata* were collected from Noakhali, Bangladesh on December, 2016. Collected

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plant parts were washed thoroughly and identified later by the Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh. The voucher specimen no. was (Accession No DACB 42765).

### Preparation of plant extract

The dried and powdered rhizomes (1 kg) were soaked in 2.5L of distilled methanol (99.8%) for about 15 days at room temperature with occasional stirring. After that the mixture was filtered using filter cloth and Whatman's filter paper. The resultant filtrates were then evaporated with the help of rotary evaporator below 40°C to dryness and thus a concentrated semisolid mass of the extract was obtained. The brown granular mass was designated as crude extract of methanol.

### Experimental animals

Swiss-albino mice of both sex, aged 4-5 weeks and weight between 20-30gm, were collected from the animal house of Jahangirnagar University, Dhaka. They were kept in standard environmental condition and fed ICDDRB formulated rodent food and water (ad-libitum). Mice were kept in normal temperature and humidity. As these animals are very sensitive to environmental changes, they were allowed to adapt with the environment for 7 days prior to experimental session.<sup>12</sup>

### Elevated plus-maze test

Elevated Plus Maze (EPM) Test have found acceptance in many laboratories. It has been proposed for selective identification of anxiolytic and anxiogenic drugs. It was designed based on the description of Lister, 1987. The EPM consists of two open arms (35 × 5 cm) crossed with two closed arms (35×5×15 cm). The arms were connected together with a central square of 5×5 cm. The apparatus was elevated to the height of 40 cm in a dimly illuminated room.<sup>13</sup> Swiss albino mice overnight fasting of four groups having 5 mice in each group were administered with four different samples respectively. After 1 hour mice were individually placed in center square facing either one of the open arms. The numbers of entries into the open and closed arms were also counted during the test recorded for 5 minutes.<sup>14</sup>

### Hole board test

In the hole board test, head dipping of mice in a hole at least up to the eye level is observed. An increase number of head dips and the number of rears compared to the controls were considered to suggest an anxiolytic like effect, while the decrease in these variables suggest a sedative effect.<sup>15</sup> The hole-board consists of a wooden box (40 × 40 × 25 cm) with 16 holes (each of diameter 3 cm) evenly distributed on

the floor. The apparatus was elevated to the height of 35 cm. Each group consists of 5 mice, where each group mice were administered different samples respectively. Then each mouse was placed in turn at one corner of the board with the animal subsequently moving about and dipping its head into the holes and the number of head dips for each mouse recorded for 5 minutes periods individually.

### Open field method

This method used for CNS depressant activity tests which were evaluated by Gupta *et al.*<sup>16</sup> Each four groups of mice were administered with four different samples respectively. Each mouse was observed at a time interval (0, 30, 60, 90 and 120 minutes after administration) for 3 minutes to note the number of fields crossed by each mouse in all groups. The mean number squares of open fields crossed by mice of each groups were compared with control group to detect CNS depressant activity. The open field apparatus was constructed of white plywood and measured 72×72 cm with 36 cm walls. One of the walls was clear plexiglas, so mice could be visible in the apparatus. The lines divided the floor into sixteen 18×18 cm squares. A central square of 18×18 cm was drawn in the middle of the open field.<sup>17,18</sup>

### Hole cross method

For measuring the CNS depressant activity in mice, we had used the method of Takagi *et al.* with slight modification.<sup>19</sup> In the middle of a cage, a wood partition was set having a size of 30×20×14 cm. A hole of 3 cm diameter was made at a height of 7.5 cm in the center of the cage. The number of passage of a mouse through the hole from one chamber to other was counted for a period of 3 min at 0, 30, 60, 90 and 120 min after oral administration of the four different samples respectively in each group of mice.<sup>20</sup>

### Tail suspension test

The method was described by Steru *et al.*<sup>21</sup> The mice were hung by the tail with the help of an adhesive tape on a plastic string of 75 cm above the surface. Mice were considered immobile when it hung inactively and completely motionless. The duration time of immobility was counted for a period of 6 minutes after oral administration of the four different samples respectively in each group of mice. Our applied tail suspension boxes (Four-Hour Day, Baltimore MD), made of plastic with the dimensions 55×60×11.5 cm. The mice are suspended in the middle of this compartment, so that it can't contact with walls. To prevent from interact during observing each animal is suspended within its own three-walled rectangular compartment 55×15×11.5 cm.

### Forced swimming test

In this experiment, we used the method of Porsolt *et al.* with minor modification.<sup>22</sup> Each animal was placed individually in a 5 L glass beaker, filled with water up to a height of 15 cm and was observed for a duration of 6 min and last 4 min values were taken for calculation after oral administration of the four different samples respectively in each group of mice. The animal was considered immobile when it floated static or keep its head above the water surface without moving.

### Statistical analysis

Data were expressed as Mean±SEM (Standard Error of Mean), (n=5) and were analyzed statically by using one way ANOVA followed by Dunnet's t-test. All the groups were compared with control. \*p<0.05 was considered to be statistically significant and \*\*p<0.001 was considered to be highly significant.

## RESULTS

### Elevated plus-maze test

This test is done for identification of anxiolytic behaviour of mice treated with methanolic extract of *Dioscorea alata*. In this test, the group treated with plant extract tends to stay more time in the open arm when compared to the control. The time spent in open arm was 28±0.95 seconds (for 250 mg/kg extract feeding group) and 82±2.02 seconds (for 500 mg/kg extract feeding group); whereas the control groups spent time was 19.0±2.17 seconds. Again, the time spent in the close arm for 250mg/kg group was 262±1.82 seconds and 500mg/kg group was 208.2±2.08 seconds, whereas the control groups spent time was 270±1.64 seconds. Results are presented in Figure 1.1 and Figure 1.2.

### Hole board test

This test is done for neophilia or anxiolytic behaviour of mice. We found that methanolic extract of *D. alata* increased the head dipping activity as compared to control group of mice. The average head dipping for methanolic extract of 250 mg/kg group was 34.8±1.66 and 500 mg/kg group was 73.6±5.99, whereas the control group was 27.8±4.97. Comparison of this test result was shown in Figure 2.

### Open field method

The locomotor activity of the group of mice feed with methanolic extract of 250 mg/kg was slightly increased in 5<sup>th</sup> observation, whereas 500 mg/kg slightly decreased the locomotor activity in 5<sup>th</sup> observation compared to the control group which is presented in Figure 3.

### Hole cross method

In this test, locomotor activity was decreased with increase of time. The decrease of motion activity was dose dependent where maximum suppression of locomotor activity was displayed by methanolic extract of 250 mg/kg and 500 mg/kg which is presented in Figure 4.

### Tail suspension test

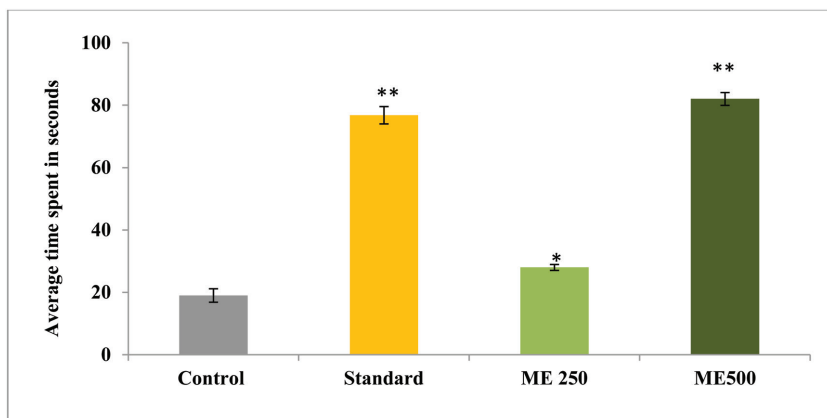
This test is done to assess the antidepressant activity. The average of duration of immobility in seconds for methanolic extract of 250 mg/kg group was 175.2±6.76 seconds and 500 mg/kg group was 137.6±2.56 seconds, whereas the control groups was 210.8±4.97 seconds. Data is represented as graphically in Figure 5.

### Forced swimming test

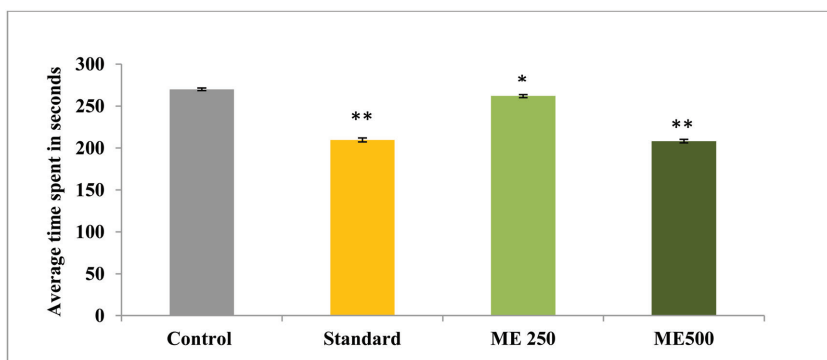
The results obtained by the depressant models tests showed that the effect was dose dependent. The average of duration of immobility in forced

**Table 1** Preparation of test samples

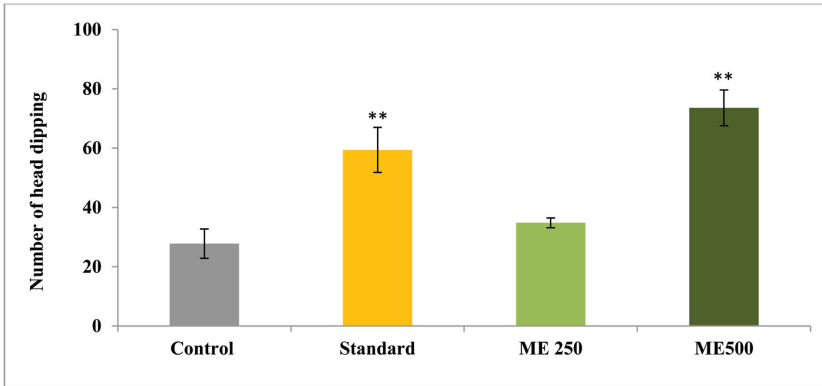
Sample name	Concentration	
Control	10 ml/kg	
Methanolic Extract (ME)	250 mg/kg	500 mg/kg
Diazepam (standard)	1 mg/kg	



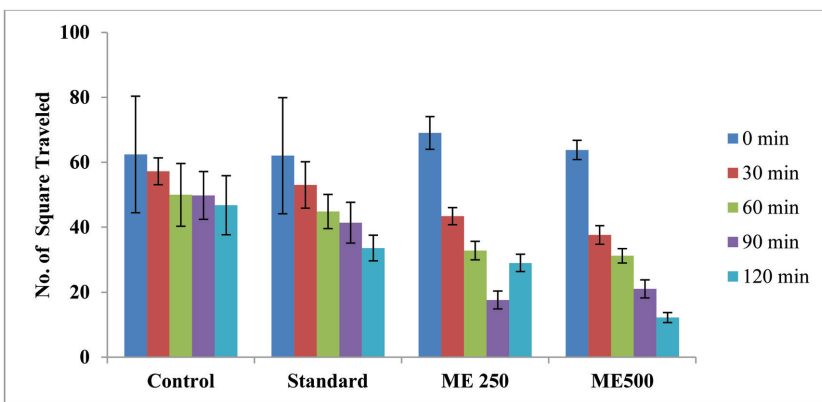
**Figure 1.1** Time spend in open arm of the EPM



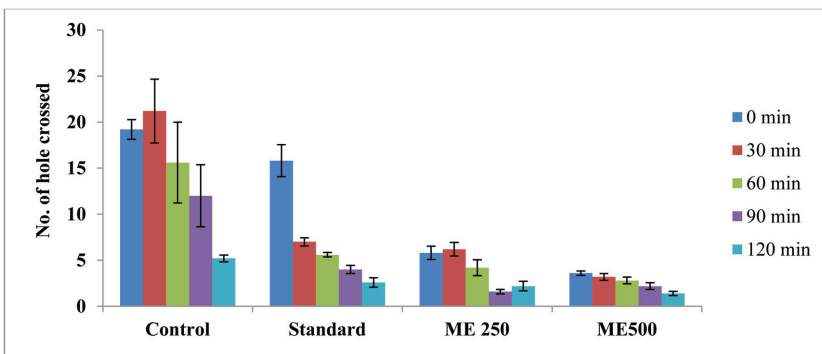
**Figure 1.2** Time spend in close arm of the EPM



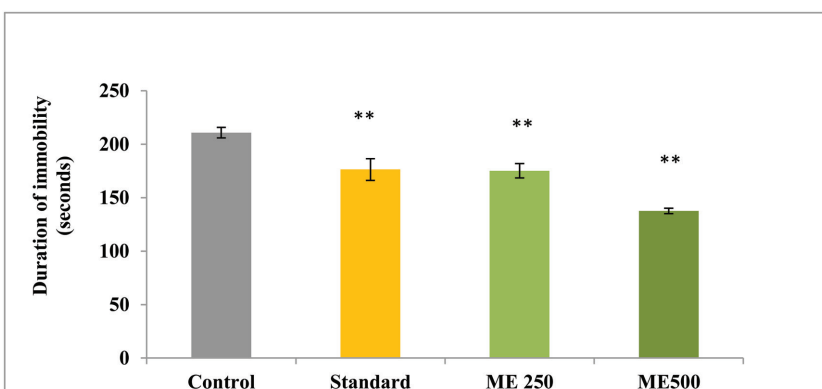
**Figure 2** Number of head dipping in hole board



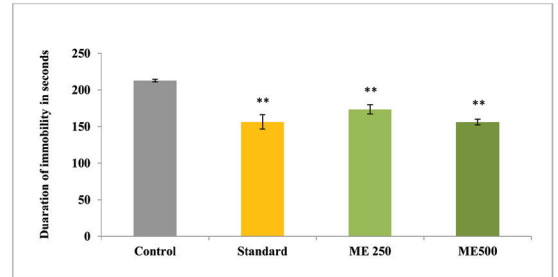
**Figure 3** Movement of mice in open field



**Figure 4** Number of hole crossed by mice



**Figure 5** Duration of immobility in tail suspension test



**Figure 6** Duration of immobility in force swimming test

swimming test for methanolic extract of 250 mg/kg group was  $173.4 \pm 6.43$  seconds and 500 mg/kg group was  $156 \pm 3.85$  seconds, whereas the control groups was  $212.6 \pm 1.72$  seconds. Results are presented in Figure 6.

## DISCUSSION

To evaluate the drug action on CNS, the most important step is to observe its effect on locomotor activity of animal. The measure of increasing activity indicates the level of excitability of the CNS and decreasing activity may be closely related to sedation resulting from depression of the CNS. Our present study was carried out to investigate the neuropharmacological properties of *D. alata* which has not been identified earlier. Here, we described the influence of methanolic extract of *D. alata* on mice behavior in different experimental models.

The EPM is based on the observation that rodents tend to avoid open area. This avoidance is termed as an anxiolytic activity. So it can be said that increase in open arm activity reflects anxiolytic behaviour.<sup>23</sup> In this test, the mice of the methanolic extract of *D. alata* group tends to stay more time in the open arm and less time in closed arm when compared to control. The high levels of head dipping are indicator of neophilia or anxiolytic behavior while low levels are assumed to result from a lack of neophilia or assumed to be reflection of high anxiety like state in the animal.<sup>24</sup> In this test, methanolic extract groups increasing the head dipping activity as compared to control group of mice. From above discussion it can be said that methanolic extract groups reflect high level of neophilia or high anxiolytic activity than the control group of mice. Usually lower the number of movements in open field test indicates locomotion activity and lack of anxiety behaviour. In open field test and hole cross test, the locomotor activity lowering effect was evident from the 2<sup>nd</sup> observation (30 min) and continued up to 5<sup>th</sup> observation period (120 min). In open field



test methanolic extract of 250 mg/kg, locomotor activity was slightly increased in 5<sup>th</sup> observation, whereas 500 mg/kg slightly decreased the locomotor activity in 5<sup>th</sup> observation compared to the control group. But in hole cross test dose dependent decrease of motion was exhibited where maximum suppression of locomotor activity was displayed by 250mg/kg and 500 mg/kg dose of methanolic extract.

The plant extract has remarkable antidepressant activity. The increase in locomotor activity indicates a stimulant effect of the plant extract. This result endorses the study of Leza *et al.*<sup>25</sup> The results obtained by the depressant models tests showed that the effect of the methanolic extract on the reduction of immobility time were more strongly in the tail-suspension model and the forced-swimming test compared to the control groups. This effect of this extract may be due to the action on inhibition of serotonin and noradrenalin which play important role in motor activity.<sup>26</sup> These results are also logical with the study of H<sub>1</sub> antagonist is showed to increase levels of noradrenalin and serotonin in the brain.<sup>27</sup> Extracellular serotonin binds with the receptor on the transporter and causes changes in transporter, serotonin, Na<sup>+</sup>, Cl<sup>-</sup> into the cell. Later binding of intracellular K<sup>+</sup> causes transporter to its original conformation and release of serotonin inside the cell.<sup>28</sup> The monoamine theory also proposed that depression is occurring due to deficiency of serotonin or noradrenaline. On the basis of our study, it can be assumed that methanolic extract of *D. alata* may decrease the serotonin level of mice which indicates the CNS depression capability of this extract.

## CONCLUSION

From the investigation of our study, it may conclude that methanolic extracts of *D. alata* tubers have significant anxiolytic-like effect in mice. It also have moderate antidepressant activity which was found in open field, hole cross, tail suspension and force swimming test. The neuropharmacological active behaviors may be due to the present of bioactive compounds in this plant extract. Advance study is recommended for isolating the bioactive compounds and find out the exact mechanism of actions of these compounds for drug development program.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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