Evaluation of Antidepressant Activity of *Dolichos Biflorus* Methanolic Seed Extract in Animal Models of Depression

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**Abstract**

**Objective:** To evaluate the antidepressant activity of methanolic extract of *Dolichos Biflorus* seeds in Swiss albino mice. **Methodology:** A total of 72 (n=72) Swiss albino male mice were used in the study. They were divided into 12 groups of six mice in each. First six groups were evaluated by Tail Suspension Test (TST) and remaining by Forced Swim Test (FST). First group of mice (control) received normal saline 10mg/kg, second group (standard) received Imipramine 10mg/kg and third, fourth, fifth and sixth groups (test) respectively received methanolic extract of *Dolichos Biflorus* seeds (MEDB) 100mg/kg, 200mg/kg, 300mg/kg and 400mg/kg dose orally for 30 days. They were evaluated for antidepressant activity using TST after 60 minutes of drug administration on 30th day. Duration of immobility was noted for six minutes for each mouse in all groups. Similarly, remaining six groups (7th to 12th) received the same drugs and evaluated for antidepressant activity using FST after 60 minutes of drug administration. **Results:** Results were analyzed by Kruskal-Wallis non-parametric test followed by Dunnet’s test for comparison between groups. The immobility periods in TST were 245±9.7, 80±7.8, 216.4±4.9, 225.3±2.3, 198.4±3.2 and 185.4±2.7 seconds respectively for control, standard and four test groups (100/200/300/400mg/kg). Similarly, immobility periods of 139.33±7.9, 71.66±4.9, 54.7±2.9, 58±5.2, 126.2±7.9 and 113.4±2.8 seconds were noted for FST for remaining six groups of mice. In TST, test drug MEDB in all the doses (50/100/200/300/400mg/kg) failed to show statistically significant antidepressant action when compared to control group (p>0.05). Whereas, in FST, MEDB in a dose of 200mg/kg has shown statistically significant (p<0.05) antidepressant action when compared to control. **Conclusion:** Present study shown methanolic extract of *Dolichos Biflorus* seeds (MEDB) has significant antidepressant activity at the dose of 200mg/kg in FST model of depression in mice.

**Keywords** Methanolic Extract of *Dolichos Biflorus* Seeds (MEDB), Forced Swim Test, Tail Suspension Test

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1. Introduction

Major depressive disorder (MDD) is a mental disorder common in psychiatry wherein patient presents with one of two major symptoms, constant sadness or anhedonia, accompanied by secondary symptoms like feelings of worthlessness, difficulty concentrating, changes in diet, and sleep patterns, for at least period of two weeks.[1] It is a relapsing, remitting illness having greater than 40% rate of recurrence over a period of two years. [2] It must be distinguished from normal grief, sadness, disappointment, and dysphoria or demoralization associated with medical illness and from bipolar disorder in which depression alters with hypomania or mania. The condition is often undiagnosed and frequently undertreated.[3]

Depression often co–exists with other illnesses like anxiety disorders, including panic-agoraphobia syndrome, generalized anxiety disorder, posttraumatic stress disorder, and obsessive-compulsive disorder. Alcohol and other substance abuse or dependence may also present with depression.

Depression results from a combination of multiple etiologic factors—genetic, biochemical, psychodynamic, and socio-environmental. The children of a depressed person are at a higher risk for depression. Monozygotic twins have a higher concordance rate (46%) than dizygotic siblings (20%).[4] Biochemical factors include decrease in the levels of neurotransmitters like nor-epinephrine and serotonin in the brain. Crucial life events, particularly the
death or loss of a loved one or an emotional trauma can precede the onset of depression. Various drugs are available for the treatment of depression. They include monoamine oxidase inhibitors, selective and non-selective monoamine reuptake inhibitors and selective serotonin reuptake inhibitors. These medications work by normalizing the levels of neurotransmitters, notably serotonin and nor-epinephrine. Approximately 60 to 70% of depressed patients respond to conventional antidepressants, if it is given in a sufficient dose for 6–8 weeks. But there is no ideal antidepressant; with rapid onset of action, moderate half-life, a low side-effect profile, minimal interaction with other drugs, and safety in overdose.[5] More over conventional antidepressant drugs have unusual side effects. The need for newer, better-tolerated and more efficacious treatments remains high.

Recently, increased pro-inflammatory cytokines (IL-6, TNF-α, NF-κB), increased Nitric Oxide (L-arginine-NO-cGMP pathway), and increased oxidative stress are implicated in the pathogenesis of depression.[6-8] Moreover, individuals with depression display lower serum antioxidant potentials, coenzyme Q10 (CoQ10), and reduced brain GSH levels as compared to matched controls.[9-12] Moreover, there is increased generation of free radicals (H2O2, O2·) in MDD individuals due to increased serum Xanthene Oxidase (XO) levels.[13,14] A recent post mortem study found increased XO activity in the thalamus and putamen of patients with recurrent MDD.[15]

It was observed that pretreatment with amitriptyline and fluoxetine associated with increased Super Oxide Dismutase activity, and no signs of cell death were observed in cultured pheochromocytoma cells suggesting neuroprotective property.[16] In another study, imipramine, fluvoxamine, or reboxetine inhibited NO production in a dose-dependent manner in an activated microglia cell culture protocol. [17] This has shown that these antidepressant drugs have additional anti-oxidant property. *Dolichos Biflorus* being an ancient ayurvedic drug with anti-oxidant action, the present study was undertaken to evaluate its antidepressant action in albino mice.

### 2. Materials and Methods

Institutional Ethical clearance was obtained before conducting the study. Male Swiss albino mice weighing 25-35 g. were used for the study. The mice were inbred in the central animal house of the Department of Pharmacology, Yenepoya Medical College, Yenepoya University, Mangalore, under suitable conditions of housing, temperature, ventilation and nutrition. The study was conducted in accordance with standard CPCSEA guidelines.

#### 2.1. Drugs
- *Dolichos Biflorus* seeds were purchased from local stores of Mangalore, India. The seeds were authenticated by Professor of Botany Department, Mangala Gangotri University, Mangalore. Dried seeds were grinded using mixer-grinder having 550 watts, 17000 rpm rotating speed electrical motor. The seeds were grinded and crushed well and uniformly for 10 minutes with highest precaution to avoid any contamination and made them as particle-sized powder (<0.5mm). The powdered material was packed in plastic pouches and stored in normal room temperature until use. Seed powder was subjected to solvent extraction with methanol in a soxhlet apparatus. After exhaustive extraction, the methanolic extract of *Dolichos Biflorus* (MEDB) was dried at low temperature under reduced pressure in a rotary evaporator to obtain brownish-black colored residue which was used for antidepressant studies.
- Pure form of imipramine was obtained from Torrent Pharmaceutical Company, Ahmadabad, India.
- 0.9% normal saline was obtained from Yenepoya Pharmacy, Yenepoya Medical College Hospital, Yenepoya University, Mangalore

#### 2.2. Acute Oral Toxicity Test

Acute oral toxicity was done in male Swiss albino mice according to Organization of Economic Co-operation and Development (OECD) guidelines, ANNEX-423. [18] Animals were administered methanolic extract of *Dolichos Biflorus* seeds in a dose of 50,100, 200 and 2000mg/kg per orally to find out safe dose range in animals. Animals were observed for 48 hours from the time of drug administration. Mice were looked for general behavior and mortality.

#### 2.3. Inclusion Criteria
- Male Swiss albino mice weighing between 25-35g.
- Age 3-4 months.
- Healthy with normal behavior and activity.

#### 2.4. Exclusion Criteria
- Mice <25g and >35g and age <3 months and > 4 months.
- Female mice will be excluded
- Animals previously used in other experiments.

A total of 72 animals (n=72) were used. They were divided into 12 groups of 6 animals each. Animals were evaluated for antidepressant activity using two models – Tail suspension test (TST) and Forced swimming test (FST). The experiment was conducted in Post Graduate Experimental Laboratory of the Department of Pharmacology, Yenepoya Medical College, Yenepoya.
University, between 8:00 A.M. to 2:00 P.M. The food and water was removed during study period. Animals were weighed and appropriate dose of drug was given per oral to the different groups for 30 days. The experiment was conducted sixty minutes after the oral administration of the drug. Sufficient gap was maintained for giving the drug so that all the animals were tested after 30 min of drug administration.

2.5. Tail Suspension Test (TST)

Steru et al. developed the Tail Suspension Test to evaluate antidepressant activity. [19] The model used in the present experiment was similar to the original method described. Animals were suspended upside down on a metal rod at a height of 55 cm from the ground level with the help of an adhesive tape placed approximately 1 cm from the tip of the tail. Initially the animals tried to escape by making vigorous movements but when unable to escape became immobile. The animal was considered immobile when it did not show any movement of body and hanged passively. The immobility displayed by rodents when subjected to this kind of unavoidable and inescapable stress has been hypothesized to reflect behavioral despair which in turn may reflect depressive disorders in humans. The total duration of immobility was noted during six minutes period. Each animal was used only once.

The animals for Tail Suspension Test were divided as follows.

- Group I: Received 10ml/kg of normal saline p.o
- Group II: Received 10 mg/kg Imipramine p.o
- Group III: Received methanolic extract of Dolichos Biflorus (MEDB) 100mg/kg p.o
- Group IV: Received methanolic extract of Dolichos Biflorus (MEDB) 200mg/kg p.o
- Group V: Received methanolic extract of Dolichos Biflorus (MEDB) 300mg/kg p.o
- Group VI: Received methanolic extract of Dolichos Biflorus (MEDB) 400mg/kg p.o

2.6. Forced Swim Test (FST)

Porsolt et al. developed the Forced Swimming model to evaluate antidepressant activity. [20] The model used in the present experiment is similar to the original method described. The animals were forced to swim in a plastic cylinder measuring 30 X 30 cm containing water to a depth of 20 cm at room temperature. After an initial two minutes of vigorous activity, each animal assumed a typical immobile posture. The mouse was considered immobile when it remained floating in the water without struggling, making only minimum movements of its limbs necessary to keep its head just above the water level. Total duration of immobility was recorded during next 4 minutes of total 6 minutes duration. The differences in the immobility period were noted after administering drugs in all the group of animals. Each animal was used only once.

Animals for Forced Swimming Test (FST) were divided as follows,

- Group VII: Received 10ml/kg of normal saline p.o
- Group VIII: Received 10 mg/kg Imipramine p.o
- Group IX: Received methanolic extract of Dolichos Biflorus (MEDB) 100mg/kg p.o
- Group X: Received methanolic extract of Dolichos Biflorus (MEDB) 200mg/kg p.o
- Group XI: Received methanolic extract of Dolichos Biflorus (MEDB) 300mg/kg p.o
- Group XII: Received methanolic extract of Dolichos Biflorus (MEDB) 400mg/kg p.o

2.7. Statistical Analysis

Statistical analysis was done by Kruskal-Wallis non-parametric test followed by Dunnet’s test for comparison between groups.

3. Results

In this study, effort was made to evaluate antidepressant activity of methanolic extract of Dolichos Biflorus (MEDB) in albino mice using tail suspension test (TST) and forced swim test (FST). Mice were divided into 12 groups and dosed with normal saline (control), Imipramine (standard) and MEDB (test-50,100,200,300, 400) for 30 days. The antidepressant activity was assessed on 31st day. The immobility time in TST and FST was recorded for each mouse. Surprisingly, the MEDB has failed to show statistically significant (p>0.05) antidepressant activity in TST for all the doses when compared to control and standard groups. However, in forced swim model, MEDB in a dose of 200mg/kg has shown statistically significant antidepressant action when compared to control group.

### Table 1. The effect of Methanolic extract of Dolichus Biflorus (MEDB) on immobility period in Tail Suspension Test

<table>
<thead>
<tr>
<th>Group No</th>
<th>Drug/Treatment</th>
<th>No of animals</th>
<th>Dose (Kg⁻¹)</th>
<th>Immobility Time in sec (Mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Control (NS)</td>
<td>6</td>
<td>10ml</td>
<td>245±9.7</td>
</tr>
<tr>
<td>02</td>
<td>Standard (Imipramine)</td>
<td>6</td>
<td>10mg</td>
<td>80.66±7.8**</td>
</tr>
<tr>
<td>03</td>
<td>MEDB</td>
<td>6</td>
<td>100mg</td>
<td>216.4±4.9</td>
</tr>
<tr>
<td>04</td>
<td>MEDB</td>
<td>6</td>
<td>200mg</td>
<td>225.3±2.3</td>
</tr>
<tr>
<td>05</td>
<td>MEDB</td>
<td>6</td>
<td>300mg</td>
<td>198.4±3.2</td>
</tr>
<tr>
<td>06</td>
<td>MEDB</td>
<td>6</td>
<td>400mg</td>
<td>185.4±2.7</td>
</tr>
</tbody>
</table>
4. Discussion

Herbal medicines have great contribution towards health care. More number of drugs which are synthesized from plants are being approved for the management of diseases. *Dolichos Biflorus* is not only edible beans but also has medicinal properties. It has been used for the treatment of piles, pain, constipation, wounds, urinary calculi, cough, edema, asthma, etc. The seeds of *Dolichos Biflorus* also have antilithiatic, antihepatotoxic and hypolipidemic activity.

Chemical constituents like streptogenin, β-sitosterol, bulbiformin, linoleic acid, polyphenols, oxalates and crude fibers have been identified in *Dolichos Biflorus* plant. A number of isoflavones have been isolated from the leaves and stems namely genistein, dalbergioidin, kievitone, phaseollidin and isoferrerin. [21] The seeds of *Dolichos Biflorus* contain 5-hydroxy-7, 3, 4-trimethoxy-8-methylisoflavone-5-neohesperidoside, genistein, β-sitosterol and 5-o-αL-rhamnopyranosyl (1-2)-β-D-glucopyranosyl. Two Ayurvedic preparations, having *Dolichos Biflorus* as an ingredient, have shown their antioxidant and free radical scavenging activity. [22]

Study by Muthu AK et al. has shown that methanol extract of *Dolichos Biflorus* Linn. has potent antioxidant action in high fat diet fed rabbits. [23] Previous studies by Batoo et al. have concluded that aqueous extract of *Dolichos biflorus* seeds have diuretic activity, helpful in nephrolithiasis. [24] It was also found that *Dolichos Biflorus* has anti-diabetic and hypolipidemic activity in experimental animals. [25] In the view of effectiveness of *Dolichos Biflorus* herb on various disease ailments, effort was made in the present study to assess the antidepressant action of methanolic seed extract of *Dolichos Biflorus* in experimental models. The results of our study have concluded that MEDB in a dose of 200mg/kg has shown statistically significant antidepressant action when compared to control group. The possible mechanism of antidepressant activity could be antioxidant action of *Dolichos Biflorus*. However, further research needs to be conducted to elucidate detailed pharmacokinetic and pharmacodynamic properties of *Dolichos Biflorus*.

5. Conclusions

Present study has shown methanolic extract of *Dolichos Biflorus* seeds (MEDB) has significant antidepressant activity at the dose of 200mg/kg in FST model of depression in mice.

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REFERENCES


