



## NEUROPHARMACOLOGICAL PROFILE OF ETHANOLIC EXTRACT OF *DALBERGIA LATIFOLIA* ROXB IN SWISS ALBINO MICE

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### ABSTRACT

The present study was carried out to evaluate CNS activity of ethanolic extract of roots of *Dalbergia latifolia* that includes general behavior studies, sedative, muscle relaxant, anxiolytic and nootropic activity in mice. The results reveal potential neuropharmacological activity of *Dalbergia latifolia* as nootropic and also having anxiolytic property. Further neurochemical investigation can unravel the mechanism of action of drug with respect to nootropic and anxiolytic activity. Preliminary investigation showed that ethanolic extract of *Dalbergia latifolia* has significant neuropharmacological activity.

**Keywords:** *Dalbergia latifolia*, Ethanolic extract, acute toxicity, Nootropic activity.

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### INTRODUCTION

Herbal medicine emphasizes prevention of disease, rejuvenation of our body systems and it extends the life span and makes healthy life, -balance and harmony<sup>1</sup> Medicinal herbs are indispensable part of traditional medicine practiced all over the world due to easy access, low cost and ancestral experience. *Dalbergia latifolia* (DL), family Fabaceae is used in traditional system of medicine and it is regarded as brain tonic<sup>2</sup> to the nervous system *Dalbergia latifolia* is a vulnerable large tree, shrubs, woody climbers which is found in different geographical locations of India.

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The parts of tree were reported for anti- oxidant<sup>3</sup>, anti bacterial activities<sup>4</sup>.

However, no investigation reports exist pertaining to central nervous system activity, hence we decided to study in experimental animal models, the neuropharmacological effects of ethanolic extract of *Dalbergia latifolia* root that includes general behavior studies, sedative, muscle relaxant, anxiolytic, nootropic studies.

### MATERIALS AND METHODS

#### Plant Material

The roots of *Dalbergia latifolia* Roxb were collected from local areas of Tirupati, chittor District, Andhra pradesh, India.

It was authenticated by Dr. T.Vijaya, Taxonomist in S.V.U College of Sciences, S.V.University Tirupati. A voucher specimen no 18/ SVUCS/2011 of the plant was deposited in the department, for further reference.

#### **Preparation of Extracts:**

The roots of *Dalbergia latifolia* was powdered (950g) and ethanolic extract was prepared using soxhelt extraction process using 2L of ethanol. The ethanolic extract was evaporated under reduced pressure using rotavapor evaporator. The yield of the extract was 18.94% g. A suspension was prepared using 2 % v/v tween 80 and administered orally.

#### **Animals:**

Healthy swiss albino mice of either sex (20–25 gm) were used and they were procured from mahaveer Enterprises, Hyderabad. The animals were housed in clean metabolic cages, maintained in controlled temperature ( $22\pm 3^\circ\text{C}$ ) and light cycle (12 hour light and 12 hour dark). They were fed with standard pellet diet and water libitum. The protocol was approved by the Institutional animal ethical committee (IAEC) of Krishna Theja Pharmacy College (1521/PO/a/11/CPCSEA).

#### **Preliminary Phytochemical Analysis:**

Preliminary phytochemical investigation was conducted as per procedure described by Kokate <sup>5</sup>.

#### **Acute toxicity study:**

Acute toxicity studies were performed according to the OECD 423 guidelines. The overnight fasted mice weighing 20-25gm was selected and divided into groups containing six animals in each group. The single dose of the ethanolic extract of DL starting from 5mg/kg, 50mg/kg, 300mg/kg, 1000mg/kg & 5000mg/kg was administered orally. The drug treated animals was carefully observed individually for the

toxicity signs and mortality. The parameters such as changes in skin and fur, eyes and mucous membranes, circulatory, respiratory, autonomic and central nervous system, behavioural pattern, tremors, convulsions salivation, diarrhoea, lethargy, sleep and coma were observed<sup>6</sup>. The observation was continued for 14 days.

### **NEUROPHARMACOLOGICAL ACTIVITY:**

#### **General Behavior Studies:**

Evaluation of general behavioral profiles was performed by the method <sup>7-8</sup>. Albino mice were divided in to four groups (n=6). Ethanolic extract of *Dalbergia latifolia* was administered for two groups at dose of 100 and 200mg/kg p.o respectively. While the last group was administered diazepam (2mg/kg) as drug control and 2% v/v tween 80 as vehicle control. The animals were under observation for their behavioral changes if any, at 30 min intervals in the first one hour and at the hourly intervals for the next 4 hour for the following parameters.

#### **Awareness, Alertness and Spontaneous Activity:**

The awareness, alertness was recorded by visual measure of the animal's response when placed in a different position and its ability to orient itself without bumps or falls. Animal usually show a moderate degree of inquisitive behavior.

#### **Righting Reflex:**

Groups of mice were treated with the test compounds on the test day. After 15, 30 and 60 min, each mice was placed gently on its back on an undulated surface made of white iron and kept at  $30^\circ\text{C}$ .

If the animal remained on its back for 30 sec, it was considered as a loss of righting reflex.

### **Pinna Reflex:**

The reflex is examined by touching the centre of pinna with a hair or other fine instrument. The unaffected mice withdraw from the irritating hair.

### **Grip Strength:**

The grip Strength test is used to assess muscular function in rodents. It was measured by allowing the animal to grasp a pencil in the horizontal position and noting the time taken by the animal to drop the pencil on the table.

**Touch Response:** The touch response was recorded by touching the mice with a pencil or forceps at the various part of the body (i.e. on the side of the neck, abdomen and groin).

**Pain Response:** The pain response was graded when a small artery clamp was attached to the base of the tail and response was noted.

**Sound Response:** Mice normally utter no sound, so vocalization may indicate a noxious stimulus.

### **Locomotor Activity:**

Locomotor activity (horizontal activity) was measured using actophotometer. Mice were divided into four groups consisting of 6 per group. Two groups received the extract at a dose of 100 & 200mg/kg body wt. The other two groups received control vehicle 2%v/v tween 80 and standard drug (Diazepam 2 mg/kg, i.p). Locomotor activity is easily measured using actophotometer which operates on photoelectric cells connected with a counter. When a beam of light falling on the photocell is cut off by the animal a count is recorded and displayed digitally. Each mice was placed individually in the activity cage floor for 10 min. The animals were placed in the actophotometer for recording the activity score after 60 min of drug and standard administration<sup>9</sup>.

### **Effect on Motor Coordination:**

Mice were divided into four groups consisting of 6 rats per group. Two groups received the extract at a dose of 100, 200 mg/kg body wt. The other two groups received control vehicle 2%v/v tween 80 and standard drug (Diazepam 2 mg/kg, i.p). All the groups of mice were trained to remain on the rota rod for three min.

Only those mice which could balance themselves were selected for the study. The animals were discarded and replaced if they failed to do so. All the group animals were placed on the rota rod and the number of falls within 3 min was noted 60 mins after test drug administration and standard administration<sup>10</sup>.

### **Assessment of Anxiolytic Activity in Mice using the Hole board Apparatus:**

Anxiety level were also evaluated in mice using a hole board apparatus. The hole board apparatus consisted of wooden box (40 x40 x25 cm) with 16 holes (Diameter, 3cm) evenly distributed in the floor. The hole board was elevated to the height of 25 cm. The test was performed 60 min after administration of ethanolic extract of DL (100, 200mg/kg p.o), control vehicle 2% v/v tween 80 and standard drug (Diazepam 2mg/kg, i.p). The number of head poking during 5 min period was recorded and the percentage decrease in head poking was also calculated<sup>11</sup>. An increase of the hole poking response reveals a positive anxiolytic like effect<sup>12</sup>.

### **Nootropic Activity using Elevated plus Maze:**

The nootropic activity was assessed using the elevated plus maze. Mice were divided into four groups consisting of 6 mice per group. Two groups received the extract at a dose of 100, 200 mg/kg body wt. The other two groups received control vehicle 2% v/v tween 80 and standard drug Diazepam (2 mg/ kg, i.p).

The elevated plus maze considered is the exteroceptive behavioral model to evaluate learning and memory. The apparatus consisting of two open arms (50 cms x 10 cms) and two covered arms (50 cms x 10 cms x 40 cms) extended from a central platform (10 cms x 10 cms) was elevated to a height of 50 cms from the floor. On the first training day, each animal was placed at the end of an open arm facing away from the central platform.

Transfer latency (TL) was taken as the time taken by the mice to move into any one of the covered arms with all its four legs. TL was recorded on the training day. If the animal did not enter into the one of the arm within 90secs, it was gently pushed into one of the covered arms and the TL was assigned as 90 secs. The animals was allowed to explore to the maze for 10 secs and then returned to its home cage. Transfer latency was examined on 6<sup>th</sup> day and after 24hrs on 7<sup>th</sup> day of drug treatment. Significant reduction in transfer latency value indicates improvement in memory<sup>13</sup>.

#### **Statistical Analysis:**

The data are expressed as mean  $\pm$  SEM. Statistical analysis was done using one way analysis of variance (ANOVA) followed by Dunnet's test.

#### **RESULTS**

The preliminary phytochemical screening carried out on ethanolic extract of *Dalbergia latifolia* revealed the presence of phytoconstituents such as alkaloids, tannins, flavonoids, carbohydrates, glycosides. The extract did not produce any toxic symptoms of mortality up to dose level of 5000 mg/kg body weight in mice and hence the drugs were considered safe for further pharmacological screening.

Mice treated with extract of *Dalbergia latifolia* and submitted to general behavioral profile studies did not show any difference in their behavior.

The animals showed no signs of depression during the observation period. However, the standard drug diazepam caused a significant depression of all these responses compared with the ethanolic extract of *Dalbergia latifolia*. The ethanolic extract of DL in a dose of (100 mg/kg and 200mg/kg, p.o) did not produce statistically any significant reduction in locomotor activity as compared to the control animals receiving only the vehicle. Diazepam treated groups revealed a statistically significant decrease in locomotor activity as compared to the control. Results were shown in table 1.

There was no statistically significant increase in number of falls within 3 min after the treatment with ethanolic extract at DL which suggests that the extract does not have muscle relaxant property (Results shown in table 2). However, diazepam treated groups showed an increase in the number of falls as compared to the control. The statistical analysis of data obtained indicated that the groups treated with ethanolic extract at dose level of 100 mg/kg and 200 mg/kg, p.o, show no significant increase in number of head poking compared to the control group. However diazepam treated group showed significant increase in exploratory activity thus indicating anxiolytic activity. Results are shown in table 3.

Transfer latency reflected retention of learned task or memory. DL treated animals at a dose of 100 mg/kg and 200 mg/kg, p.o showed dose dependant decrease in transfer latency on 6<sup>th</sup> and 7<sup>th</sup> day when compared to the control group. Higher dose of DL 200 mg/kg p.o more significantly enhanced learning and memory when subjected to elevated plus maze test.

Extract of 100,200 mg/kg p.o treated shows decrease transfer latency of time spent in the arms are compared to standard treated group. Indicating about improvement in learning and memory. Results are shown in table 4.

**Table 1:** Effect of ethanolic extract of *dalbergia latifolia* and diazepam on locomotor activity in mice using actophotometer apparatus

Treatment	Locomotor activity (scores) in 10 min	
	Before treatment	After treatment
Control (vehicle, p.o)	466.15±11.78	450.83±8.72
DL (100mg/kg p.o)	491.5 ± 5.61	416.15 ± 13.41
DL (200mg/kg p.o)	486.81 ± 12.92	403.32 ± 9.12
Standard (Diazepam 2mg/kg i.p)	499.5± 7.96	114.15± 10.76*

Statistical significance test was done by ANOVA followed by Dunnet's 't' test (n=6); Values are mean ± SEM of 6 animals per group; \*P<0.01 vs control; DL- *Dalbergia latifolia*

**Table 2:** Effect of ethanolic extract of *dalbergia latifolia* and diazepam on muscle relaxant activity in mice, studied using rota rod apparatus

Treatment	Falls of time	
	Before treatment	After treatment
Control (vehicle, p.o)	180.7±4.58	178.32±3.12
DL (100mg/kg p.o)	180.4±3.32	169.5±1.55
DL (200mg/kg p.o)	180.2±1.81	177.0±1.35
Standard (Diazepam 2mg/kg i.p)	180.5±2.71	18.4±1.75*

Statistical significance test was done by ANOVA followed by Dunnet's 't' test (n=6); Values are mean ± SEM of 6 animals per group; \*P<0.01 vs control; DL-*Dalbergia latifolia*

**Table 3:** Effect of ethanolic extract of *dalbergia latifolia* and diazepam on anxiety induced in mice using hole board apparatus

Treatment	Number of head pokings
Control (vehicle, p.o)	19.32 ± 2.34
DL (100mg/kg p.o)	18.05 ± 1.02
DL (200mg/kg p.o)	17.15 ± 1.12
Standard (Diazepam 2mg/kg i.p)	8.30 ± 0.31*

Statistical significance test was done by ANOVA followed by Dunnet's 't' test (n=6); Values are mean ± SEM of 6 animals per group; \*P<0.01 vs control; DL- *Dalbergia latifolia*

**Table 4:** Effect of ethanolic extract of *dalbergia latifolia* and diazepam on learning and memory using elevated plus maze apparatus

Treatment	Transfer latency		Entries
	Open arm	Closed arm	Open arm
Control (vehicle, p.o)	44.82±7.22	203.4±9.57	1.6±0.11
DL (100mg/kg p.o)	45.82±5.26 NS	141.72±11.33	1.82 ±0.15
DL (200mg/kg p.o)	49.65±3.76 <sup>NS</sup>	176.16±6.23	2±0.35
Diazepam (2mg/kg)	105.32± 5.21*	115.41±9.62	5.66±0.32

Statistical significance test was done by ANOVA followed by Dunnet's 't' test (n=6); Values are mean ± SEM of 6 animals per group; \*P<0.01 vs control; NS-Non significant

**DISCUSSION:**

General behavior studies suggest that ethanolic extract does not possess any neurotoxicity. The extracts of *Dalbergia latifolia* were found to have no effect on the locomotor activity. Locomotor activity is considered as an index of alertness and a decrease in the activity would indicate sedative activity. Experimental findings suggest the extracts did not demonstrate any effect on the muscle coordination, as indicated by the findings with respect to the rota rod. In our investigation, the extracts did not produce any significant change or increase in the exploratory activity of the

mice in the hole board method, hence, we can conclude that the extract does not possess anxiolytic activity. Generally most of the anxiolytic agents have an adverse effect on memory as seen with the benzodiazepines, commonly used as anxiolytics<sup>14</sup>. Our findings indicated that ethanolic extract treated mice show remarkable dose dependent reduction in transfer latency, indicating significant improvement in memory, thus demonstrating nootropic activity. This facilitatory effect on learning and memory was observed only after treatment for a period of 7 days. This probably may be attributed to the

involvement of neurotransmitters since the building of memory is augmented only when the levels of neurotransmitters are attenuated on repeated administration of the extracts. There is ample evidence demonstrating that the central cholinergic system, serotonergic transmission and noradrenaline function play a vital role in the cognitive function of the brain<sup>15</sup>. Moreover, the lack of effect on locomotor activity works to the advantage of the plant demonstrating nootropic activity. The present findings indicate improvement of learning acquisition and observed anxiolytic

property of *Dalbergia latifolia* root extract, there by validating its claim as a brain tonic in the Indian system of medicine. Considering the lack of need of drugs with proven effect in improving learning, specific memory improving and anxiolytic effect of *Dalbergia latifolia* can be of enormous interest for further neurochemical investigation which can unravel the mechanism of action of drug with respect to activity.

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