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Central nervous system depressant activity of *Barringtonia acutangula* (Linn.) Gaertn

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ABSTRACT

*In the present study Barringtonia acutangula (Linn) Gaertn. (Family: Lecythidaceae) has been selected and evaluated for its therapeutic potential. The coarsely powdered leaves of B. acutangula were extracted with ethyl alcohol by using soxhlet continuous extraction method. After performing the gross behavioral study, the CNS depressant activity was evaluated by sodium pentobarbitone induced sleeping time assay, locomotor activity assay, rota rod test and exploratory activity (y-maze test and hole board test). In these, all the values are expressed as mean Â± SEM, n=6, *p<0.001 significant compared to control. The results altogether indicates that the extract reveals the CNS depressant behavior. The ethanolic extract of B.acutangula leaves causes a maximum inhibition of neuronal activity in the central nervous system leads to its depressant activity.*

Key Words: *Barringtonia acutangula*, Sodium pentobarbitone, Sleeping time assay, Locomotor activity assay, Rota rod test and Exploratory behavior, CNS depressant activity

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INTRODUCTION

A huge number of compounds and drugs are available which depress the central nervous system (CNS) and hypotonic effects [1, 2]. Use of plant products has gained interest in various segment of the population [3]. *Barringtonia acutangula* (family: Lecythidaceae), known as Indian Oak in English, is an evergreen tree with Simple, alternate leaves, 40cm long pendulous racemes, 1.5cm across, fragrant and dark scarlet flowers with 4 lobed ovate calyx and 2 celled ovary. It has Ellipsoid to ovoid Berry, 1.5 x 0.6cm, fibrous, truncate at both ends, crowned by small persistent calyx. The berries possess one ovoid black seed. The literature survey reveals that various parts of *Barringtonia acutangula* have been used as a folklore medicine for curing various diseases cough, hemiplegia, pain in joints, spleenic disorders, stomach disorders, poisoning, anthelmintic, dyspnoea, leprosy, intermittent fever, eye diseases and diarrhoea [4,5].There were no reports on systematic and scientific study of CNS Depressant activity on leaf extracts. In the present study, the CNS Depressant activity of ethanolic extract of the leaves of *Barringtonia acutangula* being evaluated.

MATERIALS AND METHODS

Plant Collection

The fresh leaves of *Barringtonia acutangula* (Linn.) Gaertn was collected which was authenticated by Prof. P. Jayaraman, Ph.D. Director of National Institute of Herbal science Plant Anatomy Research Centre **PARC/2008/197**.

Extraction
The dried leaves were coarsely powdered and extracted with ethyl alcohol by using a Soxhlet apparatus at 60°C. The solvent was completely removed and obtained the dried crude extract which was used for investigation.

Animals
Swiss albino mice (25-35 g) breed in Central Animal House facility of the Institute, were used. They were housed under standard conditions, maintained on a 12 hours light/dark cycle and had free access to food and water up to the time of experimentation. All the protocols were approved by the Institutional Animal Ethical Committee (IAEC) and conducted according to the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

Preliminary Phytochemical Screening
Preliminary phytochemical screening was performed by using ethanolic extracts of *Barringtonia acutangula* (Linn.) Gaertn.to check the presence of various phytoconstituents [6, 7].

ACUTE TOXICITY STUDIES
Acute oral toxicity [8], study was performed as per OECD-423 guidelines (acute toxic class method). Swiss albino mice (n = 6) of either sex selected by random sampling technique were used for the study. The animals were kept fasting for overnight providing only water, after which the ethanolic extracts were administered orally at the dose level of 5mg/kg body weight by intragastric tube and observed for 14 days. If mortality was observed in 2-3 animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher dose such as 50, 100 and 2000 mg/kg body weight.

CNS DEPRESSANT ACTIVITY
Sodium Pentobarbitone induced sleeping time assay
Sleep potentiating effects of the extracts was studied in a group of mice that received Sodium pentobarbitone at a dose of 40mg/kg intraperitoneally, 30 minutes after administration of extracts [9, 10]. There were 6 mice in each group. The sleeping time was measured as the time between disappearance and recovery of the straightening reflex. The doses of the Ethanolic leaf extract were 100, 200 and 400 mg/kg.

Locomotor activity
The Spontaneous motor activity (SMA) was measured using an actophotometer. The movement of the animal cut off a beam of light falling on the photocell and a count was recorded and displayed digitally. Each mouse was placed individually in the actophotometer for 10 min and basal activity score was obtained. 1 h after treatment, mice was placed again in the actophotometer for recording the activity score [11].

Hole board assay
This assay was conducted in a floor of 60cm * 60cm and 30 cm high walls, with four centered and equally spaced holes in the floor, 2cm in diameter each. The mice were placed and released singly in the centre of the board, facing away from the observer. The number of holes explored in 2 min was noted. A decrease in the number of head – dips, reveals a sedative behavior [12].

Rota-rod test
Mice were placed on a horizontal steel rod (32mm diameter) rotating at the speed of 25 rpm. The micecapable of remaining on the top for 3 min or more, in three successive trails were selected for the study [13]. The selected animals were divided into five groups (n=6). Groups were injected intraperitoneally with the extract at 100, 200 and 400mg/kg, propylene glycol (5ml/kg) and diazepam (10mg/kg) respectively. Each group of animals was then placed on the rod at an interval of 30 min. The animals failed more than once to remain on the rotating rod for 3 min were considered as positive for muscle relaxation.

Exploratory activity (Y-maze test)
The test was performed in 5 groups of 6 albino mice at 30, 60, 90 and 120 min after injection of either propylene glycol (5ml/kg), diazepam (10 mg/kg), ethanolic extract (100, 200 and 400 mg/kg) respectively. The mice were placed individually in a symmetrical Y-shaped runway (33 × 38 × 13cm) for 3 min and the number of times a rat entered in the arm of the maze with all 4ft (an 'entry') were counted[14,15].

Statistical analysis
Data obtained from pharmacological experiments are expressed as mean±SEM. Difference between the control and the treatments in these experiments were tested for significance using ANOVA followed by Dunnet's t-test [16].

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening
The preliminary phytochemical screening of powdered drug & ethanolic extract of *Barringtonia acutangula* leaves was performed by standard methods and the results indicated the presence of carbohydrates, proteins, amino acids, steroids, glycosides, alkaloids, tannins & phenolic compounds, flavonoids, fats & oils.

Acute toxicity studies
Acute toxicity studies showed no mortality up to the doses of 2000 mg/kg body weight. So, the extracts safe for long term administration.

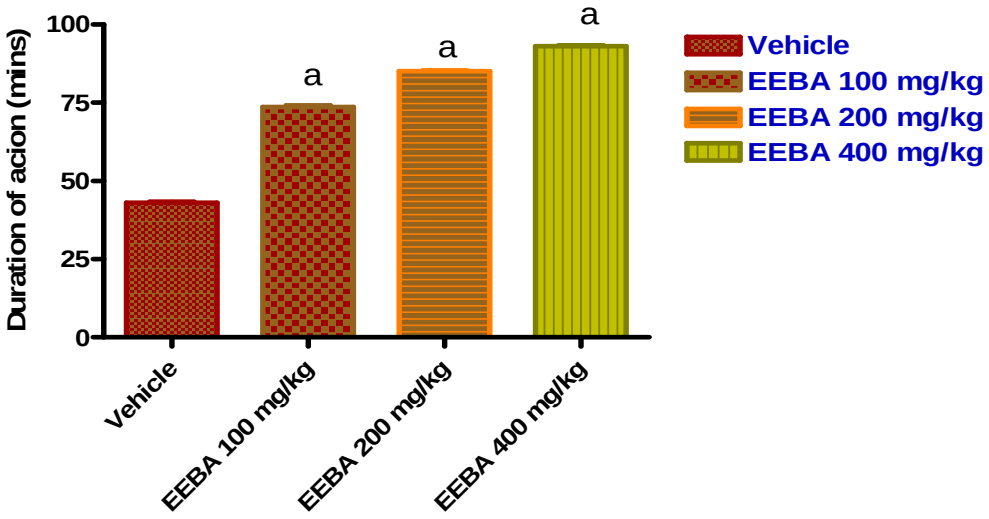
CNS Depressant activity
Sodium pentobarbitone induced sleeping time enhanced significantly when compared to the vehicle group. The extract gave a dose-dependent increase in the phenobarbitone sleeping time (Table 1& Fig 1).

Table 1: Evaluation of CNS Activity by Sodium Pentobarbitone induced sleeping time

Treatment	Dose	Duration of action
Vehicle	-	43±0.577
Ethanolic extract	100mg/kg	73.66±0.61[a]
Ethanolic extract	200 mg/kg	85.16±0.30[a]
Ethanolic extract	400 mg/kg	93±0.57[a]

Each value represents mean ±S.E.M (n=6) and was analyzed by ANOVA Dunnett test compared with control group, [a]P<0.001

Figure 1: Effect of Leaves extract of *Barringtonia acutangula* on Sodium Pentobarbitone induced sleep time in mice



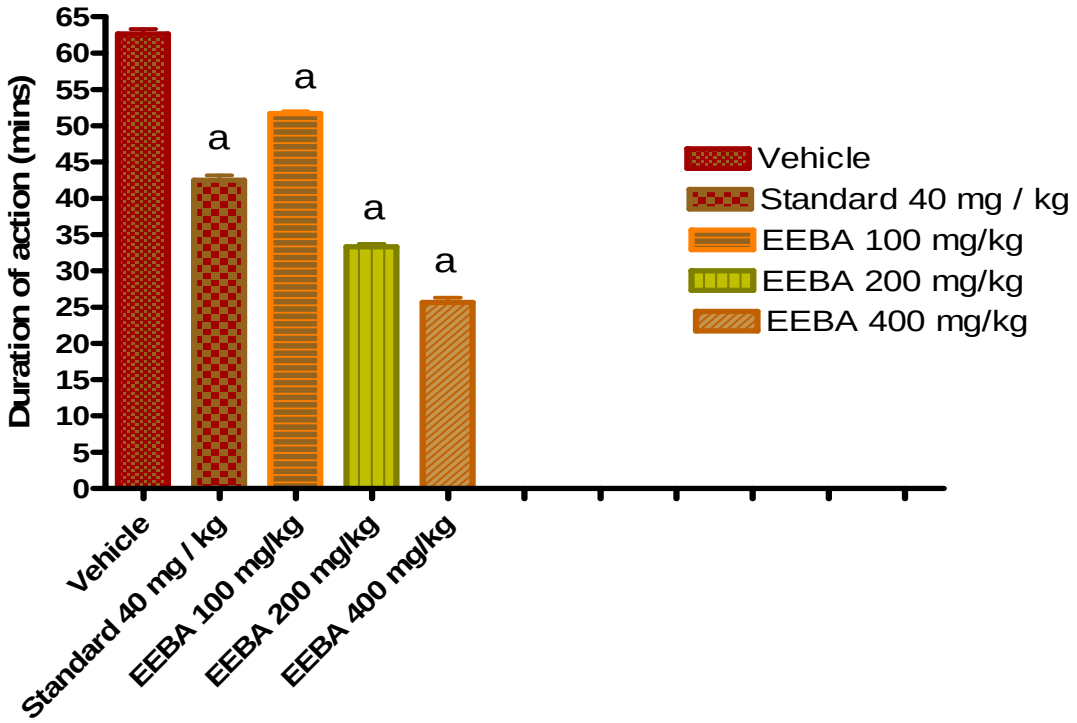
There was a significant (p<0.001) reduction in the sleep latency at the same doses relative to control.

Table 2: Evaluation of CNS Activity by Various Methods

Treatment	Dose	Locomotor activity	Hole board test
Vehicle	-	62.66±0.66	22.33±0.61
Standard (Pentobarbitone)	40 mg /kg	42.5±0.67[a]	10.16±0.47[a]
Ethanolic extract	100mg/kg	51.66±0.33[a]	22±0.36
Ethanolic extract	200 mg/kg	33.33±0.42[a]	17.16±0.60[a]
Ethanolic extract	400 mg/kg	25.66±0.66[a]	13.5±0.71[a]

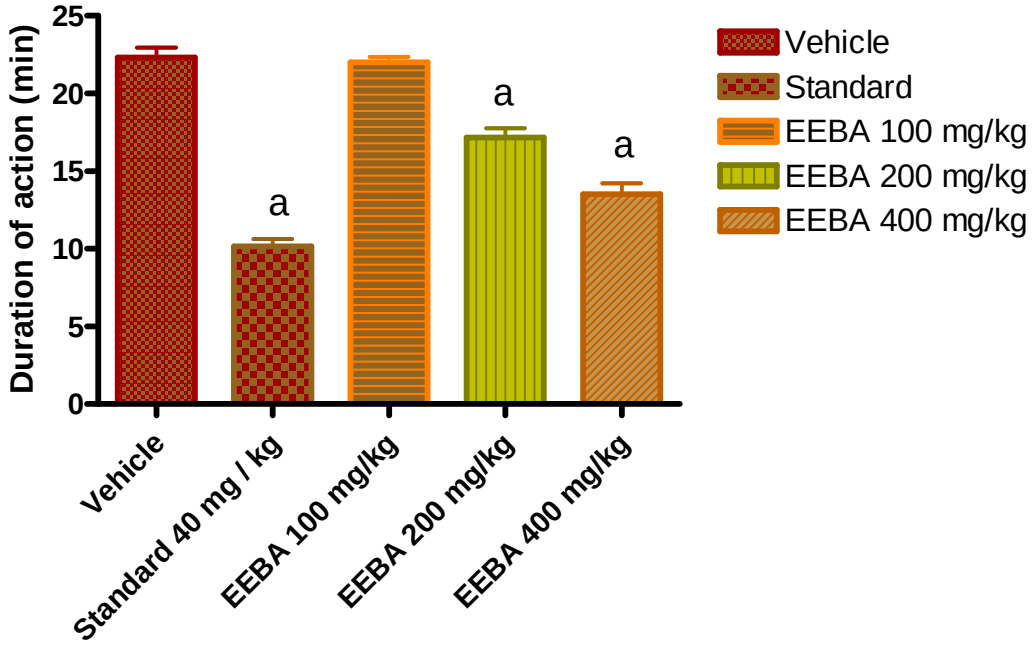
Each value represents mean ±S.E.M (n=6) and was analyzed by ANOVA Dunnett test compared with control group, [a]P<0.001

Figure 2: Effect of Leaves extract of *Barringtonia acutangula* on Locomotor activity using Actophotometer



In the **locomotor activity assay**, all the extracts produced significant ($p<0.001$) and dose dependent reduction in spontaneous motor activity. The frequency and amplitude of movements in the treatment groups was less when compared to the vehicle group (Table 2 & Fig 2).

Figure 3: Effect of Leaves extract of *Barringtonia acutangula* on Hole board assay.



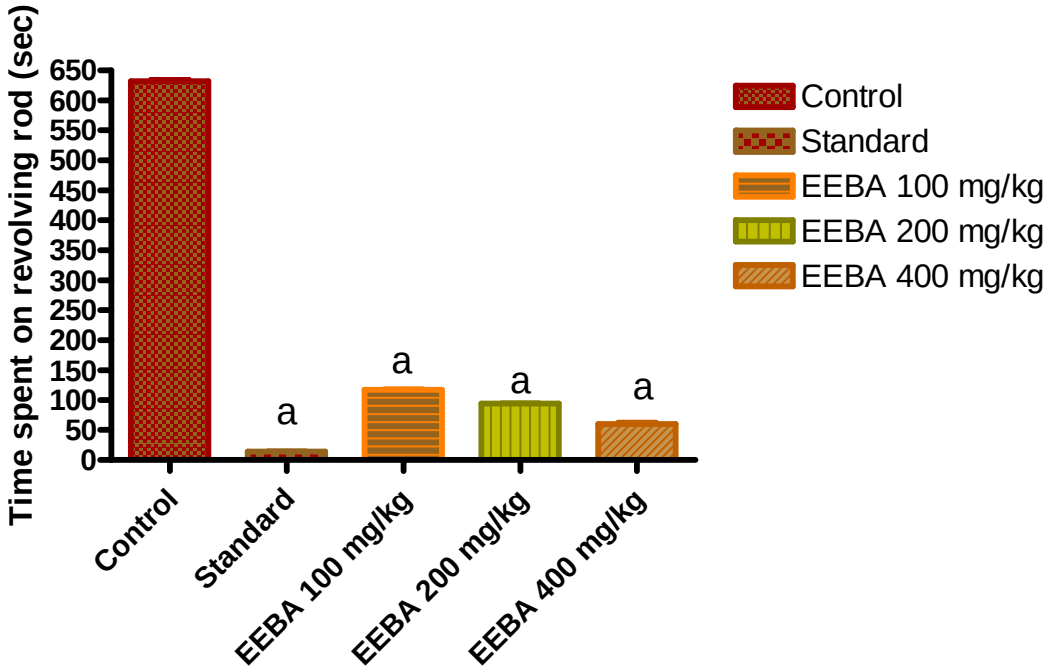
In the **hole board test,** there was a significant (p<0.001) reduction in the head dips responses occurred in mice treated with the extract, compared with the control (Table 2 & Fig: 3).

Table 3: CNS activity by rota rod test

Treatment	Dose	Time spent on revolving rod (sec)
Control	5 ml / kg	631.67 ± 3.77
Standard	10 mg/kg	14 ± 1.41[a]
Ethanolic extract	100 mg / kg	117 ± 2.28[a]
Ethanolic extract	200 mg / kg	94 ± 1.67[a]
Ethanolic extract	400 mg / kg	59.67 ± 4.08[a]

Each value represents mean ±S.E.M (n=6) and was analyzed by ANOVA Dunnett test compared with control group, [a]P<0.001

Figure 4: Effect of Leaves extract of *Barringtonia acutangula* on Rota rod method.



In the **rota rod test**, the ethanol extracts of *Barringtonia acutangula* leaves at the dose level of 100, 200 and 400 mg/kg administered orally exhibited significant (p<0.001) reduction of activity compared with control group of animals (Table 3 & Fig 4) .

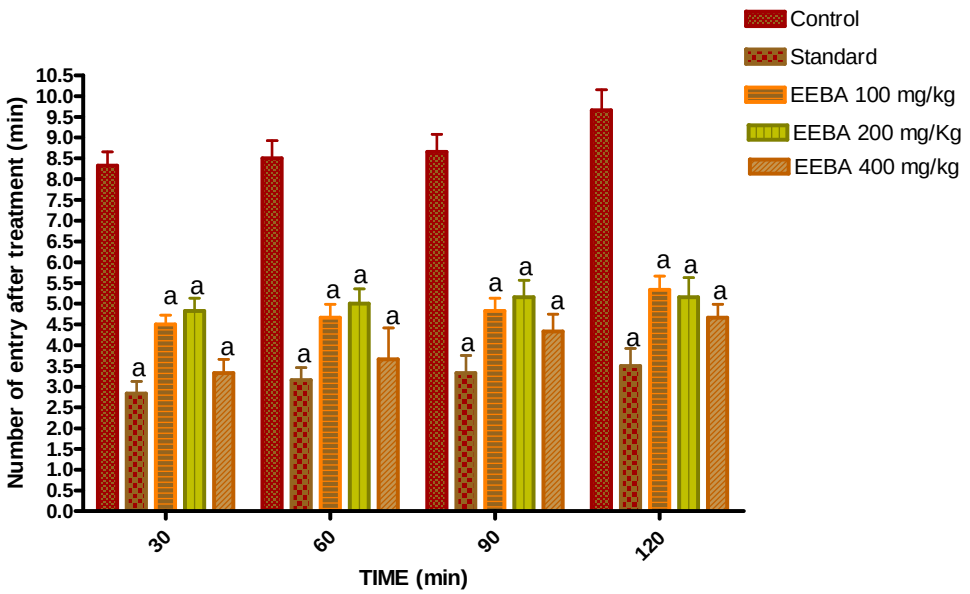
Table 4: CNS activity by y-maze test

GROUPS	DOSE	Number of entry after treatment (min)			
		30	60	90	120
Control	5 ml/kg	8.33±0.33	8.5±0.43	8.66±0.42	9.66±0.49
Standard	10 mg/kg	2.83±0.30[a]	3.16±0.30[a]	3.33±0.42[a]	3.5±0.42[a]
EEBA	100 mg/kg	4.5±0.22[a]	4.66±0.33[a]	4.83±0.30[a]	5.33±0.33[a]
EEBA	200 mg/kg	4.83±0.30[a]	5±0.36[a]	5.16±0.40[a]	5.16±0.47[a]
EEBA	400mg / kg	3.33±0.33[a]	3.66±0.76[a]	4.33±0.42[a]	4.66±0.33[a]

Each value represents mean ±S.E.M (n=6) and was analyzed by ANOVA Dunnett test compared with control group, [a] P<0.001.

In the **Y-maze test**, the animals treated with the extract in tested doses have shown a marked significant (p<0.001) decrease in exploratory behavior compared with controls (Table 4 & Fig 5).

Figure 5: Effect of Leaves extract of *Barringtonia acutangula* on Hole board assay.



To test the neuro-psychopharmacological activities of prose compound(s) animal methods are rapidly used [17].The release of oxygen free radicals has been reported during the recovery phases from many pathological noxious stimuli to the central tissues. A number of *invitro* studies have shown that antioxidants can protect nervous tissues from damage due to oxidative stress [18]. The general aim of the studies reported here was to detect the possible CNS depressant action of a number of available Flavonoids [19, 20, 21]. The general CNS depressant activity was confirmed in the spontaneous locomotion test where the ethanolic extract of *B.acutangula* significantly reduced spontaneous motor activity. The reduction in motor activity indicates the level of excitability of the CNS [22] and this may be related to sedation resulting from CNS depression [23].

The sedative effects of drugs can also be evaluated by measurement of the sleep time induced by Pento barbital in laboratory animals [24, 25]. The ability of ethanolic extract of *B.acutangula* to potentiate pentobarbital induced hypnosis could be attributed to the effects on the central mechanisms involved in the regulation of sleep [26, 27] or to an inhibition of pentobarbital metabolism [28]. The CNS depressant activity may be due to the increase in the concentration of GABA in brain [29]. Many flavonoids were found to be ligands for the gamma amino butyric acid type A (GABA A) receptors in the central nervous system (CNS), which led to the hypothesis that they act as benzodiazepine-like molecules [30]. Furthermore, a detailed investigation regarding phytochemical Isolation, Characterization is needed for explaining exact mechanism for CNS depressant activity.

CONCLUSION

From the findings of the present study it can be concluded that the ethanolic extracts of *Barringtonia acutangula*leaves possesses significant CNS depressant activity by performing sodium pentobarbitone induced sleeping time assay, locomotor activity assay, rota rod test and exploratory activity (y-maze test and hole board test).the ethanolic extracts of *B. acutangula*leaves caused a dose dependent reduction in motor activity in mice.Present work was a preliminary effort, and further detailed investigation on isolation and, characterization of active principle(s) and require for development of effective drug for clinical use.

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