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Research Article

PSYCHOPHARMACOLOGICAL EVALUATION OF ETHANOLIC EXTRACT OF LEAVES OF BAUHINIA TOMENTOSA L. IN MICE

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Abstract

Aim: The present study was designed to investigate the effects of ethanolic extract of leaves of Bauhinia tomentosa L (EEBT) in several Psychopharmacological experimental models.

Methods: Swiss albino mice of either sex, weighing about 20-25 gm were used. Anxiolytic activity was done by elevated plus maze model, Hole board test and Light dark model. Antidepressant activity was done using Actophotometer (locomotor activity), forced swim test, Diazepam induced sleeping time in mice. Nootropic activity using elevated plus maze model, Motor coordination activity using Rota rod test and Catatonic activity by Haloperidol induced catalepsy were done.

Results: The extract at doses (200 and 400 mg/Kg, p.o.) revealed the anti-anxiety activity. The depressant activity was confirmed by spontaneous locomotor activity and forced swim test at the dose levels of (200 and 400 mg/Kg, p.o.), and diazepam-induced sleeping time in mice at the dose of 200 mg/Kg, p.o. In dose of 400 mg/Kg, p.o. , the extract significantly increased the inflexion ratio, which confirmed its nootropic activity. The extract (200 and 400 mg/Kg, p.o.) has shown no significant effect on motor co-ordination and potentiated the catalepsy at 30 min. after haloperidol administration at (200 mg/Kg, p.o.).

Conclusion: The results of the present work suggested that the EEBT has an anxiolytic and Central nervous system

(CNS) depressant activity with mild antipsychotic activity which may be due to the presence of flavonoids as its important phyto constituent, exhibiting its action through $GABA_A$ receptors in CNS.

Key Words: Bauhinia tomentosa, anxiety, depression, Psychopharmacological activity.

Introduction

In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems[1-6]. Medicinal plants have been useful in the development of new drugs and continue to play an invaluable role in the drug discovery processes[7]. These herbs are selectively cheap and available and their uses are dependent on ancestral experience[8-10]. A search for novel pharmacotherapy from medicinal plants for psychiatric illnesses has progressed significantly in the past decade. This is reflected in the large number of herbal preparations for which psychotherapeutic potential has been evaluated in a variety of animal models[11-13].

Mind-altering drugs, especially plants, have always fascinated human beings surrounded by mystic superstitions, magic thoughts and religious rituals, they have always occupied man's attention among the plants used by humans, those able to alter the conscience and the sensorium have drawn special considerations[14]. Researchers even in the advanced world are exploring their traditional remedies to find a suitable cure for "Mind affecting diseases" which have been the out come of man's zest to win the nature[15].

The plant (Bauhinia tomentosa) is used in folk medicine for its Anti dysenteric, anthelmentic and aphrodisiac effects. Bruised bark ground with rice water made into a paste is applied externally to tumours and wounds such as scrofulous. Fruit is diuretic. Seeds may be eaten for their tonic action and made into paste with vinegar as an efficacious application for snake bites and scorpion sting[16].

The neuroprotective actions of dietary flavonoids involve a number of effects within the brain, including a potential to protect neurons against injury induced by neurotoxins, an ability to suppress neuroinflammation, and the potential to promote memory, learning and cognitive functions[17]. Therefore, the consumption of flavonoid-rich foods, throughout life holds a potential to limit the neurodegeneration associated with a variety of neurological disorders and to prevent or reverse normal or abnormal deteriorations in cognitive performance[18]. The literatures

indicate that the leaves of *Bauhinia tomentosa* are rich in flavonol glycosides. The present study has been made to investigate its several psychopharmacological actions in mice.

Materials and Methods

Animals

Swiss albino mice of either sex, weighing 20-25 gm were used. All animals were obtained from the Animal house, K M C H College of Pharmacy, Coimbatore, Tamil Nadu. They were allowed food and water *ad libitum* up to the experimentation period. Prior to use, the mice were housed in polypropylene cages in group of six to eight animals under natural light-dark cycle. Each animal was used only once under standard laboratory conditions. All the observations were made at room temperature in a noiseless diffusely illuminated room and were made between 9.00 to 17.00 hr in the experimental room. All the experimental protocols were approved by Institutional Animals Ethics Committee (IAEC) as per provisions of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

PLANT MATERIALS AND EXTRACTION

Fresh leaves of *Bauhinia tomentosa* L. were collected locally from Tamil Nadu, India and authenticated by P.Satyanarayana, scientist, Botanical survey of India (BSI), Coimbatore, Tamil Nadu. Leaves were separated from adulterants, shade dried and powdered coarsely.

The air dried powdered material (100 g) was taken in 1000 ml soxhlet apparatus and extracted with petroleum ether for 7 days to remove fatty material. At the end of 7^{th} day the marc was taken out and it was dried and again subjected to extraction with absolute ethanol until the colour disappeared. Then the extract was concentrated by distillation. The final solution was evaporated to remove excess of remaining ethanol. Finally the colour consistency of ethanolic extract was noted [19].

CHEMICALS AND DRUGS USED

Diazepam, Chlorpromazine Hydrochloride, Haloperidol, Normal saline.

PHARMACOLOGICAL EVALUATION

DETERMINATION OF ACUTE ORAL TOXICITY

Acute oral toxicity study was performed according to OECD-423 guidelines (acute toxic class method). Swiss albino mice (20-25 gm, n = 6) of either sex were selected by random sampling technique were used for the study. The animals were kept fasting for 4 hours prior to the experiment providing only water after which the extracts (suspended with 0.5% w/v, CMC) were administered orally at a dose of 5mg/kg to separate group of mice and mortality was observed for 3 days. If mortality was observed in 2/3 or 3/3 animals, then the dose administered was assigned as toxic dose. If mortality observed in one animal out of three animals, then the same dose was repeated again to confirm the toxic effect. If mortality was not observed, the procedure was repeated for further doses as 500, 1000 and 2000 mg/kg. The animals were observed for mortality for 24 hr after drug administration[20].

PSYCHOPHARMACOLOGICAL EVALUATION

ANXIOLYTIC ACTIVITY

1 ELEVATED PLUS MAZE MODEL:

Four groups of mice (each group containing 6 animals) were taken. A standard 5 minutes test was employed for each mouse. The elevated plus maze apparatus consists of two open arms (30×5 cm) and two closed arms (30×5×5 cm) that extend from a common central platform (5×5 cm). The floor of each arm is wooden and the walls of the closed arms are also wooden. The open arm edges are 0.5 cm in height to keep the mouse from falling down and the edges of the closed arms are 15 cm in height. The entire maze is elevated to a height of 38 cm above the floor level, as has been validated and described. Mice were placed on the maze 30 min. after diazepam (1 mg/Kg, i.p.) and 60 min. after EEBT (200 and 400 mg/Kg, p.o.) administration. The animal was placed at the centre of the maze, facing one of the closed arms and readings were taken [21-22].

2 HOLE BOARD TEST

Standard hole board apparatus was used for the test. The hole board apparatus 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 25 cm. Four groups of mice, containing six animals each were taken for the study. Mice were placed on the hole board apparatus 30 minutes after diazepam (0.5 mg/Kg, i.p.) and 60 minutes after EEBT (200 and 400 mg/Kg, p.o.) administration. Number of hole dips were recorded[23-24].

3 LIGHT-DARK MODEL

The light-dark apparatus which consist of two compartments was used. The small compartment was painted in black and illuminated with dim light, whereas the large compartment was painted in white and brightly illuminated with 60 W cold light source. Thirty min after the administration of diazepam (1 mg/Kg, i.p.) and 60 min after the administration of EEBT (200 and 400 mg/Kg, p.o.) mouse was placed individually in the centre of the light compartment (facing away from the door). Four groups of mice were used, containing six animals each. During 10-min test period, measurements were noted[25].

ANTIDEPRESSANT ACTIVITY

1. Locomotor Activity

The photocell activity cage was utilized to determine the degree of depression. The actions of plant extract on spontaneous locomotor activity were measured automatically by using Actophotometer, (Medicraft photo actometer, model No: 600-40, S. No: PA-0149, India). The units of the activity counts were arbitrary and based on the beam breaks by movement of the mice. The spontaneous locomotor activity of each mouse was recorded individually for 10 minutes using actophotometer. Basal activity score of all animals were noted before administration. Mice were re-tested for activity scores 30 min. after chlorpromazine (3 mg/Kg, i.p.) administration and 60 min. after EEBT (200 and 400 mg/Kg, p.o.) administration, respectively. Six mice were used for each treatment group[26].

2. Forced Swim Test (FST)

Forced swim test was proposed as a model for an antidepressant activity. Mice were forced to swim individually for 15 min. in glass cylinder (30 cm high, 22.5 cm in diameter) containing 15 cm water at room temperature. Animals were individually trained in 15 min sessions. This constituted the “pre-test session”. Twenty-four hours later, the animals were treated with extract (200 and 400 mg/Kg, p.o.) or vehicle (control group) and each animal was again forced to swim in similar environment for a period of 6 min in a “Test Session” and duration of

immobility time for each mouse was recorded. Each experimental group consisted of 6 mice and was chosen by means of completely randomized schedule[27].

3. Diazepam-Induced Sleeping Time

Three groups of animals (each contained 6 mice) were used to determine effect on duration of diazepam-induced sleep. The animals were subjected to pretreatment and treatment. Pretreatment was carried out 30 min prior to treatment. In pretreatment, the control group was treated with normal saline and the test groups were treated with EEBT (200 and 400 mg/Kg, p.o.). In treatment, diazepam (25 mg/Kg, i.p.) was given to all groups. Each mouse was observed and duration of sleep was recorded. Sleeping time in all cases was measured as the time interval between the loss and regaining of righting reflex[28].

NOOTROPIC ACTIVITY

ELEVATED PLUS MAZE MODEL

The elevated plus maze apparatus used was same as described earlier. Mice were placed individually at the end of an open arm facing away from the central platform and the time it took to move from the end of open arm to either of the closed arms (transfer latency, TL) was recorded. Animals were randomly divided into three groups of six mice each. On the first day, mice were allowed to explore the plus maze for 3 min after the measurement of TL. On the following day, mice received vehicle, or extract (200 and 400 mg/kg, i.p.) 1 hour before their placement on the elevated plus maze as before and TL was noted again for 90 seconds. The TL was expressed as retention after 24 hr, calculating the "inflexion ratio (IR)" using the formula described by Bhattacharya *et al.,* and Mrudula *et al.,* [29-30].

$$IR = (L1-L0)/L0$$

Where L0 = transfer latency after 24 hr and L1= initial transfer latency in seconds.

MOTOR COORDINATION ACTIVITY

ROTAROD TEST: Rotarod test was used to determine the effect of drugs on motor co-ordination. A custom build Rotarod apparatus (Medicraft Rota Rod, Model No.519/E-2C, S.NO: MRA-036, Medicraft electro medicals (P) Ltd., Luknow.) was used for the experiment. The instrument (a horizontal rotation device was set at a rate of 25 rpm. Three groups of mice (each contained 6 animals) were taken. Mice were placed on the rod and those animals that

remained on the rod for 3 minutes were selected for the study. The animals were then evaluated for motor coordination. Basal reading (the time each animal falls off from the rod) was noted before administration. Mice were re-tested for fall off time 60 min. after vehicle and EEBT (200 and 400 mg/Kg, p.o.) administration, respectively[31].

CATATONIC ACTIVITY

HALOPERIDOL (HP)-INDUCED CATALEPSY

Catalepsy, defined as a reduced ability to initiate movement and a failure to correct posture, was measured by means of the bar test. To test for catalepsy, mice were positioned so that their hindquarters were on the bench and their forelimbs rested on a 1 cm diameter horizontal bar, 4 cm above the bench. Three groups of mice (containing 6 animals each) were used. The length of time the mice maintained this position was recorded by stopwatch to a maximum of 180 s. The control group was treated with haloperidol (2 mg/Kg, i.p.) 30 min and 90 min before the experiment. The test groups were administered with EEBT (200 and 400 mg/Kg, p.o.) 30 min before haloperidol (2 mg/Kg, i.p.) treatment and the experiment were repeated. Mice were judged to be cataleptic if they maintained this position for 30 s or more[32].

RESULTS AND DISCUSSION

PHARMACOLOGICAL EVALUATION

ACUTE TOXICITY STUDY

The ethanolic extract of leaves of *Bauhinia tomentosa* L. was evaluated for its acute toxicity in mice. No mortality was observed to a dose as high as 2000 mg/Kg, p.o. of EEBT leaves and the results showed that the extract can be used safely in animals upto a dose of 2000 mg/Kg, p.o.

PSYCHOPHARMACOLOGICAL EVALUATION

ANXIOLYTIC ACTIVITY

ELEVATED PLUS MAZE MODEL

The EEBT (200 and 400 mg/Kg, p.o.) and diazepam (1 mg/Kg, i.p.) significantly increased the mean number of entries and mean time spent in the open arms and decreased the preferences to the closed arms when compared

with control. The results were given in the table 1. This indicates an anxiolytic-like activity of the extract.

Table: 1 Effect of ethanolic extract of leaves of *Bauhinia tomentosa* L. on Elevated plus maze

Treatment (mg/Kg)	Mean no of entries in		Mean time spent in (s)	
	Open arm	Closed arm	Open arm	Closed arm
Control (Vehicle)	2.666±0.4944	11.500±0.6708	52.666±2.512	180.333±6.781
Diazepam (1 mg/Kg, i.p.)	10.833±0.6009**	5.167±0.7923**	153.66±7.982**	92.83±2.344**
EEBT (200 mg/Kg, p.o.)	5.666±0.4216**	9.000±0.2582*	117.83±4.269**	144.66±8.204**
EEBT (400 mg/Kg, p.o.)	7.833±0.4773**	7.833±0.4773**	135.50±4.201**	118.16±3.701**

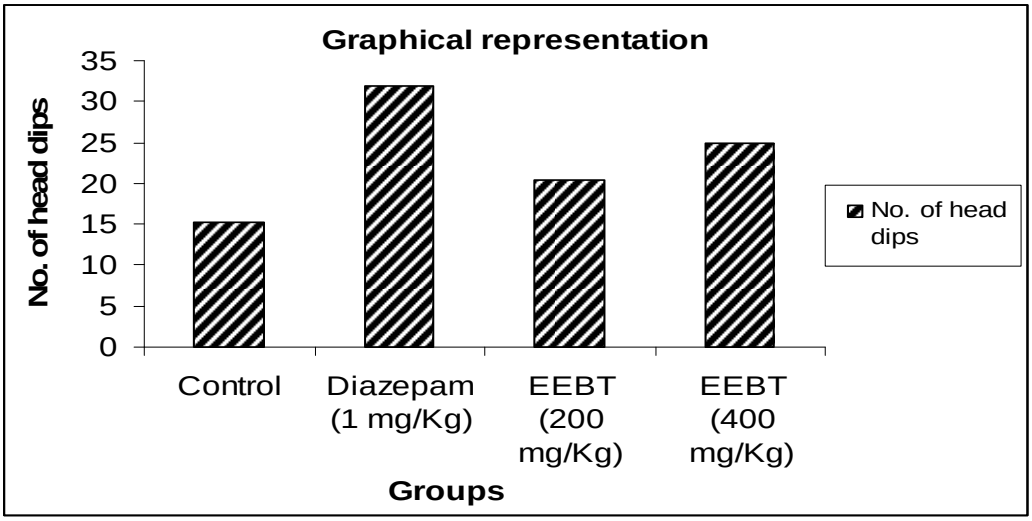
Values are mean ± SEM of 6 animals a group. *p<0.05, **p<0.01

(One way ANOVA followed by Dunnett's test as compared to control group)

Hole Board Test

The EEBT at doses 200 and 400 mg/kg show clear and consistent effects on head-dipping behavior in the hole-board test. Data were given in Fig-1. The number of exploratory head-dips were dose-dependently increased. This observation is consistent with previous reports of an increase in the frequency of exploratory head-dips exhibited on a hole-board following the injection of non-sedative doses of diazepam. However, this effect was reversed at high doses of diazepam, which induced sedation.

Fig-1 Effect of ethanolic extract of leaves of *Bauhinia tomentosa* L. on Hole Board test.



Light-Dark model

The test indicated that the pharmacological profile of EEBT (200 and 400 mg/Kg, p.o.) was similar to that induced by diazepam (1 mg/Kg, i.p.) a well known benzodiazepine agonist. Data were shown in table 2.

Table: 2 Effect of ethanolic extract of leaves of *Bauhinia tomentosa* L. on Light-Dark model.

Treatment (mg/Kg)	Mean no of entries in		Mean time spent in	
	Light chamber	Dark chamber	Light chamber(s)	Dark chamber(s)
Control (Vehicle)	6.667±0.4216	14.833±0.6009	84.667±9.404	412.83±15.469
Diazepam (1 mg/Kg, i.p.)	17.167±1.447**	6.667±0.6146**	389.33±18.390**	94.833±18.702**
EEBT (200 mg/Kg, p.o.)	11.000±0.8563*	11.167±1.046*	246.33±13.177**	165.83±21.626**
EEBT (400 mg/Kg, p.o.)	14.000±1.155**	9.500±1.176**	302.50±26.184**	117.33±13.296**

Values are mean ± SEM of 6 animals a group. *p<0.05, **p<0.01

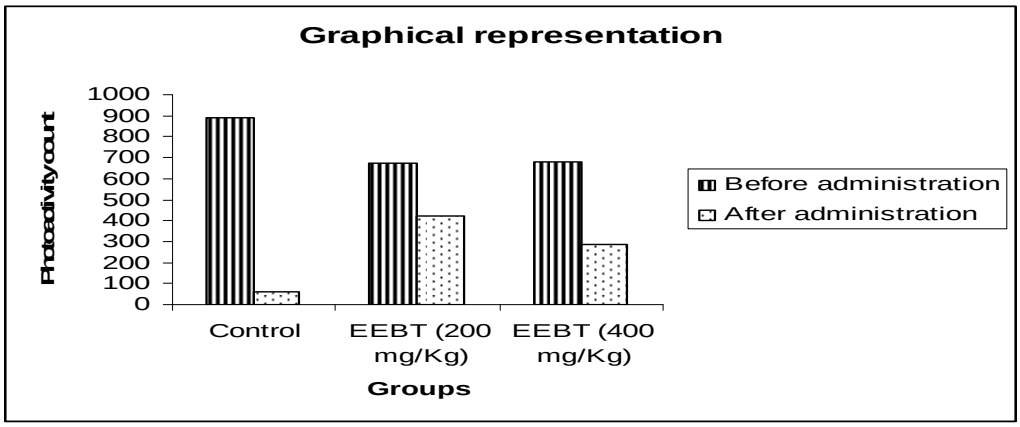
(One way ANOVA followed by Dunnett's test as compared to control group)

ANTIDEPRESSANT ACTIVITY

Spontaneous Locomotor Activity

The effect of ethanolic extract and standard drug on spontaneous locomotor activity (SLA) was summarized in Fig-2. The locomotor activity count in the extract treated groups was significantly reduced when compared to basal value. The standard treated group also revealed the same effect.

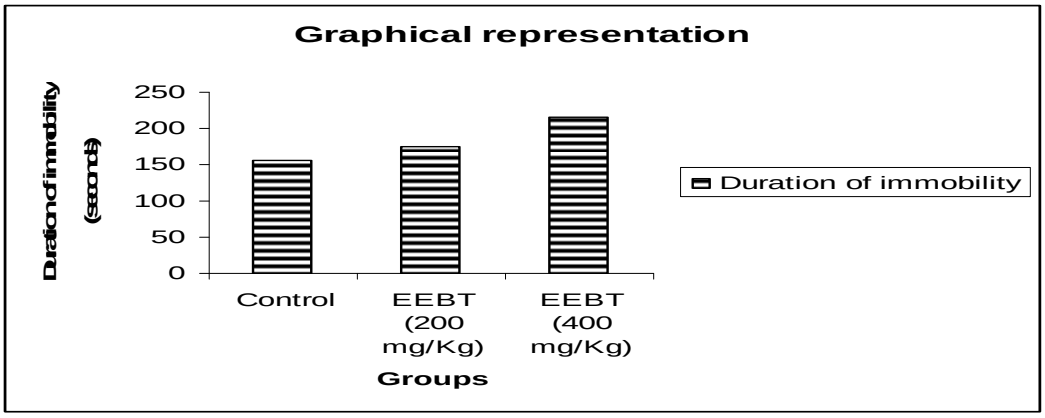
Fig-2 Showing Spontaneous Locomotor Activity



Forced Swim Test

The extract (200 and 400 mg/Kg, p.o.) shown significant increase in immobility period in a dose dependant manner, compared to control. The results were shown in the Fig-3.

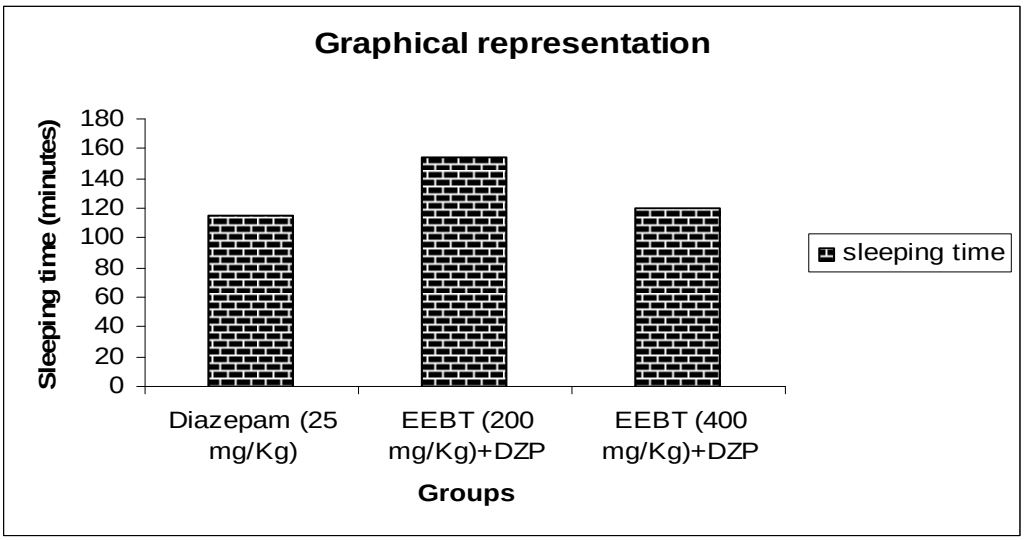
Fig-3 Showing Forced Swim Test.



Diazepam – induced sleeping time

Diazepam induced sleep has been used to elucidate CNS active properties of drugs in animals. The *Bauhinia tomentosa* leaf extract potentiated the diazepam-induced sleeping time in mice (Fig-4). The average sleeping time due to dopamine (25 mg/Kg, i.p.) alone was found to be 115 in minutes. EEBT (200 mg/Kg, p.o.) significantly potentiated the sleeping time, whereas the extract (400 mg/Kg, p.o.) did not shown any significant change in sleeping time induced by diazepam (25 mg/Kg, i.p.). The potentiation of benzodiazepine induced sleep further suggests that the plant possess some sleep inducing property.

Fig-4 Effect of ethanolic extract of leaves of *Bauhinia tomentosa* L. on Diazepam – induced sleeping time.



NOOTROPIC ACTIVITY

Elevated plus maze test

The elevated plus maze is a widely accepted model to study nootropic activity. The increase in IR by the ethanolic extract (400 mg/Kg, p.o.) proved that *Bauhinia tomentosa* possessed nootropic activity. The results were shown in table 3. This finding is interesting with regards to the previous contention that anxiogenic agents can improve cognitive behaviour and anxiolytics can impair learning and memory. Moreover it supports the view that Gamma Aminobutyric Acid (GABA) is involved in the nootropic and anxiolytic activity and that anxiolytics can improve learning and memory.

Table: 3 Effect of ethanolic extract of leaves of *Bauhinia tomentosa* L. on Elevated plus maze for Nootropic activity.

Treatment (mg/Kg)	Transfer latency in seconds		
	Day-1	**Day-2**	**Inflexion ratio**
Control (Vehicle)	37.333±1.626	16.667±1.382	1.341±0.2492
EEBT (200 mg/Kg, p.o.)	47.000±1.713**	15.667±0.8433^{ns}	2.032±0.1554^{ns}
EEBT (400 mg/Kg, p.o.)	54.167±2.535**	11.167±0.833**	4.048±0.5517**

Values are mean ± SEM of 6 animals a group. **p<0.01, ns = non significant

(One way ANOVA followed by Dunnett's test as compared to control group)

MOTOR COORDINATION TEST

Rotarod test

The rotarod method is used to determine the motor co-ordination ability of the extract. It is also used to test the neurotoxicity of the extract. The EEBT (200 and 400 mg/Kg, p.o) had no effect on the motor co-ordination. All the animals stayed on the rotarod for longer than 180 seconds, suggesting that the extract (200 and 400 mg/Kg, p.o) is devoid of neurotoxicity at these particular doses. Absence of observable effects on motor co-ordination by the extract (table 4) suggests that, the inhibitory effects seen in other studies (SLA, FST, diazepam-induced sleeping time) might be elicited centrally and not due to a peripheral neuromuscular blockade[33]. The test also demonstrated the lack of neurological impairment caused by the extract.

Table: 4 Effect of ethanolic extract of leaves of *Bauhinia tomentosa* L. on Rotarod test.

Treatment (mg/Kg)	Fall off time (Seconds)	
	Before administration	After administration
Control (Vehicle)	273.33±9.254	277.83±8.491
EEBT (200 mg/Kg, p.o.)	231.83±16.212	221.66±6.756^{ns}
EEBT (400 mg/Kg, p.o.)	223.50±17.586	234.32±6.448^{ns}

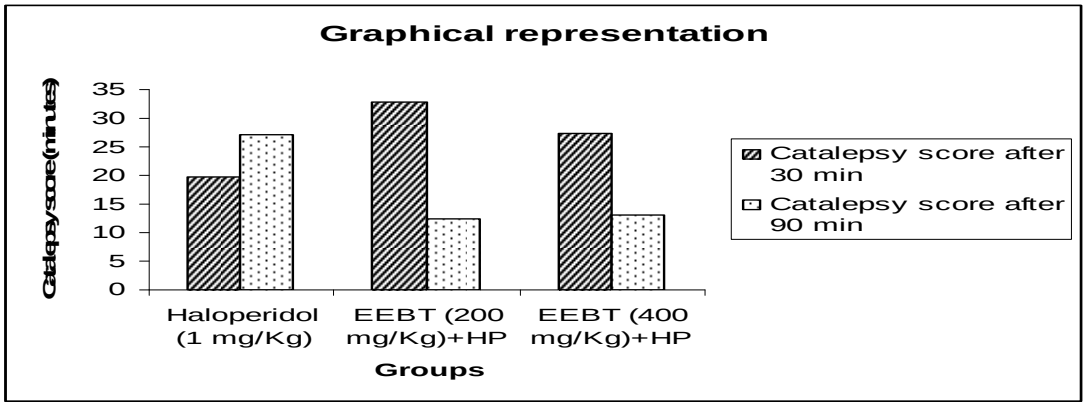
Values are mean ± SEM of 6 animals a group. ns = non significant as compared with basal value.

TEST FOR CATALEPSY

Haloperidol-induced catalepsy

The results showed that the extract (200 mg/Kg, p.o.) potentiates the haloperidol-induced catalepsy initially, after 30 minutes of haloperidol administration (1 mg/Kg, i.p.) and after 90 minutes the catalepsy score was significantly decreased. The extract (400 mg/Kg, p.o.) did not show any significant potentiation of catalepsy after 30 minutes but decreased the score significantly after 90 minutes. Results were given in the Fig-5. The results suggested that the extract act as a mild antipsychotic against schizophrenia at low doses, whereas its anti-cataleptic activity after 90 minutes may be due to its antioxidant activity.

Fig-5 Effect of ethanolic extract of leaves of *Bauhinia tomentosa* L. on Haloperidol – induced catalepsy.



The results of the present work provided evidences that the ethanolic extract of *Bauhinia tomentosa* L. leaves may contain psycho active substances that are depressant in nature. The extract was found to produce alteration in the general behavioral pattern, significant increase in open arm entry-time spent in open arm in elevated plus maze, increase in number of head dips in hole-board test and significant increase in number of entries and time spent in light chamber in light-dark model, significant reduction in spontaneous motor activity, increase in immobility time in forced swim test and potentiation of diazepam-induced sleeping time. The extract significantly increased the IR in nootropic activity, did not induced disturbances in the motor co-ordinaition in rotarod test, and in addition also potentiated the haloperidol-induced sleeping time.

The result that was obtained from anxiety - related behavior models in mice revealed that the EEBT leaves possess an anxiolytic – like effect. Flavonoids are already reported as an anxiolytic agents through the γ-aminobutyric acid type A (GABA -A) receptors in the central nervous system (CNS) [34]. Hence the reported anxiolytic activity of the extract might be due to flavonoids. Further studies are required to confirm the exact mechanism of action.

All the above anti-depressant screening models suggested the CNS-depressant activity of EEBT leaves in mice. The CNS depressant activity may be due to the increase in the concentration of GABA in brains[35]. GABA is known as an inhibitory neurotransmitter in a number of CNS pathways. Studies have also shown that GABA serves as a transmitter at about 30% of all the synapses in the CNS. The present study indicated that the leaf extract significantly increased brain GABA content in mice. According to a study conducted by Saad[36], CNS-depressant drugs increased brain GABA content in mice, and these findings are in agreement with the above anti-anxiety studies that were shown that the anxiolytic like effect of EEBT is mainly due to the presence of flavonoids since they act as a ligand for $GABA_A$ receptors and found to increase GABA content in brain.

Summary and Conclusion

The *Bauhinia tomentosa* L. leaf extracts were studied on elevated plus maze model, hole-board test and light-dark model of anxiety. The extract at doses (200 and 400 mg/Kg, p.o.) revealed the anti-anxiety activity. The CNS depressant activity of the extracts were confirmed by spontaneous locomotor activity and forced swim test at the

dose levels of (200 and 400 mg/Kg, p.o.), and diazepam-induced sleeping time in mice at the dose of 200 mg/Kg, p.o. In dose of 400 mg/Kg, p.o. the extract significantly increased the Inflexion Ratio, which confirmed its nootropic activity. The extract has shown no significant effect on motor co-ordination, hence EEBT is devoid of neurotoxicity at these doses (200 and 400 mg/Kg, p.o.). The effect of EEBT at various doses against haloperidol-induced catalepsy in mice was studied. It was found that EEBT (200 mg/Kg, p.o.) administration potentiated catalepsy in mice at 30 minutes after haloperidol administration. It also suggested that EEBT possessed Anti-anxiety and CNS depressant activity in mice. The activity may be due to the presence of flavonoids as its important phyto constituent which may exhibits its action through $GABA_A$ receptors in CNS.

Therefore the ethanolic extract of the leaves of *Bauhinia tomentosa* may be used in the treatment of CNS disorders.

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