IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
November 2022 Vol.:25, Issue:4

Investigation of Anxiolytic Activity of Ethanolic Extract of *Pithecellobium dulce* Benth. Leaves in Rats

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ISSN 2349-7203
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Submitted: 25 October 2022
Accepted: 31 October 2022
Published: 30 November 2022

Keywords: one-way ANOVA; *Pithecellobium dulce* Benth.; Anti-anxiety; Diazepam Elevated plus maze; Rota rod model; Hole board model; Open field apparatus.

ABSTRACT

The word anxiety is derived from the Latin word “anxietas”. In common words it can be said as feeling of fear may be true or that may be an imagination. Herbal medicines are receiving popularity all over the world due to their safety, efficacy especially in treating various diseases. Now a days demand for medicinal plants are increasing with growth of human needs. *Pithecellobium dulce* Benth. is an evergreen small to medium sized spiny tree belongs to family Leguminosae. Aerial parts of this plant consists of several chemical constituents like alkaloids, phenols, glycosides, flavonoids, tannins and saponins. The present study designed to investigate the effect of Ethanolic extract of *Pithecellobium dulce* Benth. leaves (EEPDBL) 2O0 and 400mg/kg by using experimental models like Elevated plus maze (EPM), Rota rod model (RRM), Hole board model (HBM) and Open field apparatus (OFA). In EPM, number of entries and time spent by the animal in open arm and centre was more significant than low dose (200mg/kg) of extract. High dose of EEPDBL (400 mg/kg) shows decrease in time spent by the animal on Rota rod at different intervals of time, increase in head dipping in hole board model, and increase in locomotory and exploratory activity by animal in Open field apparatus. The interpretation of the results was done by one-way ANOVA followed by Tukey-kramer multiple comparison tests. The Ethanolic extract of *Pithecellobium dulce* Benth. leaves showed significant anxiolytic activity in different models.

INTRODUCTION

The word anxiety is derived from Latin “anxietas” (to choke, throttle, trouble, and upset). In common words anxiety can be said as feeling of fear. Here, the reason for fear may be true or that may be just an imagination. Some general causes of anxiety are like tension of examination, fear to stand in front of crowd. [1] Anxiety disorders are highly prevalent psychiatric disorders. 25% of adult population affected by anxiety at some point during their life time. One of the large surveys found that only 14% of people with psychiatric disorders receives treatment. Anxiety disorders occurring in about 58% of patients with major depressive disorders and 93% patients with bipolar disorder. [2] It is common to be anxious or to worry about fearing things. There is difference for normal worrying and generalized anxiety. Generalized anxiety is excessive, intrusive, persistent, debilitating. [3] Now a days, anxiety becomes one of the common problems in our society, it affects about 40 million adult of age group 18 years and older. [4]

Major symptoms of anxiety are fear, nervousness, dizziness, sweating, uncontrolled urination, headache, sleeplessness, bradycardia, hypertension, isolation feelings, feeling of imminent danger, chest pain, nausea, abdominal discomfort, and heart preparations. [5] Drugs used to treat anxiety by maintaining normal calm level of body and brain is known as anti-anxiety drugs. Anti-anxiety drugs were formerly called as minor tranquilizers. Excess usage of these drugs may cause addiction and habituation. They were classified into different categories such as benzodiazepines, barbiturates, benzodiazepine antagonist and hypnotic agents. Some examples are alprazolam, lorazepam, diazepam, clonazepam and etc. [6]

GABA channels are open by anti-anxiety drugs, penetration of chloride channel increases inside cell which leads to increase negative charge inside the cell, when negativity increases it also increases polarization, but this generated polarization is comparatively longer than normal polarization, so it is also called as hyperpolarization. Hyperpolarized condition delays depolarization state and these moves the post-synaptic potential away from action threshold and inhibit action potential. [7]

Medicinal plants are more popular among rural and urban community in India. [8] Herbal medicines are receiving popularity all over the world due to their safety, efficacy, especially in treating refractory diseases, immunomodulation, and CNS related diseases. [9] Modern science acknowledged their active action and its pharmacological benefits. [10]

Pithecellobium dulce Benth. (Leguminosae) is an evergreen, small to medium sized, spiny tree composed of several chemical constituents like alkaloids, phenols, glycosides, flavonoids, tannins and saponins. [11] Leaves of the plant have been reported to be a folk remedy for leprosy, peptic ulcer, intestinal disorders, toothache, and ear ache. It is also used as emollient, abortifacient, anodyne and larvicidin in folk medicines. [11]

Objectives of this study are to collect, identify and authenticate the *Pithecellobium dulce* Benth. leaves, to prepare Ethanolic Extract of *Pithecellobium dulce* Benth. leaves by using Soxhlet apparatus, to determine the presence of phytoconstituents in crude extract of *Pithecellobium dulce* Benth. leaves, to investigate the Anxiolytic efficacy of the test extract in experimental animals by following below models. Elevated Plus Maze. Rota rod model. Hole board model. Open field apparatus.

MATERIALS AND METHODS

Plant materials

Leaves of *Pithecellobium dulce* Benth. were collected from local garden Nayakanahatty, Chitradurga, Karnataka and they were washed, then the leaves were dried in fresh circulating air under shade for seven days. The leaves material was identified and authenticated by Prof. Dr. A B Banakar, Associate Professor, Govt. Science College, Chitradurga, Karnataka.

Preparation of plant extracts [12]

The leaves were dried in fresh circulating air under shade for seven days, leaves were subjected to size reduction by dry grinder. Dried leaves powder was subjected to Soxhlet apparatus and extracted with ethanol (95% v/v) at 40°C. For each gram of powder 2 ml of solvent was used. The extract was filtered through Whatman No.1 filter paper. The residue obtained was designate as crude extract and stored in a freezer for future studies.

The stock solutions of Ethanolic extracts were prepared using Distilled water and ethanol which is used for following studies:

1. Study of Preliminary phytochemical investigation.
2. Study of anxiolytic activities.

Preliminary phytochemical screening:

Preliminary phytochemical investigations were carried out on the ethanolic extract of

cucumis melo seeds for detection of various phytochemicals by using standard methods prescribed in practical pharmacognosy by C K Kokate and R K Khandelwal.

Experimental animals:

Animal ethics clearance will be obtained from Institution Animal Ethics Committee (IAEC) for experimental purpose **(Ref no: 02/SJMCP/IAEC/2021-22)**. Healthy Adult Wistar Albino rats weighing about 150 to 200g of either sex will be used for this study. The animals will be obtained from Biogen Laboratory Animal Facility Bangalore - 562107. The animals will be acclimatized under laboratory condition with controlled environment of temperature, humidity as per committee for the purpose of control and supervision of experiments on animal (CPCSEA) guidelines. They will be provided with standard diet and water ad libitum.

EVALUATION OF ANXIOLYTIC ACTIVITY

Selection of screening dose: [13]

Screening of anxiolytic activity dose will be considered based on literature of acute toxicity studies of ethanolic extract of Pithecellobium dulce Benth. Leaves, is given by,

Low dose: 200 mg/kg

High dose: 400mg/kg **Experimental Design:**

The obtained wistar rats weighing 150-250g were randomly divided into 4 groups each containing 6 animals. The schedule of dose administration in experimental groups was followed as:

Animals were divided into four groups with six rats each,

Group 1: - Treated with saline (10ml/kg)

Group 2: - Treated with Diazepam (standard drug) (1mg/kg, i.p.).

Group 3: - Treated with low dose of EEPDBL. extract (200mg/kg).

Group 4: - Treated with high dose of EEPDBL. extract (400mg/kg).

1) Elevated Plus maze [14]

Animals were grouped and divided into four groups each consisting of six rats**.** Elevated plus maze test was performed according to Handley and Mithani. Animals of each group control,

standard and treated was given a single oral dose of normal saline, standard drugs and extracts of *Pithecellobium dulce* Benth. at 200mg/kg and 400mg/kg respectively one hour prior to testing. Rats were placed at the junction of the four arms of the maze, facing an open arm. Dose schedule was adjusted in a way that every animal has its 5 min session after hour of administration of drug. Number of entries/durations in each arm was recorded for 5 min. An increase in open arm activity (duration and entries) reflects anti-anxiety behaviour. Precautions were taken to avoid any provoked and abnormal response by maintaining noise free environment.

2) Rota rod model [15]

Animals were grouped and divided into four groups each consisting of six rats. The effect on motor coordination was assessed using a Rota-rod apparatus. Rota rod consist of a base platform and an iron rod of 3 cm diameter and 30 cm length, with a non-slippery surface. The rod was divided into four equal sections by three disks. The animals were pre-selected in a training session 24 h before the test, based on their ability to remain on the bar (at 12 rpm) for 2 min. Then allowing four rat to walk on the rod at the speed of 12 rpm at the same time observed over a period of 30-, 60-, and 90-min. Animals were injected with saline or extract or standard drug one hour prior to testing. Intervals between the mounting of the animal on the rotating bar and falling off of it was registered automatically as the performance time. Time spent in the apparatus was observed for 5 min duration (300 s).

3) Hole board model [16, 17]

Animals were grouped and divided into four groups each consisting of six rats. Hole board model was performed according to Boissier and Simon. The hole board consisted of a wood box (60 x 60 x 30 cm) with four 2-cm diameter holes equidistant in the floor box. Animals were injected with saline or extract or standard drug. 10mins after the injection they were allowed to explore hole board. The number of head dips and the time spent head-dipping was counted during 10 mins. To ensure the cleanliness, hole board was cleaned with 10% alcohol after each trial. Head dipping was considered to take place when the head will be dipped into holes at least to level of eyes. An increase in the number and the time spent head-dipping implies a greater exploratory activity. A decrease of both parameters reveals a sedative behaviour. In this test an anxiolytic-like state may be reflected by an increase in head-dipping.

4) Open field model [18]

Animals were grouped and divided into four groups each consisting of six rats. Open field model was performed according to hall. The apparatus consisted of a wooden box (60 × 60 × 30 cm). The floor of the box was divided into 16 squares (15 × 15 cm). The apparatus is illuminated with a 40-W lamp suspended 100 cm above. Rats were treated with *Pithecellobium dulce* Benth. extract or vehicle. After 30 min, animals were placed individually in one of the corner squares. The number of rearing, assisted rearing (forepaws touching the walls of the apparatus), and the number of squares crossed will be counted for 5 min. Diazepam (1 mg/kg, i.p.) was used as the standard drug. Increase in square crossings indicated the locomotory activity.

Statistical analysis:

The data obtained from the above findings was subjected to statistical analysis using one-way ANOVA followed by Tukey's Kramer Multiple Comparison Test to assess the statistical significance of the results.

RESULTS

Percentage yield of ethanolic extract: 15.70%.

Preliminary phytochemical screening:

Table No.1: Preliminary phytochemical screening of EEPDBL

Constituents	Ethanolic extract of EEPDBL
Carbohydrates	+
Triterpenoids	-
Flavonoids	+
Tannins	+
Glycosides	-
Proteins	+
Resins	+
Steroids	+

(+) Present, (-) Absent

1) Evaluation of Anxiolytic activity of Ethanolic Extract of *Pithecellobium dulce* Benth. leaves by using Elevated Plus Maze.

In Elevated Plus Maze, parameters like time spent and number of entries to open arm, closed arm and centre of control group (10 ml/kg) was compared with standard drug Diazepam (1mg/kg), EEPDBL (200mg/kg) and EEPDBL (400mg/kg). Diazepam (1mg/kg) treated group showed significant increase (***P<0.001) in time spent and significant increase (***P<0.001) in number of entries in open arm and centre. Low dose of EEPDBL (200mg/kg) treated group shows significant decrease in number of entries and time spent in open arm and centre when compared to control group. High dose of EEPDBL (400mg/kg) treated group shows significant increase (***P<0.001) in both number of entries and time spent in open arm and centre compared to low dose (200mg/kg) and control, but this group is not much significant when compared to standard. Animals treated with high dose (400mg/kg) shows more significant results when compared with low dose (200mg/kg) and less significant results when compared with standard group (Diazepam 1mg/kg). Effect of EEPDBL statistically shown in below table and graph.

Table No.2: Results of Elevated Plus Maze

Sl. No	Treatment	Number of entries (counts/5min)			Time spent (sec)in 5mins		
		Open arm	Closed arm	Centre	Open arm	Closed arm	Centre
1	Control (Saline) 10 ml/kg	2.50 ± 0.71	4.33 ± 1.11	2.67 ± 0.49	20.00 ± 7.61	265.5 ± 10.74	14.50 ± 4.121
2	Standard (Diazepam) 1mg/kg	7.50 ± 0.92^{***}	12.67 ± 0.71^{***}	10.50 ± 0.76^{***}	154.8 ± 8.53^{***}	95.20 ± 6.779^{***}	50 ± 9.135^{*}
3	Low dose (EEPDBL) 200mg/kg	5.50 ± 0.84^{ns}	8.66 ± 0.71^{*}	5.00 ± 0.85^{ns}	96.50 ± 5.33^{ns}	162.2 ± 16.53^{**}	41.30 ± 6.04^{ns}
4	High dose (EEPDBL) 400mg/kg	5.66 ± 0.49^{*}	10.50 ± 0.99^{***}	6.83 ± 1.04^{**}	115.6 ± 22.40^{**}	125.15 ± 24.17^{***}	59.25 ± 5.200^{*}

Values were expressed as Mean ± SEM (n=6); significance values are ***P< 0.001, **P<0.01, *P<0.05, ^{ns}P>0.05. Control vs all groups (By one-way ANOVA followed by Tukey-kramer Multiple Comparison tests).

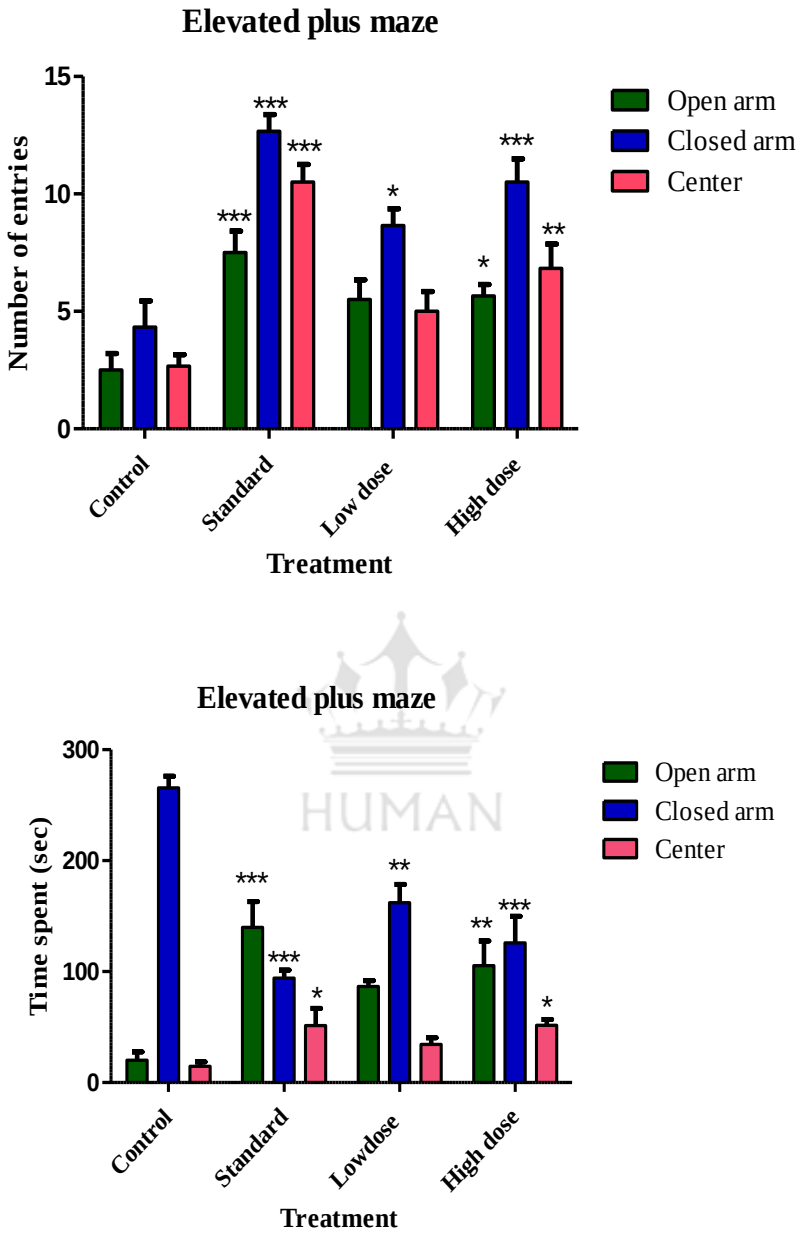


Figure no 1: Graphical representation of results of Elevated Plus Maze

2) Evaluation of Anxiolytic activity of ethanolic Extract of *Pithecellobium dulce* Benth. Leaves by using Rota Rod Model.

Diazepam (1mg/kg) treated group it shows a significant decrease in the locomotor score when compared with control group (***P<0.001) in 30 mins and 60 mins time interval except 90 mins. Low dose (200mg/kg) treated group shows highly increase in locomotor activity and animals maintain equilibrium and stayed on rotating rod for a longer period of time without falling, less significance (*P<0.05) was showed by this group in 60 mins time interval. High dose (400mg/kg) treated group shows decrease in locomotion on rota rod, and it is moderately significant (**P<0.01) when compare with control group in all time intervals. Dose dependent activity was showed by animals in Rota rod model. Effect of EEPDBL statistically shown in below table and graph.

Table no 3: Results of rota rod model

Sl. No	Treatment	Falling time(sec)		
		30 min	60min	90min
1	Control (saline) 10ml/kg	293±3.493	288.3±3.393	270±7.97
2	Standard (diazepam) 1mg/kg	$81.67±3.547^{***}$	$51.33±3.373^{***}$	$179.7±17.45^{**}$
3	Low dose (EEPDBL)200 mg/kg	$271.0±5.574^{ns}$	$193.2±34.28^{*}$	$229.2±16.96^{ns}$
4	High dose (EEPDBL) 400mg/kg	$236.7±15.96^{**}$	$183.3±15.69^{**}$	$177.7±24.15^{**}$

Values were expressed as Mean ± SEM (n=6); significance values are ***P< 0.001, **P<0.01, *P<0.05, ^{ns}P>0.05. Control vs all groups (By one-way ANOVA followed by Tukey-kramer Multiple Comparison tests).

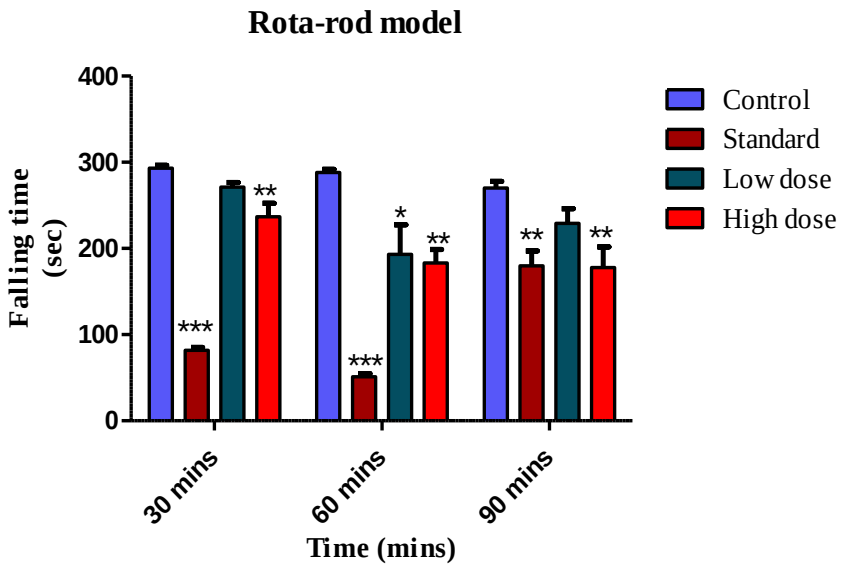


Figure No 2: Results of rota rod model

3) Evaluation of Anxiolytic activity of ethanolic Extract of *Pithecellobium dulce* Benth. leaves by using Hole Board Model.

Head dipping behaviour was sensitive to changes in the emotional state of the animal and recommended that the expression of an anxiolytic state in animals may be reflected by an increase in head dipping behaviour. In hole board model, animals were treated with two doses of EEPDBL, in which high dose (400 mg/kg) shows moderately significant effect (**P<0.01) by increase in number of head dip. Similarly, in animals treated with diazepam (1mg/kg), as expected it showed a significant increase (***P<0.001) in head dip counts. Animals treated with high dose (400 mg/kg) shows more significant results.

Table No 4: Results of Hole board model

Sl. no.	**Treatment**	**Number of head dipping In 5mins**
1	Control (Saline) 10ml/kg	11.17 ± 1.167
2	Standard (Diazepam) 1mg/kg	23.83 ± 1.641***
3	Low dose EEPDBL) 200mg/kg	14.60 ± 1.461ns
4	High dose (EEPDBL) 400mg/kg	19.50 ± 1.784**

Values were expressed as Mean ± SEM (n=6); significance values are ***P< 0.001, **P<0.01, *P<0.05, ^{ns}P>0.05. Control vs all groups (By one-way ANOVA followed by Tukey-kramer Multiple Comparison tests).

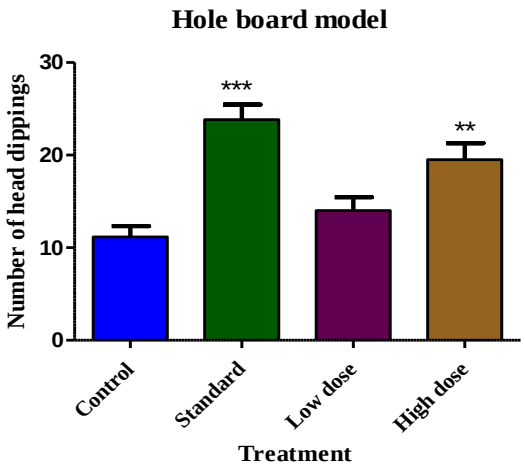


Figure No 3: Results of Hole Board Model

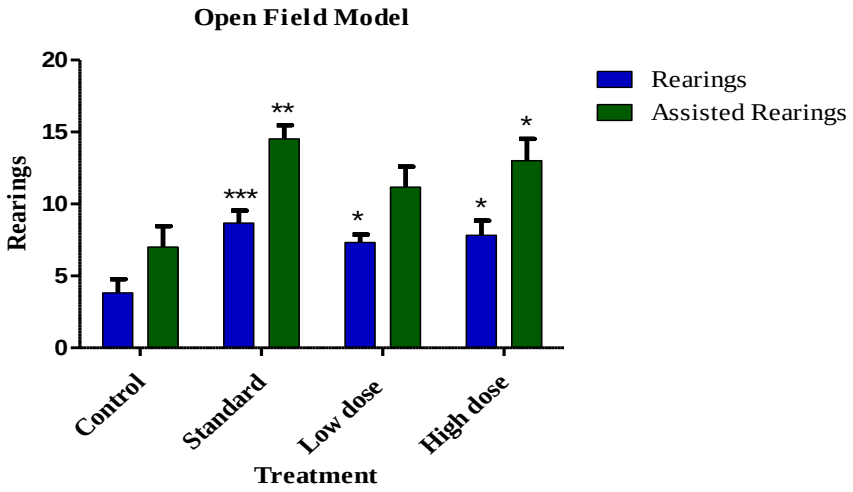
4) Evaluation of Anxiolytic activity of ethanolic Extract of *Pithecellobium dulce* Benth. by using Open Field Apparatus.

Diazepam treated group shows highly significance value (***P<0.001) increase in locomotion and exploratory activity compared to remaining three group of animals indicating anxiolytic behaviour. In a low dose of EEPDBL (200 mg/kg) it shows less significance value (*P<0.05) when compare with control group. In a high dose of EEPDBL (400 mg/kg) it shows moderately increase in significance value (**P<0.01) when compare with control group. Dose dependent activity was showed by animals in open field model. Effect of EEPDBL statistically shown in below table and graph.

Table No 5: Results of Open field apparatus

Sl. No.	Treatment	Rearings in 5 mins	Assisted Rearings in 5 mins	Number of squares traversed in 5 mins
1	Control (Saline) 10ml/kg	3.833±0.945	7.00±1.461	69.50±7.361
2	Standard (diazepam) 1mg/kg	8.667±0.881***	14.52±0.96**	117.7 ± 5.408***
3	Low dose (EEPDBL) 200 mg/kg	7.33±0.55*	11.17±1.42ns	71.17± 9.71ns
4	High dose (EEPDBL) 400mg/kg	7.83±1.01*	13.00±1.52*	103.5± 6.21*

Values were expressed as Mean ± SEM (n=6); significance values are ***P< 0.001, **P<0.01, *P<0.05, nsP>0.05. Control vs all groups (By one-way ANOVA followed by Tukey-kramer Multiple Comparison tests).



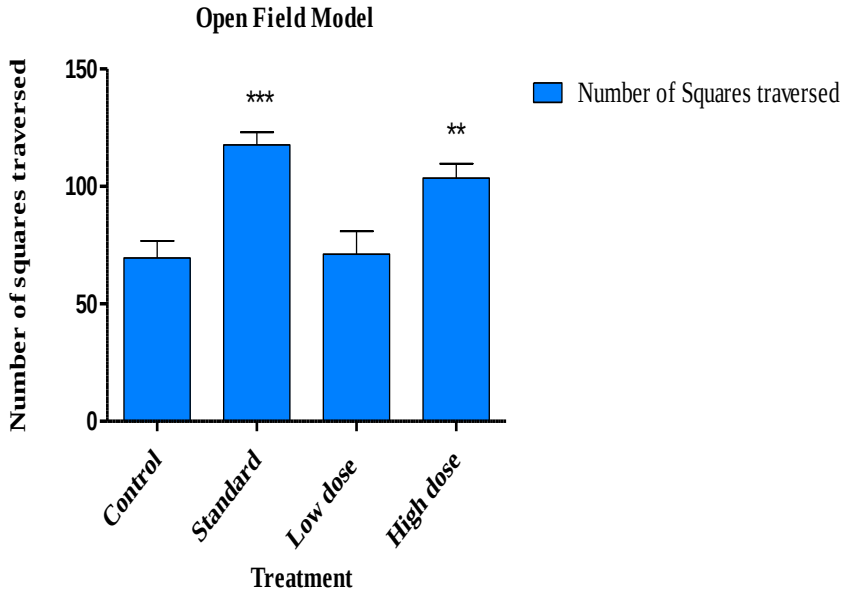


Figure No 4: Results of Open field apparatus

DISCUSSION

Now a days, medicinal plants are considered as importance source to prevent or treat various kind of diseases. Every plant in the world consists numerous medical constituents that can be used in the development of different kind of drugs. [19] *Pithecellobium dulce* Benth. is an evergreen small to medium sized spiny tree belongs to the family Leguminosae, leaves of the plant have been reported to be a native medicine to treat ear ache, leprosy, peptic ulcer, tooth ache and veneral diseases. [20] Presence of flavonoids, alkaloids, glycosides. Saponins, triterpenoids, tannins and phenolic compounds in the ethanolic extract is supported by the previous phytochemicals study. The elevated plus maze is currently one of the most widely used animal models to evaluate anxiety. Number of entries and time spent by the animals in open arm, closed arm and centre. Readings of control group is compared with standard, low dose and high dose of leaf extracts. The sensitivity to agents acts via Gamma-aminobutyric acid receptor complex justifying the use of diazepam as standard drug (1 mg/kg) shows highly significance when compare with control and extract treated groups. [19] Low dose of EEPDBL (200 mg/kg) shows less significance, High dose of EEPDBL (400 mg/kg) shows significant increase in time spent and number of entries in elevated plus maze. In rota rod model results shown that standard drug (Diazepam 1mg/kg) have increased significance value, Low dose of EEPDBL (200 mg/kg) shown decrease in significance value, but in high dose of EEPDBL (400 mg/kg) it shows moderately significant when compare with control

and low dose treated group. Therefore, the observed reduction of the spontaneous activity may be related to the muscle-relaxant effect of the extract. On the other hand, the anxiolytic effect of the extract reached significance only at high dose (400 mg/kg). In Hole board model the number of head dipping was increased significantly in case of standard group (Diazepam 1 mg/kg) as compared to animals of control group. Low dose of EEPDBL (200 mg/kg) shows significant decrease in number of head dipping, in case of high dose of EEPDBL (400 mg/kg) it shows moderately significant when compare with of control group. The open field test revealed that in standard group (Diazepam 1mg/kg) the animals spent significantly more time to explore and locomotion when compare to control group. In high dose of EEPDBL (400 mg/kg) it shows increase in significance value when compare with control and low dose of EEPDBL (200 mg/kg).

These findings from above models raised the possibilities that the anxiolytic effects of the extract may be exerted due to the presence of different phytoconstituents possibly acting through different receptor sub types or having different affinity for the relevant receptors. Further study regarding isolation and characterization of active principle responsible for the pharmacological activity is needed. The results of this study showed that the EEPDBL has anxiolytic effects. The phytoconstituents responsible for the observed anxiolytic effects has to be isolated and identified from *Pithecellobium dulce* Benth. leaves in future studies. The present research study concludes that the effect of Ethanolic extract of leaves of *Pithecellobium dulce* Benth. possess Dose-dependent anxiolytic activity.

ACKNOWLEDGEMENT:

First and foremost, I would like to thank Almighty for giving me the courage, knowledge, ability to undertake this research and complete it satisfactorily. I express my heartfelt gratitude and respectful thanks to my guide Dr. Nataraj G R for his support and guidance.

CONCLUSION:

The present research study concludes that the effect of Ethanolic extract of leaves of *Pithecellobium dulce* Benth. possess Dose-dependent anxiolytic activity. Anxiolytic activity of Ethanolic extract of *Pithecellobium dulce* Benth. leaves were carried out by four models i.e., Elevated plus maze, Rota rod model, Hole board model and Open field apparatus. Considering results of above-mentioned experimental models, it reveals that leaves extract showed good anxiolytic activity. The results from different experimental models shown that

higher dose (400 mg/kg) of Ethanolic extract of *Pithecellobium dulce* Benth. leaves consist higher significance value than that of low dose (200 mg/kg) of EEPDBL leaves. Hence, this research studies justifies that Ethanolic extract of *Pithecellobium dulce* Benth. leaves can be used in the treatment of anxiety as Anti-anxiety drug.

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