

ANXIOLYTIC PROPERTY OF *CAESALPINIA PULCHERRIMA* LEAVES EXTRACT IN
EXPERIMENTAL ANIMALS

Miss. Asmath Tabasuum*

Dept. of Pharmacology, DSR Institute of Pharmaceutical Sciences, Kalaburagi/Gulbarga-585102, Karnataka, India.

***Corresponding Author: Miss. Asmath Tabasuum**

Dept. of Pharmacology, DSR Institute of Pharmaceutical Sciences, Kalaburagi/Gulbarga-585102, Karnataka, India.

DOI: <https://doi.org/10.5281/zenodo.23032440>**How to cite this Article:** Miss. Asmath Tabasuum*. (2026). Anxiolytic Property of *Caesalpinia Pulcherrima* Leaves Extract In Experimental Animals. *European Journal of Pharmaceutical and Medical Research*, 13(10), 251–262.
This work is licensed under Creative Commons Attribution 4.0 International license.

Article Received on 31/08/2026

Article Revised on 21/09/2026

Article Published on 01/10/2026

ABSTRACT

Objectives: To evaluate the anxiolytic property of ethanolic extract of leaves of *Caesalpinia pulcherrima* in Albino Wistar rats and Swiss Albino mice. **Methods:** The anxiolytic activity was evaluated using behavioral models of anxiety like elevated plus maze test, light-dark transition test, hole board test and open field test. The ethanolic extract was screened for toxicity by acute oral toxicity studies according to OECD guidelines 425 for selection of dose. **Results:** Compared to control group, animals in diazepam (2mg/kg i.p) treated group and the extract (ethanolic 800mg/kg p.o) treated groups revealed a statistically significant increase in anxiolytic activity. (ethanolic extract at 400mg/kg was statistically not significant except in light/dark transition test). **Conclusion:** Ethanolic extract showed significant dose dependent anxiolytic effect. Phytochemical screening revealed presence of flavonoids, and saponins as major constituents. The results suggest that the ethanolic extract (800mg/kg) of CP leaves seems to possess pronounced anxiolytic effect with lower sedative activity than the ethanolic extract (400mg/kg) when compared to diazepam. The results could be attributed to the flavonoids content (quercetin and sitosterol) of the ethanolic extract.

KEYWORDS: Ethanolic extract, anxiolytic, *Caesalpinia pulcherrima*, elevated plus maze, open field test, light/Dark test, hole board test.

INTRODUCTION

Anxiety is a normal reaction to stress and can actually be beneficial in some situations. For some people, however, anxiety can become excessive. While the person suffering may realize their anxiety is too much, they may also have difficulty controlling it and it may negatively affect their day-to-day living.^[1]

Anxiety can be a result of a known or unrecognized medical condition, or substance-induced (over-the-counter medications, herbal medications, substances of abuse). Genetic factors are also known to significantly influence risk for many anxiety disorders.^[2]

Genetic factors significantly influence risk for many anxiety disorders. Environmental factors such as early childhood trauma can also contribute to risk for later anxiety disorders. The debate whether gene or environment is primary in anxiety disorders has evolved

to a better understanding of the important role of the interaction between genes and environment. Some individuals appear resilient to stress, while others are vulnerable to stress, which precipitates an anxiety disorder.^[2]

Norepinephrine, serotonin, dopamine, and gamma-aminobutyric acid (GABA) are known to be the major mediators of the symptoms of anxiety disorders.^[2]

Anxiety related illnesses are bipolar disorder, eating disorder, headache, irritable bowel syndrome (IBS), sleep disorder, substance abuse.^[3] In general, anxiety disorders are treated with medication, specific types of psychotherapy, or both.

Selective serotonin reuptake inhibitors (SSRI), tricyclic antidepressants (TCAs), Mono amino oxidase inhibitors

(MAOIs) and benzodiazepines are used in anxiety disorders.^[1]

Antianxiety drugs are known to produce untoward effects on higher cerebral functions and also lead to dependence.^[4]

Natural drug from the plants are gaining popularity because of several advantages such as often fewer side effect, better patient tolerance, relatively less expensive and acceptance due to a long history of use, especially herbal medicines provide rational means for the treatment of many diseases that are obstinate and incurable in other system of medicine.^[5]

Recent study on anxiety claims that the flavonoids, alkaloids and terpenoids are responsible for anxiolytic (anti anxiety) and sedative activity.^[6,7,8]

The literature reveals that the plant *Caesalpinia pulcherrima* possesses various bioactive compounds along with flavonoids (quercetin, sitosterol) and there is no scientific data on anxiolytic activity of *Caesalpinia pulcherrima* leaves. In view of this, in this study we investigated the possible anxiolytic activity of *Caesalpinia pulcherrima* leaves extract in swiss albino mice and albino wistar rats.

MATERIALS AND METHODS

Plant material collection and extraction

Plants sample were collected from surrounding of Lufman College of Pharmacy. It was authenticated by Joint Director, and Botanical Survey of India, Pune, Maharashtra. The leaves were shade dried at room temperature and pulverized. The powder was passed through sieve and stored in container. The powdered material was then subjected to successive extraction with soxhlet apparatus using solvents of increasing polarity like; petroleum ether, chloroform, ethanol and distilled water.

Animals

The study was performed on adult Albino Wistar rats of either sex weighing between 150 to 200gm and adult Swiss Albino mice of either sex weighing between 20 to 30gms. The animals were procured from registered breeders (146/999 CPCSEA, Mahavir Enterprises, Hyderabad). The animals were housed under standard conditions of temperature ($25 \pm 2^\circ\text{C}$) and relative humidity (30-70%) with a 12:12 light-dark cycle. All the animals were acclimatized for seven days before the study. The animals were housed individually in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellets as basal diet V.R.K Nutritional solutions, Pune and water ad libitum. Institutional animal ethics committee (IAEC) approval was obtained for these studies.

Acute toxicity

The ethanolic extract was screened for acute oral toxicity as per OECD guideline 425. Female Swiss Albino mice weighing 20-30 gm were used in the study. Animals were maintained on normal diet and water prior to and during the course of experiments.

Experimental protocol

Group I: Animals (-veControl) were administered with 2% gum acacia; p.o.

Group II: Animals were administered with Diazepam 2mg/kg; p.o suspended in 2% gum acacia

Group III: Animals were administered with ethanolic extract of CP at 400 mg/kg; p.o

Group IV: Animals were administered with ethanolic extract of CP at 800 mg/kg; p.o

Anti-anxiety tests

Elevated Plus-Maze Test in mice^[9,10,11,12]

Elevated plus maze (EPM) one of the commonly used animal model (exteroceptive aversion stimulus model) for testing anti-anxiety drugs was employed to assess anxiolytic activity of sample under study. EPM is based on the apparent natural aversion of rodents to open and high spaces animals have tendency to spend more time in enclosed arms than in the open arms.

Swiss Albino mice of either sex weighing between 20-30 gm were dividing into four experimental groups of six animals each.

Standard drug (Diazepam) was administered 30 min prior to testing and extract was administered p.o 30 min prior to testing. Anxiolytic activity was measured using the elevated plus maze test. The maze consisted of two open (28 cm x 5 cm) and two closed (28 cm x 5 cm x 14 cm) arms, extending from the central platform (5 cm x 5 cm) and elevated up to the height of 25 cm above the floor. The entire maze was made of clear Plexiglass. Mice were individually placed on the centre of the maze facing an open arm, and the number of entries and the time spend in closed and open arm were recorded during a 5 min observation period. Arm entries were defined as entry of all four paws in to the arm.

The following parameters were calculated for each animal.

1. % of open arm time
2. % of close arm time
3. % open arm entry
4. % closed arm entry

Hole-board test in rats^[13]

Standard drug (Diazepam) was administered 30 min prior to testing and extract was administered p.o 30 min prior to testing. The hole-board apparatus was an open-field arena with 16 equally spaced holes of 3 cm diameter in the floor, similar to the box used in social interaction test. Rats were placed singly in the centre of the hole-board, and during a 5-min trial.

The following parameters were recorded.

1. Total time spend with the head dips
2. Number of head dips in the holes
3. Total locomotors activity (numbers of squares crossed)
4. Number of assisted rearing

A head dip was scored if both eyes disappeared into the hole. The arenas were wiped with a damp cloth after each trial and any faeces removed.

Light-dark transition test in mice^[14,15,16,17]

The light/dark transition test is based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behavior of rodent in response to mild stressors, that is, novel environment and light. A natural conflict situation occurs when an animal is exposed to an unfamiliar environment or novel objects. The conflict is between the tendency to explore and the initial tendency to avoid the unfamiliar (neophobia). The exploratory activity reflects the combined result of these tendencies in novel situations. Thus, in the light/dark test, drug induced increase in behavior in the white part of a two compartment box, in which a large white compartment is illuminated and a small black compartment is darkened, is suggested as an index of anxiolytic activity.

Standard drug (Diazepam) was administered 30 min prior totesting and extracts were administered p.o 45 min prior to testing.

The apparatus for light/dark transition test consist of two compartments: one light area (27 L x 27 W x 27 H cm), illuminated by 100 W desk lamp was painted white, and the other dark area (18 L x 27 W x 27 H cm) was painted black. The two compartments were separated by a partition with a tunnel (7.5 x 7.5 cm) to allow passage from one compartment to the other. The experiments were performed between 09:00 and 14:00. Animal was placed in the centre of the light area with its back to the opening.

The following parameters were recorded during 5 min

1. Total time spen in the light area.
2. Total time spen in the dark area.

Open field test in mice^[18,19]

Standard drug (Diazepam) was administered 30 min prior to testing and extracts were administered p.o 30 min prior to testing.

The apparatus consists of a wooden box (60X60X30cm). The apparatus is illuminated with 40-W lamp suspended 100cm above it. Mice will be fed orally with extract(s), vehicle (normalsaline) or diazepam (1mg/kg;p.o).

After 30 minutes following parameters are observedand recorded during the test session of 5 minutes.

1. Number of entry in central square.
2. Time spent in Central Square.
3. Number of squares crossed.
4. Number of assisted rearing.

Statistical analysis

All data were expressed as mean±SEM and were analyzed by one-way ANOVA followed by Dunnet's test. GraphPad Prism 5 was used for data analysis.

RESULTS**Acute Toxicity Study**

Acute toxicity studies for petroleum ether, chloroform, etanolic and aqueous extracts of *Caesalpinia pulcherrima* leaves (Fabaceae) were conducted as per OECD guidelines 425 using female swiss albino mice. Each animal was administered ethanolic extract by oral route. The animals were observed for any changes continuously for the first 2 h and up to 24 h for mortality, there were no mortality and noticeable behavioural changes in all the groups tested. The extracts were found to be safe up to 2000 mg/kg body weight.

Elevated Plus Maze Test

The effects of ethanolic and extract and diazepam, on the behaviour of mice in the elevated plus maze test were summarized in Table 1. and Figure 1,2,3,4.

Diazepam has increased the percentage of entries in open arms (** $P < 0.01$,) and time spent in open arms (** $p < 0.01$,), percentage of entries in close arm (*** $P < 0.001$,), and percentage of time spent in closed arms (* $P < 0.05$) has decreased significantly compared to control group.

It was seen that the ET1 is statistically nonsignificant in all the parameters of EPM test.

While ET2 has significantly increased the percentage of open arm entry (* $p < 0.05$) and time spent in open arms (* $p < 0.05$) when compared to control group.

Table 1: Effects of ethanolic extract of CP leaves from elevated plus maze test in mice.

Treatment	% open arm entry	% open arm time	% close arm entry	% close arm time
Control	6.333±1.085	77.17±10.41	12.33±1.054	214.5±11.20
Diazepam (2 mg/kg)	17.33±2.445**	155.5±20.06**	6.500±0.6708***	136.5±20.26*
ET1 (400 g/kg)	9.667±1.116	80.33±10.58	11.83±1.078	205.5±10.52
ET2 (800mg/kg)	14.33±2.445*	151.3±20.02*	8.333±0.4216*	150.5±23.76*

All values are mean ± SEM, (n =6), one way ANOVA, followed by Dunnet's test.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, when compared to vehicle treated group.

ELEVATED PLUS MAZE

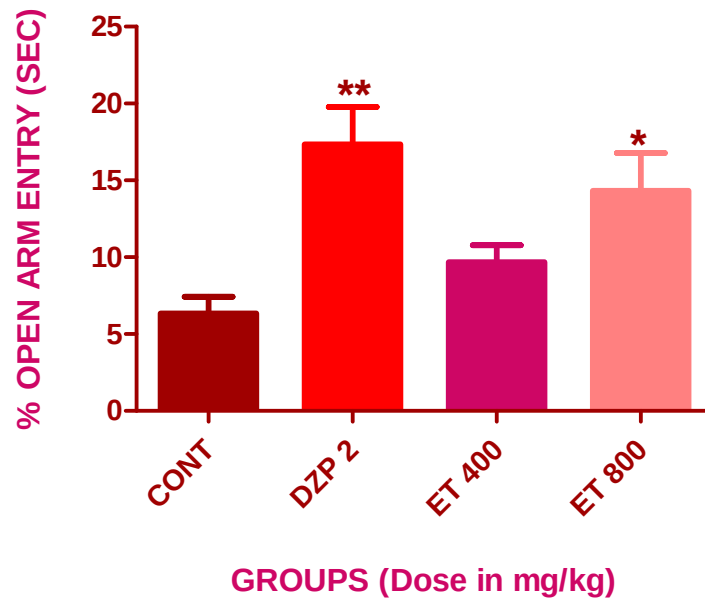


Figure 1: Percentage of open arm entry in 5-min EPM test of Ethanolic Extract.

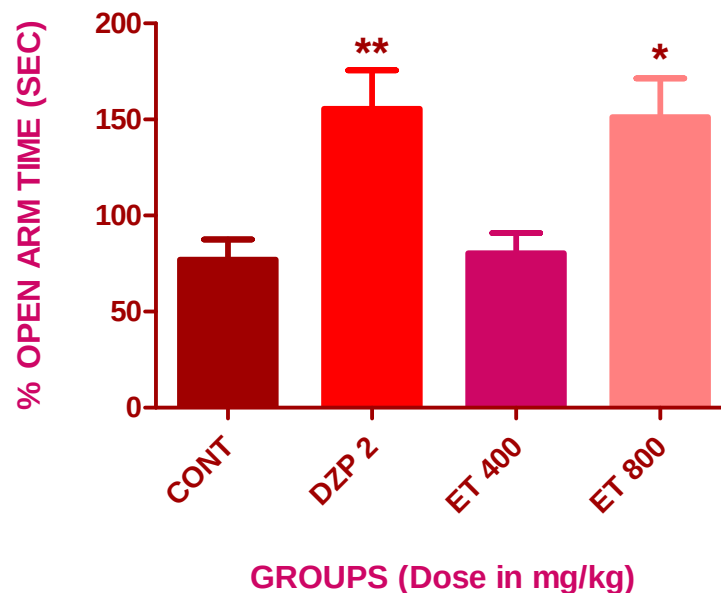


Figure 2: Percentage of open arm time in 5-min EPM Test of Ethanolic Extract.

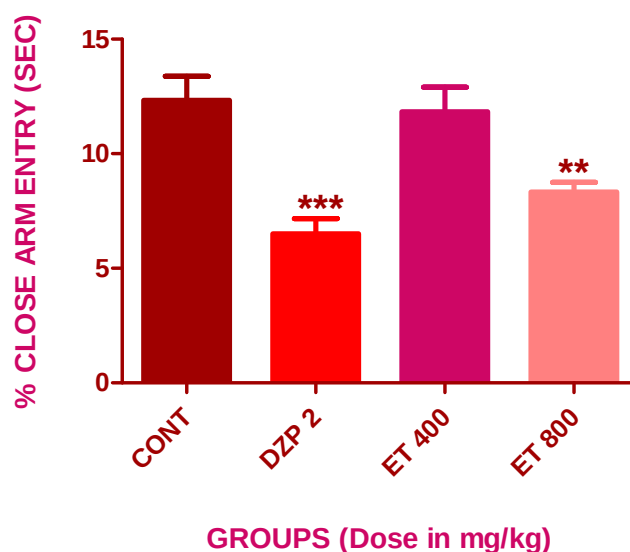


Figure 3: Percentage of close arm entry in 5-min EPM Test of Ethanolic Extract.

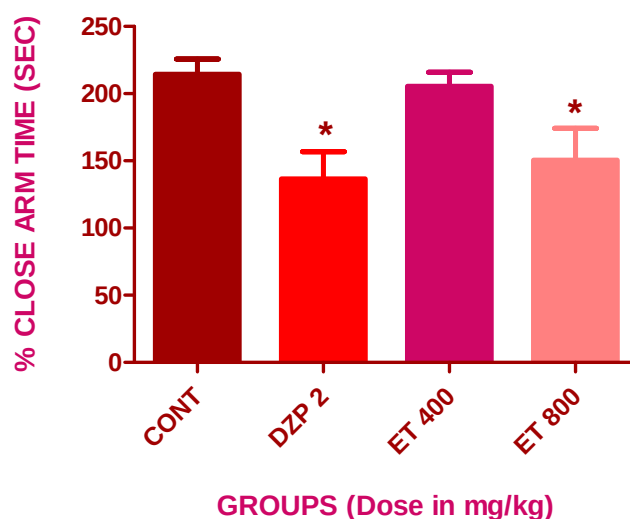


Figure 4: Percentage of close arm time in 5-min EPM Test of Ethanolic Extract.

Hole Board Test

5.5.1. Ethanolic extract of CP leaves in hole board test

The effects of ethanolic and pet.ether extracts and diazepam ummerised in Table 2 and Figure 5,6,7,8.

Diazepam has showed significant increase in all the parameters of HBT. It was found moderately significant in head dip count, and no. of assisted rearing (** $p < 0.01$).

0.01), while it was more significant in head dip latency and no. Of squares crossed (** $p < 0.001$).

The ET1 extract was found to be statistically nonsignificant in all the parameters of HBT. ET2 was found moderately significant in number of squares crossed (** $p < 0.01$) and less significant in head dip count, head dip latency and no. of assisted rearing (* $p < 0.05$).

Table 2: Effect of ethanolic extract of CP leaves from hole board test in rat.

Treatment	Head dip count	Head dip latency	Locomotion	No. Of Assisted Rearing
Control	11.67±1.430	16.67±1.994	30.33±3.221	15.50±2.837
Diazepam 2 mg/kg	25.67±3.818**	32.67±3.528***	76.50±7.796***	31.33±4.616**
ET1 (400 mg/kg)	14.33±1.358	20.67±1.498	40.67±2.362	15.67±2.028
ET2 (800 mg/kg)	21.67±2.092*	28.33±3.169*	66.17±9.119**	27.00±3.454*

All Values are mean ± SEM, (n =6), one way ANOVA, followed by Dunnet's test.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, when compared to vehicle treated group.

HOLE BOARD TEST

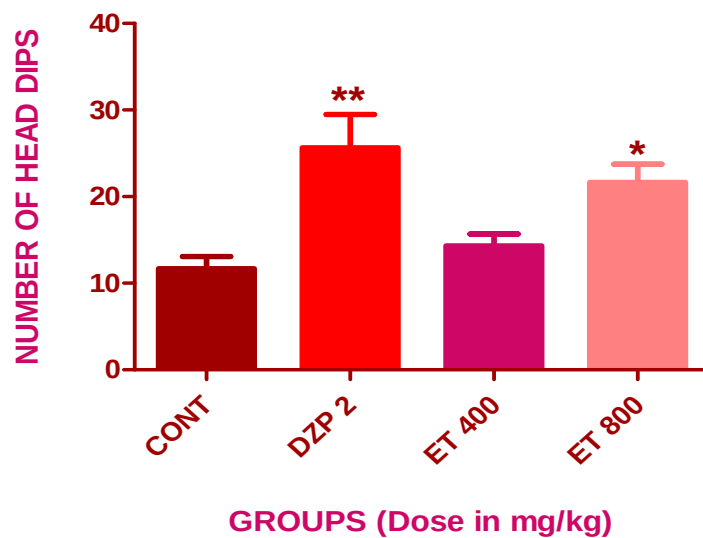


Figure 5: Head dip count in 5-min Hole Board Test of Ethanolic Extract.

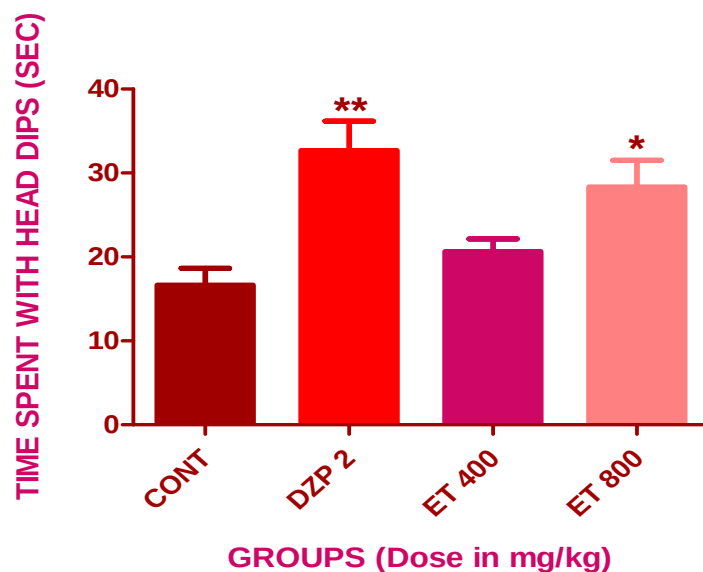


Figure 6: Head dip latency in 5-min Hole Board Test of Ethanolic Extract.

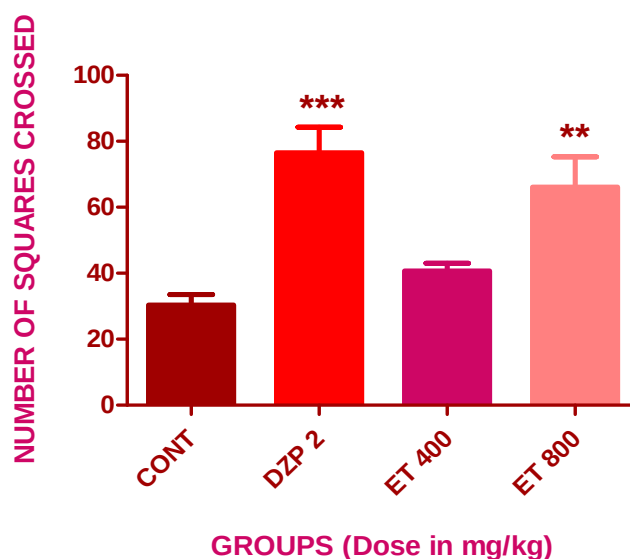


Figure 7: Locomotion in 5-min Hole Board Test of Ethanolic Extract.

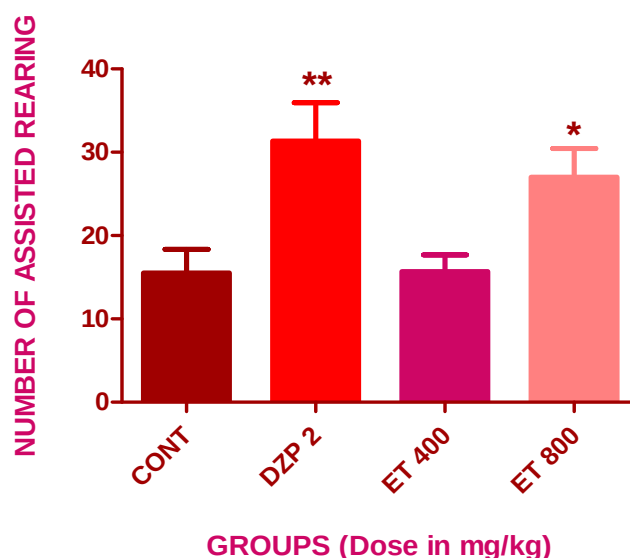


Figure 8: Assisted rearing in 5-min Hole Board Test of Ethanolic Extract.

Light/Dark Transition Test

5.6.1. Ethanolic extract of CP leaves in Light/Dark transition test

Results obtained from ethanolic extract and diazepam have been revealed in Table no 3. and Figure no. 9,10.

Diazepam and ethanolic extract (ET2) have more significantly increased the time spent in light area

(*** $p < 0.001$) and decreased the time spent in dark area more significantly (*** $p < 0.001$) when compared to control group.

While ET1 was moderately significant in the time spent in light area (** $p < 0.001$) and less significantly decreased the time spent in dark area (* $p < 0.01$).

Table 3: Effect of ethanolic extract of CP leaves from light/dark transition test in mice.

Treatment	Time in dark area	Time in light area
Control	190.7±9.646	103.0±9.487
Diazepam 2mg/kg	93.83±5.043***	199.7±4.485***
ET1 (400 mg/kg)	158.3±7.762*	136.0±7.123**
ET2 (800 mg/kg)	129.3±6.525***	165.2±5.963***

All values are mean ± SEM, (n =6), one way ANOVA, followed by Dunnet's test.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ when compared to vehicle treated group.

LIGHT/DARK TRANSITION TEST

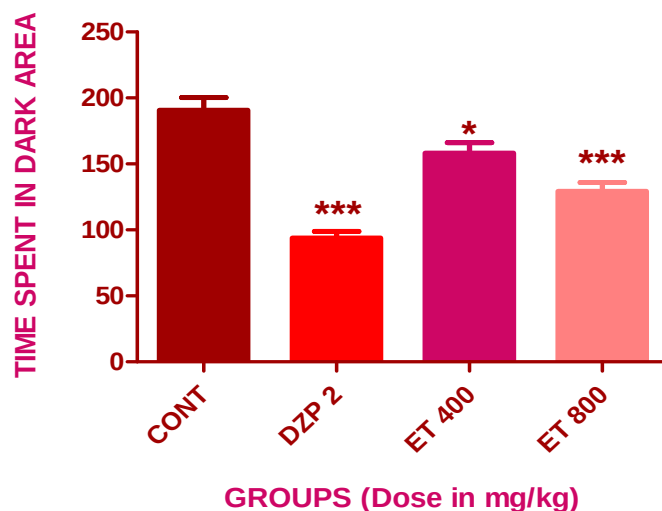


Figure 9: Time spent in dark area in 5-min Light / Dark Test of Ethanolic Extract.

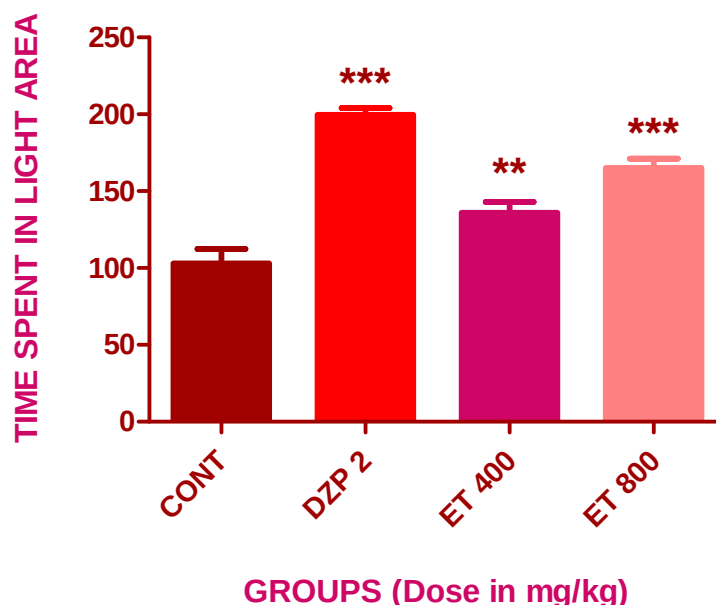


Figure 10: Time spent in Light area in 5-min Light / Dark Test of Ethanolic Extract.

5.7 Open Field Test

5.7.1. Ethanolic extract of CP leaves in Open field test

Results of open field test obtained from diazepam and both the extracts are shown in Table no 4 and Figure no. 11, 12, 13, 14.

In the OF test diazepam has increased the locomotion and no. of assisted rearing more significantly ($***p < 0.001$).

And entries in the central square and time spent in central square increased were moderately significant ($**p < 0.01$) when compared to control group. Whereas ET2 was highly significant in locomotion ($***p < 0.001$), moderately significant in time spent in central square and no. Of assisted rearing ($**p < 0.01$) and was found less significant in no. Of entry in central square ($*p < 0.05$). While ET1 was non significant in all the parameters.

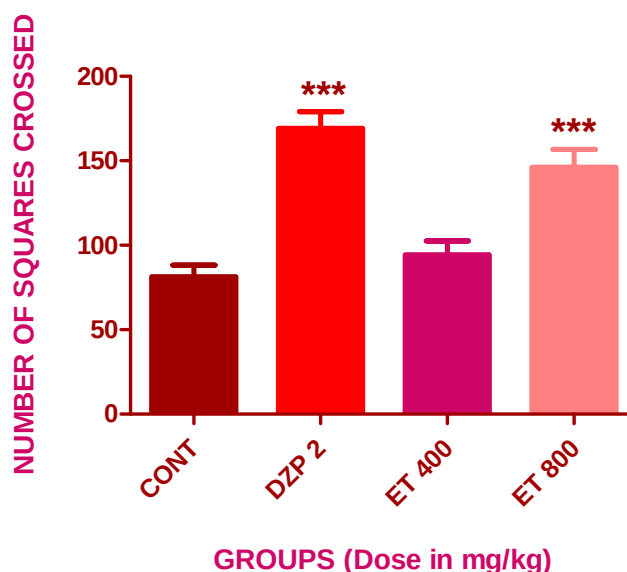
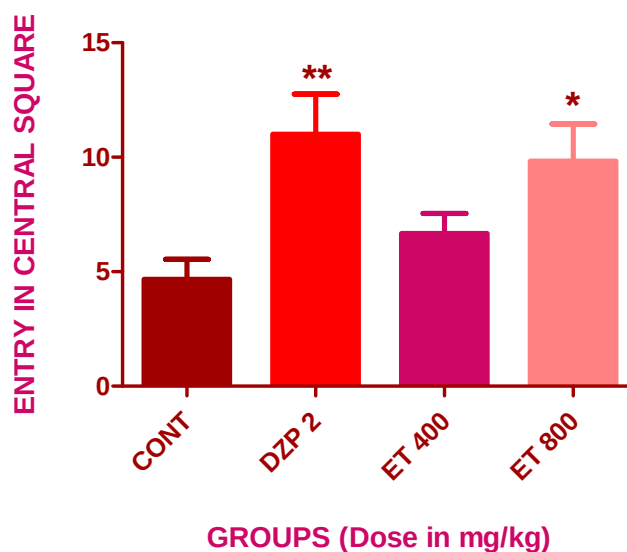
Table 4: Effects of ethanolic extract of CP leaves from Open field test in mice.

Treatment	Locomotion	No. Of assisted rearing	Entry in central square	Time spent in central square
Control	81.33±6.89	17.17±0.9458	4.667±0.8819	35.00±4.351
Diazepam (2mg/kg)	169.3±9.708***	33.83±2.613***	11.00±1.751**	72.33±8.644*
ET1 (400 mg/kg)	94.33±8.297	20.17±0.948	6.667±0.8819	37.83±4.206
ET2 (800 mg/kg)	146.2±10.55***	29.17±2.676***	9.833±1.621*	67.83±7.391**

All values are mean ± SEM, (n = 6), one way ANOVA, followed by Dunnet's test.

* $p < 0.05$, ** $p < 0.01$, When compared to vehicle treated group.

OPEN FIELD TEST

**Figure 11: Locomotion in 5-min open field Test of Ethanolic Extract.****Figure 12: Entry in Central square in 5-min open field Test of Ethanolic Extract.**

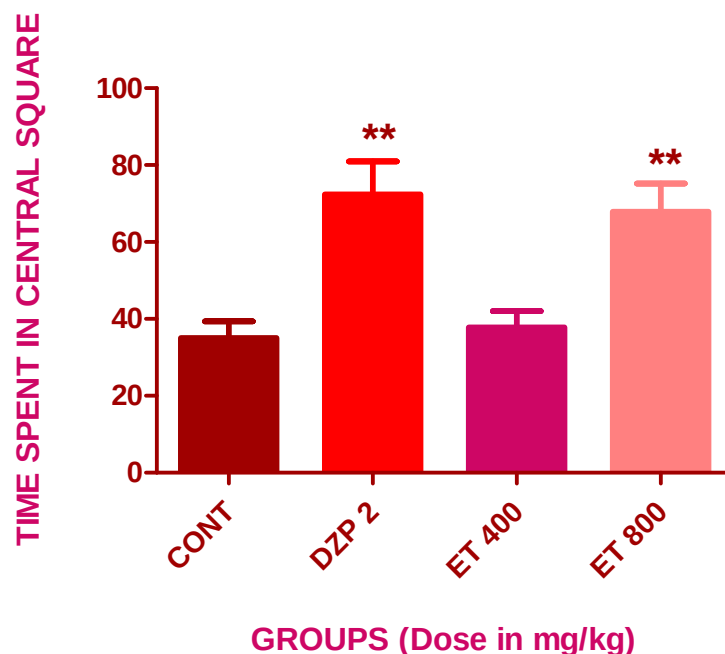


Figure 13: Central square latency in 5-min open field Test of Ethanolic Extract.

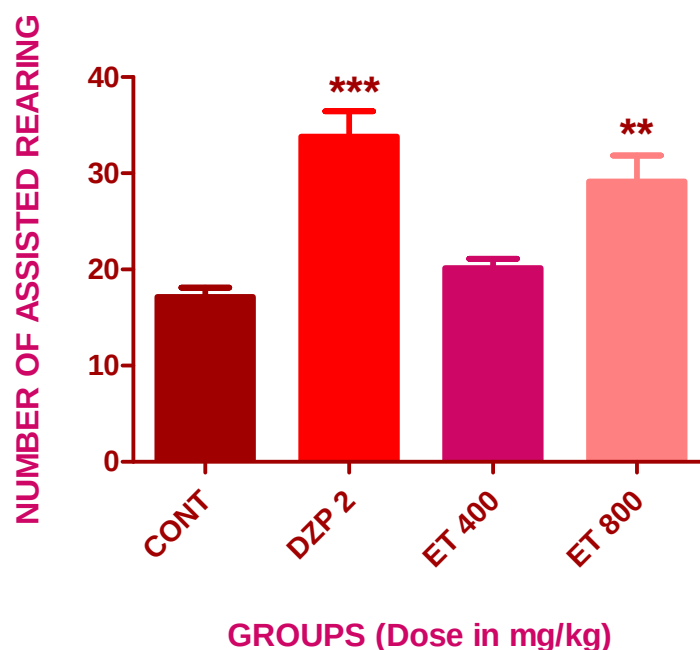


Figure 14: Assisted rearing in 5-min open field Test of Ethanolic Extract.

DISCUSSION

Despite the widespread traditional use of *Caesalpinia pulcherrima* for treating various disorders there are no reports of scientific evaluation of its anxiolytic activity, therefore the present study was undertaken to explore anxiolytic potential of the leaves of *Caesalpinia pulcherrima* using anxiety models like, Elevated plus maze, Hole board test, Light/Dark transition test and Open field test. Acute toxicity study was conducted for ethanolic extract and after 21 days study it was concluded that the extract was safe upto

2000 mg/kg body weight with fewer side effects like grooming and hair loss and no adverse effects.

The elevated plus maze is currently one of the most widely used model of animal anxiety and it's validated and use in rats and mice.^[20] The EPM test is based on a premise where the exposure to an EPM evoked an approach-avoidance conflict that was considerably stronger than evoked by the exposure to an enclosed arm.^[21] The decrease in aversion to the open arm is the result of an anxiolytic effect, expressed by the increased

time spent and entries in to the open arm are sensitive to agents thought to act via the GABAA receptor complex, justifying the use of diazepam as a positive control in this study^[22], even when the compound being screened does not act via benzodiazepine receptors.

Diazepam has increased the percentage of entries in open arms ($***P < 0.001$) and time spent in open arms ($**p < 0.01$), the percentage of entries in close arms ($***P < 0.001$), and percentage of time spent in closed arms ($*P < 0.05$) has decreased significantly compared to control group. It was seen that the ethanolic extract (400 mg/kg) is statistically non-significant in all the parameters of EPM test. While ethanolic extract (800mg/kg) has significantly increased the percentage of open arm entry ($*p < 0.01$) and time spent in open arms ($*p < 0.05$) when compared to control group.

The hole-board test provides a simple method for measuring the response of an animal to an unfamiliar environment and is widely used to assess emotionality, anxiety and/or responses to stress in animals. The head dipping behavior was sensitive to changes in the emotional state of the animal, and suggested that the expression of the anxiolytic state in animals may be reflected by an increase in head dipping behaviour.^[23]

Diazepam has showed significant increase in all the parameters of HBT. It was found moderately significant in head dip count and no. of assisted rearing ($**p < 0.01$), while it was more significant in head dip latency and locomotion ($***p < 0.001$). The ethanolic extract (400 mg/kg) was found to be statistically non-significant in all the parameters of HBT. Ethanolic extract (800 mg/kg) was found moderately significant in head dip latency and locomotion ($**p < 0.01$) and less significant in head dip count and no. of assisted rearing ($*p < 0.05$). when compared to control group.

Light/dark box is another widely used rodent anxiety model for screening anxiolytic or anxiogenic drugs. It is based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behavior of rodents in response to mild stressors that is novel environment and light.^[16] Drugs induced increase in behavior in the white part of a two compartment box, in which a large white compartment is illuminated and a small black compartment is darkened, is suggested as an index of anxiolytic activity.^[17]

Diazepam and ethanolic extract (800mg/kg) have more significantly increased the time spent in dark area ($***p < 0.001$) and decreased the time spent in light area more significantly ($***p < 0.001$) when compared to control group. While ethanolic extract (400 mg/kg) increased the time spent in dark area less significantly and the time spent in light area was decreased less significantly ($*p < 0.01$).

The Open Field Test (Walsh & Cummins, 1976) provides simultaneous measures of locomotion, exploration and anxiety. The number of line crosses and the frequency of rearing are usually used as measures of locomotor activity, but are also measures of 4 exploration and anxiety. A high frequency of these behaviours indicates increased locomotion and exploration and/or a lower level of anxiety. The number of central square entries and the duration of time spent in the central square are measures of exploratory behaviour and anxiety. A high frequency/duration of these behaviours indicates high exploratory behaviour and low anxiety levels.^[24]

In the open field test diazepam and ethanolic extract at a dose of 800mg/kg increased the locomotion and no. of assisted rearing more significantly ($***p < 0.001$). And entries in the central square and time spent in central square increased were statistically less significant ($**p < 0.01$) when compared to control group. While ethanolic extract at a dose of 400mg/kg were non-significant in all the parameters.

Earlier reports on the chemical constituents of plants and their pharmacology suggests that plants containing flavonoids, alkaloids, phenolic acids, essential oils, saponins and tannins possess activity against many CNS disorders.^[144] Investigations on the phytochemical screening of *Caesalpinia pulcherrima* leaves extracts revealed the presence of glycosides, saponins, tannins and flavonoids. From the literature survey it was also found that *Caesalpinia pulcherrima* leaves contains flavonoids like sitosterol and quercetin which have been reported earlier to possess anxiolytic activity.

Thus, in this study the ethanolic extract of *Caesalpinia pulcherrima* leaves at a dose of 800mg/kg and diazepam (2mg/kg) significantly possessed anxiolytic property in a dose dependent manner (400; 800mg/kg) in all the parameters of anxiety models mentioned, when compared to control group.

CONCLUSION

The results of the entire study revealed that the ethanolic extract (800mg/kg) of *Caesalpinia pulcherrima* leaves possessed pronounced significant dose dependent anxiolytic effect when compared to control group. This effect may be due to the interaction of the extract with the neural substrates or chemical mediators like noradrenaline, serotonin, GABA and BZD which are implicated to be responsible for anxiety-like condition. Also, there could be a linkage in the interaction of the extract with serotonergic pathway since serotonin had been widely implicated in aggressive behavior.

Our results demonstrated the anxiolytic effect of *Caesalpinia pulcherrima* leaves extracts and suggested that it might be used as a novel therapeutic strategy for anxiety. However, further isolation, characterisation and pharmacological investigations for elucidating the exact

mechanism of the active principles of the extract responsible for the reported activity are still warranted.

REFERENCES

1. Available from: http://www.nimh.nih.gov/health/topics/anxiety_disorders.
2. Yates WR. Anxiety disorders [internet]. [Updated 2012 Jan 23; cited 2014 Jan 7]. Available from: <http://emedicine.medscape.com/article/286227overview>.
3. Available from: [http://www.adaa.org/Understanding the Facts](http://www.adaa.org/Understanding_the_Facts).
4. Edwards JG. Adverse effects of antianxiety drugs [Internet]. [1981 Dec; 22(6): 495-514. Cited 2014 Jan 10]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/6119192>
5. Ashwanikumar. Natural drugs from the plants [2010 Jan 22. Cited 2014 Jan 10]. Available from: <http://www.Science20.com> Public Health Humboldt fellow and Science.
6. Houghton PJ. The scientific basis for the reputed activity of valerian. J. pharm pharmacol, 1999; 51: 505–12.
7. Dhawan K, KumarS, SharmaA. Anti anxiety studies on extracts of *Passiflora incarnata* Linn. J. Ethnopharmacol, 78: 165–70.
8. Carlini EA. Plants and the central nervous system. Pharmacol Biochem Behav, 2003; 75: 501-12.
9. Rang HP, Dale MM, Ritter JM, Moore PK. Pharmacology. 5th ed. Published by Elsevier, 2006.
10. Vogel GH. Drug Discovery and Evaluation Pharmacological Assays 2nd revised updated and Enlarged Edition.
11. Lister RG. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology, 1987; 92: 180-85.
12. Hogg S. A review of the validity and variability of the elevated plus - maze as an animal model of anxiety. Pharmacol Biochem Behav, 1996; 54: 21-30.
13. Pellow S, File SE. Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze a novel test of anxiety in the rat. Pharmacol Biochem Behav, 1986; 24: 525-529.
14. Kulkarni SK. Handbook of Experimental pharmacology. 3rd edition. New Delhi; Vallabha Prakashan, 1999; 135.
15. Rodgers RJ, Johnson NJT. Behaviorally selective effects of neuroactive steroids on plus-maze anxiety in mice. Pharmacol Biochem Behav, 1998; 59: 221-232.
16. Soman I, Mengi SA, Kasture SB. Effects of leaves of *Butia frondosa* on stress, anxiety and cognition in rats. Pharmacol Biochem Behav, 2004; 79: 11-16.
17. Zanolli P, Avallone R, Baraldi M. Behavioral characterization of the flavonoids apigenin and chrysin. Fitoterapia, 2000; 71: S117-S123.
18. Maribel HR. Antidepressant and anxiolytic effects of hydroalcoholic extract from *Salvia elegans*. J Ethnopharmacol, 2006; 107: 53-58.
19. Crawley J, Goodwin FK. Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. Pharmacol Biochem Behav, 1980; 13: 167-70.
20. File SE. Usefulness of animal models with newer anxiolytic. Clin Neuropharmacol, 1992; 15(1): 525A-526A.
21. Sonovane GS, Sarveiya VP, Kasture VS. Anxiolytic activity of *Myristica fragrans* seeds. Pharmacol biochem behave, 2002; 71: 239.
22. Bhattacharya SK, Satyan KS. Experimental methods for evaluation of psychotropic agents in rodents: antianxiety agents. Indian J Exp Biol., 1997; 35: 575.
23. Takeda H, Tsuji M, Matsumiya T. Changes in head dipping behaviour in the hole board test reflect the anxiogenic and/or anxiolytic state in mice. Eur. J. Pharmacol, 1998; 350: 21-29.
24. Available from: <http://www.uonbi.ac.ke/Projects/libro/images/---/Open-field-methods>, 2005.