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Evaluation of anxiolytic activity of ethanolic extract of *Pisonia grandis* R. Br leaves in mice

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ABSTRACT

In the present study an attempt was made to evaluate the anxiolytic activity of ethanolic extract of Pisonia grandis R.Br leaves (EEPGL) in mice. The anxiolytic activity was evaluated by Elevated plus maze, Y-maze, Hole-board and Actophotometer behavior models. The efficacy of the extract (150 and 300 mg/kg, p. o) were compared with the standard drug Diazepam (2 mg/kg, p. o). EEPGL showed significantly increased the number of entries and time spent in the open arm in the elevated plus maze, increased the number of head dipping and line crossing in Hole board, decrease in locomotor in their respective Behavior Models. This study provides scientific basis of anxiolytic activity of Pisonia grandis R.Br.

Keywords: *Pisonia grandis* R.Br, Anxiolytic, Elevated plus Maze, Y-maze, Hole-board.

INTRODUCTION

Pisonia grandis R.Br is an important plant belonging to the family Nyctaginaceae and is one of the most widespread large shrubs in the forests of India, usually occurring in deciduous forests. It is an important Ayurvedic medicinal herb and its synonym is *Pisonia morindaefolia* R.Br. The phytochemical study reveals that the presence of steroids likes octocosanol, betositosterol, alphaspinosterol, dulcitol and flavonoids in the leaves of the plant [1]. According to traditional use, the different parts of the plants of Pisonia grandis are used as diuretics and purgative. The anti inflammatory [2], antifungal [3], wound healing [4], diuretic [5], anti-diabetic [6] were reported earlier.

No major investigated reports were found for its CNS activity; therefore, we undertook the present study to determine anxiolytic activity. Anxiety affects one in every eight population worldwide and has become an important research area in the field of psychopharmacology [7]. Benzodiazepines barbiturates, tricyclic antidepressants have been used for long time to treat anxiety disorders. The serious side effects associated with these drugs, namely rebound insomnia, sedation, muscle relaxation, withdrawal and tolerance, sexual dysfunction, anticholinergic, antihistaminic effects have limited their use in patients. Due to this many research studies are going to find an alternative medicine or plant-derived medications with more specific anxiolytic effects [8].

EXPERIMENTAL SECTION

Collection and Authentication of Plant Material

Fresh leaves of *Pisonia grandis* R.Br were collected from Anakaputhur, Chennai, Tamil Nadu, India. The plant was identified and authenticated by Botanist, Dr. P. Jayaraman, Plant Anatomical Research Centre (PARC), Tambaram, Chennai and the voucher specimen (PARC/2010/654) have been kept in the Department of Pharmacognosy, SRM College of Pharmacy, Chennai, for future reference. Care was taken to select the healthy plants.

Preparation of ethanolic extract Pisonia grandis R.Br leaves (EEPGL)

The freshly collected leaves were chopped, shade dried and coarsely powdered (40 mesh size). The powder was defatted with petroleum ether (60 - 80°C) and then extracted with 90% ethanol in a soxhlet extractor. The extract was dried under reduced pressure using a rotary vacuum evaporator and the percentage yield was 20.90% w/w. The extract was stored in refrigerator at 4^{o}C until used for treatment.

Preliminary Phytochemical Screening

Standard methods [9, 10] were used for preliminary phytochemical screening of the ethanolic extract to know the nature of phyto-constituents present in it. The obtained ethanol extract was suspended in 3% gum acacia for the pharmacological screening.

Drugs and Chemicals

Diazepam (Ranbaxy Laboratories Ltd., Mumbai) used as the standard anxiolytic drug was purchased form Retail Pharmacy in Chennai and Ethanol was provided from the store SRM College of Pharmacy, Chennai.

Animals

Inbred Swiss albino mice (20-25 gm.) of either sex used in experiment were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. Standard pellet feed and drinking water was provided *ad libitum.* Animals were acclimatized to laboratory conditions one week prior to initiation of experiments. The animals were maintained as per the norms of CPCSEA and the experiments were cleared by CPSEA and the institutional ethics committee. The voucher number is IAEC/135/2010.

Acute Oral Toxicity Study

The procedure was followed as per OECD 423 guidelines. The extract was administered orally at a dose 2000 mg/kg body weight to different groups of mice and observed for signs of behavioral, Neurological toxicity and mortality 14 days. [11].

Experimental design

On the 1^{st} day of the experiment, the animals were divided randomly into four groups of six animals in each.

Group I: Vehicle, (3% acacia)
Group II: EEPGL 150 mg /kg b. w
Group III: EEPGL 300 mg /kg b.w
Group IV: Diazepam 2mg/Kg b.w

The extracts were suspended in the vehicle (3% acacia) and all the treatments were given orally by using intragastric catheter at dose (10ml/kg b.w).

Elevated plus Maze Model [12]
The plus-maze apparatus, consisting of two open arms (16 x 5 cm) and two closed arms (16 x 5 x 12 cm) having an open roof. The EEPGL (150 and 300 mg/kg) and vehicle were administered for 5 days once daily p.o. and the last dose was given on the 5th day, 60 min prior to experiment. The standard drug was given at a dose of 2 mg/kg p.o. 60 min before starting the experiment. After proper treatment each mouse was placed at the center of the maze with its head facing the open arm. During the 5 min experiment, the behavior of the mouse was recorded as: the number of entries into the open or closed arms and time spent by the mouse in each of the arms. An arm entry was defined as the entry of all four paws into the arm.

Y – Maze Model [13,22]
Y- Maze is made of black painted wood or grey plastic. Mice were treated with the EEPGL (150 and 300 mg / kg p.o.) or vehicle for 5 days once daily p.o. and the last dose was given on the 5th day, 60 min prior to experiment and kept individually in one arm of the apparatus. The standard drug was given at a dose of 2 mg/kg p.o. 60 min before starting the experiment. For a period of 10 min. the total numbers of visits to different arm were measured.

Hole –Board Model [14]
The Hole-board apparatus consists of a wooden box (40 x 40 x 25 cm) with 16 holes (each of diameter 3 cm) evenly distributed on the floor. The EEPGL(150 and 300 mg/kg) and vehicle were administered for 5 days p.o. once daily and the last dose was given on the 5th day, 60 min before starting the experiment. The standard drug was given at a dose of 2 mg/kg p.o. 60 min before starting the experiment. For a period of 10 min. the number of line crossing and number of head dipping were calculated.

Locomotor Activity [15, 21]
The locomotor activity was measured by using an Actophotometer. The movement of the animal interrupts a beam of light falling on a photocell, at which a count was recorded and displayed digitally. The EEPGL (150 and 300 mg/kg) and vehicle were administered for 5 days once daily p.o. and the last dose was given on the 5th day, 60 min before starting the experiment. The standard drug was given at a dose of 2 mg/kg p.o. 60 min before starting the experiment and the animals were kept in the Actophotometer individually. The locomotor activity was measured for a period of 10 min.

Statistical Analysis
The data were expressed as mean ± standard error mean (SEM). The data were analyzed by using Graph pad software version5 by one way analysis of variance (ANOVA). The test was followed by Dennett's't'-test, p values less than 0.05 were considered as significance.

RESULTS AND DISCUSSION

Phytochemical Screening
The results of the preliminary phytochemical screening of the EEPGL revealed the presence of phytoconstituents such as alkaloids, steroids, flavonoids, phenolic compounds, tannins and glycosides.

Acute toxicity Study

Acute oral toxicity studies revealed the non-toxic nature of EEPGL. There was no morbidity observed or any profound toxic reactions found at a dose of 2000 mg/Kg p.o. which indirectly pronouns the safety profile of the plant extract.

Elevated plus Maze Model

The results showed that the number of open arm entries and time spent in the open arms were increased and number of closed arm entries and time spent in the closed arms were decreased significantly in the extract treated groups which was comparable with the standard Diazepam.

Y-Maze Model

A significant decrease in the number of visits in the three arms of the Y-maze was observed in the Diazepam treated animals as compared to the control animals. Both the doses of EEPGL showed a significant decrease in the number of visits in the three arms of the Y-maze which was comparable with the standard Diazepam.

Hole-Board Model

The number of line crossing and head dipping was increased significantly in case of Diazepam treated animals as compared to the control animals. The EEPGL at both dose levels showed an increase in the number of line crossing and head dipping significantly as compared to the control animals.

Locomotor Activity

A significant decrease in the locomotor score was observed for Diazepam when compared to the control animals. Both the doses of EEPGL showed significant decrease in the locomotor score when compared to the control animals.

Table-1: Effect of EEPGL in EPM model

Groups	Treatment	Time spent in the open arm (s)	Time spent in the enclosed arm (s)	No. of entries in open arm	No. of entries in enclosed arm
I	Vehicle	32.67± 3.68	267.3± 3.68	4.50± 0.42	15.83± 1.07
II	EEPGL 150 mg/kg	59.33± 3.76**	240.7±3.76**	7.16± 0.60^{ns}	13.00± 1.46^{ns}
III	EEPGL 300 mg/kg	72.17± 5.78***	227.8± 5.78***	8.83± 0.54**	12.17± 1.04^{ns}
IV	Diazepam 2mg/Kg	103.5± 5.34***	196.5±5.34***	10.67± 1.45***	11.67± 0.88*

Table-2: Effect of EEPGL in Y-maze model

Groups	Treatment	Number of visits
I	Vehicle	57.67± 3.65
II	EEPGL 150 mg/kg	47.17± 3.75^{ns}
III	EEPGL 300 mg/kg	42.00± 2.74**
IV	Diazepam 2mg/Kg	29.33± 2.06***

Table- 3: Effect of EEPGL in Hole-board model

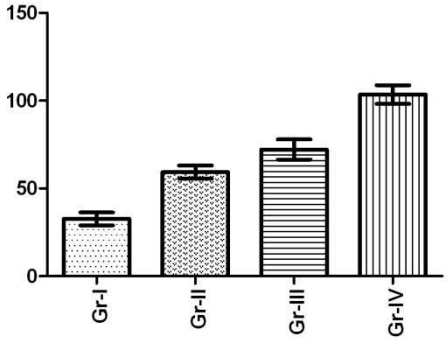
Groups	Treatment	No. of head dipping	No. of line crossing
I	Vehicle	21.17± 2.44	62.00± 3.80
II	EEPGL 150 mg/kg	28.00± 2.53^{ns}	69.17± 3.93^{ns}
III	EEPGL 300 mg/kg	32.50± 3.73*	83.00± 3.83**
IV	Diazepam 2mg/Kg	41.83±3.20***	116.8± 3.36***

Table-4: Effect of EEPGL in locomotor activity

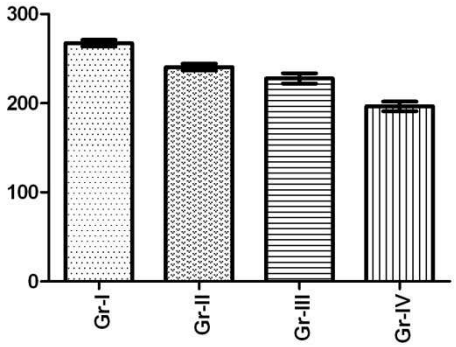
Group	Treatment	Locomotor readings for 10 min.
I	Vehicle	764.2± 25.39
II	EEPGL 150 mg/kg	690.7± 19.38*
III	EEPGL 300 mg/kg	653.3± 16.25**
IV	Diazepam 2mg/Kg	353.3± 16.80***

*Values represented in (Mean ±S.E.M. (n=6), [ns] Non significant, *p<0.05, **p<0.01, ***p<0.001, p compared vs. Group I*

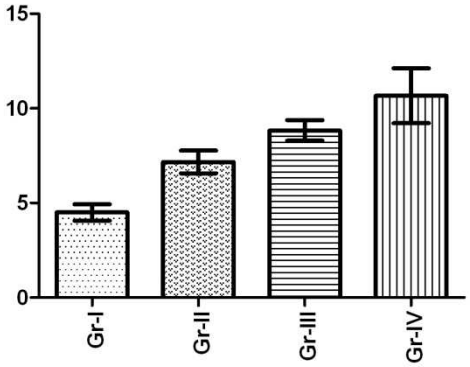
Graph 1: Effect of EEPGL in EPM model



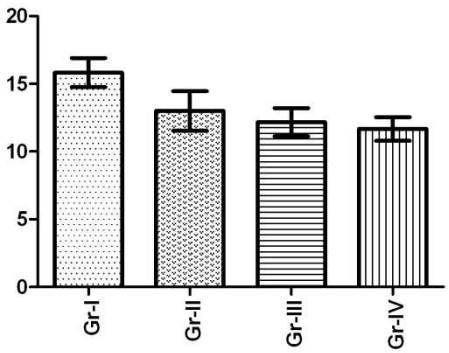
a. Time spent in open arm in (sec) Vs Groups



b. Time spent in enclosed arm (sec) Vs Groups

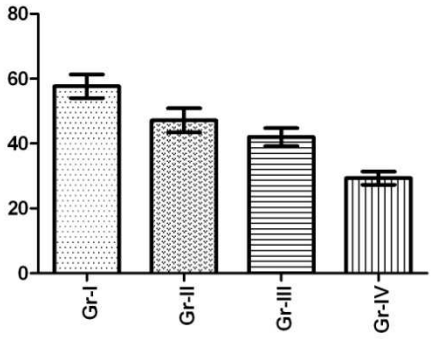


c. No. of entries in open arm Vs Groups



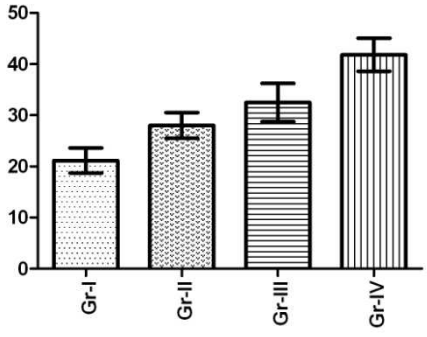
d. No. of entries in enclosed arm

Graph 2: Effect of EEPGL in Y-Maze model



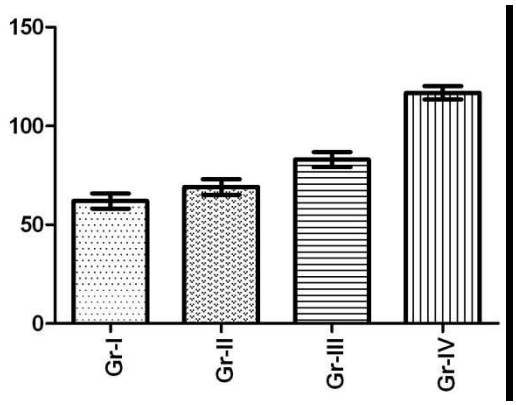
No of visits Vs Groups

Graph 3: Effect of EEPGL in Hole-board

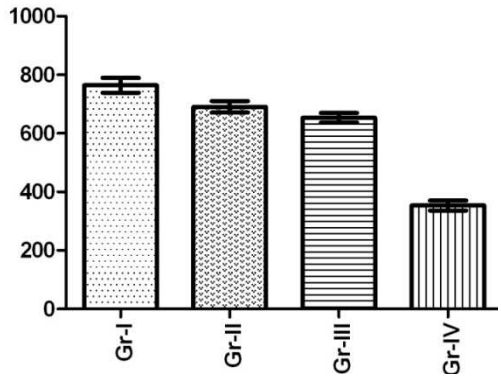


No. of head dipping Vs Groups

Graph 3: Effect of EEPGL in Hole-board **Graph 4: Effect of EEPGL in Actophotometer**



No. of Line crossings Vs Groups



Locomotor readings Vs Groups

The etiology of most anxiety disorders are not fully understood, but various studies has shown the involvement of GABAergic, serotonergic neurotransmission in etiology, expression and treatment of anxiety [16]. The adrenergic and dopaminergic systems have also been shown to play a role in anxiety [17]. Despite the widespread traditional use of *Pisonia grandis* R.Br for treating various disorders there are no reports of scientific evaluation of its anxiolytic activity. The present work demonstrated that the EEPGL has anxiolytic activity in mice in several animal models of anxiety like by EPM, Y-maze, Hole-board and Actophotometer .The EPM is highly sensitive to the influence of both anxiolytic and anxiogenic drugs acting at the $GABA_A$-benzodiazepine complex [18]. This animal model is considered one of the most widely validated tests for assaying sedative and anxiolytic substances such as the benzodiazepines In EPM, normal mice will normally prefer to spend much of their allotted time in the closed arms. This preference appears to reflect an aversion towards open arms that is generated by the fears of the open spaces. Drugs that increase open arm exploration are considered as anxiolytic and the reverse holds true for anxiogenics. In this study, we observed that EEPGL induced significant increases in the both number of entries and time spent in the open arms and the number of entries and time spent in the closed arms were reduced in the EPM model. The results obtained in the Y-maze model showed that the number of visits in the three arms decreased significantly for all groups when compared to the control animals, which supports the anxiolytic activity of EEPGL. In the Hole-board model a significant increase in the exploratory head-dipping and line crossing behavior were observed after treatment with EEPGL thus reinforcing the hypothesis that it has anxiolytic activity. Locomotors activity is considered as an index of alertness and a decrease in that indicates a sedative effect [19]. Both the doses of the EEPGL showed a decrease in the locomotors score, thus indicating the sedative effect of the extract.

It may possible that the mechanism of anxiolytic action of EEPGL could be due to the binding of any of the phytoconstituents to the $GABA_A$-BZD complex. In support of this, it has been found that flavones bind with high affinity BZD site of the $GABA_A$ receptor [20].

CONCLUSION

From the above observations we can conclude that Ethanolic extract of *Pisonia grandis* R.Br leaves possesses anxiolytic activity at both the dose level which is comparable with the standard diazepam. However further studies are required to know the exact mechanism of action of EEPGL as anxiolytic.

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