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Original Article

Anxiolytic Activity Of Seed Extract Of Caesalpinia Bonducella (Roxb) In Laboratory Animals

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Abstract

In the traditional system of Indian medicine C.bonducella is widely used for its antipyretic, antiperiodic, anticonvulsant and antiparalytic activities. The present study was aimed to explore the anxiolytic activities of seed extract of C.bonducella in experimental animals, mice and rats. In Stair-case model, all the three doses i-e low, medium and high 400, 600 and 800mg/kg of PECB had showed a significant and dose dependent Anxiolytic activity by increasing the number of steps climbed, without any significant effect on rearings by all these three doses. Similarly in EPM model medium and high doses, but not the low dose of PECB had significantly enhanced both number of entries and time spent in open arms and decreased in number of entries and time spent in closed arms. In Hole- board model, medium and high doses 600 and 800mg/kg but not the low dose 400mg/kg of PECB had significantly enhanced the number, latency and the duration of head dipping but not the rearings. However in LDT model high doses 800mg/kg of PECB had significantly exhibited anxiolytic activity by increasing time spent, number of crossings in light compartment and decreased the time spent in dark compartment and decreased the number of rearings in both light and dark compartments. In OFT models, medium and high doses 600 and 800mg/kg but not the low dose 400mg/kg of PECB had significantly enhanced total locomotion, central locomotion, number of grooming but the immobility time has drastically reduced. All doses of PECB have not exerted any significant effect with rearing, defecation and urination. Moreover in Mirror-chamber model of anxiety, both medium and high doses 600 and 800mg/kg but not the low dose 400mg/kg of PECB had significantly reduced the time latency to enter in to the mirror chamber and increased the number of entries and time spent in the chamber. Thus the result recorded with above experimental models confirms the anxiolytic activity of PECB.

Introduction

In day today life of stress and strain there is a dire need for agents having neuroprotective and neuropharmacological activity enhancing learning and memory caliber of the brain [1]. Stress involves complex biochemical, neurological and immunological mechanisms and plays a crucial role in the genesis/progression of a variety of disease states ranging from psychiatric disorders like depression and anxiety, immunosupression, endocrine disorders including diabetes mellitus, impotency and cognitive dysfunctions [2].

Anxiety related disorders such as generalized anxiety, panic, obsessive-compulsion, phobias or post traumatic stress disorders are common and major cause of disability [3] and 1/8 th of the total population worldwide affected with anxiety and became a very important area of research interest in psychopharmology 4 . Anxiety is also an obvious component of many psychiatric and medical conditions 5 .

During the last two decades, pharmacotherapy with psychoactive drugs has become management of anxiety, stress and psychomotic disorders. Traditionally pharmacological research in the area of anxiety and stress treatment is very much influenced by the availability of anxiolytic drugs. Throughout history recorded, ethanol was and is the standard drug for treatment of feelings of discomfort, tension, anxiety and stress [6] .

Though barbiturates were dominant agents from 1900-1950 because of considerable concern about their safety lead to the search of better alteration. Moreover benzodiazepines (bdz) as anxiolytic agents have brought tremendous progress in understanding the physiological, biochemical and pathological status of the disease. However the use of tranquillizer and psychotropic drugs leads to

variety of autonomic, neurologic and hematopoietic disorder, but these agents primarily relieve the symptoms and offer a palliative relief of a temporary nature [7].

In recent years use of alternative medicine in particular, derived from plant have been increased in a number of patient with condition that affect the mind [8].

In traditional system of indian medicine (Ayurveda) caesalpinia bonducella (roxb.) is widely used for its antipyretic, antiperiodic, anthelmintic, anti-inflammatory, antimalarial and also for various ailments like skin diseases, leprosy, hydrocele, orchitis, convulsions, paralysis and similar nervous complaints [9].

Materials and Methods

Animals

Swiss albino mice of either sex weighing between 20-30g were procured from Shri Venkateswara Enterprises, Bangalore for experimental purpose. All the animals were acclimatized for seven days under standard husbandry conditions i.e.; room temperature of $24^0 \pm 10^0$ C; relative humidity 45-55% and a 12:12h light/ dark cycle. [40,41] The animals had free access to standard rat pellet diet (Amrut laboratories, Pranava Agro Industries Ltd., Sangli, India), with water provided ad libitum under strict hygienic conditions. Each experimental group had separate set of animals and care was taken to ensure that animals used for one response were not employed elsewhere. Animals were habituated to laboratory conditions for 48 hours prior to experimental protocol to minimize if any of non-specific stress. The approval of the Institutional Animal Ethical Committee (IAEC) of V. L. College of Pharmacy Raichur (Karnataka) was taken prior to the experiments. All the protocols and the experiments were conducted in strict compliance according to ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), with registration number 557/02/c/CPCSEA.

Preparation of seed extracts of

Seed powder of C.bonducella will be successively extracted with petroleum ether, ethanol, methanol and water. Each time before extracting with the next solvent, marc will be dried in Hot air-oven below 50^0 C. Finally the marc will be macerated with chloroform water (i.e.; chloroform acts as a preservative) for 24 hrs to obtain the aqueous extract. Each extract will be concentrated by distilling off the solvent and then evaporating to dryness on the water bath.

Drugs

Diazepam [Ranbaxy Laboratories Ltd, Mumbai, India], Tween-80 [s.d.fine Chem Ltd. Mumbai], Petroleum Ether [The Ugar Sugar Works Ltd. Ugar Khurd, Belgaum] and Distilled water [Mysore Petro Chemicals, Raichur, India].

Preliminary phytochemical Screening „

The preliminary phytochemical investigations will be carried out with the seed extract of C.bonducella (PE) for qualitative identification of phytoconstituents.

Experimental design

Acute toxicity test of

The acute toxicity of C. bonducella will be determined by using albino mice of either sex (20-25 g), maintained under standard conditions. The animals will be fasted for 3 hr prior to the experiment. Animals will be administered with single dose of seed extract of C. bonducella and observed for its mortality upto 48 hr study period (short term toxicity). Based on the short-term toxicity profile, the next dose will be determined as per OECD guidelines No 425. From the LD_{50} dose 1/20, 1/10 and 1/5 th doses are to be selected and considered as low, medium and high dose respectively.

Grouping of animals and treatment schedule

Male albino mice (22-25g) and wistar rats (150-200g) were divided into following groups each consisting of six animals.

Group A - Normal control (2% gum acacia p.o)

Group B - Standard (Diazepam 3mg/kg p.o)

Group C - Seed extract of C.bonducella (400mg/kg p.o)

Group D - Seed extract of C.bonducella (600 mg/kg p.o)

Group E - Seed extract of C.bonducella (800 mg/kg p.o)

All the drugs were administered to the respective groups in all the models for a period of eight days and experiments were performed one hour after the administration of last dose.

All the experiments were carried in a sound attenuated dark room. After test with each animal, all the apparatus was cleaned with 5% alcohol in order to eliminate any olfactory cues which might modify the behavior of the next animal.

Staircase test in mice

The staircase is composed of five identical steps 2.5 cm high, 10 cm wide and 7.5 cm deep. The internal height of the walls is constant along the whole length of the staircase. Each animal is used only once.ment. At the end of experimental period mice were placed individually on the floor of the box with its back to the staircase. Total number of steps climbed and total number of rearings were recorded over a period of 3 min. A step is considered to be climbed only if the mouse has placed all four paws on the step[15] .

Elevated Plus-Maze Test in mice

The plus-maze apparatus comprises of two open arms (16×5cm) and two closed arms (16×5×12cm) that extend from a common central platform (5×5cm). The entire maze is elevated to a height of 25cms above the floor level. Mice were placed individually in the center of the maze facing one of the enclosed arms for recording various parameters in a period of 5 min.[16,17,18,19]

Hole-board test in rats

The apparatus used in this model consists of wooden chamber (40x40x25 cm) with 16 holes (diameter 3 cm) on the floor, elevated from the ground so that the rats could peep through the holes each rat will be placed individually in the apparatus for recording following parameters, latency to the first head dips, no. of head dips in the holes, total time spent with the head dips, no. of rearings,

no. of defecation units.[20]

Light-dark model transition test in mice

The light-dark apparatus consists of two-compartment chamber (40×60×20cm/h) comprising of a brightly illuminated area (40×40cm) and a dark area (40×20 cm) separated by a wall with a round hole (7 cm diameter) will be used. Mice were placed individually in the illuminated part of the cage and following parameters were recorded during the test session of 5 min, total no. of crossings, no. crossings between the light and dark area, total time spent in the illuminated part of the cage, time spent in the dark part of the cage, no. of rearings in illuminated part of the cage, no. of rearings in dark part of the cage, no. of defecation units. [21,22,23]

Statistical analysis

The values were expressed as mean ± SEM from 6 animals. The results were subjected to statistical analysis by using ANOVA followed by Dennett's- t -test test to calculate the significance difference if any among the groups. $P<0.05$ was considered significant.

Results

Effect of on mice in Stair-case test

Three different doses of PECB (400, 600 and 800mg/kg) were subjected for anxiolytic activity using Stair-case test in mice. These doses when administered orally daily once for 7 days, high and medium doses (800&600mg/kg) has showed significant increase in no. of steps climbed and rearings dose dependently when compared to control. However, PECB at 400 mg/kg had not shown any significant effect with no. of rearings. Standard drug diazepam (2mg/kg) had exhibited significant anxiolytic effect.

Figure 1

Table 1: Anxiolytic effect of PECB on stair-case model in mice

Treatment	No. of rearing	No. of steps climbed
Control	7.667 ±1.667	7.833 ±0.4773
Diazepam	33.000**±6.335	20.667** ±2.499
PECB (400mg/kg)	13.667^{ns} ±3.106	14.333* ±1.585
PECB (600mg/kg)	23.333* ±5.377	16.000** ±0.9309
PECB (800mg/kg)	27.833** ±1.621	19.500** ±1.727
One-way ANOVA F df	6.357 29	10.027 29
*P<0.05, **P <0.01 when compared to control		

Effect of on mice in Elevated plus maze

Diazepam has long been reported for its anxiolytic activity in mice with the EPM model. In our study also, a significant anxiolytic effect was recorded with diazepam as increased number of entries in to open and decreased number of entries in to closed arms and with increased time spent in open and central platform but not in closed arms. Insignificant effect was recorded with total

number of entries in both the arms when compared to control. In chronic study when different doses of PECB i.e. 400, 600 and 800mg/kg were administered orally daily once for seven days, it was found that lower dose (400mg/kg) increased the number of entries and time spent in the open arm ,central platform and decreased the time spent in closed arm as compared to control group. Where as medium and high doses (400 and 800mg/kg) had increased the number of entries and time spent in the open arm, central platform and decreased the number of entries and time spent in closed arm as compared to control group and exhibited statistically significant activity. No significant effect was observed with total number of entries with all doses. Among these, low dose (400mg/kg) had increased time spent in central platform when compared to medium, high and standard doses respectively.

Figure 2

Table 2: Anxiolytic effect of PECB with elevated plus maze model in mice

Groups	Number of entries (Counts/5min)		Time spent in (5min)			Total number Of entries
	Open arm	Closed arm	Open arm	Closed arm	Central platform	
Control	5.000 ±0.5164	15.333 ±1.476	36.667 ±5.024	191.00 ±18.007	72.333± 15.607	20.333 ±1.726
Diazepam	12.833** ±1.249	6.167** ± 0.9098	106.67** ±11.652	82.167** ±16.899	111.17± 25.988	19.000 ±1.125
PECB(400mg/kg)	8.000ns ± 0.3651	15.833ns ± 2.023	42.667ns ± 16.399	140.17ns ±10.628	117.17± 13.826	23.833 ±2.072
PECB(600mg/kg)	11.667** ±1.282	8.500* ± 2.141	109.00** ± 16.813	113.67** ±14.375	77.333± 15.549	20.167 ±1.939
PECB(800mg/kg)	12.333** ± 0.8819	7.667** ± 0.4944	132.17** ± 8.731	115.67** ±12.614	52.167± 11.353	20.000 ± 0.8944
One way ANOVA F	12.902	8.636	11.722	7.549	2.542	1.299
df	29	29	29	29	29	29

*P<0.05, **P <0.01 when compared to control

Effect of on mice in Hole- board model

Three different doses of PECB (400, 600 and 800mg/kg) were subjected for anxiolytic activity using HB- model in mice. These doses when administered orally daily once for 7 days, high and medium doses (800&600mg/kg) but not low dose (400mg/kg) has shown significant increase in latency to the 1 st head dips, number of head dips and time spent in head dips. A significant increase in number of rearing was observed in both medium and high doses as compared to control group. Defecation units have significantly reduced with high dose but not with low and medium doses as compared to control group. Standard drug diazepam (2mg/kg) had exhibited significant anxiolytic activity.

Figure 3

Table 3: Anxiolytic effect of PECB with Hole Board Model in rats

Treatment	No of head dips	Time spent in head dips	Latency to first head dips	No. of rearings	No. of defecation
Control	5.833 ±1.470	10.333 ±2.290	38.333 ± 5.321	6.500 ±0.9916	3.667 ±0.6146
Standard	14.833** ±0.7032	42.833** ±3.851	12.833** ± 0.7923	22.000** ±3.044	0.8333* ±0.4773
PECB (400mg/kg)	9.000ns ±3.327	19.000 ns ±5.825	29.500ns ±2.705	15.000ns ±1.807	2.667ns ±0.8433
PECB (600mg/kg)	13.000* ± 1.000	23.333* ±0.9545	20.833** ±1.493	17.833* ±3.719	1.667ns ±0.5578
PECB (800mg/kg)	14.000* ± 1.238	23.833* ±1.327	14.500** ±2.172	19.833** ±2.688	1.167* ±0.6540
One way ANOVA F df	4.447 29	12.502 29	13.237 29	5.244 29	3.277 29

n = 6, *P<0.05, **P <0.01 when compared to control

Effect of on mice in Light-Dark transition test

Three different doses of PECB (400, 600 and 800mg/kg) were subjected for anxiolytic activity using LDT model in mice. These doses when administered orally daily once for 7 days, high dose (800mg/kg) but not the low and medium doses (400 & 800mg/kg) had produced an increase in number of crossings and time spent in light box and decrease in the number of rearings in both light and dark compartments. Defecation boli were not significantly altered with these different doses of PECB when compared to control group. High dose had statistically showed significant anxiolytic activity and Standard drug diazepam (2mg/kg) had exhibited significant anxiolytic activity.

Figure 4

Table 4: Anxiolytic effect of PECB in mice in Light-dark model

Treatment	No. of Crossings	Time(sec) spent in light box	Time(sec) spent in Dark box	No. of rearings in L.box	No. of rearings in D.box	No. of defecation units
Control	4.833 ±1.108	83.500 ±3.971	223.67 ±11.589	7.000 ±3.864	21.667 ±5.371	0.5 ±0.2236
Diazepam	11.333* ±1.726	197.67* ±32.893	102.33* ±32.893	0.1667** ±0.1667	2.333* ±1.961	1.16 ±0.6540
PECB (400mg/kg)	7.000ns ±1.653	111.67ns ±28.992	188.33ns ±28.992	6.500ns ±4.161	15.500ns ±7.334	0.5 ±0.2236
PECB (600mg/kg)	8.333 ns ±2.011	147.33ns ±9.680	142.67ns ±8.739	3.000ns ±1.155	6.000* ±0.6831	0.5 ±0.3416
PECB (800mg/kg)	10.667* ±1.022	186.17* ±28.268	113.83* ±28.268	2.667* ±1.856	1.833** ±1.222	1.16 ±0.5426
ANOVA F df	2.954 29	3.889 29	4.452 29	1.101 29	4.331 29	0.7101 29

*P<0.05, **P <0.01 when compared to control

Discussion

The stair-case test has been proven as a simple and reliable method for screening of anxiolytics in several laboratories. The stair-case test for evaluating anxiolytic activity was originally described for rats [24] . When introduced into a novel environment, rodents experience anxiety manifested by increased vigilance and behavioral activity. In the stair-case paradigm, step-climbing is purported to reflect exploratory or locomotor activity, while rearing behaviors is an index of anxiety state. The number of rearing and steps climbed are recorded in a 5 min period. The test was modified for rapid

screening of anxiolytic activity in mice [25].

When compared to control PECB with all the three doses i-e, low, medium and high (400, 600 and 800mg/kg) tested had showed a significant increase in number of steps climbed up and rearings dose dependently. However, PECB at 400 mg/kg had not shown any significant effect with no. of rearings. Standard drug diazepam (2mg/kg) had exhibited significant anxiolytic effect.

The elevated plus maze is a well-established animal model for testing anxiolytic drugs [26,27]. A standard anxiolytic drug diazepam used clinically is also employed in behavioral pharmacology as a reference compound for inducing anxiolytic like effects [28], even when the compound being screened does not act via benzodiazepine receptors.

Although orginal validation of the EPM was performed in rats [35], it has also been found to be selectively sensitive to the effects of anxiolytic and anxiogenic drugs in mice [30].

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 [34]. 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. The primary index is spatiotemporal in nature: it is reduced by anxiolytic drugs and can be increased by anxiogenic compounds [32]. The decrease in time spent on the central platform is another indication of reduced “decision-making” behaviour. Both parameters are accepted as reliable indicators of anxiety and fearfulness [33].

PECB at medium and high doses (600 and 800mg/kg), but not the lower dose (400mg/kg) had increased the time spent in and number of entries into open arms with a decreased time spent and number of entries into closed arms. After treatment with all three doses in different groups the total numbers of entries into both open and closed arms were decreased insignificantly as compared to control group but except with the low dose (400mg/kg). It can be suggest that, the higher doses of PECB may have the sedative effect more than the standard drug and as a result animals spent more time in open, closed arms & less time in central platform, while standard drug treated animals spent more time in open arm & central platform but less time in closed arm. Lower dose of PECB (400mg/kg) didn't alter the above parameters significantly; suggesting that 400mg/kg of PECB had not exhibiting anxiolytic effect. Therefore, behavioral alterations induced by medium and higher dose of PECB (600 and 800mg/kg) were consistent with anxiolytic activity. The three dose of PECB (400,600 and 800mg/kg) showed dose depended anxiolytic profile. The lower dose of PECB (400mg/kg) didn't exhibit any anxiolytic activity.

Standard drug diazepam act selectively on GABAA receptors which mediate fast inhibitory synaptic transmission throughout the CNS. Benzodiazepines (BDZs) bind to the gamma sub-unit of the GABA-A receptor, that causes an allosteric (structural) modification of the receptor results with an increase in $GABA_A$ receptor activity. BDZs do not substitute for GABA, which bind at the alpha sub-unit, but increase the frequency of channel opening events which leads to an increase in chloride ion conductance and inhibition of the action potential. These drugs also exert a marked taming effect, allowing animals to be handled more easily [34].

Hole-board model indicated that head-dipping behaviour was sensitive to changes in the emotional state of the animal, and suggested that the expression of an anxiolytic state in animals may be reflected by an increase in head-dipping behaviour [36].The PECB medium and high doses (600 & 800mg/kg) shows increase in number, latency and duration of head dipping and the number of rearing in the hole board test. It could be argued that the increased head-dipping in rats is merely an artifact of the hyperactivity induced by the drug. But the effect of low dose (400mg/kg) was found to be insignificant when compared with control. There is a significant reduction in defecation units seen with the high dose (800mg/kg), but not with medium and low doses (600& 400mg/kg)

respectively. Diazepam a putative anxiolytic agent increased the number, latency and duration of head dipping and the number of rearing with reduction in defecation units as also noted with PECB medium & high (600 & 800mg/kg) doses. Therefore the increased number of head dippings & rearings are not only a representation of hyperactivity but also a reflection of anxiolytic effect.

It has been suggested that some animal models based on spontaneous behaviour or ethologically based models [37] like the light-dark test, may be more sensitive to the behavioural responses than conditioned paradigms [38].

The light-dark test may be useful to predict the anxiolytic like activity of drugs in mice. It has the advantages of being quick and easy to use without food and water deprivation prior training of animals and natural stimuli are used. Transitions have been reported to be an index of activity exploration because of habituation over time and the time spent in each compartment to be a reflection of aversion [39].

In Light-Dark Transition test, the apparatus contains two compartments i.e. light and dark. Animals always try to spend more time in dark compartment because of fear about new environment. In this model, four behavioral events were observed i.e. number of crossings to light compartment, time spent in light and dark box, number of rearings in light and dark box and defecation units. In this study high doses (800mg/kg) of PECB had significantly increased the time spent in light compartment along with reduced time spent in dark compartment, number of crossings and decreased the number of rearings in light as well as dark compartments, indicating that high dose of PECB had produced significant anxiolytic effect but not medium & lower dose (600 & 400mg/kg). Medium dose (600mg/kg) had shown decreased rearings in dark compartment, but the effects on remaining parameters were insignificant as compared with control.

It is well known that BZDs have sedative, anticonvulsant, anxiolytic and hypothermic effect. Anxiolytics are known to exert pharmacological action by causing an increase in GABA content in the cerebral hemisphere [40].

Several lines of evidence show that natural and synthetic flavonoids are potent anxiolytic agents without sedative, myorelaxant or amnestic effects. It is known the participation of GABA in these effects [41].

Conclusion

The results obtained from these experimental models clearly confirmed the anxiolytic activities of PECB. Phytoconstituents like flavonoids and saponins were reported for their anxiolytic effect and these two were present in petroleum ether extract. So these active principle might be responsible for anxiolytic effects.

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