

ANXIOLYTIC ACTIVITY OF *FLACOURTIA INDICA* USING STAIR CASE AND LIGHT DARK EXPLORATION METHODS IN MICE

N. Gnanasekar¹, C. Uma Maheswara Reddy², N. Narayanan³, C. Chamundeeswari⁴, T.K. Gopal⁵

¹Department of Pharmacology, Kamalakshi Pandurangan College of Pharmacy, Tiruvannamalai.

²Faculty of Pharmacy, Sri Ramachandra University, Chennai, Tamil Nadu, India.

³Director, Jaya College of Pharmacy, Chennai, Tamil Nadu, India.

⁴Faculty of Pharmacy, Sri Ramachandra University, Chennai, Tamil Nadu, India.

⁵Faculty of Pharmacy, Sri Ramachandra University, Chennai, Tamil Nadu, India.

*Corresponding author: E.Mail:nata67@rediffmail.com

ABSTRACT

Traditional system of medicine has been used to treat various diseases including anxiety. *Flacourtia Indica* has been found to have many medicinal properties and the present study aims at evaluating its use as an anxiolytic. The staircase and light dark exploration methods have been used to study its anxiolytic activity. In both the methods, alcoholic extract of *Flacourtia Indica* seems to have significant anxiolytic activity.

KEY WORDS: *Flacourtia Indica*, Staircase, light-dark exploration, anti anxiety.

1. INTRODUCTION

Anxiety which is one of the modern man's disease, affects 1/8th of population in developing countries. Anxiety is defined as a feeling of uncertainty, apprehension or tension. Benzodiazepines (BZD) are drugs used for reducing anxiety. Their intake has become necessary as anxiety disrupts day to day life. BZD's like other allopathic medicines has many side effects which includes physical dependence, withdrawal syndromes, muscle fatigue etc., so a new and safe drug with no side effects has to be discovered from plant kingdom. Plants have long been used to treat CNS disorders and folk medicines particularly values plants that "calm down," tranquilize and raise mood (Medina, 1990).

Flacourtia Indica is a small deciduous thorny shrub, found throughout India in scrub forests and rocky hills up to 900m. The roots are used in treating poisonous bites, skin diseases, nephropathy and psychopathy. The leaves are used for treating pruritus and scabies. Fruits are useful for treating jaundice, gastropathy and splenomegaly (Orient Longman, 1995). Hence the present study is used for evaluating the anxiolytic property of the alcoholic extract of leaves of *Flacourtia Indica*.

2. MATERIALS AND METHOD

Collection and authentication of plant material: Plant material was collected from the hilly regions of Tirupati, Andhra Pradesh. It was identified and authenticated by Dr. K. Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupati..

Preparation of alcoholic extract of *F. indica*: The leaves were shade dried and powdered. The powdered leaf was extracted with ethanol using Soxhlet apparatus for 2 days. The obtained extract was concentrated by distillation and stored in a desiccator.

LD₅₀ value of *Flacourtia indica* (Gnanasekar, 2013): LD₅₀ of the test drug was found to be greater than 2000 mg/kg body weight. Hence, the test drug falls in the "category-5" or "unclassified" in accordance to the Globally Harmonized System

Grouping of Animals and Treatment Schedule: Male albino mice (22-25g) were divided into following groups each consisting of six animals.

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

Group B - Standard (Diazepam 2mg/kg p.o.)

Group C - Test drug 100mg/kg p.o.

Group D - Test drug 200mg/kg p.o.

Staircase test in mice: The staircase is composed of five identical steps 2.5 cm high and 10 cm deep. The internal height of the walls is constant along the whole length of the staircase. Each animal is used only once. At the end of experimental period mouse 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 3min. A step is considered to be climbed only if the mouse has placed all four paws on the step (Vogel, 2005).

Light - dark model transition test in mice: The light - dark apparatus consists of two - compartment chamber (40x60x20cm) comprising of a brightly illuminated area (40x40cm) and a dark area (40x20cm) separated by a wall with a round hole (7cm diameter) . 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. of 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 and no. of defecation units (Zanoli, 2000; Maribel, 2006; Crawl, 2008).

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

3. RESULTS AND RESULTS

Effect of Test drug on mice in stair-case test: Test drug (100mg/kg and 200mg/kg) were subjected for anxiolytic activity using Stair-case test in mice. The dose when administered orally daily once for 7 days, 100mg/kg and 200mg/kg has shown significant effect with no. of rearings. Standard drug Diazepam (2mg/kg) has exhibited significant anxiolytic effect.

Table No 1: Effect of Test drug on mice in Rearing test

Groups	Treatment	Rearing test value	Significant Value
1	Control	16.29 \pm 0.77	-----
2	Diazepam	11.80 \pm 0.46***	P<0.001
3	Test drug (100mg/kg)	14.69 \pm 1.00**	P<0.01
4	Test drug (200mg/kg)	12.08 \pm 1.05***	P<0.001

***P<0.001; **P<0.01 when compared to control

Table No 2: Effect of Test drug on mice in Climbing test

Groups	Treatment	Climbing test value	Significant Value
1	Control	8.61 \pm 2.21	-----
2	Diazepam	23.41 \pm 1.34***	P<0.001
3	Test drug (100mg/kg)	11.69 \pm 1.94**	P<0.01
4	Test drug (200mg/kg)	20.32 \pm 1.06***	P<0.001

***P<0.001; **P<0.01 when compared to control

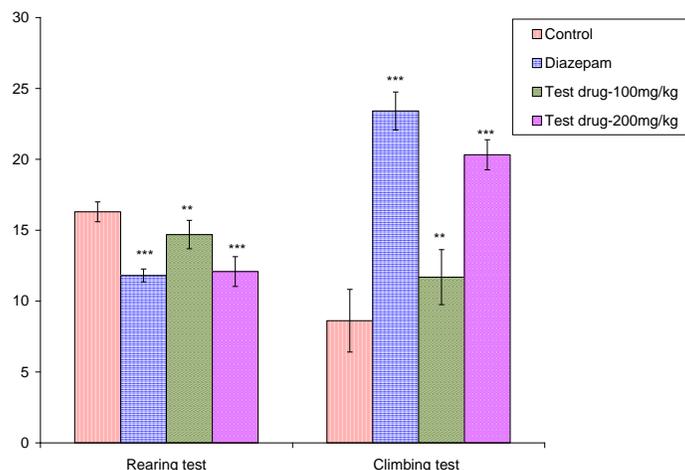


Figure.1.Effect of Test drug on mice in stair-case test

Effect of Test drug on mice in Light - dark transition test: Test drug was subjected for anxiolytic activity using LDT model in mice. The dose was administered orally daily once for 7 days. Test drug 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 the dose of test drug compared to control groups. The selected dose statistically showed significant anxiolytic activity and standard drug Diazepam (2mg/kg) exhibited significant anxiolytic activity.

Table.3.Effect of Test drug on mice in Light - dark transition test

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	197.678* ± 32.893	102.33* ± 32.893	0.1667*** ± 0.1667	2.333* ± 1.961	1.16 ± 0.6540
Test drug (100mg/kg)	7.000* ± 1.653	111.67* ± 28.992	188.33* ± 28.992	6.500*** ± 4.161	15.500* ± 7.334	0.5 ± 0.2236
Test drug (200mg/kg)	8.02 ± 1.822	108.58 ± 30.124	192.00* ± 25.120	6.22*** ± 3.980	14.02* ± 6.422	0.5 ± 0.280

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

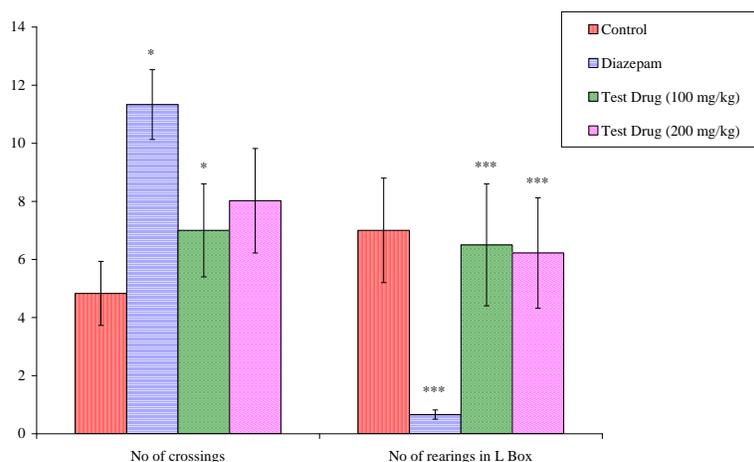


Figure.2. Effect of Test drug on mice in Light - dark transition test

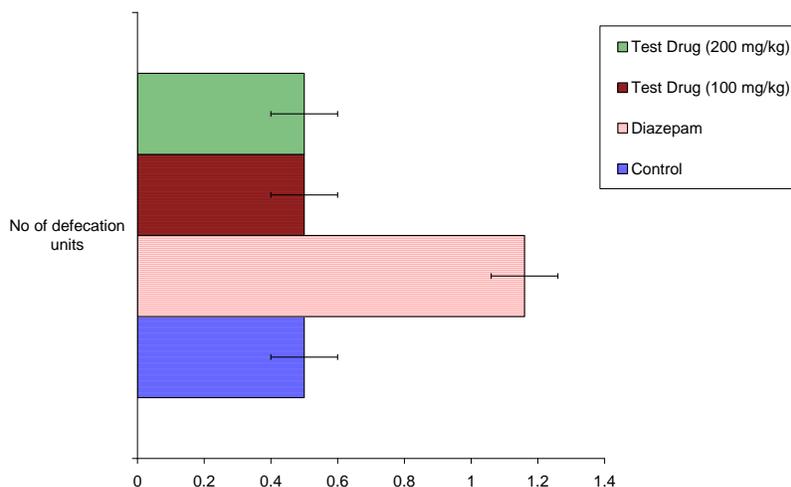


Figure.3. Effect of Test drug on mice in Light - dark transition test

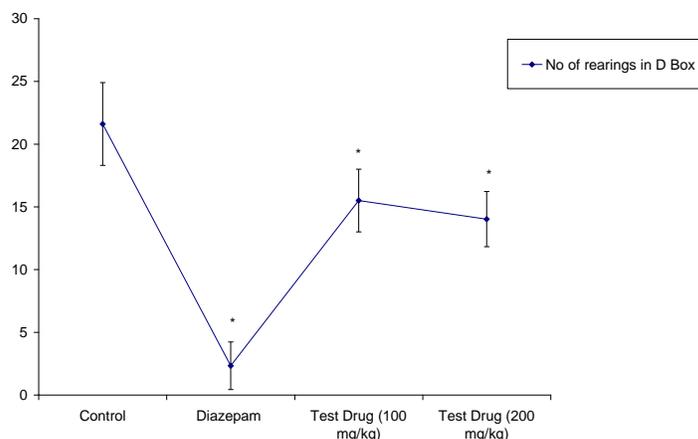


Figure.4. Effect of Test drug on mice in Light - dark transition test

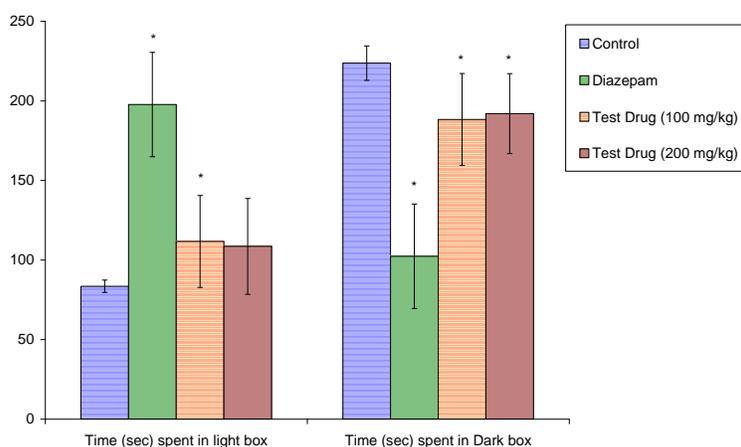


Figure.5. Effect of Test drug on mice in Light - dark transition test

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 (Thiebot, 1973). When introduced in to 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 loco motor activity, while rearing behavior 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 (Simiand, 1984). The test drug 100mg/kg and 200mg/kg has shown significant effect with no. of rearings. Standard drug Diazepam (2mg.kg) had exhibited significant anxiolytic effect. The light-dark test may be more sensitive to the behavioral responses than conditioned paradigms (Griebel, 1996). The light-dark test may be useful to predict the anxiolytic 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 (Belzung, 1987). 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 the test dose (100mg/kg and 200mg/kg) had shown decreased rearings in dark compartment, but the effects on remaining parameters were insignificant as compared with control.

4. CONCLUSION

From the above study it may be inferred that alcoholic extract of *flacourtia indica* has significant anxiolytic activity. Further investigations are necessary for isolation of compounds in *flacourtia indica* for anxiolytic activity.

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