APPENDIX 1

SRI VENKATESWARA UNIVERSITY
TIRUPATI - 517 502, A.P., INDIA

Dr. K. MADHAVA CHETTY
M.Sc., M.Ed., M.Phil., Ph.D., PG DPD
Assistant Professor
Department of Botany

Phone: +91-877-2233685 (R)
Cell: + 91-9490486654
Email : madhavachetty@gmail.com

Date:- 27-07-2009

AUTHENTICATION CERTIFICATE

I hereby certify that the following plant species for pharmacognostical / pharmaceutical / pharmacological / phytochemical investigation research work is identified and their botanical name and family name is given.

<table>
<thead>
<tr>
<th>Botanical Name</th>
<th>Family</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flacourtia indica Linn.</td>
<td>Flacourtiaeae</td>
</tr>
</tbody>
</table>

Authenticated by

K. Madhava Chetty
(Dr. K. MADHAVA CHETTY)
DR. K. MADHAVA CHETTY
M.Sc., M.Ed., M.Phil., Ph.D., PG DPD
ASSISTANT PROFESSOR
DEPARTMENT OF BOTANY
SRI VENKATESWARA UNIVERSITY
TIRUPATI-517 502, A.P. India
APPENDIX 2

SRI RAMACHANDRA UNIVERSITY
(Established Under Section 3 of the UGC Act, 1956)
Purur, Chennai - 600 116.

INSTITUTIONAL ANIMAL ETHICS COMMITTEE

Chairman
Prof. S. Venkataraman

Director - Animal Research
Prof. S. Thaniakachalam

Ex-Officio Member
Prof. S.P. Thyagarajan

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Members
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Expert Member

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Assoc. Prof. CMC Research Officer
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HOD - Pathology

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HOD Representative

Mrs. Girija Kumaravelu
Social Worker

Legal Advisor
Mr. V. Swaminathan

Ph: 91-44-24768991 (Dd)
91-44-24765512
Ext. 232
Fax: 91-44-24768580

LETTER OF APPROVAL


This is to certify that the proposal entitled "Phytochemical and
Pharmacological Evaluation of Flacourtia indica Linn for Anti-anxiety
Activity" has been approved by the Institutional Animal Ethics Committee
(IAEC) in the XIth minutes of IAEC meeting held on 25th February 2008 at Sri
Ramachandra University. The proposal number is IAEC-XII/SRU/77/2008.

<table>
<thead>
<tr>
<th>Species</th>
<th>Rat</th>
<th>Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals approved</td>
<td>-6- (Six)</td>
<td>-18- (Eighteen)</td>
</tr>
</tbody>
</table>

Dr. S. Venkataraman
Chairman-IAEC.

Dr. S. Thaniakachalam
Director-Animal Research.

Place : Chennai.

Date : 25th February 2008.

To

Mr. N. Gnanasekar,
Principal,
K.P. College of Pharmacy,
Thiruvannamalai-3
APPENDIX 3

LIST OF PAPERS PUBLISHED.


STUDY OF ANTI-ANXIETY ACTIVITY OF FLACOURTIA INDICA LINN BY ELEVATED PLUS MAZE AND HOLE BOARD (HEAD DIPPING) METHODS

Gnanasekar. N. ¹, Dr. C. Uma Maheswara Reddy², Dr. N. Narayanan³
¹Department of Pharmacology, Kamalakshi Pandurangan, College of Pharmacy, Tiruvannamalai, Tamil Nadu, India,
²Professor & Head, Sri Ramachandra University, Chennai, Tamil Nadu, India,
³Director, Jaya College of Pharmacy, Chennai, Tamil Nadu, India.

ARTICLE INFO

Keywords
Flacourtia Indica,
Anti-anxiety,
Elevated plus maze,
Hole board,
Acute toxicity.

ABSTRACT
Benzodiazepines (BZD’S) are commonly used anxiolytics but they have many side effects. Therefore new anxiolytics from traditional system of medicine have to be developed with no side effects. Among various medicinal plants Flacourtia Indica has been used to cure various illnesses including psychopathy. But it lacks scientific validation. Therefore the present study aims to evaluate the anti-anxiety activity using Elevated plus maze and Hole board method by using the alcoholic extract of leaves of Flacourtia Indica along with acute toxicity tests. A dose of 100 mg. / kg. body wt. showed significant anti-anxiety activity and acute oral toxicity test performed revealed that LD₅₀ of the test drug was found to be greater than 2000 mg/kg body wt. The results suggest that Flacourtia Indica could be used as a potential anti-anxiety drug in future.

Corresponding author
Gnanasekar. N.
¹Department of Pharmacology, Kamalakshi Pandurangan, College of Pharmacy,
Tiruvannamalai, Tamil Nadu, India,
E.mail: nata67@rediffmail.com

Please cite this article in press as Gnanasekar. N.et.al. Study of anti-anxiety activity of Flacourtia indica linn by elevated plus maze and hole board (head dipping) methods. Indo American Journal of Pharm Research.2013:3(9).
INTRODUCTION

Nearly 4 – 6% of population suffers from anxiety which may be defined as a feeling of apprehension, uncertainty or tension\(^1\). Since anxiety disrupts routine day to day life, consumption of anxiolytic drugs becomes necessary. BZD’s have been used as anxiolytics for more than 3 decades but have many side effects including psychological and physical dependence, withdrawal symptoms fatigue, muscle weakness etc. BZD’s also said to have adverse prenatal effect\(^2\). Studies have been carried out to find out an alternative traditional medicine for treating anxiety as they have no side effects, easily available and are cost effective. Many pharmaceutical companies are in search of more safe plant derived anxiolytics\(^3\). The plant *Flacourtia Indica* belonging to family Flacourtiaceae is also known as Governor’s plum or Madagascar plum in English and Sottaikala, Mallukarai in Tamil\(^4\). Various parts of the plant has different medicinal values and the roots of *Flacourtia Indica* are useful in vitiated conditions of pitta and vata, poisonous bites, skin diseases, nephropathy and psychopathy\(^4\). The survey of literature on *Flacourtia Indica* reveals the use of this plant in psychopathy and hence our presents study has been designated to carry out anti-anxiety activity.

MATERIALS AND METHOD:

Collection and authentification 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 dessicator\(^5\).

Animals:

(i) Acute toxicity studies

Female Sprague drawly rats of 160 – 180 g b.wt were used for the study. The animals were grouped into two (control and test group) of 3 animals / group.

(ii) Anxiolytic activity:

Male Swiss albino mice of 25 – 30 g b.wt were used. They were grouped into three of 6 / group. The animals were housed in a well-ventilated poly-propylene cages. A 12 hr. light / 12 hr. dark artificial photo period were maintained in the room. Room temperature of 22\(\pm\)3 and relative humidity 50-70\% were maintained. Animals had free access to rodent pelleted feed (Nutri lab rodent, tetragon chemic Pvt. Ltd., India) and Reverse Osmosis (Rios, USA) purified water ad libitum.

EXPERIMENTAL PROTOCOL

Acute oral toxicity\(^6\):

Control - 3 animals / group

Test-3 animals / group. Received test drug orally via gastric intubation at a dose level of 2000 mg / kg b. wt. Acute oral toxicity was performed according to the OECD test guideline 423-Acute toxic class method. The test drug was administered once orally via gastric intubation at a dose level of 2000 mg/kg b.wt. Lethality, abnormal clinical signs and body weight changes were observed on the day of dosing and thereafter for 13 days. Gross pathological changes were also observed on the day of termination and classified the drug according to Globally Hormonised System(GHS)

Anxiolytic activity:

Group I - Vehicle control(0.5% CMC)

Group II - Reference control – Diazepam 2 mg / kg / p.o.

Group III - Test drug alcoholic extract. *F.indica* Linn 100 mg/kg/p.o.

The animals were administered with their respective drugs for 5 consecutive days. On day 5, 60 min after drug administration, the anxiolytic activity of *Flacourtia indica* Linn was analysed using elevated plus maze (EPM) and hole board maze in mice.

A. Elevated plus Maze

Elevated plus-maze was a wooden, cross shaped maze, consisting of four arms arranged in the shape of a plus sign. Two of the arms have no side or end walls (open arms; 16x5cm). The other two arms have side and end walls, but are open on the top (closed arms; 16x5x12cm). Where the four arms intersect, there is a square platform of 5x5cm. The maze was elevated to a height of 25cm. After 1h of drug administration; mice was individually placed in the center of the maze facing open arm and allowed to explore for 5 min. The number of entries into open and closed arms and the total time spent in both the arms were recorded\(^7\).
B. Hole board test
Hole-board apparatus was used to assess the anxiolytic behavior of mice. The 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 mice was placed on the center of the maze and allowed to explore free for 5 min. The immobility period, number of head dips and the number of rearing was recorded.

STATISTICAL ANALYSIS:
Statistical Data analysis was performed using Graph pad prism 4.0 version. Data were expressed in mean ± SEM. Mean difference between groups were analysed by one way ANOVA followed by Turkey’s multiple comparison test as posthoc test.

RESULTS AND DISCUSSION:

i. Acute oral toxicity
There were no treatment related death; abnormal clinical signs, remarkable body weight or gross pathological changes were observed in the experimental animals.

From the above results, LD₅₀ of the test drug was found to be greater than 2000 mg/kg b.wt. Hence, the test drug falls in the “category-5” or “unclassified” in accordance to the Globally Harmonised System.

From the toxicity studies it was observed that a oral dose of 2000 mg / kg / b.wt. did not induce drug related toxicity and mortality in the animals and therefore it can be concluded that alcoholic extract of F. indica was safe up to the dose of 2000 mg / kg / b. wt. and hence the drug falls in the “category-5” or “unclassified” according to the globally harmonized system.

ii. Anti-anxiety by Elevated plus Maze
The results reveal that the test compound significantly increases entries in open arm and also the time spent in open arm. Similarly the time spent in closed arm and number of entries in closed arm is reduced. The rationale behind this technique is that anxiolytics increase the time spent in open arm and entries in open arm. Anxiolytics increases fear in elevation on height. This is seen in control animals as they tend to avoid open spaces, exploration into open arm and gets themselves confined to closed spaces. A standard anxiolytic drug increases exploration into open spaces and motor activity which is measured by the time spent by the animal in open arm. The alternation in the time spent in open arm is considered more sensitive to the drug than the number of entries. Similarly our test drug also significantly increases the number of open arm entries and time spent in open arm which is a clear indication that anxiety has been considerably reduced.

iii. Anti-anxiety by Hole board
Our test compound increased head dips and immobility period and rearing got reduced. Immobility and rearing are signs of anxiety which is shown in control animals. The standard anxiolytic used for treatment has increased head dips which are a sign of exploration and reduced immobility period and rearing which is comparable to our test compound.

CONCLUSIONS
Alcoholic extract of F. indica showed no treatment related deaths, abnormal clinical signs, remarkable body weight or gross pathological changes upto a dose of 2000 mg / kg. In elevated plus maze and hole board method alcoholic extract of F. indica showed pronounced anxiolytic activity. Anxiolytic effect of F. indica may be due to different phyto constituents possibly acting though different receptors. Therefore use of F. indica as an anxiolytic agent similar to that of diazepam may be considered with further studies of its phyto constituents.

ACKNOWLEDGEMENTS
We would like to thank Dr. Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupati for plant identification and authentication and faculty members of Animal house Department of Sri Ramachandra University, Porur, Chennai for anxiolytic activity studies.

REFERENCES:

Table 1: Body weight of the experimental animals

<table>
<thead>
<tr>
<th>Treatment</th>
<th>-1 day</th>
<th>-1 hr before test substance administration Day 1</th>
<th>6h on Day 1</th>
<th>Day 2</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>162.33±3.76</td>
<td>152.00±4.36</td>
<td>158.00±4.73</td>
<td>159.33±4.26</td>
<td>166.33±3.28</td>
<td>183.67±0.88</td>
</tr>
<tr>
<td>Test drug</td>
<td>160.33±6.06</td>
<td>149.67±5.55</td>
<td>157.00±4.93</td>
<td>158.00±6.08</td>
<td>160.00±5.20</td>
<td>173.33±4.48</td>
</tr>
</tbody>
</table>

Values expressed in mean ±SEM; n=3

Table 2: Clinical observation of the individual animals

<table>
<thead>
<tr>
<th>Treatment (Dose/route)</th>
<th>H</th>
<th>B</th>
<th>CL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (min/hr)</td>
<td>0.5% CMC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-35</td>
<td>60-65</td>
<td>120-125</td>
<td>240-245</td>
</tr>
<tr>
<td>Abnormal Gait(rolling)</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Abnormal Gait(tip toe)</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Aggressiveness</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Convulsion</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Defecation</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Excitation</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Exophthalmos</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Head twitches</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Lacrimation</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Loss of righting reflex</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Loss of Corneal reflex</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Piloerection</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Ptosis</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Reactivity to touch</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Respiration abnormality</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Salivation</td>
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<td>X</td>
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<tr>
<td>Scratching</td>
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<td>X</td>
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<tr>
<td>Sedation</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Stereotypes (chewing)</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Stereotypes (Head movement)</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Stereotypes(sniffing)</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Straub</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>
Table 3: Anxiolytic Activity by elevated plus maze test

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Number of entries</th>
<th>Time Spent (Sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Open arm</td>
<td>Closed Arm</td>
</tr>
<tr>
<td>I</td>
<td>0.5% CMC</td>
<td>3.23 ±1.72</td>
<td>14.50 ±1.61</td>
</tr>
<tr>
<td>II</td>
<td>Diazepam (2mg/kg)</td>
<td>14.91 1.10***</td>
<td>7.82±1.54**</td>
</tr>
<tr>
<td>III</td>
<td><em>Flacourtia Indica Linn</em> (100mg/kg)</td>
<td>10.40 ±1.55**</td>
<td>9.60 ±1.15*</td>
</tr>
</tbody>
</table>

Values were expressed in mean±SEM, n=6/ group; ***,**,,* denotes p<0.001,0.01,0.05, respectively vs 0.5% CMC group

Table 4: Anxiolytic Activity by Head board test

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. of head dips</th>
<th>Immobility Periods</th>
<th>Rearing</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.5% CMC</td>
<td>19.65±1.33</td>
<td>39.60± 4.15</td>
<td>7.25 ±1.75</td>
</tr>
<tr>
<td>II</td>
<td>Diazepam (2mg/kg)</td>
<td>42.67 ±1.58***</td>
<td>14.33 ±3.80***</td>
<td>23.00 ±1.36**</td>
</tr>
<tr>
<td>III</td>
<td><em>Flacourtia Indica Linn</em> (100mg/kg)</td>
<td>36.80± 0.56***</td>
<td>21.34 ± 3.67*</td>
<td>18.67 ±3.78*</td>
</tr>
</tbody>
</table>

Values were expressed in mean±SEM, n=6/ group; ***,**,,* denotes p<0.001,0.01,0.05, respectively vs 0.5% CMC group

Figure 1: Body weight of the experimental animals

Figure 2: Anxiolytic Activity by elevated plus maze test
Figure 3: Anxiolytic Activity by Head board test
ANXIOLYTIC ACTIVITY OF FLACOURTIA INDICA USING STAIR CASE AND LIGHT DARK EXPLORATION METHODS IN MICE

N. Gnanasekar\(^1\), C. Uma Maheswara Reddy\(^2\), N. Narayanan\(^3\), C. Chamundeeswari\(^4\), T.K. Gopal\(^5\)

\(^1\)Department of Pharmacology, Kamalakshi Pandurangan College of Pharmacy, Tiruvannamalai.
\(^2\)Faculty of Pharmacy, Sri Ramachandra University, Chennai, Tamil Nadu, India.
\(^3\)Director, Jaya College of Pharmacy, Chennai, Tamil Nadu, India.
\(^4\)Faculty of Pharmacy, Sri Ramachandra University, Chennai, Tamil Nadu, India.
\(^5\)Faculty 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 1/8\(^{th}\) 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.

LD\(_{50}\) value of *Flacourtia indica* (Gnanasekar, 2013): LD\(_{50}\) 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 defection units (Zanoli, 2000; Maribel, 2006; Crawl, 2008).

**Statistical analysis:** The values were expressed as mean ±SEM from 6 animals. The results were subjected to statistical analysis by using ANOVA followed by Dunnet’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**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Rearing test value</th>
<th>Significant Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>16.29 ± 0.77</td>
<td>------------</td>
</tr>
<tr>
<td>2</td>
<td>Diazepam</td>
<td>11.80 ± 0.46**</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>3</td>
<td>Test drug (100mg/kg)</td>
<td>14.69 ± 1.00**</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>4</td>
<td>Test drug (200mg/kg)</td>
<td>12.08 ± 1.05***</td>
<td>P&lt;0.001</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Climbing test value</th>
<th>Significant Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>8.61 ± 2.21</td>
<td>------------</td>
</tr>
<tr>
<td>2</td>
<td>Diazepam</td>
<td>23.41 ± 1.34***</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>3</td>
<td>Test drug (100mg/kg)</td>
<td>11.69 ± 1.94**</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>4</td>
<td>Test drug (200mg/kg)</td>
<td>20.32 ± 1.06***</td>
<td>P&lt;0.001</td>
</tr>
</tbody>
</table>

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

![Figure.1. Effect of Test drug on mice in stair-case test](image)
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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of crossings</th>
<th>Time (sec) spent in light box</th>
<th>Time (sec) spent in Dark box</th>
<th>No. of rearings in L Box</th>
<th>No. of rearings in D Box</th>
<th>No. of defecation units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.833 ± 1.108</td>
<td>83.500 ± 3.971</td>
<td>223.67 ± 11.589</td>
<td>7.000 ± 3.864</td>
<td>21.667 ± 5.371</td>
<td>0.5 ± 0.2236</td>
</tr>
<tr>
<td>Diazepam 100mg/kg</td>
<td>11.333* ± 1.726</td>
<td>197.678* ± 32.893</td>
<td>102.33* ± 32.893</td>
<td>0.1667*** ± 0.1667</td>
<td>2.333* ± 1.961</td>
<td>1.16 ± 0.6540</td>
</tr>
<tr>
<td>Diazepam 200mg/kg</td>
<td>7.000* ± 1.653</td>
<td>111.67* ± 28.992</td>
<td>188.33* ± 28.992</td>
<td>6.500*** ± 4.161</td>
<td>15.500* ± 7.334</td>
<td>0.5 ± 0.2236</td>
</tr>
<tr>
<td>Test drug 200mg/kg</td>
<td>8.02 ± 1.822</td>
<td>108.58 ± 30.124</td>
<td>192.00* ± 25.120</td>
<td>6.22*** ± 3.980</td>
<td>14.02* ± 6.422</td>
<td>0.5 ± 0.280</td>
</tr>
</tbody>
</table>

*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
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 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.

REFERENCES


Medina JH, Paladini AC, Wolfman C, Levidestein M, Calvo D, Daiz LE, Pen AC, Chrysin (5, 7, di OH flavones) a naturally occurring ligand for benzodiazepine receptors with anticonvulsant properties, Biochem pharmacol, 4010, 1990, 2227.-31


