A Study Of The Anxiolytic-Like Activity Of Dillenia Indica LINN. Leaves In Experimental Models Of Anxiety In Mice

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Citation


Abstract

Aim

The purpose of this study was to characterize the putative anxiolytic-like activity of hydroethanolic extract prepared from the leaves of Dillenia indica. Materials and methods: Hydroethanolic extracts of Dillenia indica leaves at 50, 100 and 200 mg/kg (p.o.) was administered to study anxiolytic effect. Different models of anxiolytic activity viz. hole board, open field, elevated-plus maze and light/dark exploration models were used. Diazepam (2 mg/kg i.p.) was used as the standard drug. Observations: In the hole-board model, there was dose-dependent (at 100 and 200 mg/kg) and significant (p<.05) increase in the number of headpokes and the time of head dipping in treated groups in comparison to vehicle. In open-field test, the number of rearing and squares traversed increased significantly (p<.05) at doses 100 and 200 mg/kg. In the elevated–plus maze, there was significant increase (p<.05) in time spent and number of entries into the openarms at doses of 100 and 200 mg/kg. In light-dark exploration test the extract at 100 mg/kg and those at 200 mg/kg also showed significant activity (p<.05). Conclusion: Hydroethanolic extract of Dillenia indica leaves shows prominent anxiolytic activity in mice.

INTRODUCTION

Anxiety affects one-eighth of the total population worldwide and has become a very important area of research interest in psychopharmacology during this decade. According to the World Health report (1), approximately 450 million people suffer from a mental or behavioral disorder, yet only a small minority of them receives even the most basic treatment. This amounts to 12.3% of the global burden of disease, and will rise to 15% by 2020 (2).

Anxiety, a state of excessive fear, is characterized by motor tension, apprehension sympathetic hyperactivity and vigilance syndromes. Benzodiazepines are considered the drug of choice in the treatment of anxiety that has been used for the last 45 years to treat several forms of anxiety(3). Benzodiazepines, barbiturates, alcohol and tricyclic antidepressants (TCA,s) have been used for long time to treat anxiety disorders (4,5). Although benzodiazepines had well-known benefits, their side-effects are prominent including sedation, muscle relaxation, anterograde amnesia and physical dependence(6). BZD:s produce their therapeutic and undesirable effects by allosterically modulating the action of GABA via the central benzodiazepine receptor (CBR), located on the extracellular domain of the GABAa receptor(7).

In recent years, the development of new anxiolytics has been an area of interest. Various types of herbal medicines have been used as anxiolytic agents in different parts of the world. Dillenia indica L.(DI) is a common evergreen tree that grows widely in tropical forests in the western peninsula, Bihar, SubHimalayan tracts, Assam, Bengal, and central and southern India from Sylhet to Sri Lanka. It has been grown in gardens for its handsome foliage and attractive flower as an ornamental plant. The plant is locally known as Karambel or Karmal in Marathi, Chalta in Hindi, and Ramphal in Nepal (8,9,10).

The leaves, bark, and fruit of the plant are used in the indigenous system of medicine. The fruit juice is mixed with sugar and water and used as a cooling beverage in the treatment of fever. The fruit juice is used as a cardiotonic (11). The leaves and bark are used as a laxative and astringent.
Bruised bark is applied as a cataplasm for patients with arthritis(12). The alcoholic extract of the Dillenia indica leaves is reported to possess central nervous system (CNS) depressant activity. Phytochemical studies showed the presence of the lupeol group of triterpene such as betulinic acid and betulin and flavonol such as myricetin. Flavonoids such as Kaempferol, Quercetin, Isorhamnetin, Naringenin, and phenolic materials are also present (13,14). On the basis of the above considerations it was the purpose of this study to characterize the anxiolytic-like activity of a methanolic extract prepared from the leaves of Dillenia indica .

MATERIALS AND METHODS PREPARATION OF PLANT MATERIAL

The leaves of DI were collected from the rural districts surrounding Guwahati during the month of June 2010 and authenticated by Dr. T.R. Sarma , Lecturer in Botany , Swadeshi Academy Junior College , Guwahati-781005 . The fresh leaves were cleaned and washed thoroughly with water and rewashed with distilled water. Washed fresh leaves were dried under shade in clean dust-free environment, then grinded and powdered with a electric mixer and then stored in an airtight container . The powder ( around 250 grams) was then extracted with 500 ml of hydroethanol (50:50) in the Soxhlet extractor at a temperature of 40-50 degree centigrade . After complete extraction, the hydroethanolic extract was concentrated under vacuum to obtain a thick extract which was then used subsequently.

Acute toxicity studies were done with the extract and the animals were observed for gross behaviour and the median lethal dose (MLD). The No Observed Adverse Effect Limit (NOAEL) was subsequently found to be 2000 mg/kg/day . Three different doses of the extract ( 100, 200 and 400 mg/kg ) were administered to the animals(11). The respective doses were administered to the animals for a period of 7 days.

EXPERIMENTAL MODELS OF ANXIETY

For the evaluation of the anxiolytic activity of Dillenia indica , the activity of its hydroethanolic extracts were carried out against four models of anxiety testing :

the Elevated Plus Maze , the Light-Dark Exploration model , the Hole Board model and the Open Field test.

HOLE-BOARD TEST (15)

The hole board apparatus consists of a wooden box (40×40×25 cm) with 16 holes (each of diameter 3 cms ) evenly distributed on the base of the box . The apparatus was elevated to a height of 25 cms. Mice were fed orally with extract ( 100,200 and 400 mg/kg PO) or vehicle 30 minutes before they were placed in the apparatus . The number of head pokes and time of head dipping during a 5 minute period were recorded.

OPEN FIELD TEST(15)

The apparatus consists of a wooden box (60×60×30 cms). The base of the box was divided into 16 squares (15×15 cms ). The apparatus was illuminated with a 40 W lamp suspended 100 cms above it. The mice were fed orally with the extract (100,200 and 400 mg/kg PO), vehicle or diazepam. After 30 mins. They were placed in one of the corner squares , the number of rearings, assisted rearings (forepaws touching the walls of the apparatus) and the number of squares crossed were counted for 5 mins.

ELEVATED PLUS MAZE (EPM)(16)

The EPM apparatus consists of two open arms (35×5 cms) crossed with two closed arms (35×5×20 cms ). The arms were connected together with a central square of 5×5 cms . The apparatus was elevated to a height of 25 cms in a dimly illuminated room . Mice were treated with the extract doses (100,200 and 400 mg/kg), diazepam or vehicle 30 mins.
before being placed individually in the center of the EPM, facing a closed arm. The time spent in both open and closed arms, and the number of entries into both open and closed arms were counted for a period of 5 mins. An entry was defined as having all four paws within the arm.

**LIGHT-DARK EXPLORATION TEST (17)**

The apparatus consists of two (25×25×25 cms.) joined together. One box was made dark by covering its top with plywood, whereas a 40 W lamp illuminated the other box. The light source was placed 25 cms above the open box. The mice were placed individually in the center of the lit box and observed for the next 5 mins for the time spent in the lit and dark boxes. The mice were orally administered with extract (100, 200 and 400 mg/kg PO), diazepam or vehicle 30 mins before being placed in the lit box.

**GROUPING**

A total of 30 animals were divided into 5 groups (I, II, III, IV and V). Group I: normal control group- will receive distilled water

Group II: positive control group- will receive diazepam 2mg/kg i.p.

Group III: will receive hydroethanolic extract of D.Indica leaves in dose of 100mg/kg p.o.

Group IV: will receive hydroethanolic extract of D.Indica leaves in dose of 200 mg/kg p.o.

Group V: will receive hydroethanolic extract of D.Indica leaves in a dose of 400 mg/kg p.o.

The respective doses will be administered to the animals for a period of 7 days. On the 7th day, 1 hour after administration of the last dose experiments were carried out on the mice in all the 4 different models.

**STATISTICAL ANALYSIS**

The data was analyzed by one-way ANOVA followed by Dunnett-t test by using SPSS software version 16.0. A P-value < 0.01 was considered to be statistically significant.

**RESULTS AND OBSERVATIONS**

Data were expressed as mean ± SEM. Statistical calculations were done with SPSS 16.0 software. Results were analyzed by one way ANOVA P-values <0.05 were considered as significant.

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**Table 1:** Table showing results for Elevated-Plus Maze Test

<table>
<thead>
<tr>
<th>GROUP</th>
<th>NUMBER OF ENTRIES IN OPEN ARMS (ENTRY O)</th>
<th>NUMBER OF ENTRIES IN CLOSED ARMS (ENTRY C)</th>
<th>TIME SPENT IN OPEN ARMS (TIME O)</th>
<th>TIME SPENT IN CLOSED ARMS (TIME C)</th>
<th>TOTAL NUMBER OF ENTRIES (ENTRY T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) CONTROL</td>
<td>10±0.26</td>
<td>5±0.52</td>
<td>12±0.45</td>
<td>7±0.58</td>
<td>6±0.04</td>
</tr>
<tr>
<td>2) DIAZEPAM (2 mg/kg i.p.)</td>
<td>18±1.03*</td>
<td>12±0.58*</td>
<td>21±1.15*</td>
<td>7±0.63*</td>
<td>30±1.51*</td>
</tr>
<tr>
<td>3) DI extract (100 mg/kg p.o.)</td>
<td>8±0.58 (e)</td>
<td>6±0.45 (e)</td>
<td>15±0.90 (e)</td>
<td>8±0.90 (e)</td>
<td>23±1.09 (e)</td>
</tr>
<tr>
<td>4) DI extract (100 mg/kg p.o.)</td>
<td>15±1.06 ®</td>
<td>9±0.90 ®</td>
<td>19±0.45 ®</td>
<td>8±0.45 ®</td>
<td>25±1.09 ®</td>
</tr>
<tr>
<td>5) DI extract (400 mg/kg p.o.)</td>
<td>17±1.06 ®</td>
<td>10±0.26 ®</td>
<td>20±0.58 ®</td>
<td>6±0.46 ®</td>
<td>27±1.03 ®</td>
</tr>
</tbody>
</table>

ANOVA (one-way) :- ANOVA followed by Dunnett ‘t’ test is done

**Figure 2**
A Study Of The Anxiolytic-Like Activity Of Dillenia Indica LINN. Leaves In Experimental Models Of Anxiety In Mice

**Figure 3**

TABLE 2: TABLE SHOWING RESULTS FOR LIGHT-DARK EXPLORATION TEST

<table>
<thead>
<tr>
<th>GROUP</th>
<th>NUMBER OF CROSSINGS</th>
<th>TIME SPENT IN LIGHT BOX (sec.)</th>
<th>TIME SPENT IN DARK BOX (sec.)</th>
<th>NUMBER OF REAINGS IN LIGHT BOX</th>
<th>NUMBER OF REAINGS IN DARK BOX</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. CONTROL</td>
<td>410±0.45</td>
<td>240±21.55</td>
<td>200±21.55</td>
<td>76±21.55</td>
<td>64±21.55</td>
</tr>
<tr>
<td>2. DIAZEPAM (2 mg/kg p.o.)</td>
<td>64±0.58*</td>
<td>240±21.55</td>
<td>54±21.55</td>
<td>14±1.06*</td>
<td>14±1.06*</td>
</tr>
<tr>
<td>3. DI extract (100 mg/kg p.o.)</td>
<td>30±1.15 (c)</td>
<td>140±1.29 (c)</td>
<td>160±1.29 (c)</td>
<td>18±1.06 (c)</td>
<td>18±1.06 (c)</td>
</tr>
<tr>
<td>4. DI extract (500 mg/kg p.o.)</td>
<td>58±0.58 B</td>
<td>234±0.86 B</td>
<td>66±0.86 B</td>
<td>17±1.06 B</td>
<td>13±1.07 B</td>
</tr>
<tr>
<td>5. DI extract (1000 mg/kg p.o.)</td>
<td>62±1.32 B</td>
<td>234±0.86 B</td>
<td>66±0.86 B</td>
<td>17±1.06 B</td>
<td>13±1.07 B</td>
</tr>
</tbody>
</table>

Df = degrees of freedom, F = F-value, P = P-value, * = Pa < 0.05 when compared with control group, (±)=Pb < 0.05 when compared with std. drug (Diazepam) group, ® = Pb > 0.05 when compared with std. drug (Diazepam) group

**Figure 5**

ANOVA (one way) :- ANOVA followed by Dunnett ‘t’ test is done

**Figure 6**

TABLE 3: TABLE SHOWING RESULTS FOR HOLE BOARD TEST

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>NUMBER OF REAINGS</th>
<th>NUMBER OF ASSISTED REAINGS</th>
<th>NUMBER OF SQUARES CROSSED</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. CONTROL</td>
<td>14±0.58</td>
<td>6±0.82</td>
<td>232±1.15</td>
</tr>
<tr>
<td>2. DIAZEPAM (2 mg/kg p.o.)</td>
<td>22±0.71*</td>
<td>13±0.82*</td>
<td>261±2.03*</td>
</tr>
<tr>
<td>3. DI extract (100 mg/kg p.o.)</td>
<td>16±0.71 (c)</td>
<td>7±1.06 (c)</td>
<td>234±1.69 (c)</td>
</tr>
<tr>
<td>4. DI extract (200 mg/kg p.o.)</td>
<td>18±1.53 B</td>
<td>9±1.57 B</td>
<td>248±2.05 B</td>
</tr>
<tr>
<td>5. DI extract (400 mg/kg p.o.)</td>
<td>10±1.59 B</td>
<td>11±1.46 B</td>
<td>253±1.53 B</td>
</tr>
</tbody>
</table>

Df = degrees of freedom, F = F—value, P = P-value, * = Pa < 0.05 when compared with control group, (±)=Pb < 0.05 when compared with std. drug (Diazepam) group, ®= Pb > 0.05 when compared with std. drug (Diazepam) group

**Figure 7**

ANOVA (one way) :- ANOVA followed by Dunnett ‘t’ test is done.

**Figure 8**

TABLE 4: TABLE SHOWING RESULTS FOR OPEN FIELD TEST

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>NUMBER OF REAINGS</th>
<th>NUMBER OF ASSISTED REAINGS</th>
<th>NUMBER OF SQUARES CROSSED</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. CONTROL</td>
<td>73±0.82</td>
<td>6±0.82</td>
<td>232±1.15</td>
</tr>
<tr>
<td>2. DIAZEPAM (2 mg/kg p.o.)</td>
<td>22±0.71*</td>
<td>13±0.82*</td>
<td>261±2.03*</td>
</tr>
<tr>
<td>3. DI extract (100 mg/kg p.o.)</td>
<td>16±0.71 (c)</td>
<td>7±1.06 (c)</td>
<td>234±1.69 (c)</td>
</tr>
<tr>
<td>4. DI extract (200 mg/kg p.o.)</td>
<td>18±1.53 B</td>
<td>9±1.57 B</td>
<td>248±2.05 B</td>
</tr>
<tr>
<td>5. DI extract (400 mg/kg p.o.)</td>
<td>10±1.59 B</td>
<td>11±1.46 B</td>
<td>253±1.53 B</td>
</tr>
</tbody>
</table>

Df = degrees of freedom, F = F—value, P = P-value, * = Pa < 0.05 when compared with control group, (±)=Pb < 0.05 when compared with std. drug (Diazepam) group, ®= Pb > 0.05 when compared with std. drug (Diazepam) group

**Figure 9**

ANOVA (one way) :- ANOVA followed by Dunnett ‘t’ test is done.

**DISCUSSION**

Anxiety may be regarded as a particular form of behavioural inhibition that occurs in response to environmental events that are novel. It has been established that there are lots of plant secondary metabolites being employed in the treatment of psychotic disorders especially for anxiety in traditional medicine practice, most of which directly or indirectly affect the central nervous system, noradrenaline, serotonin, GABA and BZD neurotransmitter activities (18,19).
The hole-board model indicates that the head-dipping behaviour is sensitive to the emotional state of animals and suggests that the expression of the anxiolytic state in animals may be reflected by an increase in head-dipping behaviour(20). The Dillenia indica extract at doses 200 and 400 mg/kg (po) showed significant increase in the number of head poking and the time of head dipping comparable to that of the standard drug diazepam at a dose of 2 mg/kg (ip).

In the Open Field Model, the confrontation with the situation induces anxiety behavior in mice. The anxiety behavior is triggered by two factors, i.e., individual testing as the animal was separated from its social group and agoraphobia, as the arena is very large, relative to the animals breeding or the natural environment. In such situations rodents show thigmotaxic behavior identified by spontaneous preference to the periphery of the apparatus and reduced ambulation(21). This model showed that the administration of the Dillenia indica extract increased rearing, assisted rearings and number of squares traversed at doses 200 and 400 mg/kg which was comparable with the standard drug diazepam.

The elevated plus maze (EPM) is a well established animal model for testing anxiolytic drugs(22,23). This test is based on a premise where the exposure to an EPM evokes an approach-avoidance conflict that is stronger than that evoked by an exposure to an enclosed arm(24). The decrease in aversion to the open arm is the result of an anxiolytic effect, expressed by the increase in time spent and entries into the open arm. The primary index is spatiotemporal in nature: it is reduced by anxiolytic drugs and can be increased by anxiogenic compounds(25). Administration of extract in mice significantly increased the number of entries in open arm along with increase in duration in time spent at doses 200 and 400 mg/kg comparable to that of standard drug diazepam.

The light-dark test may be useful to predict the anxiolytic-like activity of drugs. 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 (19,26). The administration of Dillenia indica extract at doses 200 and

400 mg/kg showed significant increase in the time spent in the lit box, number of crossings and the time of latency with decrease in the time spent in the dark box.

Mechanism of anxiolytic action of plants may be by interaction with some of the natural endogenous mediators in the body as reported by various workers (27,28). Effect of most of the anxiolytic agents is to enhance the response to GABA, by facilitating the opening of GABA-activated chloride channels. Thus, the present study showed that the hydroethanolic extract of Dillenia indica leaves possessed potent anxiolytic activity which was evidenced by all the models as described above. At doses of 200 and 400 mg/kg it showed activity comparable to that of standard drug diazepam (at doses of 2 mg/kg ip).

CONCLUSION

The present study elucidated the beneficial effects of Dillenia indica in various models of experimentally-induced anxiety in mice at doses of 200 mg/kg and 400 mg/kg. It does not give a statistically significant result at doses of 100 mg/kg. This appears to be a promising approach that may be considered to be a complementary remedy for anxiety.

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