Consequences of sub acute administration of aqueous extract of *Terminalia belerica* fruit pulp on controlling anxiety in swiss albino mice

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Abstract

**Background:** The objective was to find the consequence of sub acute administration of Aqueous extract of *Terminalia belerica* fruit pulp (AETBFP) on controlling anxiety by using Dark and light arena and Elevated plus maze test.

**Methods:** Five groups of naïve male Swiss albino mice were used. Each group had six animals. I Group received 1% Gum acacia suspension 3ml/kg orally(vehicle); II Group received Diazepam 1mg/kg (standard drug) and III, IV and V Groups received AETBFP 9, 18 and 36 mg/kg (test drug) orally respectively once daily for ten days. On tenth day one hour after administering the drug, the anxiolytic activity was evaluated using screening methods. The parameters observed in dark and light arena are number of entries, duration of stay and rears in light arena. The parameters observed in elevated plus maze test are number of entries, time spent and rears in open arm. Percentage ratio of open/total arm entries was calculated. Appropriate statistical analysis was carried on.

**Results:** When compared to control group, AETBFP (36mg/kg) group showed significant increase in number of entries and duration of stay in light arena in dark and light arena model. When compared to control group, AETBFP (18mg/kg and 36mg/kg) groups showed significant increase in number of entries, time spent in open arm and percentage ratio of open/total arm entries in elevated plus maze model.

**Conclusions:** The study has shown that sub acute administration of AETBFP at a dose of 18mg/kg and 36mg/kg had significant control on anxiety.

**Keywords:** Dark and light arena, Diazepam, Elevated plus-maze, Gum acacia, Swiss albino mice, *Terminalia belerica*

Introduction

Anxiety is defined as boundless worriment and sense of fear of environment, article and living being. Different varieties of anxiety exist consisting of social phobia, specific phobias, obsessive-compulsive disorder, delayed stress syndrome, panic disorder, generalised anxiety disorder (GAD) and agoraphobia. (1) About one third of societies are found to suffer due to anxiety disorder in entire their life span. Anxiety commonly affects female gender. The age group commonly affected is 45 to 60. (2) Anxiety is found expensive to our community. The health care offered for anxiety is much more less when compared to those given for other affective disorders. (3)

A psychiatrist will encounter an annoying experience while treating anxiety. Patients affected by anxiety will demand for early and acute relaxation from anguish in contrast to patients affected by other psychiatric disorders, who are more compliant. This will naturally reduce the time required to exhibit the competency of the expert. So most often the psychiatrist ends up by treating the patient with Benzodiazepines. (4,5) Though they offer quick relief, the therapeutic window of benzodiazepines are very much narrowed leading to adverse effects such as reduced coordination, motor disturbances, unwanted sedation and cognitive decline due to abuse or misuse. So it is high time we develop a newer herbal drug molecule which is deficient of all these drawbacks. (6,7)

*Terminalia belerica* comes under the family Combretaceae. (8) In sanskrit it is named as vibhitak. (9) The deciduous tree *Terminalia belerica* is 50 m tall. Fruits are broadly ellipsoid to sub-globular in shape measuring 2.4 x 1.8-2.2 cm. (10) Fruits are astringent, laxative, antipyretic and antihelminthic; useful in treating hepatitis, asthma, bronchitis, piles, dyspepsia, coughs, diarrhoea, eye diseases, voice hoarseness, and scorpion sting. (11) The important phytoconstituents are ellargic acid, Tannins, ethyl gallate, chebulagacin acid, galloyl glucose, β-sitosterol, glucose, rhamnose, phenylemblin, mannitol, 7-hydroxy 3′4′ flavones and fructose. (12,13) *Terminalia belerica* has shown analgesic and antidepressive property in previous central nervous system studies. (14,15) On the grounds of this the present study was undertaken to find the effect of sub acute administration of aqueous extract of *Terminalia belerica* fruit pulp on controlling anxiety.

Methods

The research was performed as stated by the CPCSEA (Committee for the Purpose of Control and Supervision of Experiment on Animals) guidelines.

**Animals used:** Male naïve Swiss albino mice aged 3 months weighing around 25 -30grams were procured from our Central animal house’s breeding stock, A.J. Institute of Medical Sciences and Research Centre, Mangaluru, Karnataka, India. The animals were housed at a temperature of 24±2°c with 12:12 hour light and...
dark cycle. Food and water were provided ad libitum. Before starting the study, the animals were acclimatized for a period of 14 days. Such that they were placed in bright light in night time and placed in dark room in day time.

**Authentication of Terminalia belerica fruit pulp:** Dr. Krishna Kumar G, Chairman, Department of Applied Botany, Mangaluru University, Mangaluru, Karnataka, India authenticated the test drug (Terminalia belerica fruit pulp).

**Aqueous extraction of Terminalia belerica fruit pulp:** The crude powder of Terminalia belerica fruit pulp was air dried. The Soxhlet extractor was used to extract 1000 g of Terminalia belerica fruit pulp powder with water for a period of 36 hours. Later rotator evaporator was used to dry and reduce it under a controlled pressure at a temperature of around 40-50°C. The yield of this procedure was, 145g of brownish mass of aqueous extract. The brownish mass acquired was 14.5% w/w with respect to crude powder of Terminalia belerica fruit pulp.(16)

**Sample Size and Grouping of animals:** Five groups of Swiss albino mice were used. Each group had six animals making a total of thirty animals (Table 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug</th>
<th>Dose (Route)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Group</td>
<td>Control (1%Gumacacia)</td>
<td>10ml/kg (oral)</td>
</tr>
<tr>
<td>II Group</td>
<td>Standard drug (Diazepam)</td>
<td>1 mg/kg (oral)</td>
</tr>
<tr>
<td>III Group</td>
<td>AETBFP</td>
<td>9mg/kg (oral)</td>
</tr>
<tr>
<td>IV Group</td>
<td>AETBFP</td>
<td>18mg/kg (oral)</td>
</tr>
<tr>
<td>V Group</td>
<td>AETBFP</td>
<td>36mg/kg (oral)</td>
</tr>
</tbody>
</table>

AETBFP - Aqueous extract of Terminalia belerica fruit pulp.

**Drugs:** Diazepam was procured from the institutional pharmacy. The dried fruit of Terminalia belerica was obtained from Sri Lakshmi ayurvedic dispensary, Mangaluru. Gum acacia was provided by Department of Pharmacology, A.J. Institute of Medical Sciences, Mangaluru, Karnataka, India.

**Procedure**

**Dimensions:** The elevated plus-maze apparatus consists of two opposite open arms (30 cm × 8 cm), crossed with two closed arms of same dimensions with 16 cm high wall. The arms are connected with Central Square (8 cm × 8 cm). The dark and light arena apparatus measures 55 × 33 × 35 cm. The apparatus is divided into a 2/3rd of light chamber where we use bright light to illuminate and 1/3rd of dark chamber where we spray black paint on the inner side and use a red light. There are trays in the bottom of the apparatus to collect the faeces.

**Behavioural assessment:** After grouping of mice they were administered with the respective drugs once daily for a period of ten days. On tenth day, the animals were deprived of food for 12 hours to prevent drug food interaction. The last dose of the drug was then administered. One hour after administering the respective drugs, each mouse was experimented in both the screening models, such as light and dark arena model followed by elevated plus-maze model in one setting. The procedure was conducted in a sound attenuated room under red light. The cage was cleaned with cotton and spirit each time before placing the mouse. Stop clock was used to measure the time. In elevated plus maze test, the mouse was placed in the central platform facing one of the closed arm. The parameters observed during next 5 minutes were number of entries; time spent and rears in open arm (Fig. 1 and 2). Percentage ratio of open/total arm entries was then calculated. Next the mouse was placed in light arena of the dark and light arena model. The parameters observed during next 5 minutes were number of entries, duration of stay and rears in light arena.(17-19)

**Statistical analysis:** The mean ± SEM values of all the groups were calculated and compared to control group by using one-way ANOVA accompanied by Dunnet’s multiple comparison test, p value < 0.05 was assessed as statistically significant.

**Results**

Dose dependant control on anxiety was noted. In elevated plus maze model, when AETBFP was administered in the dose of 9mg/kg, there was no significant change in number of entries, time spent, rears in the arms and Percentage ratio of open/total arm entries compared to control. When AETBFP was administered in the dose of 18mg/kg, the time spent in open arm (35.3±2.1), the number of entry into open arm (8.5±0.7) and Percentage Ratio of open/total arm entries (41.3±5.4) was significant when compared with control. When AETBFP was administered in the dose of 36mg/kg, the time spent in open arm (54.8±3.1) and the number of entry into open arm (10.1±0.7) was highly significant when compared with control; number of total arm entries (30.1±0.4) was significant when compared with control. In the dark and light arena model, When AETBFP was administered in the dose of 9mg/kg and 18 mg/kg there was no significant difference in number of entries, duration of stay and rears in light arena compared with control. When AETBFP was administered in the dose of 36mg/kg, the time spent in light arena (210.1±28.1) and the number of entry into light arena (13.3±1.2) was highly significant when compared with control (Table- 2-4) (Fig. 3-7).
Table 2: Effect of sub acute administration of AETBFP on mice behaviour in elevated plus maze

<table>
<thead>
<tr>
<th>Dose/Group</th>
<th>Time spent in open Arm (Sec)</th>
<th>Time spent in closed Arm (Sec)</th>
<th>Number of rears in open Arm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%Gum Acacia 10ml/kg</td>
<td>26.6±1.6</td>
<td>207±6.6</td>
<td>1.3±0.2</td>
</tr>
<tr>
<td>Diazepam 1mg/kg</td>
<td>42±1.6***</td>
<td>150±7.7**</td>
<td>0.5±0.2**</td>
</tr>
<tr>
<td>AETBFP 9mg/kg</td>
<td>30.6±2.2*</td>
<td>222.1±6.1*</td>
<td>1.5±0.2*</td>
</tr>
<tr>
<td>AETBFP 18mg/kg</td>
<td>35.3±2.1**</td>
<td>191±7.1*</td>
<td>1.3±0.2*</td>
</tr>
<tr>
<td>AETBFP 36mg/kg</td>
<td>54.8±3.1***</td>
<td>132.8±3.5*</td>
<td>0.6±0.5*</td>
</tr>
</tbody>
</table>

AETBFP - Aqueous extract of Terminalia belerica fruit pulp. n= 6. All values are mean ± SEM; statistical analysis by ANOVA followed by Dunnet’s Multiple Comparison tests; *P>0.05 - not significant, **P< 0.05 – significant, ***P < 0.01- highly significant as compared with control.

Table 3: Effect of sub acute administration of AETBFP on mice behaviour in elevated plus maze

<table>
<thead>
<tr>
<th>Dose/Group</th>
<th>Number of open arm entries</th>
<th>Number of total arm entries</th>
<th>Percentage Ratio of open/total arm entries</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%Gumacacia 10ml/kg</td>
<td>5.3±0.8</td>
<td>23.8±2.0</td>
<td>22.9±3.7</td>
</tr>
<tr>
<td>Diazepam 1mg/kg</td>
<td>13±0.3***</td>
<td>28.1±2.2**</td>
<td>47.8±4.5***</td>
</tr>
<tr>
<td>AETBFP 9mg/kg</td>
<td>6.5±0.7*</td>
<td>30.6±1.8*</td>
<td>21.8±3.0*</td>
</tr>
<tr>
<td>AETBFP 18mg/kg</td>
<td>8.5±0.7**</td>
<td>21.4±1.4*</td>
<td>41.3±5.4**</td>
</tr>
<tr>
<td>AETBFP 36mg/kg</td>
<td>10.1±0.7***</td>
<td>30.1±0.4**</td>
<td>33.7±2.4*</td>
</tr>
</tbody>
</table>

AETBFP - Aqueous extract of Terminalia belerica fruit pulp. n= 6. All values are mean ± SEM; statistical analysis by ANOVA followed by Dunnet’s Multiple Comparison tests; *P>0.05 - not significant, **P< 0.05 – significant, ***P < 0.01- highly significant as compared with control.

Table 4: Effect of sub acute administration of AETBFP on mice behaviour in light and dark arena

<table>
<thead>
<tr>
<th>Drugs/ Groups</th>
<th>Time spent in Light arena (sec)</th>
<th>Number of entries into Light arena</th>
<th>Number of rears in Light arena</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%Gumacacia 10ml/kg</td>
<td>86.8±7.7</td>
<td>5.5±0.7</td>
<td>0.3±0.2</td>
</tr>
<tr>
<td>Diazepam 1mg/kg</td>
<td>212.3±7.8***</td>
<td>14.6±0.6***</td>
<td>0.8±0.4*</td>
</tr>
<tr>
<td>AETBFP 9mg/kg</td>
<td>80.5±31.3*</td>
<td>5.3±1.3*</td>
<td>1.1±0.6*</td>
</tr>
<tr>
<td>AETBFP 18mg/kg</td>
<td>153.8±28.1*</td>
<td>7.6±0.7*</td>
<td>0.5±0.2*</td>
</tr>
<tr>
<td>AETBFP 36mg/kg</td>
<td>210.1±28.1***</td>
<td>13.3±1.2***</td>
<td>1±0.3*</td>
</tr>
</tbody>
</table>

AETBFP - Aqueous extract of Terminalia belerica fruit pulp. n= 6. All values are mean ± SEM; statistical analysis by ANOVA followed by Dunnet’s Multiple Comparison tests; *P>0.05 - not significant, **P< 0.05 – significant, ***P < 0.01- highly significant as compared with control.

Discussion

Anxiety is considered as the frequent disorder among all the psychiatric disorders. Anxiety is characterized by a diffuse, unpleasant, vague sense of apprehension. Anxiety is characterised by autonomic symptoms consisting of mild discomfort in stomach, headache, chest tightness palpitation and sweating. Since Benzodiazepines and azapirones have their own draw backs of adverse effects and delayed response respectively. The search for newer molecule has begun to fight against anxiety. Terminalia belerica has two chief compounds such as tannins and 7-hydroxy 3’4’ flavones. The neurotoxicity induced by 6- hydroxydopamine can be revered using tannins and thus are found to have protective role against many neurological disorders. Numerous studies prove that flavones are the ligands of benzodiazepine receptors. In rodents, both the synthetic and natural flavones act as anxiolytic agents, since they are high affinity ligands to the benzodiazepine GABA receptors. Considering these facts the present study was undertaken. Two commonly used screening methods, performed in this study are elevated plus maze and light and dark arena. Elevated plus maze is useful to screen both anxiogenic and anxiolytic drugs. The open arms tend to
provoking anxiety due to the open and elevated spaces. Normally the control group rodent animals tend to stay in the closed arm. When anxiogenic compounds are administered the number of entries into open arms, time spent in open arms, percentage ratio of open arm/ total arm and number of rears in open arm decreases when compared to control.\(^{(23,24)}\) When anxiolytic compounds are administered the number of entries into open arms, time spent in open arms, percentage ratio of open arm/ total arm and number of rears in open arm increases when compared to control. In our present study, AETBFP’s anxiolytic activity was observed in two doses (18mg/kg and 36mg/kg). When AETBFP was administered in the dose of 18mg/kg, the time spent in open arm (35.3±2.1), the number of entry into open arm (8.5±0.7) and Percentage Ratio of open/total arm entries (41.3±5.4) was significant when compared with control. When AETBFP was administered in the dose of 36mg/kg, the time spent in open arm (54.8±3.1) and the number of entry into open arm (10.1±0.7) was highly significant when compared with control; number of total arm entries (30.1±0.4) was significant when compared with control.

Dark and light arena are used to assess the anxiolytic properties of the drugs. Those drugs proved to have anxiolytic property in this model by showing increased exploratory behaviour definitely shows increased potency in the clinical trials. The control group animals shows little crossings between dark and light arena; time spent in light arena, number of entries into light arena and number of rears in light arena decreases. When anxiolytics are administered, there is increase in the time spent in light arena, number of entries into light arena and number of rears in light arena.\(^{(25)}\) In our present study, AETBFP’s anxiolytic activity was observed in the dose of 36mg/kg. When AETBFP in the dose of 36mg/kg administered, the time spent in light arena (210.1±28.1) and the number of entry into light arena (13.3±1.2) was highly significant when compared with control.

This study effectively substantiate that subacute administration of aqueous extract of *Terminalia bellerica* fruit pulp (AETBFP) at doses of 18mg/kg and 36mg/kg can effectively control anxiety in Swiss albino mice. The forthcoming studies will aim to find the structure, pharmacokinetics, pharmacodynamics of *Terminalia bellerica* fruit pulp as it can replace the present treatment of anxiety, with least side effects and early control on anxiety.

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**Declarations**

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**Conflict of interest:** None declared

**Ethical approval:** (Approval No. IAEC/02/2013CP/SEA) dated 5/10/2013

**References**