Evaluation of Anti-anxiety Potential of *Tinospora cordifolia* (Willd.) Leaf Extract in Mice Using Experimental Models

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**ABSTRACT**

**Objectives:** The present study was conducted to evaluate the anxiolytic potential of ethanolic leaf extract of *Tinospora cordifolia* Linn. *Tinospora cordifolia*, the versatile herbal drug is the distinctive source of constituents which is having antidiabetic, immunomodulatory, antioxidant, antimicrobial, antitoxic and anticancer activity. **Methods:** Leaves of *Tinospora cordifolia* was collected, dried and powdered used for 7 days cold maceration process. Ethanolic extract was subjected to the preliminary phytochemical screening to analyse the presence of active constituents. At a dose of 100mg/kg, 200mg/kg, 400mg/kg body weight of animal was evaluated for antianxiety activity in mice using 3 experimental designs; elevated plus maze, open field, light/dark transition model. Test group animals were compared with group of animal treated with diazepam (1mg/kg) as standard drug. Ethanolic extract at 100, 200, and 400mg/kg showed activity in dose dependent manner and at a dose of 400mg/kg showed significant increase; in elevated plus maze, number of entries in open arm and time spent in open arm; number of entries into light chamber and time spent in light chamber in light and dark transition model and in open field; number of square crossed, number of rearing and time spent in central square. **Results:** The results supported traditional use of *Tinospora cordifolia* in nervous disorders. **Conclusion:** Study concluded that ethanolic extract of leaves of *Tinospora cordifolia* at a dose of 400mg/kg has significant antianxiety activity. **Key words:** Anti-anxiety activity, Diazepam, Elevated plus maze, Light and dark model, Open field *Tinospora cordifolia* Linn.

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**INTRODUCTION**

Emotional breakdown, stress and discomfort are commonly seen in the present generation. Anxiety is almost always correlated with many health and medical problems and sometimes is a symptom of mental illness.1 Anxiety is mainly due to stress but the possibility to be anxious is linked to genetic factors like trait anxiety.2 Some studies have found that people engaged in more physical activities are at reduced risk of anxiety disorder and less prone to serious anxiety symptoms.3 Certain drugs also induce anxiety like CNS stimulant, withdrawal of alcohol, drugs and metabolic changes like hyperglycaemia, thyrotoxicosis, hyperventilation.4 From the studies it was confirmed that the likely cause of anxiety is the imbalance in brain chemistry. The problems associated with the number of neurotransmitters like norepinephrine, serotonin, GABA are expressed in individuals having anxiety disorders.5 Benzodiazepine is one of the common medications for the treatment of general anxiety. Widely prescribed by physicians and used by patients because of their fast start up, effectiveness and tolerance.6 It is also prescribed for treating anxiety, acute withdrawal of alcohol, skeletal muscular spasm and seizures.7 Benzodiazepines are the GABA-receptor modulators. All commonly used benzodiazepines can facilitate the binding to GABA-receptors of the key G-aminobutyric acid inhibitors neurotransmitter (GABA), thus increasing ionic currents caused by GABA through conducting channels.8 *Tinospora cordifolia*, the versatile herbal drug is the distinctive source of constituents which is having antidiabetic, immunomodulatory, antioxidant, antimicrobial, antitoxic and anticancer activity.9 Number of investigations and research has been done on chemical constituents of guduchi.10 It also has potentiating activity on other substances in the form of shodhan – vidhi. “Guduchi” is the Sanskrit name which indicates that one protect from diseases.11 In hindu mythology, hindi name of *Tinospora cordifolia* “giloya” is believed to be the heavenly elixir which prevents aging.12 There are many studies which report various therapeutic potential of this herb. However, no published scientific datas are available on the anti-anxiety activity of leaves of *Tinospora cordifolia*. Hence the present study was undertaken.

**MATERIALS AND METHODS**

**Preparation of Extract**

The leaves of *Tinospora cordifolia* Linn. were collected and shade dried until it was moisture free. The coarse powder of dried leaves was made by using electric grinder. Ethanolic extract of *Tinospora cordifolia* leaves was prepared by 7 days cold maceration process. By using solvent ethanol, maceration process was carried out in an extraction chamber. For a period of 7 days the dried coarse powder of leaves and ethanol mixture was kept aside at room temperature along with occasional stirring. After 7 days, the content was filtered by using a muslin cloth. The separated content was kept in boiling water bath to remove ethanol and stored in a desiccator for experimental use.

**Preliminary Qualitative Phytochemical Investigation**

Qualitative phytochemical examination of Ethanolic leaf extract of *Tinospora cordifolia* Linn. was performed for identifying the presence of active constituents in it.13 Standard methods were followed to perform test.14

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### Table 1: Effect of *Tinospora cordifolia* Linn. ethanolic leaf extract on number of entries and Time spent in open arm in elevated plus maze.

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Groups</th>
<th>Number of entries into open arm Mean±SEM</th>
<th>Time spent in open arm Mean±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>1±0.365b</td>
<td>6.1±2.2a</td>
</tr>
<tr>
<td>2</td>
<td>Standard</td>
<td>9±0.89c</td>
<td>289±5.5b</td>
</tr>
<tr>
<td>3</td>
<td>Ethanolic test extract (100 mg/kg)</td>
<td>2.83±0.401a</td>
<td>41.4±5.7a</td>
</tr>
<tr>
<td>4</td>
<td>Ethanolic test extract (200 mg/kg)</td>
<td>3.6±0.55a</td>
<td>52.3±7.4a</td>
</tr>
<tr>
<td>5</td>
<td>Ethanolic test extract (400 mg/kg)</td>
<td>7.2±1.39a</td>
<td>137±17a</td>
</tr>
</tbody>
</table>

All the values are expressed as mean±SEM (n=6). a=p<0.05 when compared with control, b=p<0.05 when compared with standard. P <0.05 was considered as statistically significant.

### Table 2: Effect of *Tinospora cordifolia* Linn. Ethanolic leaf extract on number of entries and time spent in light chamber in light/dark transition model.

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Groups</th>
<th>Number of entries into light chamber Mean±SEM</th>
<th>Time spent in light chamber Mean±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>2±0.516b</td>
<td>22±2.92a</td>
</tr>
<tr>
<td>2</td>
<td>Standard</td>
<td>22.5±2.09b</td>
<td>433±41.51a</td>
</tr>
<tr>
<td>3</td>
<td>Ethanolic test extract (100 mg/kg)</td>
<td>3.1±0.65a</td>
<td>51.9±5.29b</td>
</tr>
<tr>
<td>4</td>
<td>Ethanolic test extract (200 mg/kg)</td>
<td>6±0.44b</td>
<td>90±8.56a</td>
</tr>
<tr>
<td>5</td>
<td>Ethanolic test extract (400 mg/kg)</td>
<td>13±1.064a</td>
<td>222.8±17.9a</td>
</tr>
</tbody>
</table>

All the values are expressed as mean±SEM (n=6). a=p<0.05 when compared with control, b=p<0.05 when compared with standard. P <0.05 was considered as statistically significant.

### Table 3: Effect of *Tinospora cordifolia* Linn. Ethanolic leaf extract on number of rearing, Number of square crossed and Time spent in central square in open field.

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Groups</th>
<th>Number of square crossed in open field Mean±SEM</th>
<th>Number of rearing in open field Mean±SEM</th>
<th>Time spent in central square Mean±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>7±6.93b</td>
<td>7.1±1.07b</td>
<td>15.5±6.10b</td>
</tr>
<tr>
<td>2</td>
<td>Standard</td>
<td>190.3±12.8a</td>
<td>31.6±3.37a</td>
<td>355±29.1a</td>
</tr>
<tr>
<td>3</td>
<td>Ethanolic test extract (100 mg/kg)</td>
<td>101±10.63b</td>
<td>13.1±1.19b</td>
<td>58.7±8.1a</td>
</tr>
<tr>
<td>4</td>
<td>Ethanolic test extract (200 mg/kg)</td>
<td>119±9.6b</td>
<td>16.4±1.16b</td>
<td>115±2.63b</td>
</tr>
<tr>
<td>5</td>
<td>Ethanolic test extract (400 mg/kg)</td>
<td>127.4±8.43b</td>
<td>24±1.69b</td>
<td>216.3±14.01b</td>
</tr>
</tbody>
</table>

All the values are expressed as mean±SEM (n=6). a=p<0.05 when compared with control, b=p<0.05 when compared with standard. P <0.05 was considered as statistically significant.

### Evaluation of Anti-anxiety Activity

#### Dosing
Antianxiety potential of leaves of *Tinospora cordifolia* Linn was evaluated by 3 dose levels.

1/10<sup>th</sup> of the higher dose in acute oral toxicity trials was the mid dose i.e, 1/10<sup>th</sup> of 2000mg/kg - 400mg/kg of body weight of animal. Double the 1/10<sup>th</sup> of dose will be the higher dose i.e, 400mg/kg of mice body weight. The lower dose was half of the 1/10th dose which was 100mg/kg of the mice body weight. Albino mice of approximately 20–30g was divided into five classes of 6 animal each.

#### Elevated Plus Maze
Exploratory behavior, anxiety characters and motor activity were assessed by using elevated plus maze. The apparatus is about 25 cm height from the ground level, consists of 2 open arm (16x5cm) and 2 closed arm (16x5cm) which are extended from common central arena. Individual animals from each group of animals were placed at central platform by facing to open arm. Group I was orally treated with 0.6% w/v CMC. Diazepam (1mg/kg) i.p treated animals served as group II. For 7 consecutive days, group III, group IV, group V was treated with ethanolic leaf extract at a dose of 100mg/kg, 200mg/kg, 400mg/kg. On 8<sup>th</sup> day, 1 hr after administration of standard and ethanolic extract in each groups, individually mice were placed in central platform of apparatus facing open arm. The parameters assessed for evaluation of activity during 5 min of test duration was evaluated by using ORCHID-All maze video tracking software.

#### Light and Dark Transition Test
The light/dark apparatus is divided into two chambers (40cm × 60cm × 20cm). The light chamber is white in colour and separated from dark chamber by a wall which is connected with a small opening at bottom. 20-30g weighing albino mice were divided into 5 groups, each group having 6 animals. Group I was orally treated with 0.6% w/v CMC. Diazepam (1mg/kg) i.p treated animals served as group II. For 7 consecutive days, group III, group IV, group V was treated with ethanolic leaf extract at a dose of 100mg/kg, 200mg/kg, 400mg/kg. On 8<sup>th</sup> day, 1 hr after administration of standard and ethanolic extract in each groups, individually mice were placed over illuminated area of chamber, and the parameters such as time spent in light chamber of the cage and number of entries in illuminated chamber is been noted for 10 min duration.

#### Open Field Model
Open field apparatus is a wooden box of dimension 40cm × 40cm × 30 cm. Apparatus consists of 16 squares of equal size and central zone is marked to separate squares from others. Lamp of 60W is suspended 100 cm above the apparatus. Group I was orally treated with 0.6% w/v CMC. Diazepam (1mg/kg) i.p treated animals served as group II. For 7 consecutive days, group III, group IV, group V was treated with ethanolic leaf extract at a dose of 100mg/kg, 200mg/kg, 400mg/kg. On 8<sup>th</sup> day, 1 hr after administration of standard and ethanolic extract in each groups, individually mice were placed in central zone of apparatus, 10 min of test duration evaluated parameters such as number of rearing, number of square crossed and time spent in central square.

#### Statistical Analysis
All the data were expressed as mean±SEM (n=6) and analyzed by one-way ANOVA followed by post hoc Schiff’s test using SPSS software version 10. P value less than 0.05 was considered as statistically significant.
RESULTS

Phytochemical Analysis
The preliminary qualitative phytochemical analysis performed revealed the presence of flavanoid, saponins and proteins.

Effect of Ethanolic Extract of Leaf of Tinospora cordifolia (Willd) in Mice

Elevated Plus Maze Model (Table 1)

a) Number of Open Arm Entries
The group of animals treated with standard drug (diazepam 1mg/kg) showed significant increase (p<0.05) in open arm of elevated plus maze, compared to other groups. While assessing the exploratory behavior, ethanolic extract at dose 100mg/kg, 200mg/kg and 400mg/kg exhibited activity in dose dependent manner. High dose 400mg/kg, significantly increased (p<0.05) entries into open arm.

b) Time Spent in Open Arms
Significant increase (p<0.05) in time spent in open arm by the standard drug treated group (diazepam 1mg/kg) compared to control group. The ethanolic extract of Tinospora cordifolia at a dose of 100mg/kg, 200mg/kg, 400mg/kg exhibit the potential in dose dependent manner. High dose 400 mg/kg showed significant increase (p<0.05) of time spent in open arm.

Light and Dark Transition Test (Table 2)

a) Number of Entries into Light Chamber
There was a significant increase (p<0.05) in entries into light chamber by the group of animal treated with diazepam (1mg/kg) as standard drug. At a dose of 100mg/kg, 200mg/kg, 400mg/kg it showed increase in number of entries in light chamber in dose dependent manner and 400mg/kg exhibited significant increase (p<0.05) in activity.

b) Time Spent in the Light Chamber
When compared to control group, the standard drug (diazepam 1mg/kg) treated group of animals showed significant increase (p<0.05) in time spent in light chamber. It was found that test extract of dose 100, 200 and 400 mg/kg exhibit exploratory potential in dose dependent manner. Ethanolic extract of high dose 400mg/kg showed significant increase (p<0.05) in time spent in light chamber compared to other groups.

Open Field Test (Table 3)

a) Number of Square Crossed
Significant increase (p<0.05) in number of square crossed in open field was observed in the group of animals treated with diazepam (1mg/kg) as standard drug. At a dose 100mg/kg, 200mg/kg and 400mg/kg of ethanolic extract showed number of square crossed in dose dependent manner and 400mg/kg showed significant increase (p<0.05) in number of square crossed in open field comparing to other dose.

b) Number of Rearing
There was a significant increase (p<0.05) in number of rearing showed by group of animals treated with diazepam (1mg/kg) as standard drug. Number of rearing showed by test group animals at a dose of 100mg/kg, 200mg/kg, 400mg/kg in dose dependent manner. Ethanolic extract of 400mg/kg exhibit significant (p<0.05) activity in number of rearing.

c) Time Spent in the Central Square
Group of animals treated with diazepam (1mg/kg) as standard drug showed significant increase (p<0.05) in time spent in central square of open field. Ethanolic test extract of dose 100mg/kg, 200mg/kg and 400mg/kg showed activity in dose dependent manner and high dose (400mg/kg) of ethanolic extract exhibit significant increase (p<0.05) in time spent in central square of open field.

DISCUSSION

The results from the present study showed that ethanolic extract of leaves of Tinospora cordifolia possessed significant anti-anxiety activity in dose dependent manner. Investigation on Tinospora cordifolia stem, leaf, whole plant part confirmed the vast medicinal properties. The experimental model elevated plus maze is commonly used for the screening of anxiolytic drugs. In many other experimental models the elevated plus maze exhibits more acceptance than Y-maze, radial maze. Anxiolytic and anxiogenic drugs successively identified by using this models. Chemical/extract having anti-anxiety activity shows increase in number of entries into open arm and time spent in open arm. The anxiolytic property is exerted through GABA_A receptor complex and evaluated by using diazepam (1mg/kg) as standard drug. Study was conducted by 7 days consecutive oral treatment of ethanolic extract at a dose of 100, 200, 400mg/kg and on 8th day of dosing, after 1 hr animals are placed in elevated plus maze showed anxiolytic property by increase in number of open arm entry and time spent in open arm. Activity is confirmed by comparing parameters with control group.

In light/dark transition model, animal spent more time in dark chamber than in light chamber due to fear for facing new environmental condition. Parameters such as; number of entries into light chamber and time spent in light chamber are assessed for the evaluation of antianxiety property. The ethanolic leaf extract of Tinospora cordifolia at a dose of 100, 200, 400mg/kg was given orally to mice of respective groups for 7 days. After 1 hr, of oral administration on 8th day activity was evaluated by keeping animal on model. The antianxiety activity exhibited by increase in number of entries and time spent in light chamber as compared to the control treated group.

In open field, animal shows emotional disturbance, fear and anxiety. Animals having anxiety spent more time towards outer zone, corners/ peripheral arenas of the open field. In open field, parameters like number of square crossed, rearing rate(standing on hind limb) and time spent in central square are assessed for evaluating activity. Oral treatment of 7 consecutive day with ethanolic extract and on 8th day, after 1 hr of dosing, respective groups of animals showed increase in square crossed, number of rearing and time spent in central square as compared to the normal control group.

In summary, from the results of study it was confirmed that the ethanolic leaf extract of Tinospora cordifolia treated animal produced anxioalytic effect in dose dependent manner and at high dose 400mg/kg showed significant anti-anxiety activity, when compared with normal control group.

CONCLUSION

Study was conducted to evaluate the ethanolic extract possess antianxiety activity. Presence of flavanoids, saponins and proteins are established by phytochemical analysis. The antianxiety potential was evaluated by elevated plus maze, light/dark transition test and open field test. It was found that ethanolic leaf extract of Tinospora cordifolia shows anxiolytic property in dose dependent manner. At a dose of 400mg/kg showed significant increase in parameters compared with control treated group of animals; in open arm entries and time spent in open arm of elevated plus maze, number of entries into the light chamber and time spent in light chamber. In light/dark transition model; number of square crossed, rearing rate and time spent in Central Square in open field. From the results, it is confirmed that ethanolic extract possess significant antianxiety property and it can be used as anxiolytic agents.
CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES