EVALUATION OF ANXIOLYTIC ACTIVITY OF BARK AND ROOT EXTRACTS OF GARCINIA GUMMI GUTA (L.) N.ROBSON

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ABSTRACT

Objective: To study the effect of ethanolic bark and root extract of Garcinia gummi guta (L.) N.Robson has anxiolytic activity on Swiss albino mice. Methods: Bark and root of Garcinia gummi guta (L.) N.Robson was collected and extracted using ethanol by cold maceration. The crude extracts were screened for the biological activity through the assay of anxiolytic activity. Anxiolytic activity evaluated by using in vivo methods such as plus maze, hole board and Y-maze. Result: Ethanolic bark and root extract Garcinia gummi guta (L.) N.Robson has anxiolytic activity. The result of the study showed that bark extract was more anxiolytic agent than the root extract. Conclusion: The result indicates that Garcinia gummi guta (L.) N.Robson was an anxiolytic agent.

KEYWORDS: Anxiolytic activity, Garcinia gummi guta (L.) N.Robson Swiss albino mice.

INTRODUCTION

According to WHO (1978) traditional medicine is defined as the sum total of knowledge or practice whether explicable or inexplicable, used in diagnosing, preventing or eliminating a physical, mental or social disease which may rely exclusively on past experience or observations handed down from generation, verbally or in writing. It also comprises the therapeutic practices that have been in existence often for hundreds of years before the development of modern scientific medicine.[1] Anxiety is an emotional state commonly caused by the perception of real or perceived danger that threatens the security of an individual. It allows a person to prepare for or react to environmental changes. Everyone
experiences a certain amount of nervousness and apprehension when faced with a stressful situation. This is an adaptive response and is transient in nature.\[^2\]

*Garcinia gummi guta* (L.) N.Robson is an evergreen, small to medium-sized tree. It is belongs to the family clusiaceae. It is a wild subtropical and tropical plant. The fruits of plant are commercially important for its valuable chemical components. Mostly these species are found in forest. Fruit extract is used for various treatments such as astringent, rheumatism, bowel complains and purgative. Bark and leaf extract contain alkaloid used to bowel complaints. It helps to promote digestion, and a decoction (kashayam) made out of it is used against Arthritis and some uterine diseases. It is also known to cure ulcers. The roots of plant used swelling whole body due to wiper bite.\[^3\]

**MATERIAL AND METHODS**

**Collection and authentication of plant materials**

Stem bark and roots were harvested from Pathanamthitta, kerala, India. The authenticity of the selected plant material was duly identified and confirmed by comparison with reference specimen preserved in the herbarium at Government college Nattokom, Kottayam, The voucher specimens (Voucher No.105) were lodged in the departmental herbarium for further reference. The plant materials were cleaned, washed with copious amount of distilled water, shade dried, chopped into bits and coarsely powdered in a mill for extraction.

**Preparation of crude plant extracts**

10g of coarsely powdered plant samples were exhaustively extracted with ethanol. Extraction was done by cold maceration (70C for 3 d).The extracts were filtered through Whatmann filter Paper no.1 and concentrated to dryness under room temperature. The samples were stored at 10°C till further use. At the time of administration, a suspension was prepared using the extract in 1% w/v of sodium carboxy methyl cellulose (1% sodium CMC).\[^4\]

**Phytochemical analysis**

Ethanolic extract of *Garcinia gummi guta* (L.) N.Robson was subjected to preliminary phytochemical screening.\[^5\]

**Experimental animals**

*Swiss Albino* mice of either sex 20-30 g of body weight obtained from animal house, Department of Pharmacology, Pushpagiri college of pharmacy, thiruvalla. Animals were kept
in standard animal house condition. Mice were housed in groups of six per cage. All the animals were maintained under standard conditions; that is room temperature 26±1°C, relative humidity 45-55% and 12:12 h light-dark cycle. The cages were maintained clean, and all experiments were conducted between 9 am and 4 pm.

**Acute toxicity study**
Female Swiss Albino mice (20-25 g weight) were used for acute oral toxicity study. The study was carried out as per the guidelines set by OECD 423 and animals were observed for mortality and behavioral changes.[6]

**Ethical approval**
The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of Pushpagiri college of pharmacy (Ref.No.PCP/2015/IAEC/1776/01) and all the experiments were conducted according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

**Evaluation of anxiolytic activity**
- *Elevated plus maze test*
  The apparatus consist of two open arms (5cm × 10 cm) and two closed arm (5cm×15cm×30cm) radiating from a platform (5cm×5 cm) to form a plus sign figure. The apparatus was situated 40 cm above the floor. The open - arm edges were 1 cm in height. Swiss albino mice (20-25 g) were taken and divided into four groups, each group comprised of six animals. Group A served as control and was administered with 1% sodium CMC, Group B with diazepam (2 mg/Kg P.O) and served as standard. Group C with bark extract (400mg/kg P.O) and Group D with root extract (400 mg/kg P.O) for 5 days.

  On the 5th day, 1hr after oral administration of 1% sodium CMC/standard/bark extract/root extract to respective groups, animal was placed at the center facing the open arm. During 5 minutes test period the following measures were taken.

  The number of entries into open and closed arms during the test.

  Time spent on the open and closed arm.
• **Hole-Board test**

Hole-board apparatus consist of metallic box with (70×70×25 cm) 16 holes (each of diameter 3 cm) evenly distributed on the base of box. The apparatus was elevated to height of 25 cm. Swiss albino mice (20-25 g) were taken and divided into four groups, each group comprising six animals. Group A served as control and was administered with 1% sodium CMC, Group B with diazepam (2 mg/Kg P.O) and served as standard. Group C with bark extract (400 mg/kg P.O) and Group D with root extract (400 mg/kg P.O) for 5 days. On the 5th day 1 hr after oral administration of 1% sodium CMC/standard/bark extract/root extract to respective groups. The animals placed in the apparatus. The numbers of head dips during a 5 min period were recorded.

• **Y-Maze model**

Y- Maze is made up of black printed wood or plastic. The *Swiss albino* mice (20-25g) was selected. The animals were divided into groups, each group consisting of six animals. Mice was treated with 1% CMC, Diazepam (2mg/kg), bark extract (400mg/kg) and root extract (400mg/kg) was administered for 5 days once daily P.O and the last dose was given on the 5th day, 60 min prior to the experiment and kept individually in one arm of apparatus for a period of 10 min. Then, the number of visits to the different arm was measured.[7-9]

**Statistical analysis**

Data expressed as means ± S.E.M. All result obtained from different tests were compared against the control group by using analysis of variance (ANOVA) and followed by Dunnett’s test.

**RESULTS**

**Phytochemical analysis**

**Table 1: The results of the chemical tests performed**

<table>
<thead>
<tr>
<th>TESTS</th>
<th>BARK</th>
<th>ROOT</th>
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<tbody>
<tr>
<td>Carbohydrate</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Amino acids</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>Cardiac glycoside</td>
<td>+</td>
<td>_</td>
</tr>
<tr>
<td>Coumarins</td>
<td>_</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
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</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>_</td>
</tr>
</tbody>
</table>

+ indicate presence, - indicate absence.

**Acute toxicity studies**

Acute toxicity studies of bark and root extract of *Garcinia gummi guta* (L.) N.Robson was performed according to OECD guideline 423 using female Swiss albino mice. At the dose 2000mg/kg, the bark and root extracts were produce neither mortality nor the sign of morbidity. Hence the dose 400mg/kg (1/5th of 2000mg/kg) was selected for further studies.

**Anxiolytic activity**

- Elevated plus maze (EPM) method

The ethanolic extracts of bark and root extracts of *Garcinia gummi guta* (L.) N.Robson at dose of 400mg/kg P.O and a standard drug Diazepam 2mg/kg P.O significantly increased the time spent in open arm and number of entries in open arm, when compared to control groups.

**Fig. 3: Effect of ethanolic bark extract (400 mg/kg), root extract (400mg/kg) and standard (diazepam 2 mg/kg) is, compared with vehicle treated group (1% CMC). (Each value represents the mean ±S.E.M (n=6). **P < 0.01,*P<0.05 significant, when compared with control group. Statistical analysis was done by one-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison test.**
Fig. 4: Effect of bark extract (400 mg/kg), root extract (400mg/kg) and standard (diazepam 2 mg/kg), is compared with vehicle treated control groups (1% CMC). (Each value represents the mean ±S.E.M (n=6). **P < 0.01, *P<0.05 significant, when compared with control group. Statistical analysis was done by one-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison test.)

- **Hole board**

The ethanolic extracts of bark and root extracts of *Garcinia gummi guta* (L.) N.Robson at dose of 400mg/kg.P.O and a standard drug Diazepam 2mg/kg P.O significantly increased the number of head dips, when compared to control groups.

Fig. 4: Effect of bark extract (400 mg/kg), root extract (400mg/kg) and standard (diazepam 2 mg/kg), is compared with vehicle treated control (1% CMC). (Each value represents the mean ±S.E.M (n=6). **P < 0.01, *P<0.05 significant, when compared with control group. Statistical analysis was done by one-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison test.)
Y –maze model
A significant decrease in number of visit in three arms of Y-maze was observed in standard (diazepam 2mg/kg) treated animals when compared to the control animals (1% CMC). The bark extract (400mg/kg) and root extract (400mg/kg) showed significant decrease in the number of visits in the three arms of Y –maze which was comparable with the control group.

![Y-MODEL](image)

Fig. 5: Effects of Ethanolic extract of bark and root on anxiolytic activity using Y-maze model. (Each value represents the mean ±S.E.M (n=6). **P < 0.01,*P<0.05 significant, when compared with control group. Statistical analysis was done by one-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison test.).

DISCUSSION
Preliminary phytochemical screening of bark and root extracts of *Garcinia gummi guta* (L.) N.Robson
The *Garcinia gummi guta* (L.) N.Robson barks and roots extracts where subjected to phytochemical screening. The result indicated that, bark extract shows the presence of carbohydrates, proteins, cardiac glycoside, flavonoid and tannins. In the root extract shows the presence of carbohydrates, proteins, coumarin, flavonoid and tannins.

Evaluation of Anxiolytic activity
The present work demonstrated that the ethanolic extract of bark and root of *Garcinia gummi guta* (L.) N.Robson had anxiolytic activity in mice by Elevated plus maze, Hole board, Y-maze models. Elevated plus maze is used for psychomotor performance and emotional aspects of rodents. The conventional plus maze is highly sensitive to influence both anxiolytic and anxiogenic drug acting at the GABA\_A – benzodiazepine complex. In EPM,
native mice will normally prefer to spend much for their allotted time in the closed arms. The preference appears to reflect an aversion towards open arms that is generated by the fears of open the spaces. Drugs produce the effect to increase the open arm exploration are considered as anxiolytics and the reverse holds true for anxiogenics. In the present study, we observed that bark extract (400mg/kg) and root extract(400mg/kg) induced significant increases in the both number of entries and time spent in the open arm and reduced entries and time spent in closed arm in the EPM model. Hole-board test provide a simple method for measuring the response of animals to unfamiliar environment and is widely used to assess emotionality, anxiety and/or response to stress in animals. It has been showed that head –dipping behavior was sensitive to change in the emotional states of animal. It suggested that expression of anxiolytic state in animal may be reflected by an increase in head-dip behavior. In this study, in the hole board model significant increase in the head dipping behavior were observed after treatment of bark extract (400mg/kg) and root extract (400mg/kg) of *Garcinia gummi guta* (L.) N.Robson, thus reinforcing the hypothesis that it has anxiolytic activity. Y–maze is widely used to assess exploratory behavior, learning and memory function in rodents. A significant decrease in the number of visits in the three arms of Y maze was observed in the standard (diazepam 2mg/kg) treated animals as compared to that control animals. The barks extract (400mg/kg) and root extract (400mg/kg) of *Garcinia gummi guta* (L.) N.Robson showed significant decrease in the number of visits in three arms of the Y-maze which are comparable with control animals, which supports the anxiolytic activity of extracts.

Earlier reports on the chemical constituents of the plants and their pharmacology suggest that plant containing flavonoids, tannins, alkaloids, hydroxy citric acid, xanthones, coumarins, garcinol, isogarcinol etc. were possess activity against CNS disorder. Phytochemical test of barks extract revealed the presence of flavonoid, tannins, alkaloid and root extract revealed the presence of flavonoid, tannins, coumarin.

**CONCLUSION**

Preliminary phytochemical screening revealed the presence of carbohydrates, proteins, cardiac glycoside, alkaloid, tannin flavonoid in the bark extract. The root extract revealed the presence of carbohydrate, protein, tannins, flavonoid and coumarin. These constituents may be represented for the biological activities of the plant.

Evaluation of anxiolytic activity of bark and root extract of *Garcinia gummi guta* (L.) N. Robson was studied indifferent animal models. The results of the work provided evidence
that, the bark and root may contain phytoconstituents that can suppress anxiety. In the anxiolytic property, the bark and root extracts (400mg/kg) was found to produce significant increase in the time spent and number of entries in open arms in the elevated plus maze model and increased number of head dipping in hole-board model and reduction in number of visiting in stair case model. The result showed that ethanolic bark extract have significant anxiolytic activity as compared to the ethanolic root extract.

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REFERENCES