ORIGINAL ARTICLE

Anxiolytic activity of aqueous extract of *Garcinia indica* in mice

Manish Patel, Bhavesh Antala, Chandana Barua[1], Mangala Lahkar
Departments of Pharmacology and Toxicology, National Institute of Pharmaceutical Education and Research-Guwahati, Guwahati Medical College, Bhangagarh, [1]College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India

Background: Anxiety or depressed mood is associated with low levels of serotonin in the brain. A hydroxycitric acid (HCA), constituent of *Garcinia indica* (GIA), increases serotonin release from isolated rat brain cortex. **Aim:** To evaluate the anxiolytic activity of aqueous extract of dried fruits of GIA in mice. **Materials and Methods:** The anxiolytic-like effects of aqueous extract of dried fruits of GIA were evaluated by using elevated plus maze (EPM), hole board and light/dark exploration models in Swiss albino mice. Control mice were treated with an equal volume of saline, and positive control mice were treated with diazepam (1 mg/kg). **Results:** GIA administered orally, 30 min before the test in different doses (125, 250 and 500 mg/kg of body weight), was able to increase significantly ($P < 0.05$) the time-spent and entries into open arms of the EPM and reduced the time-spent and entries into closed arms versus control. In the hole-board test, treatment with GIA (250 and 500 mg/kg) significantly increased the number of head-dips and duration of head dipping ($P < 0.05$). In the light–dark paradigm test, number of transitions and the time spent in the light box increased with reduction in time spent in the dark box and immobility period significantly ($P < 0.05$) after treatment with GIA. However, no significant changes in locomotor activity were observed versus control. **Conclusion:** The results of the present study suggest that aqueous extract of dried fruits of GIA is an effective anxiolytic agent for behavioural models in mice.

Key words: Anxiolytic, elevated plus maze, *Garcinia indica,* light/dark test, locomotor activity

INTRODUCTION

Anxiety-related disorders such as generalised anxiety, obsessive-compulsive disorder, phobias or post-traumatic stress are the major causes of disability in the world.[1] Currently, the most widely prescribed medications for anxiety disorders are benzodiazepines. However, the clinical uses of benzodiazepines are limited by their side effects such as psychomotor impairment, potentiating activity of other central depressant drugs and dependence liability.[2]

Garcinia indica (GIA) (Kokum) is a traditional home remedy in case of constipation, heart diseases, dysentery and pains.[3] Garcinol, a polyisoprenylated benzophenone purified from GIA fruit rind has an antioxidant and anti-ulcer properties.[4,5] Apart from hydroxycitric acid (HCA) and garcinol, kokum contains other compounds with potential antioxidant properties. These include citric acid, malic acid, polyphenols, carbohydrates, anthocyanin pigments and ascorbic acid. Antioxidant and anti-glycation activity of garcinol from GIA fruit rind has also been reported.[5]

Researchers have found that those individuals producing low levels of serotonin in the brain have a greater chance of experiencing anxiety or depressed mood.[6,7] A HCA increases serotonin release from isolated rat brain cortex.[8] The fruit rinds of GIA contain 8% (-)-HCA.[9,10] Therefore, the present study was undertaken to evaluate the anxiolytic effects of aqueous extract of dried fruits of GIA in mice. In addition, in an attempt to distinguish between specific and un-specific changes in animals' activity, the effect of GIA on mice's motility was assessed in a locomotor activity test.

Access this article online	
Quick Response Code:	Website: www.greenpharmacy.info
	DOI: 10.4103/0973-8258.122089

MATERIALS AND METHODS

Plant Material

The dried fruits of GIA (Kokum) were obtained from an herbalist supplier in Guwahati, Assam, India and authenticated by a pharmacognosy expert in the Department of Pharmacy, NIPER, Guwahati, India. A voucher specimen (voucher no. 651) representing this collection has been retained in our laboratory for future reference.

Address for correspondence: Mr. Manish Patel, National Institute of Pharmaceutical Education and Research-Ahmedabad, C/O B. V. Patel PERD Centre, SG Highway, Thaltej, Ahmedabad - 380 054, Gujarat, India. E-mail: patel_manish22002@yahoo.com
Received: 31-05-2013; **Accepted:** 13-09-2013

Preparation of the Extract

Aqueous extract of GIA was prepared by boiling grinded powder of dried fruits in 10 volumes of distilled water for 15 min. Aqueous solution so obtained was filtered through a Whatman No. 1 filter paper and concentrated on a water bath under vacuum. After which water was evaporated by rotary evaporator and all traces of water were eliminated by leaving the extract under vacuum overnight (yield 19%w/w), which was then stored at −20°C until required.

Phytochemical Screening

Phytochemical analysis of the extract was performed according to the previously described methods.[11] The extract was screened for the presence of alkaloids, saponins, flavonoids, tannins, glycosides, steroids and terpenoids using conventional protocols. Preliminary phytochemical screening revealed the presence of saponins, flavonoids, tannins, glycosides, steroids and terpenoids.

Animals

Swiss albino mice were procured from M/s Chakraborty enterprise, Kolkata, India. Groups of six female Swiss albino mice (20-24 g) were housed in microloan cages in a controlled environment (temperature 25 ± 2°C with 45% relative humidity and 12 h dark/light cycle) with standard laboratory diet and water *ad libitum*. The project protocols have been approved by the institutional animal ethics committee of Gauhati medical college (Approval No. 04/ NIPER/CPCSEA/351), Guwahati, Assam, India.

Acute Toxicity Study

The acute oral toxicity study was carried out as per guidelines set by Organisation for Economic Co-operation and Development (OECD) guideline 423 (adopted in December 2001). The purpose of the acute toxicity study is to allow selection of the appropriate starting for the main study. Female Swiss albino mice (8-12 weeks old) were taken for acute toxicity study. The GIA was administered to a group of three female Swiss albino mice in a sequential manner (increasing dose levels from starting dose 5 to 50, 300, 2000 mg/kg body weight as per OECD 423). Based on the results, the extracts did not produce any mortality at the doses tested. To optimise the dose levels, $1/16^{th}$ (125 mg/kg body weight), $1/8^{th}$ (250 mg/kg body weight) and $1/4^{th}$ (500 mg/kg body weight) of the maximum dose (2000 mg/kg body weight), given for the acute toxicity study as per OECD 423, were selected for the evaluation.

Drugs and Experimental Groups

Diazepam (1.0 mg/kg, Sigma) was used as the standard anxiolytic drug. Normal saline solution (NaCl 0.9%) was used to treat control group and aqueous extract of dried fruits of GIA was used for the treated groups. Different doses of GIA (125, 250 and 500 mg/kg of body weight, p.o.); diazepam (1 mg/kg, i.p.) and normal saline were administered 30 min before each behavioural test.

Behavioural Paradigms

Elevated plus maze test

The elevated plus maze (EPM) consisted of two open arms (35 × 5 cm) crossed with two closed arms (35 × 5 × 20 cm). The arms were connected together with a central square of 5 × 5 cm. The apparatus was elevated to the height of 25 cm in a dimly illuminated room. Mice ($n = 6$) were treated with GIA (125, 250 and 500 mg/kg, p.o.), diazepam (1 mg/kg, i.p.) or normal saline 30 min before being placed individually in the centre of the EPM, facing a closed arm. The time spent in both the open and closed arms was recorded for 5 min. The numbers of entries into open and closed arms were counted during the test. An entry was defined as having all four paws within the arm.[12]

Hole Board Test

The hole board apparatus consisted of a wooden box (40 × 40 × 25 cm) with 16 holes (each of diameter 3 cm) evenly distributed on the base of box. The apparatus was elevated to the height of 25 cm. Mice ($n = 6$) were treated with GIA (125, 250 and 500 mg/kg, p.o.), diazepam (1 mg/kg, i.p.) or normal saline 30 min before they were placed in the apparatus. The numbers of head dips and the time of head dipping during a 5 min period were recorded.[13]

Light-dark Test

The apparatus consisted of two boxes (25 × 25 × 25 cm) 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 cm above the open box. The mice were placed individually in the centre of the light box and observed for the next 5 min for the time spent in the light and dark box. The mice ($n = 6$) were administered with GIA (125, 250 and 500 mg/kg, p.o.), diazepam (1 mg/kg, i.p.) or normal saline 30 min before being placed in the light box.[14]

Locomotor Activity

The locomotor activity was measured using an actophotometer. The movement of the animal interrupts a beam of light falling on a photocell, at which a count was recorded and displayed digitally. Each mouse was placed individually in the actophotometer for 10 min and the basal activity score was obtained. Subsequently, the animals were divided into groups, each consisting of six animals. GIA (125, 250 and 500 mg/kg, p.o.), diazepam (1 mg/kg, i.p.) or normal saline was administered and after 30 min the mice were placed again in the actophotometer for recording the activity score.[15]

Statistical Analysis

Data are expressed as means ± S.E.M. All results obtained from the different tests were compared against the

control group by using analysis of variance (ANOVA) and followed by a *post-hoc* Dunnett's test. Differences between experimental groups were considered statistically significant when P was less than 0.05.

RESULTS

Elevated Plus-maze Test

The results are shown in Table 1. All the doses of GIA (125, 250 and 500 mg/kg) and diazepam (1 mg/kg) resulted in a significant increase in the time spent and number of entries into open arms ($P < 0.05$ and $P < 0.01$) in dose-dependent manner. However, some difference was observed, for example GIA 125 mg/kg have not significantly increased the number of entries into open arms.

Hole-board Test

The data are summarised in Table 2. GIA (250 and 500 mg/kg) significantly increased head-dip counts ($P < 0.05$ and $P < 0.01$) and GIA (125, 250, 500 mg/kg) significantly increased duration of head-dipping ($P < 0.05$ and $P < 0.01$). In the same manner diazepam (1 mg/kg) has significantly increased number of head-dips and duration of head-dipping ($P < 0.01$).

Light/Dark Test

The results of the light/dark test are shown in Table 3. GIA (125, 250 and 500 mg/kg) and diazepam (1 mg/kg) induced a significant increase in the time spent in the light box ($P < 0.01$) with significant decrease in the time spent in the dark box. Moreover, GIA (250 and 500 mg/kg) and diazepam (1 mg/kg) significantly increased number of transitions with significantly decreased the immobility period.

Locomotor Activity

To determine whether a possible stimulatory effect of GIA modified exploratory behaviour, we performed a locomotor activity test. However, GIA (125, 250 and 500 mg/kg) produced no significant changes in locomotor activity as compared with the control animals, which was also similar in case of diazepam (1 mg/kg) [Table 4].

DISCUSSION

Based on pharmacological knowledge and previous research about this plant would allow us to presume that it has an anxiolytic activity on central nervous system (CNS), which could be oriented to decrease anxiety or depression states in patients. In the present work, a clear anxiolytic-like activity of aqueous extract prepared with dried fruits from GIA has been demonstrated.

The EPM is considered to be an aetiologically valid animal model of anxiety because it uses natural stimuli, that is the fear of a new, brightly light open space and the fear of balancing on a relatively narrow raised platform.[16] Moreover, it is known that anxiolytic agents increase the frequency of entries and the time spent in open arms of the EPM.[17] In the present study, aqueous extract of GIA (250 and 500 mg/kg) markedly increased the frequency

Table 1: Effects of aqueous extract of *Garcinia indica* on the number of entries into and the time spent in each arm in elevated plus-maze over 5 min test period

Treatment (Dose: mg/kg)	Time spent in open arms (s)	Time spent in closed arms (s)	No. of entries in open arms	No. of entries in closed arms
Control	38.2±8.5	215.2±7.2	4.3±0.9	15.5±0.8
Diazepam	147.0±8.3**	111.3±7.3**	10.2±0.9**	6.8±0.5**
GIA 125	120.5±6.3**	136.2±4.8**	6.3±0.4	8.8±1.1**
GIA 250	134.2±10.5**	124.2±9.7**	7.7±0.7*	7.5±0.6**
GIA 500	138.2±12. 8**	120.3±12.0**	9.5±1.3**	6.5±0.6**

Data represent the mean±S.E.M. n=6 no. of animals per group, **P<0.01; *P<0.05 vs control group, GIA – *Garcinia indica*

Table 2: Effects of aqueous extract of *Garcinia indica* on number of head dips and duration of head dipping in hole-board test over 5 min test period

Treatment (Dose: mg/kg)	No. of head dips	Duration of head dipping (s)
Control	25.3±2.5	25.8±2.3
Diazepam	45.7±4.7**	59.7±4.7**
GIA 125	37.2±2.4	40.0±2.8*
GIA 250	39.3±3.2*	40.7±3.5*
GIA 500	44.2±2.7**	48.8±3.7**

Data represent the mean±S.E.M. n=6 no. of animals per group, **P<0.01; *P<0.05 vs control group, GIA – *Garcinia indica*

Table 3: Effects of aqueous extract of *Garcinia indica* on number of transitions, immobility period and the time spent in each box in light-dark test over 5 min test period

Treatment (Dose: mg/kg)	No. of transitions	Immobility period (s)	Time spent in light box (s)	Time spent in dark box (s)
Control	11.7±1.5	25.5±1.4	72.3±2.7	172.2±3.7
Diazepam	22.7±2.1**	11.5±1.1**	160.5±3.3**	88±3.9**
GIA 125	13.0±2.5	18.2±1.4**	118.2±7.2**	130.3±8.1**
GIA 250	19.7±1.6*	17.7±1.3**	128.2±12.8**	119.2±13.2**
GIA 500	21.7±2.7*	13.0±1.8**	136.7±8.1**	105.3±9.7**

Data represent the mean±S.E.M. n=6 no. of animals per group, **P<0.01; *P<0.05 vs control group, GIA – *Garcinia indica*

Table 4: Effects of aqueous extract of *Garcinia indica* on locomotor activity using actophotometer over 10 min test period

Treatment (Dose: mg/kg)	Locomotor activity (score) in 10 min	
	Before treatment	After treatment
Control	520.7±15.7	494.0±8.7
Diazepam	493.7±10.5	468.7±12.5
GIA 125	488.0±10.9	459.3±14.2
GIA 250	496.8±9.3	473.3±12.1
GIA 500	507.3±13.5	501.7±14.6

Data represent the mean±S.E.M. n=6 no. of animals per group, GIA – *Garcinia indica*

of entries and the time spent by the animals in the open arms.

An essential pre-requisite in testing for anxiolytic activity is that the test drug should not induce significant motor deficit in anxiolytic doses, since that could adversely affect the validation of the test results.[18,19] None of the doses of GIA used in this study, and that of diazepam, induced any significant degree of motor deficit as was evidenced by their insignificant effects on locomotor activity [Table 4].

Moreover, the hole-board test provides a simple method for measuring the response of an animal to an unfamiliar environment and is widely used to assess emotionality, anxiety and/or responses to stress in animals.[20] It has been showed that head-dipping behaviour was sensitive to changes in the emotional state of the animal, and suggested that the expression of an anxiolytic state in animals may be reflected by an increase in head-dipping behaviour. In the present study, GIA (250 and 500 mg/kg) increased head-dip counts and GIA (125, 250 and 500 mg/kg) increased head-dip duration without changing locomotion. These results indicate that GIA has a significant anxiolytic effect in this paradigm.

The light/dark test is a useful procedure to predict anxiolytic-like or anxiogenic-like activity in rodents.[21] The GIA (125, 250 and 500 mg/kg) significantly increased time spent in light box with significant reduction in immobility period. This paradigm is limited by its ability to yield false positive results due to a drugs' ability to affect general activity. This, however, can probably be ruled out, since locomotor activity levels displayed by GIA treated mice were not different from those exhibited by control animals. Based on the present results it could be concluded that GIA reduced the anxiety of animals exposed to the light/dark procedure without influencing motor activity of animals.

The pharmacological mechanism(s) that might account for the anxiolytic effects of GIA has yet to be determined. Further studies will be required to assess the generality of the present findings to other species and behavioural paradigms. Finally, the current findings, for the first time, to our knowledge, demonstrate a possible implication of active constituents of GIA in anxiety.

REFERENCES

1. Barua CC, Talukdar A, Begum SA, Borah P, Lahkar M. Anxiolytic activity of methanol leaf extract of *Achyranthes aspera Linn* in mice using experimental models of anxiety. Indian J Pharmacol 2012;44:63-7.
2. Emamghoreishi M, Khasaki M, Aazam MF. *Coriandrum sativum*: Evaluation of its anxiolytic effect in the elevated plus-maze. J Ethnopharmacol 2005;96:365-70.
3. Kirtikar KR, Basu BD. Guttiferae. In: Blatter E, Caius JF, Mhaskar KS, editors. Indian Medicinal Plants. 2nd ed. Vol. 1. Calcutta: Prabasi press; 1991. p. 263.
4. Selvi AT, Joseph GS, Jayaprakasha GK. Inhibition of growth and aflatoxin production in *Aspergillus flavus* by *Garcinia indica* extract and its antioxidant activity. Food Microbiol 2003;20:455-60.
5. Yamaguchi F, Ariga T, Yoshimura Y, Nakazawa H. Antioxidative and anti-glycation activity of garcinol from *Garcinia indica* fruit rind. J Agric Food Chem 2000;48:180-5.
6. Blaine B. Does depression cause obesity? A meta-analysis of longitudinal studies of depression and weight control. J Health Psychol 2008;13:1190-7.
7. Wurtman JJ. Depression and weight gain: The serotonin connection. J Affect Disord 1993;29:183-92.
8. Ohia SE, Awe SO, LeDay AM, Opere CA, Bagchi D. Effect of hydroxycitric acid on serotonin release from isolated rat brain cortex. Res Commun Mol Pathol Pharmacol 2001;109:210-6.
9. Jena BS, Jayaprakasha GK, Sakariah KK. Organic acids from leaves, fruits, and rinds of *Garcinia cowa*. J Agric Food Chem 2002;50:3431-4.
10. Mote S, Misra TS. Estimation of hydroxycitric acid (-)-HCA in *Garcinia indica* Choisy by HPLC method. Anal Chem: An Indian J [Internet]. 2008 [cited 2009];7. Available from: http://www.tradescienceinc.com/index.php?option=com_content and view=article and id=436 and Itemid=104 [Accessed on 2013 May 19].
11. Odebiyi OO, Sofowora EA. Phytochemical screening of Nigerian medicinal plants II. Lloydia 1978;41:234-46.
12. Adeyemi OO, Yemitan OK, Taiwo AE. Neurosedative and muscle-relaxant activities of ethyl acetate extract of *Baphia nitida* AFZEL. J Ethnopharmacol 2006;106:312-6.
13. Sonavane GS, Sarveiya VP, Kasture VS, Kasture SB. Anxiogenic activity of *Myristica fragrans* seeds. Pharmacol Biochem Behav 2002;71:239-44.
14. Jain NN, Ohal CC, Shroff SK, Bhutada RH, Somani RS, Kasture VS, *et al.* Clitoria ternatea and the CNS. Pharmacol Biochem Behav 2003;75:529-36.
15. Kulkarni SK. Experiments on intact preparations. In: Kulkarni SK, editor. Practical Pharmacology and Clinical Pharmacy. 1st ed. Delhi: Vallabh Publications; 2008. p. 131-3.
16. Dawson GR, Tricklebank MD. Use of the elevated plus maze in the search for novel anxiolytic agents. Trends Pharmacol Sci 1995;16:33-6.
17. Pellow S, Chopin P, File SE, Briley M. Validation of open: Closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 1985;14:149-67.
18. Bhattacharya SK, Satyan KS. Experimental methods for evaluation of psychotropic agents in rodents: I--Anti-anxiety agents. Indian J Exp Biol 1997;35:565-75.
19. Treit D. Animal models for the study of anti-anxiety agents: A review. Neurosci Biobehav Rev 1985;9:203-22.
20. Takeda H, Tsuji M, Matsumiya T. Changes in head-dipping behavior in the hole-board test reflect the anxiogenic and/or anxiolytic state in mice. Eur J Pharmacol 1998;350:21-9.
21. Crawley J, Goodwin FK. Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. Pharmacol Biochem Behav 1980;13:167-70.

How to cite this article: Patel M, Antala B, Barua C, Lahkar M. Anxiolytic activity of aqueous extract of *Garcinia indica* in mice. Int J Green Pharm 2013;7:332-5.

Source of Support: This research project was fully sponsored by National Institute of Pharmaceutical Education and Research (NIPER)-Guwahati with grant number 04/NIPER/CPCSEA/351, **Conflict of Interest:** None declared.