www.ThePharmaJournal.com

The Pharma Innovation

Pratik Rane
Post-Graduate Resident,
Department of Pharmacology,
Dr. D. Y. Patil Medical College,
Hospital and Research Centre,
Dr. D. Y. Patil Vidyapeeth,
Pune, Maharashtra, India

Seema Bhalerao
Professor, Department of
Pharmacology, Dr. D. Y. Patil
Medical College, Hospital and
Research Centre, Dr. D. Y. Patil
Vidyapeeth, Pune, Maharashtra,
India

Rahul Bhalsinge
Associate Professor, Department
of Pharmacology, Dr. D. Y. Patil
Medical College, Hospital and
Research Centre, Dr. D. Y. Patil
Vidyapeeth, Pune, Maharashtra,
India

Aakash Kewlani
Post-Graduate Resident,
Department of Pharmacology,
Dr. D. Y. Patil Medical College,
Hospital and Research Centre,
Dr. D. Y. Patil Vidyapeeth,
Pune, Maharashtra, India

Sayan Das
Post-Graduate Resident,
Department of Pharmacology,
Dr. D. Y. Patil Medical College,
Hospital and Research Centre,
Dr. D. Y. Patil Vidyapeeth,
Pune, Maharashtra, India

Samiksha Shelar
Post-Graduate Resident,
Department of Pharmacology,
Swami Ramanand Tirth Rural
Government Medical College,
Maharashtra University of
Health science, Ambajogai,
Beed, Maharashtra, India

Correspondence
Rahul Bhalsinge
Associate Professor, Department
of Pharmacology, Dr. D. Y. Patil
Medical College, Hospital and
Research Centre, Dr. D. Y. Patil
Vidyapeeth, Pune, Maharashtra,
India

Comparison of anxiolytic activity of *Garcinia indica* (Kokum) with diazepam in rats using elevated plus maze test

Pratik Rane, Seema Bhalerao, Rahul Bhalsinge, Aakash Kewlani, Sayan Das and Samiksha Shelar

Abstract
Background: Anxiety is a universal human emotion, closely allied with fear, presumably serving psycho-biologically adaptive purposes. Drugs are available in modern medicine to treat anxiety disorders, Diazepam being drug of choice but it produces various systemic side effects or exhibit tolerance upon chronic use. In Ayurveda, many plant products have been claimed to be free from side effects and less toxic than synthetic drugs, one of them is *Garcinia indica* which was evaluated for its anxiolytic activity.
Methodology: Wistar albino rats of either sex (150-250g) were divided into four groups with eight rats in each group. Different groups were given distilled water (0.5ml p.o.), Diazepam (1mg/kg p.o.), *Garcinia indica* (1.75gm/kg p.o.) and *Garcinia indica* (3.5gm/kg p.o.) respectively. Effect of test drugs on anxiety was evaluated using elevated plus maze test. Parameters such as number of entries in open arm, time spent in open arm were assessed. Results (mean±standard deviation) were analysed using one-way ANOVA test followed by Tukey's test.
Result: Statistically significant increase ($p<0.05$) in time spent in open arm was seen in *Garcinia indica* (3.5gm/kg) and Diazepam groups compared to control group; Diazepam and *Garcinia indica* (3.5gm/kg) groups showed comparable effect. Number of open arm entries was significantly increased ($p<0.05$) in *Garcinia indica* (both doses) and Diazepam groups as compared to control group. Diazepam did not show any significant difference ($p>0.05$) when compared to both doses of *Garcinia indica.*
Conclusion: *Garcinia indica* (3.5gm/kg) have more significant anxiolytic activity showing comparable effects with Diazepam.

Keywords: Anxiety, anxiolytic activity, elevated plus maze test, diazepam, *Garcinia indica,* Kokum

Introduction
Anxiety disorders are among the most common psychiatric disorders that affect all age groups of the general population [1]. It is a cardinal symptom of many psychiatric disorders and an inevitable component of many medical and surgical conditions. It is also a universal human emotion, closely allied with Worry and fear, presumably serving psycho biologically adaptive purposes [2]. Worry and fear are completely natural human feelings. If these feelings occur in completely natural stimuli and are experienced for an extended period, it affects the physical and mental health and leads to clinical anxiety disorders. It is a response to perceived immediate threat or significant stress. It may be acute or chronic. It can be a short term "state" or a long term "trait", whereas trait anxiety means worrying about the future events, close to the concept of neuroticism [3].

Neurochemicals such as Serotonin, GABA, Dopamine, Norepinephrine and many others have also recently been linked to anxiety disorders. Each chemical plays a very different, but equally important, role in anxiety regulation [4].

Many drugs are available in modern medicine to treat anxiety disorders. Most commonly used drugs are benzodiazepines. Diazepam being the drug of choice for anxiety disorders also have sedative, anti-convulsant activities. It may also increase the effects of other sedatives like that of alcohol and may lead to development of tolerance to the anxiolytic effects on its long-term use [5]. Sudden withdrawal of benzodiazepines after chronic treatment can cause anxiety and seizures. Chronic use of benzodiazepines may lead to habituation, dependence and abuse.

In Ayurveda, many plant products have been claimed to be free from side effects and less toxic than synthetic drugs, one of them is Kokum. *Garcinia indica* (Kokum) is a fruit tree of culinary, pharmaceutical, nutraceuticals and industrial uses. Kokum is one of the important indigenous tree spice crops originated and grown in Western Ghats of India.

For most indigenous system listed medicinal plants such as siddha, Ayurveda, Unani and allopathy which *Garcinia indica* is one of the traditional medicinally imported deciduous plants available all over India [6]. Researchers have found that those individuals producing low levels of serotonin in the brain have a greater chance of experiencing anxiety or depressed mood [7, 8]. The main active component in kokum is hydroxycitric acid (HCA), mainly found in leaves and rinds as HCA and some quantity is present as HCA lactone. HCA increases serotonin release from isolated rat brain cortex which help in reducing anxiety [9].

Other uses of *Garcinia indica* are
1. Traditional home remedy in case of constipation, heart diseases, dysentery and pains [10].
2. Garcinol, a polyisoprenylated benzophenone purified from *Garcinia indica* fruit rind has an antioxidant and anti-ulcer properties [11, 12].
3. HCA is also a potential anti-obesity agent.6
4. Anthocyanins pigment found in GI are well known for their antioxidant, anti-inflammatory and anti-carcinogenic activity [6].
5. It also has anti-microbial activity [13].

In order to find a drug which, have a potent anxiolytic activity comparable to that of Diazepam but having less adverse effects than it, this comparative study of above-mentioned drugs was conducted. Objectives of this study are to evaluate anxiolytic activity of *Garcinia indica* and to compare it with Diazepam using elevated plus maze test in rats.

Materials and Methods
The study was commenced after IAEC (Institutional Animal Ethics Committee) approval and was conducted in accordance with CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines and was done in a period of one year from 1st January 2018 to 31st December 2018. Wistar Albino rats weighing 150-250 gm of either sex were procured from the animal house located in Dr. D. Y. Patil Medical College, Pimpri, Pune-18, Maharashtra, India. Rats were housed under standard conditions in polypropylene cages.

Plant material
Dried seeds of *Garcinia indica* were obtained from Konkan region of the western ghats in Maharshtra from Sindhudurg district Ghonsari village which were verified and certified by Dr D.Y. Patil College of Ayurveda, Pimpri, Pune and an aqueous extract of seeds was obtained from the same institute.

Standard drug
Diazepam obtained from Intas pharmaceutical, India was used as standard drug given in the dose of 1mg/kg orally.

Test drug
Extract of *Garcinia indica* obtained from Dr D.Y. Patil College of Ayurveda, Pimpri, Pune was used as test drug given in the dose of 1.75gm/kg and 3.5gm/kg orally.

Study design
There were four groups containing eight rats in each group. They were administered:
Group I: Distilled water 0.5 ml p.o. (Control group)
Group II: Diazepam 1mg/kg p.o.
Group III: *Garcinia indica* 1.75gm/kg p.o.
Group IV: *Garcinia indica* 3.5gm/kg p.o.

Elevated plus maze test
The elevated plus maze (EPM), perhaps the most employed animal model of anxiety in current practice, was first proposed by Handley & Mithani and further validated by File *et al.* EPM is an unconditioned spontaneous behavioural conflict model. It is a useful test to investigate both anxiolytic and anxiogenic agents.
Apparatus: EPM apparatus consisting of two open arms (43 cm x 15 cm x 0.5 cm) and two enclosed arms (43 cm x 15 cm x 23 cm), extending from a central platform (5 cm x 5 cm x 0.5 cm) and raised 50 cm above floor level. The EPM is in the form of a plus" with two open elevated arms facing opposite to each other and separated by a central square and two arms of the same dimensions, but enclosed by walls [14-17]. The maze is raised off the ground so that the open arms combine elements of unfamiliarity, openness and elevation. The EPM is based on the natural aversion of rodents for open spaces and uses conflict between exploration and aversion to elevated open places. Provoked behaviour profiles in the EPM appear to include elements of neophobia, fear of height, exploration, agoraphobia and approach/avoidance conflict. Rats generally taken from their home cages will show a pattern of behaviour characterized by open-arm avoidance with a consistent preference for the closed arms. The rank order preference profile is closed > centre > open, indicative of a penchant for relatively secured sections of the maze [14-17]. Procedure: For testing, vehicle or drug treated rats were placed in the elevated plus maze individually, on the platform joining the four arms and the rat was observed for a period of 3 min. The number of open arm entries and the amount of time spent in open arms were recorded [14-17].

Statistical Analysis
The study observations made were recorded in a tabulated format in the excels master charts then mean and standard deviation was calculated. The data was compiled and analyzed using the statistical package, Primer of biostatistics, version 5.0. When the data passed normality test, the parametric test one-way analysis of variance (ANOVA) followed by post-hoc test which is Tukey's multiple comparisons test was used. p-value less than 0.05 was considered statistically significant.

Results

Table 1: Time spent in open arms

Group	Dose (per oral)	Time spent in open arm (secs) (Mean ± SD)
Control (DW)	0.5ml	25 ± 2.50
Diazepam	1mg/kg	35.88 ± 2.9***
Garcinia indica	1.75gm/kg	30.25 ± 4.33*
Garcinia indica	3.5gm/kg	37.12 ± 2.35***$$$

Compared with control
* p value < 0.05, *** p value < 0.001
Compared with Diazepam
$ p value < 0.05, $$$ p value < 0.001

This table shows
- *Garcinia indica* have significant anxiolytic activity in both (1.75gm/kg and 3.5gm/kg) doses as compared to control group.
- *Garcinia indica* administered in a dose of 3.5gm/kg and

Diazepam showed highly significant result as compared to control.

- Diazepam showed significant increase in time spent in open arm as compared to *Garcinia indica* in low dose.
- Diazepam and *Garcinia indica* in high dose showed comparable effect.
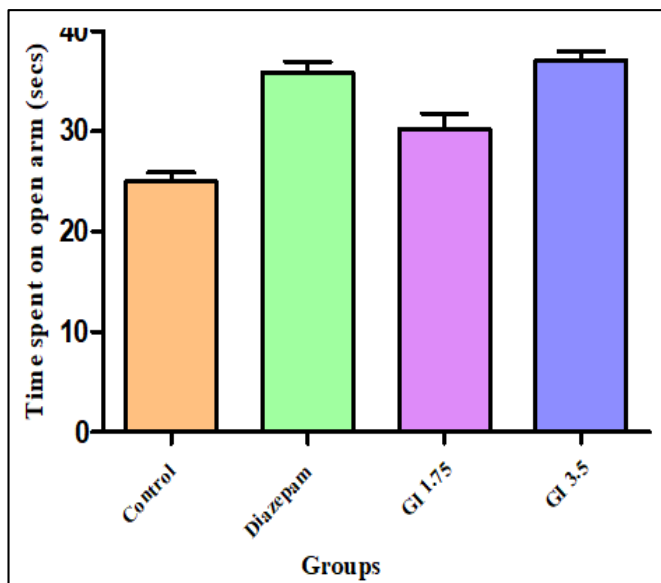
Graph 1: Time spent in open arms

This graph shows

- Both the doses of *Garcinia indica* showed significant increase in time spent in open arms as compared to control group.
- High dose *Garcinia indica* showed comparable effect to that of Diazepam.
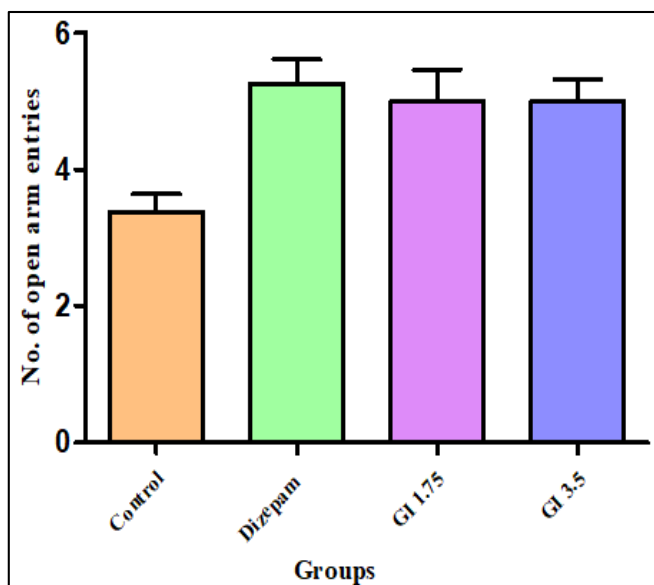
Table 2: Number of open arm entries

Group	Dose (per oral)	No. of open arm entries (Mean ± SD)
Control (DW)	0.5ml	3.375 ± 0.744
Diazepam	1mg/kg	5.25 ± 1.035**
Garcinia indica	1.75gm/kg	5 ± 1.309*
Garcinia indica	3.5gm/kg	5 ± 0.9258*

Compared with control
* denotes p value < 0.05, ** denotes p value < 0.01

This table shows

- Number of open arm entries was significantly increased in Diazepam and both doses of *Garcinia indica* treated rats 1.75gm/kg and 3.5gm/kg respectively compared to control group.
- Diazepam treated rats did not show any significant difference when compared to both doses of *Garcinia indica.*

Graph 2: Number of open arm entries

This graph shows

- Diazepam treated rats did not show any significant difference in open arm entries when compared to both the doses of *Garcinia indica.*

Discussion

Anxiety disorders are among the most common psychiatric disorders that affect all age groups of the general population. Benzodiazepines are still frequently used in spite of their side effect profile. Newer drugs as well as compounds derived from traditional herbs have possible therapeutic relevance in treatment of anxiety.

In depression, decreased levels of monoamines such as serotonin and norepinephrine in central nervous system may be due to increase in monoamine oxidase-MAO activity (Brigitta *et al.*, 2002) [18].

The fruit rind of *Garcinia indica,* widely consumed in the form of sharbat, contains high amount hydroxycitric acid (HCA), which is an established booster of serotonin liberator from rat brain (Ohia *et al.*,2001) [9].

For most indigenous systems such as Siddha, Ayurveda, Unani and allopathy, it is one of the traditional medicinally important deciduous plants available all over India [6]. Hence*,* in a present study *Garcinia indica* was selected as a potential anxiolytic herb.

EPM test is one of the most popular procedure for screening anti-anxiety compounds. It is used to evaluate psychomotor performance and emotional aspects of rodents [2]. When

Garcinia indica was given in a high dose, it showed comparable anxiolytic activity to that of Diazepam. It was better than Diazepam in preserving the locomotor activity and had less sedating effect as compared to Diazepam.

Anxiolytic activity of *Garcinia indica* was evaluated in 0.5 to 2% w/w range of concentrations by Dhamija *et al.* (2017). *Garcinia indica* at these concentrations significantly enhanced (*p*<0.05) both the number of entries and time spent in open arms when compared with control group in EPM [19].

Anxiolytic activity of *Garcinia indica* was also evaluated in the doses of 125mg/kg, 250mg/kg and 500mg/kg by Patel *et al.* (2013). *Garcinia indica* at these doses significantly enhanced (*p*<0.05) both, the number of entries and time spent in open arms, when compared with control group in EPM except in a dose of 125mg/kg, where there was no significant increase in number of entries observed in open arm [20].

In this study, we found that *Garcinia indica* when given in the dose of 1.75gm/kg and 3.5gm/kg had shown significant anxiolytic activity when compared with control group which are consistent with results of above two studies. In this study, we also found that *Garcinia indica* possess comparable anxiolytic activity with Diazepam when given in the dose of 3.5gm/kg. These findings revealed antianxiety potential of *Garcinia indica*.

Anti-depressant effect of *Garcinia indica* may be due to its adrenergic (α1) and dopaminergic (D2-receptors) pathways. *Garcinia indica* is known to inhibit monoamine oxidase enzyme in brain causing increased concentration of norepinephrine and serotonin in brain. It produces favourable effect in depression where these monoamine concentrations are considerably reduced. It is also known to have antioxidant effect. In anxiety and depression there is oxidative stress which leads to generation of free radicals like reactive oxygen species (ROS), catalase, superoxide anion, peroxide, hydroxyl radicals, singlet [oxygen and nitric oxide] in excess (Coyle *et al.*, 1993) [21]. ROS & Malondialdehyde (MDA) causes initiation of lipid peroxidation and disorientation of membrane proteins which leads to destruction of brain defence system causing disruption of neurons and cell death.

Further oxidation of nucleic acid and neurotransmitter also produce detrimental effects on neuronal function (Bouayed *et al.*, 2009) [22]. Antioxidant action of *Garcinia indica* when used for a long time i.e 14 days is known to reduce brain MDA levels and thus protect the brain from oxidative damage. Its phytoconstituent, garcinol due to the presence of phenolic hydroxyl group and β-diketone moiety causes the activation of GABAergic receptors and produces anti-anxiety and antidepressant effects (Padhye *et al.*, 2009) [23]. Garcinol and anthyocynins which are present in *Garcinia indica* act as potential free radical scavengers and produce antioxidant activity (Baliga *et al.*, 2011) [24].

Conclusion
This study shows that seed extract of *Garcinia indica* in a high dose has comparable anxiolytic activity with Diazepam, though not superior, but it has less sedating effect on rats as compared to Diazepam. *Garcinia indica* was also found to be preserving the locomotor activity in rats suggestive of its anxiolytic action.

Furthermore, increase in open arm entries and time spent in open arm with high dose *Garcinia indica* is also suggestive of improvement in psychomotor performance and emotional aspects of rats in addition to anxiolytic activity. Further study involving large number of animals is required to understand the precise mechanism of anxiolytic activity *Garcinia indica*.

References
1. Jung YH, Ha RR, Kwon SH, Hong SI, Lee KH. Anxiolytic effects of Julibroside C1 isolated from *Albizia julibrissin* in mice. Prog. Neuropsychopharmacol. Biol. Psychiatry. 2013; 44:184-192
2. Singh P, Rahul MK, Thawani V *et al.* Anxiolytic Effect of Chronic Administration of Gallic acid in Rats. Journal of Applied Pharmaceutical Science. 2013; 3(07):101-104.
3. Diagnostic and Statistical Manual of Mental Disorders American Psychiatric Association (5th ed.). Arlington: American Psychiatric Publishing, 2013, 189-195.
4. Mathew SJ, Price RB, Charney DS. Recent advances in the neurobiology of anxiety disorders: Implications for novel therapeutics. Am J Med Genet Part C Semin Med Genet. 2008; 148C:89-98.
5. O'Donnell JM, Shelton RC. Drug Therapy of Depression and Anxiety Disorders. In: Brunton LL, Chabner BA, Knollmann BC, editors. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 12th ed. New York. The McGraw-Hill Companies, 2011.
6. Baslingappa S *et al.* Kokum (*Garcinia indica*) and its Many Functional Components as Related to the Human Health: A Review. Journal of Food Research and Technology. 2014; 2(4):130-142.
7. Blaine B. Does depression cause obesity? A meta-analysis of longitudinal studies of depression and weight control. J Health Psychol. 2008; 13:1190-7.
8. Wurtman JJ. Depression and weight gain: The serotonin connection. J Affect Disorder. 1993; 29:183-92.
9. Ohia SE, Awe SO, Bagchi D *et al.* Effect of hydroxycitric acid on serotonin release from isolated rat brain cortex. Res Commun Mol Pathol Pharmacol. 2001; 109:210-6.
10. Kirtikar KR, Basu BD. Guttiferae. Indian Medicinal Plants. 2nd ed. Calcutta: Prabasi press, 1991; 1:263.
11. 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.
12. Yamaguchi F, Ariga T, Yoshimura Y, Nakazawa H. Antioxidativeand anti-glycation activity of garcinol from *Garcinia indica* fruit rind. J Agric Food Chem. 2000; 48:180-5.
13. Varalakshmi KL *et al.* Antimicrobial and Cytotoxic Effects of *Garcinia indica* Fruit Rind Extract. American-Eurasian J Agric. & Environ. Sci. 2010; 7(6):652-656.
14. Thierry Steimer. Animal models of anxiety disorders in rats and mice: some conceptual issues. Dialogues in Clinical Neuroscience. 2011; 13(4):495-506.
15. Campos AC, Fogaça MV, Aguiar DC, Guimaraes FS. Animal models of anxiety disorders and stress. Rev Bras Psiquiatr. 2013; 35(2):S101-111.
16. Joachim Uys DK, Dan Stein J, Willie Daniels MU, Brian Harvey H. Animal Models of Anxiety Disorders. Current Psychiatry Reports. 2003; 5:274-281.
17. Vogel HG. Ed. Drug Discovery and Evaluation: Pharmacological Assays. 3rd ed. Springer-Verlag Berlin, 2008, 585-660.
18. Brigitta B. Pathophysiology of depression and mechanism of treatment. Dialogues Clin Neurosci. 2002; 4(1):7-20.
19. Dhamija *et al.* Antidepressant and anxiolytic effects of

Garcinia indica fruit rind via monoaminergic pathway. 3 Biotech. 2017; 7(2):131.

20. 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.

21. Coyle JT, Puttfarcken P. Oxidative stress, glutamate and neurodegenerative disorders. Science. 1993; 262:689-695.

22. Bouayed J, Rammal H, Soulimani R. Oxidative stress and anxiety: relationship and cellular pathways. Oxid Med Cell Longev. 2009; 2:63-67.

23. Padhye S, Ahmad A, Oswal N, Sarkar FH. Emerging role of Garcinol, the antioxidant chalcone from *Garcinia indica* Choisy and its synthetic analogs. J Hematol Oncol. 2009; 2:1-13.

24. Baliga MS, Bhat HP, Pai RJ, Boloor R, Palatty PL. The chemistry and medicinal uses of the underutilized Indian fruit tree *Garcinia indica* Choisy (Kokum): A Review. Food Res Int. 2011; 44:1790-1799. doi: 10.1016/j.foodres.2011.01.064.