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Original Research Article

Evaluation of anti-anxiety activity of *Garcinia indica* using Vogel water lick conflict test in rats

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ABSTRACT

Background: Anxiety is usually described as a psychological, physiological and behavioural state induced in animals and humans by a threat to well-being or survival, either actual or potential. In modern medicine, benzodiazepines are still commonly used anti-anxiety agents in spite of their unfavourable adverse effect profile. Traditional herbs like *Garcinia indica* (Kokum) known to have possible therapeutic relevance in treatment of anxiety as a potential anxiolytic herb. So, this study was conducted to evaluate anti-anxiety effect of *Garcinia indica* in both, low and high doses, using Vogel's conflict test which is of broad significance to clinical anxiety.

Methods: Wistar albino rats of either sex (150-250 g) were divided into four groups with eight rats in each group. Different groups were given distilled water (0.5 ml p.o.), Diazepam (1 mg/kg p.o.), *Garcinia indica* (1.75 gm/kg p.o.) and *Garcinia indica* (3.5 gm/kg p.o.) respectively. Effect of test drugs on anxiety was evaluated using Vogel's water lick conflict test. Parameters such as number of water licks, total number of shocks tolerated were assessed. Results expressed in mean and standard deviation were analysed using one-way analysis of variance (ANOVA) test followed by Tukey's test.

Results: Diazepam and high dose *Garcinia indica* (3.5 gm/kg) showed statistically significant increase in number of water licks and number of shocks tolerated but low dose *Garcinia indica* (1.75 gm/kg) did not show any significant increase in these parameters.

Conclusions: This study shows that high dose *Garcinia indica* possess significant anti-anxiety effect comparable to that of diazepam.

Keywords: Anxiety, Anti-anxiety activity, Diazepam, *Garcinia indica*, Vogel's water lick conflict test

INTRODUCTION

Anxiety disorders are described anxiety feelings as an abnormal response to different stress factors (adaptive disorders), psychological reactions to traumatic events

(acute stress disorder and post-traumatic stress disorder) The anxiety can take different aspects. It can be perceived as an inexplicable feeling of eminent death, as an unfounded and exaggerated worry from daily life (like health of children, and professional or financial problems)

or an unjustified fear of certain situations (like traveling by bus) or an activity (like driving the car) or of an object (like fear of sharp objects, or of animals).^{1,2}

Diazepam is a benzodiazepine, a class of drugs that has anxiolytic, sedative, hypnotic and anticonvulsant properties.³⁻⁵ Diazepam is the prototype of the benzodiazepine class of drugs. Earlier, benzodiazepines superseded the class of drugs called the barbiturates, as well as the carbamates (meprobamate), owing to safety and wider therapeutic range. It was so effective that barbiturates, once popular as sedative hypnotics were no longer used after the popularity of diazepam. But because of various adverse events like that of sedation, impairment of motor control, abuse potential and withdrawal effects, there is a need to find a much safer drug with same potential against anxiety disorders.

Since the past decade, many herbal medicines have been used in people with anxiety disorders. Due to the increasing popularity of herbal medications majority of the patients are consulting herbalists, naturopaths, and other healers, in addition to physicians.⁶ Various herbs and ayurvedic plant extracts have shown anxiolytic activity in various animal models of anxiety.⁷ Some of them are *Actaea spicata* Linn, *Gelsemium sempervirens*, *Afrormosia laxiflora*, *Mimosa pudica* Linn, and *Garcinia indica*.

Garcinia indica commonly known as kokum found commonly in Western Ghats of India along the western coast, is known to have potential against anxiety without any harmful adverse effects. It is one of the most commonly used herbs in western coast of India which is used as antianxiety, antidiabetic, hypolipidemic, and antimicrobial activity.⁸⁻¹⁰

In the view to find a drug which would be as potential as that of diazepam against anxiety but safer in terms of adverse effects, this study was conducted which evaluated antianxiety activity of *Garcinia indica* in comparison with diazepam.

METHODS

The study was commenced after Institutional Animal Ethics Committee (IAEC) approval and was conducted in accordance with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines. Wistar Albino rats weighing 150-250 gm of either sex were procured from the animal house located in Dr. D. Y. Patil Medical College, Hospital and Research Centre, Pimpri, Pune, Maharashtra, India. Rats were housed under standard conditions in polypropylene cages.

Period of study

The study was conducted from 01 January 2018 to 30 June 2018.

Plant material

Dried seeds of *Garcinia indica* were obtained from Konkan region of the Western Ghats in Maharashtra 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

The standard drug was diazepam (1 mg/kg p.o.) obtained from Intas Pharmaceutical, India.

Test drug

Extract of *Garcinia indica* (1.75 gm/kg and 3.5 gm/kg p.o.) was obtained from Dr. D.Y. Patil College of Ayurveda, Pimpri, Pune.

Study design

Four study groups were designed containing eight rats in each group. Distilled water (vehicle) was administered to control group while diazepam and obtained extract were dissolved in distilled water before their administration into test groups. Vehicle and drugs were administered per orally to rats. Groups were divided based on the vehicle or drug administered to the rats - group I: distilled water 0.5 ml (control group), group II: diazepam 1 mg/kg, group III: *Garcinia indica* 1.75 gm/kg, and group IV: *Garcinia indica* 3.5 gm/kg.

Vogel water lick conflict test

This study was done by using Vogel's apparatus which consists of polycarbonate cage of size (43×29×20 cm) with 250 ml water bottle, top and floor made of stainless steel grill and lickometer plus shocker with circuit and software installed to deliver and count shock.¹¹⁻¹⁴

Procedure

Rats were water-deprived for 24 hours. Two hours before testing, each rat was placed in the cage and allowed to locate the metallic drinking spout of the water bottle; the whole exercise took less than 2 min. For testing, vehicle or drug treated rats were placed in the cage individually, for a period of 5 min. Electric shock (0.5 mA, 1 sec) was given through the spout on each contact of the snout of the rat with the metallic spout. The total number of such drink contacts was recorded during the 5 min observation period.

RESULTS

Table 1 shows that diazepam and *Garcinia indica* in a dose of 3.5 gm/kg showed significant increase in the number of water licks while *Garcinia indica* in a dose of 1.75 gm/kg showed slight increase in the number of water licks as compared to control group.

Figure 1 implies that *Garcinia indica* (3.5 gm/kg) showed statistically significant increase in the total number of water licks and was comparable to diazepam.

Table 1: Total number of water licks.

Group	Dose (P.O)	Total no. of water licks (mean±SD)
Control (distilled water)	0.5 ml	72.25±4.09
Diazepam	1 mg/kg	105.2±5.44
<i>Garcinia indica</i>	1.75 gm/kg	78.5±2.26
<i>Garcinia indica</i>	3.5 gm/kg	104.2±6.49

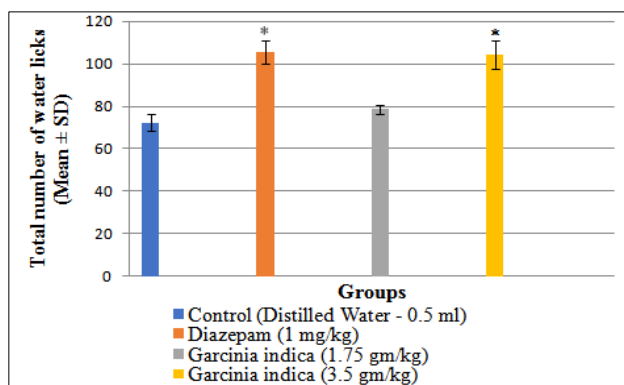


Figure 1: Difference in various groups in total number of water licks.

*P value <0.05 as compared to control group

Table 2: Total number of shocks received.

Group	Dose (P.O)	Total no. of shocks received (mean±SD)
Control (distilled water)	0.5 ml	4.875±2.03
Diazepam	1 mg/kg	9±1.06
<i>Garcinia indica</i>	1.75 gm/kg	6.375±0.74
<i>Garcinia indica</i>	3.5 gm/kg	7.5±1.19

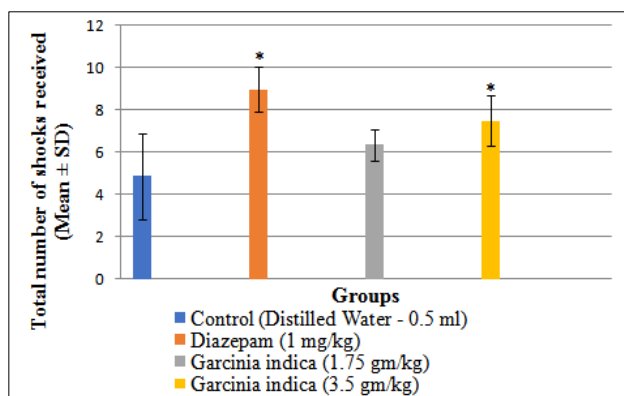


Figure 2: Difference in various groups in number of shocks received.

*P value <0.05 as compared to control group

Table 2 shows that diazepam and *Garcinia indica* in a dose of 3.5 gm/kg showed significant increase in number of shocks received while *Garcinia indica* in a dose of 1.75 gm/kg showed slight increase in number of shocks received as compared to control group.

Figure 2 implies that *Garcinia indica* (3.5 gm/kg) showed statistically significant increase in the shock tolerating capacity of rats but less as compared to that of diazepam.

DISCUSSION

Anxiety disorders are extensively co-morbid, not only with depression, but also with each other and with many other conditions such as substance abuse, attention deficit hyperactivity disorder, bipolar disorder, pain disorders, sleep disorders, and more.¹⁵ If not given proper attention to its symptoms it may lead to panic attacks in patient.

Nor-adrenergic overactivity, serotonin receptor (5-HT1A, 5-HT2C) dysregulation and a decrease in the number of benzodiazepine sites on the GABAA - benzodiazepine receptor complex are known to be associated with pathophysiology of anxiety disorders.¹⁶ Benzodiazepines bind to the benzodiazepine receptor complex located on the postsynaptic neuron which augments the effect of GABA and cause the opening of chloride ion channels, resulting into influx of the chloride ions into the cell leading to neuronal membrane stabilization.¹⁷

GABA may also influence anxiety levels by mediating the release of other neurotransmitters such as cholecystokinin and suppressing neuronal activity in the serotonergic and noradrenergic systems. Although it is likely that differing pathophysiologies underlie various anxiety disorders, it is widely believed that GABA circuits are integrally involved in anxiety disorders.¹⁸ Diazepam being one of the most potent benzodiazepines are most commonly used in anxiety disorders.

Garcinia indica (Kokum), a traditional plant found mainly in Konkan region of Western Ghats of India, is known to have various important medicinal properties. It consists of hydroxy citric acid (HCA) known to increase serotonin release from isolated rat brain cortex which could be the probable mechanism in its anti-anxiety activity.^{19,20} 23% of HCA is found on a dry basis in *Garcinia indica*. Major part of HCA is found in leaves and fruit rinds and is also known as Garcinia acid. Its fruit rinds contain about 8% (-) -HCA.^{21,22}

Vogel's water lick conflict test is a widely used screening test in industry setting which measures anticipatory anxiety and correlates with panic disorder in humans. It involves the animal's conditioned responses to stressful and often painful events. It produces conditioned fear in animals requires the pairing of a previously neutral stimulus with an electric shock. Subsequent presentations of the stimulus disrupt ongoing behaviour and produce avoidance or defence. Prior training of subjects to attain

specific levels of response decreases individual variability. Automated evaluation of the investigated parameters, together with a rigorous control and methodological manipulation of the experimental variables, also facilitates the use of conditioned models.²³

In this study, extract of *Garcinia indica* given in the dose of 1.75 gm/kg and 3.5 gm/kg was evaluated for its anti-anxiety activity against Diazepam given in the dose of 1mg/kg and placebo given distilled water in the dose of 0.5 ml and two parameters were evaluated which were difference in number of water lick and number of shocks tolerated by rats.

There was significant increase in number of water licks in both diazepam and *Garcinia indica* at the dose of 3.5 gm/kg when compared with placebo and no such significant increase was seen in *Garcinia indica* in the dose of 1.75 gm/kg as compared to placebo. Increase in water licks in both diazepam and *Garcinia indica* (3.5 gm/kg) were comparable with each other. Significant increase in number of shocks tolerated were seen in both *Garcinia indica* at the dose of 3.5 gm/kg and diazepam but not in *Garcinia indica* in the dose of 1.75 gm/kg as compared to placebo. However, in this parameter increase observed in *Garcinia indica* (3.5 gm/kg) and diazepam were not comparable and it was high in diazepam.

This study shows that *Garcinia indica* at 3.5 gm/kg possess significant anti-anxiety activity like that of diazepam but are devoid of adverse effects like diazepam. As far as known, no study has been done previously which compare *Garcinia indica* (3.5 gm/kg and 1.75 gm/kg) and diazepam using Vogel water lick conflict test.

Limitations

There are some limitations of this study like Vogel's water lick conflict test is a simple and specific model of evaluating anticipatory anxiety but other elements of anxiety like cognitive based symptoms or some phobia like neophobia, and acrophobia cannot be evaluated from this model. Furthermore, studies in clinical setting are still required to evaluate the safety and efficacy of *Garcinia indica* before it can be used as an anti-anxiety drug.

CONCLUSION

Garcinia indica given in the dose of 3.5 gm/kg possess significant anti-anxiety activity which is comparable to that of diazepam. However, more studies are required to establish this fact but it can prove to be an add-on therapy to already existing anxiolytic therapies.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Akiyoshi J. The pathophysiology and diagnosis of anxiety disorder. *Seishin Shinkeigaku Zasshi*. 2012;114(9):1063-9.
2. Sher L. Etiology and pathogenesis of anxiety disorders. *Med Hypotheses*. 2001;57(1):101-2.
3. Mihic SJ, Mayfield M, Harris RA. Hypnotics and sedative. In: Hilal-Dandan R, Brunton LL, Knollmann BC. Goodman and Gilman's The pharmacological basis of therapeutics. 13th ed. New York McGraw Hill Education. 2018;339-54.
4. Trevor AJ. Sedative –hypnotic drugs. In Katzung BG, Trevor AJ. Basic and Clinical Pharmacology. 13th edition. New York McGraw Hill Education. 2015;369-83.
5. Rang HP, Ritter JM, Flower RJ, Henderson G. Rang & Dales Pharmacology. 8th edition. London: Elsevier Ltd. 2016.
6. Liu L, Liu C, Wang Y, Wang P, Li Y, Li B. Herbal Medicine for Anxiety, Depression and Insomnia. *Curr Neuropharmacol*. 2015;13(4):481-93.
7. Awad R, Levac D, Cybulska P, Merali Z, Trudeau VL, Arnason JT. Effects of traditionally used anxiolytic botanicals on enzyme of the gamma-aminobutyric acid (GABA) system. *Can J Physiol Pharmacol*. 2007;85(9):933-42.
8. Brigitta B. Pathophysiology of depression and mechanism of treatment. *Dialogues Clin Neurosci*. 2002;4(1):7-20.
9. Deore AB, Sapakal VD, Naikwade NS. Antidiabetic, Antihyperlipidemic and Renal Protective Activities of *Garcinia indica* Linn. Clusiaceae. *Int J Pharm Sci Drug Res*. 2018;10(1):39-45.
10. Varalakshmi KN, Sangeetha CG, Shabeena AN, Sunitha SR, Vapika J. Antimicrobial and Cytotoxic Effects of *Garcinia indica* Fruit Rind Extract. *American-Eurasian J Agric Environ Sci*. 2010;7(6):652-6.
11. Steimer T. Animal models of anxiety disorders in rats and mice: some conceptual issues. *Dialogues Clin Neurosci*. 2011;13(4):495-506.
12. Campos AC, Fogaça MV, Aguiar DC, Guimaraes FS. Animal models of anxiety disorders and stress. *Rev Bras Psiquiatr*. 2013;35(2):S101-11.
13. Joachim Uys DK, Dan Stein J, Willie Daniels MU, Brian Harvey H. Animal Models of Anxiety Disorders. *Curr Psychiatr Rep*. 2003;5:274-81.
14. Vogel HG. Drug Discovery and Evaluation: Pharmacological Assays. 3rd edition. Springer-Verlag Berlin. 2008;585-660.
15. Stahl SM. Chapter 9: Anxiety disorders and anxiolytics. *Stahl's Essential Psychopharmacology: Neuroscientific Basis and Practical Application*. 4th edition. New York: Cambridge University Press. 2013;388-419.
16. Nuss P. Anxiety disorders and GABA neurotransmission: a disturbance of modulation. *Neuropsychiatr Dis Treat*. 2015;11:165-75.

17. Ressler KJ, Nemeroff CB. Role of serotonergic and noradrenergic systems in the pathophysiology of depression and anxiety disorders. *Depression Anxiety*. 2000;12:2-19.
18. Feldman S, Weidenfeld J. Involvement of endogenous glutamate in the stimulatory effect of norepinephrine and serotonin on the hypothalamo-pituitary-adrenocortical axis. *Neuroendocrinology*. 2004;79(1):43-53.
19. Holm M. Prescription of benzodiazepines in general practice in the county of Arhus, Denmark. *Dan Med Bull*. 1988;35(5):495-9.
20. Rane P, Bhalerao S, Bhalsinge R, Kewlani A, Das S, Shelar S. Comparison of anxiolytic activity of *Garcinia indica* (Kokum) with diazepam in rats using elevated plus maze test. *Pharma Innovation*. 2019;8(4):918-22.
21. Swami BB, Thakor NJ, Patil SC. Kokum (*Garcinia Indica*) and its Many Functional Components as Related to the Human Health: A Review. *J Food Res Technol*. 2014;2(4):130-42.
22. Ramachandran HD. Plant Profile, Phytochemistry and Pharmacology of *Garcinia indica*: A Review. *Int J Pharm Sci Rev Res*. 2014;27(2):376-81.
23. Bourin M. Animal models for screening anxiolytic-like drugs: a perspective. *Dialogues Clin Neurosci*. 2015;17(3):295-303.

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