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Original Research Article

Evaluation of anxiolytic effect of aqueous extract of *Garcinia indica* seeds using open field test

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ABSTRACT

Background: Anxiety is a common psychiatric condition frequently encountered in medical practice. While benzodiazepines like Diazepam are widely used as anxiolytics, they come with long-term adverse effects. *Garcinia indica* (Kokum), a natural herb, is believed to have anxiolytic properties without these drawbacks. This study aimed to evaluate its anxiolytic potential as a safer alternative.
Method: Wistar albino rats (n=32) were divided into four groups (8 per group) and administered orally with distilled water (0.5 ml), Diazepam (1 mg/kg), *Garcinia indica* (1.75 gm/kg), and *Garcinia indica* (3.5 gm/kg), respectively. Anxiety was assessed using the open field test, measuring parameters like the number of lines crossed, central square entries, rearing, grooming, immobility, and urination. Data were analyzed using one-way ANOVA followed by Tukey's test.
Results: Both doses of *Garcinia indica* and Diazepam significantly increased central square crossings and improved behavior such as rearing, grooming, and immobility. However, *Garcinia indica* did not significantly affect urination or number of lines crossed compared to the control. Diazepam significantly reduced number of lines crossed compared to *Garcinia indica* (3.5 gm/kg).
Conclusion: *Garcinia indica* demonstrated significant anxiolytic effects comparable to Diazepam, suggesting its potential as a safer alternative for anxiety management.

Keywords: Anxiolytic effect, Diazepam, *Garcinia indica*, Open field test, Locomotor activity and behavioural response

INTRODUCTION

Anxiety is a prevalent and complex psychological condition characterized by persistent worry, fear, and apprehension. It can manifest in various forms, ranging from generalized anxiety disorder (GAD) to specific phobias and panic attacks.[1] Affecting millions of individuals worldwide, anxiety has become a significant public health concern, influencing emotional well-being and overall quality of life. The condition is often accompanied by physical symptoms such as increased heart rate, sweating, and muscle tension, which can further

exacerbate distress. In large measure, the current understanding of pathophysiological mechanisms underlying depression and anxiety has been inferred from the mechanisms of action of psycho-pharmacological compounds, notably their actions on neurotransmission involving serotonin (5HT), NE, and GABA. While anxiety disorders comprise a wide range of symptoms, including changes in mood, behavior, somatic function, and cognition, some progress has been made in developing animal models that respond with some sensitivity and selectivity to anxiolytic drugs.[2]

The impact of anxiety extends beyond the individual, contributing to higher rates of absenteeism, reduced productivity, and a strain on healthcare systems. While anxiety disorders are among the most common mental health conditions, the mechanisms underlying anxiety remain complex and multifactorial, involving genetic, environmental, and neurobiological factors. Current treatment options, including pharmacological therapies, such as selective serotonin reuptake inhibitors (SSRIs), and cognitive-behavioural therapy (CBT), are widely used, yet these approaches may not provide adequate relief for all patients or may have undesirable side effects.[3-5]

In recent years, there has been growing interest in alternative and complementary approaches to anxiety management, including mindfulness, yoga, herbal remedies, and lifestyle interventions.

As a result, there is an increasing demand for alternative, safe, and effective treatments for various health conditions. The current trend is shifting towards the use of herbal compounds, nutrients, and supplements as preferred therapies over traditional allopathic medicines. In Ayurveda, many plant products have been claimed to be free from side effects and less toxic than synthetic drugs.[6]

Garcinia indica, commonly known as kokum, is a fruit-bearing tree native to India and the surrounding regions. Traditionally, it has been used in various forms of Ayurvedic medicine for its numerous health benefits, including its potential to aid in digestion, weight management, and skin health.[7]

Garcinol, a polyisoprenyl benzophenone purified from GI fruit rind has an antioxidant and anti-ulcer properties. The bioactive compounds present in *Garcinia indica*, such as hydroxycitric acid (HCA), have been investigated for their potential to influence neurotransmitter activity in the brain, which plays a key role in regulating mood and stress responses.

HCA is believed to impact the serotonin and cortisol levels, which are closely linked to anxiety and stress. By enhancing serotonin production and reducing cortisol levels, *Garcinia indica* may help alleviate symptoms of anxiety and promote a sense of calm.[8]

Therefore, the present study was conducted to assess the anxiolytic effects of the dried extract of *Garcinia indica* (GI) seed in rats. Additionally, to differentiate between specific and non-specific changes in animal activity, the impact of GI on mice motility was evaluated through a locomotor activity test using the open field test.

METHODS

The study was conducted as per CPCSEA (committee for the purpose of control and supervision of experiments on animals) guidelines after IAEC (Institutional Animal Ethics Committee) approval. Study was conducted from 01/01/2018 to 30/06/2018. Wistar Albino rats were procured from the animal house located in Dr. D. Y. Patil Medical College, Hospital and Research Centre, Pimpri, Pune-18, Maharashtra, India. Rats of either gender and weighing between 150-250 gm were separated for study from other rats and where the rats were housed under standard condition of temperature (25±5°C) and relative humidity (55±10%) and 12/12-hour light/dark cycle.

Extraction of plant material

Dried seeds of *Garcinia indica* were obtained from Konkan region of the Western Ghats in Maharashtra. An aqueous extract of seeds was prepared at Dr D.Y. Patil College of Ayurveda, Pimpri, Pune the seeds of *Garcinia indica* were shade dried and grinded to a coarse powder and then passed through sieve no. 22. The dried finely powdered material was then exhaustively extracted with distilled water by soxhletion, concentrated under controlled temperature and was stored in dry and cool place until it was used for the pharmacological investigation.

Standard comparator and placebo

Diazepam obtained from Intas pharmaceutical, India was dissolved in distilled water and was administered orally to rats at a dose of 1 mg/kg to one group (comparator group) and 0.5 ml of distilled water (vehicle) was administered to one group (control group).

Test drugs

Aqueous extracts of *Garcinia indica* were dissolved in distilled water and were administered orally to rats at a dose of 1.75 gm/kg and 3.5 gm/kg respectively in two different groups (test groups).

Study groups

Four study groups were designed having eight rats in each group. Groups were divided on the basis of the drugs or vehicle that they received. Group I: Distilled water 0.5 ml. Group II: Diazepam 1 mg/kg. Group III: *Garcinia indica* 1.75 gm/kg. Group IV: *Garcinia indica* 3.5 gm/kg.

Open field test

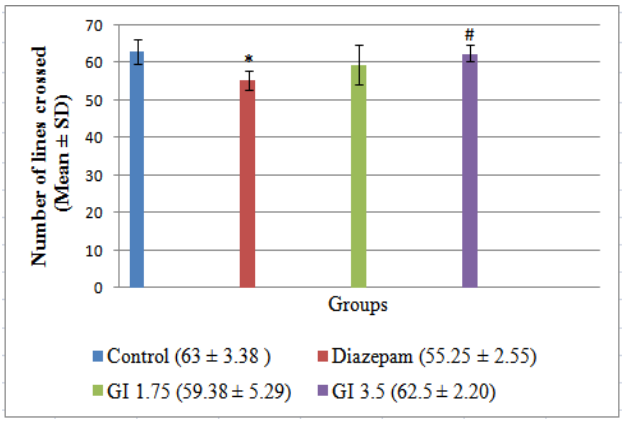
Open Field apparatus consists of a multiple unit enclosure. Each enclosure is surrounded by walls of 15 inches and the length and breadth of the base measuring 19X19 inches. The open field is divided into symmetric squares of 4X4 with 4 central squares and 12 peripheral squares. Infra-red-light beams have been used for measuring the locomotion, as they are interrupted by moving rats that can be measured. Rats were placed individually in a single unit of peripheral square of the open field enclosure and the movement of rats is observed and recorded. The rat behaviour and activity were recorded with the help of a video camera for accuracy, reassessment and reproducibility. Total number of lines and central squares crossed were recorded. Various behavioural responses such as rearing, grooming, immobility and urination if done by rats during the test were recorded.

Statistical analysis

The data was compiled and analyzed using the statistical package, Primer of biostatistics, version 5.0. If the data passed normality test, the parametric test used was one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test.

RESULTS

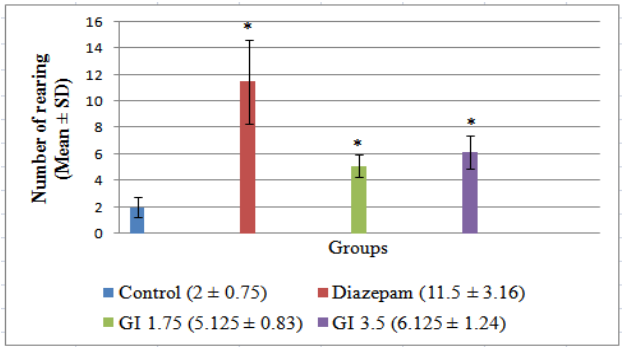
In this section, observed values in control group, standard comparator and test groups are displayed in graphical form and values are also represented in numerical form at the bottom of all the figures. The test groups that received *Garcinia indica* in the dose of 1.75 mg/kg and 3.5 mg/kg are abbreviated as GI 1.75 and GI 3.5 respectively for representation in the figures.



*Compared with control p value <0.05
#Compared with Diazepam p value <0.05

Figure 1: Comparison of number of lines crossed by rats in different groups.

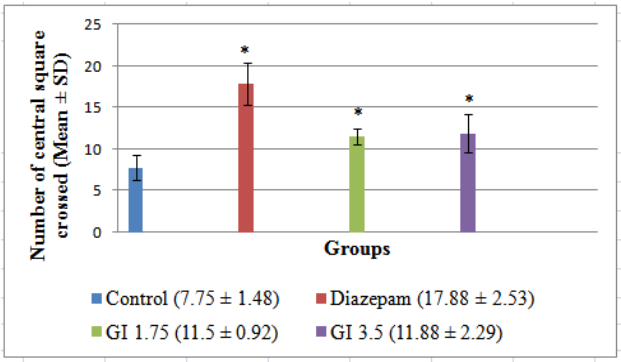
Figure 1 shows that diazepam treated rats shown significant decrease in number of lines crossed (locomotor activity) as compared to both control and *Garcinia indica* in dose of 3.5gm/kg treated rats. However, there was no significant difference observed in number of lines crossed in control group and both the groups of *Garcinia indica* treated rats.



*Compared with control p value <0.05

Figure 2: Comparison of number of rearing done by rats in different groups.

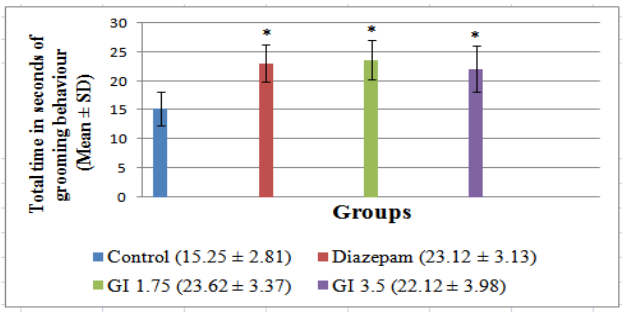
Figure 2 shows that both the groups of *Garcinia indica* and diazepam treated rats showed significant increase in number of rearing as compared to control group.



*Compared with control p value <0.05

Figure 3: Comparison of number of central squares crossed by rats in different groups.

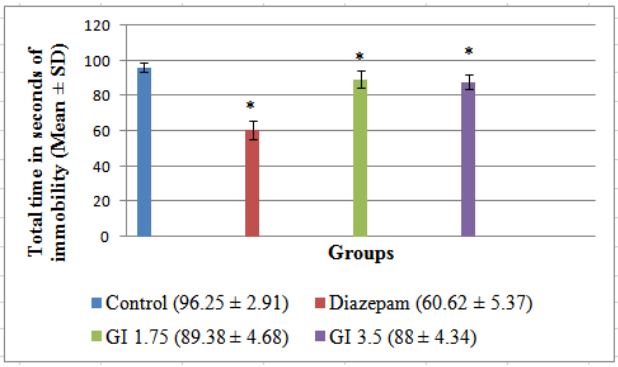
Figure 3 shows that both the groups of *Garcinia indica* and diazepam treated rats showed significant increase in number of central squares crossed (locomotor activity) as compared to control group.



*Compared with control p-value < 0.05

Figure 4: Comparison of time of grooming behaviour shown by rats in different groups.

Figure 4 shows that both the groups of *Garcinia indica* and diazepam treated rats showed significant increase in grooming behaviour as compared to control group.



*Compared with control p-value < 0.05

Figure 5: Comparison of time of immobility exhibited by rats in different groups.

Figure 5 shows that both the groups of *Garcinia indica* and diazepam treated rats showed significant decrease in duration of immobility as compared to control group.

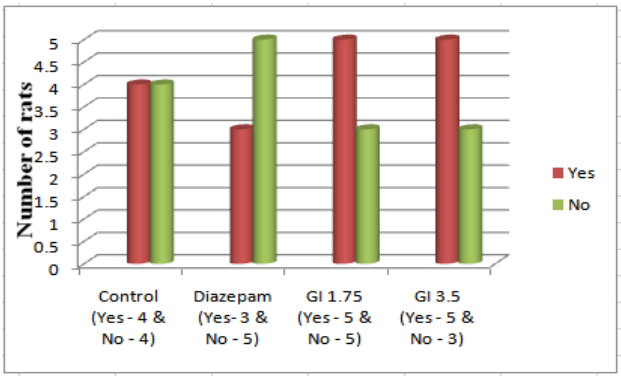


Figure 6: Comparison in number of rats that passed urine in different groups.

Figure 6 shows number of rats that passed urine in which yes indicates number of rats that urinated and no indicates number of rats that didn't urinate in each group. No significant decrease in urination was observed in both the groups of *Garcinia indica* and diazepam treated rats as compared to control group.

DISCUSSION

In the world with cut throat competition and increasing complexity, changing world, rapid industrialisation, growing population, relative scarcity of resources, immigration and emigration and all such factors only increase anxiety causing feeling of unease, such as worry or apprehension, dread or foreboding, can indicate a primary psychiatric condition or a reaction to a primary medical disease.[1] Three major neurotransmitters are involved in anxiety: serotonin, norepinephrine and gamma-aminobutyric acid. GABA plays a role in helping to induce relaxation and sleep, and in preventing overexcitation.

Dysfunctions of various neurotransmitters and receptors in the brain have been implicated in anxiety disorders.[9] The 3 neurotransmitters primarily implicated in anxiety are GABA, serotonin (5-HT) and noradrenaline. Dysregulations in the noradrenergic systems are hypothesized to occur in anxiety disorders. Noradrenaline modulates autonomic arousal mechanisms, including increased heart rate and respiration. This leads to a physiological cascade resulting in panic symptoms such as paraesthesia, numbness and tightness in the chest. GAD is associated with noradrenergic overactivity, serotonin receptor (5-HT1A, 5-HT2C) dysregulation and a decrease in the number of benzodiazepine sites on the GABAA-benzodiazepine receptor complex.[10]

Pharmacotherapy is the first line treatment of these disorders, but it can impose some problems, including sedation and amnesia, causing tolerance, psychomotor effects, and dependence.[11] Inspite of such long-term adverse effect profile, drugs like Benzodiazepines (e.g. Diazepam) known to enhance the activity of GABA, producing a calming effect, are still frequently used but there is always a scope for new drugs with better efficacy and predictability along with fewer side effects. Ayurvedic and herbal treatments are likely to be a source of good anxiolytic drugs. The treatment of anxiety in Ayurveda is holistic and not aimed only at calming the patient or psychotherapy, but also aimed at improving the overall brain function and wellbeing of the patient.[12]

It has been found that those individuals producing low levels of serotonin in the brain have a greater chance of experiencing anxiety and depression.[13,14] *Garcinia indica* also known as kokum consumed majorly in Kokan region of western Ghats of Maharashtra is known to contain hydroxycitric acid (HCA), which is an established booster of serotonin liberator from rat brain as per Ohia et al which can be the cause of its anxiolytic effect.[15]

This study is focussed in assessing the anxiolytic effect of *Garcinia indica* using open field test, a reliable animal model for analysis of anxiolytic activity of any drug. This test, originally designed by Hall in 1934 on rats, consists in placing an animal in an unknown environment with surrounding walls, so as to observe a number of behavior patterns, including the tendency to stay on the periphery of the field without entering the centre (called thigmotaxis and often interpreted as anxious behaviour), levels of defecation and urination. It is an exploration-based model that is useful to observe behavioural cues and assess the locomotor activity of animal. This model tests for neophobia and agoraphobia and also correlates with phobias and generalized anxiety disorder in humans. It is sensitive to the anxiolytic-like effects of Benzodiazepines and 5-HT1A receptor agonists.[16-19]

In this study we assessed number of lines and central square crossed for evaluation of locomotor activity in rats and also assessed grooming, rearing, immobility and urination habits for evaluation of behavioural cues. Number of lines crossed was decreased in diazepam treated rats as compared to control group and *Garcinia indica* (3.5 gm/kg) treated rats but number of central squares crossed was increased significantly in diazepam and both group of *Garcinia indica* treated rats as compared to control group while no significant difference was observed in the later parameter as we compared diazepam with both the groups of *Garcinia indica*.

A study was done by Patel et al which showed that *Garcinia indica* in various doses produced no significant changes in locomotor activity as compared with the control animals, which was also similar in case of diazepam8. Another study conducted by Dhamija et al reported that *Garcinia indica*, when admixed with diet of mice in different concentrations did not exhibit any significant difference on locomotor activity in comparison with control group.[7]

In present study, duration of immobility was significantly decreased in all drug treated rats as compared to control group but no significant difference was observed while they were compared with each other. A study conducted by Ibrahim et al using *Garcinia cambogia* (having similar bioactive compound hydroxycitric acid) reported a dose dependent increase in the immobility time, although statistically significant in the high dose group only, was observed compared to the control group.[11]

Some particular behaviour like grooming, resting and sleeping are highly frequent in situation where animal have no fear. Rearing, in which the animal temporarily stands on its hind legs to sample the environment, is indicative of vigilance and a measure of vertical locomotio.[20] Grooming is a complex process which includes licking of fur, paws, legs, washing movements of head and cleaning of genitals and tail and it is found to be increased in low as well as high conditions of stress.[21] Grooming is highly sensitive to various stressors and drugs.[22] In the current study, rearing and grooming behavior were significantly increased in diazepam and both group of *Garcinia indica* treated rats as compared to control group but no such significant difference was observed while comparing diazepam with both the groups of *Garcinia indica.*

There was no significant difference observed for urination habits in any drug treated rats when compared with control group. As far as we know, no studies were done previously which assessed grooming, rearing and urination behaviour in rats using *Garcinia indica.*

It was interpreted from the present study that *Garcinia indica* in both doses have significant anxiolytic effect comparable to that of diazepam and better than diazepam in preserving the locomotor activity in high dose.

This study has some limitations which indicate a need for human models of anxiety, like many clinically important, especially cognitive-based, symptoms of anxiety cannot be directly modelled in this animal study, behavioral measures might reflect changes in general activity and which may often be confounded with results of study and often poor correlation was noted between different behavioural measures taken in the same test.

CONCLUSION

Garcinia indica have a significant anxiolytic effect which was even comparable to that of diazepam and can prove to be an additional drug therapy for treatment of anxiety, identification of major components like hydroxycitric acid could be used as prototype compounds to design new drug but still clinical studies are required for assessing its safety and efficacy as an anxiolytic drug.

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

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