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Original Research Article

An in vivo study analyzing the anxiolytic activity of *Garcinia indica* fruit rind in preclinical models

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ABSTRACT

Anxiety disorder is a chronic psychiatric condition associated with increased morbidity and mortality, personal distress and reduced quality of life. Currently used anxiolytic drugs only have modest efficacy and are associated with significant untoward effects which leads to low compliance.
Traditional ayurvedic medicine describes *Garcinia indica* as a home remedy for many conditions. Hydroxycitric acid, a phytochemical constituent of Garcinia indica, has shown to elevate brain serotonin levels in the rat. The main aim of the present study is to evaluate the anxiolytic activity of Garcinia indica fruit rind in preclinical models.
Materials and Methods: Male and female wistar albino rats were divided into four groups with six animals in each group: Control(0.1% carboxymethylcellulose 10ml/kg), *Garcinia indica* ethanolic extract (GIEE1- 200mg/kg), *Garcinia indica* ethanolic extract (GIEE2- 400mg/kg) and Diazepam (1mg/kg). All the drugs were administered orally for a period of 14 days. On the 14th day after one hour of drug administration, animals were evaluated using two models- Elevated plus maze and Light dark arena.
Statistical analysis: Statistical significance was interpreted using one way ANOVA (Analysis of Variance) followed by Tukey Krammer Test.
Results: GIEE1 and GIEE2 treated groups showed significant increase in time spent in open arms and time spent in light arena in elevated plus maze and light dark arena model respectively, compared to control.
Conclusion: *Garcinia indica* ethanolic extract demonstrated significant anxiolytic activity compared to the control.

1. Introduction

Anxiety is defined by an unpleasant emotional situation associated with troubled feelings and uneasiness. Anxiety is one way by which the person reacts to a particular threat like a scary sight, loud noise.[1] This kind of 'normal' anxiety can also be described by the term 'fear' and is a part of each and every human being. 'Pathological' anxiety is described when the anxiety is excessively severe and increased in frequency and if it interferes with the person's day to day activities. 'Pathological' anxiety is a chronic psychiatric condition associated with increased morbidity and mortality, personal distress and reduced quality of life. It also possesses a significant social and economic burden. Currently used anxiolytic drugs only have modest efficacy associated with significant untoward effects which leads to low compliance.[2] This situation necessitates a research in search of a novel anxiolytic drug with no adverse effects.

Garcinia indica (Thours) Choisy, commonly known as kokum is a slender evergreen tree grown in the humid region of the Western ghat region of South India.[3–5] Traditional ayurvedic medicine describes *Garcinia indica* as a home remedy for many conditions like diarrhoea, dysentery, hemorrhoids, asthma, gastritis, indigestion problems, flatulence, dermatitis, ulcer and helminthic infestations. It is also being used as an anti obesity agent, anti inflammatory and analgesic agent, cardiotonic, hepatotonic, antitumor, anti perspirant, astringent, demulcent and emollient since

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ages.[3,6,7]

Hydroxycitric acid, a phytochemical constituent of *Garcinia indica*, has shown to elevate brain serotonin levels in the rat.[8] It is widely recognized and accepted information that

abnormality in serotonergic transmission is one of the etiological factor in anxiety disorder.

Therefore this study has been conducted to evaluate the activity of *Garcinia indica* (Thours) Choisy fruit rind in preclinical models of anxiety for validating its effectiveness scientifically.

2. Materials and Methods

Ethics clearance was obtained from the Institutional Animal Ethics Committee before initiating the study.

2.1. Plant material

Garcinia indica fruits were collected in the month of March and April from the kokum tree grown in Dakshina Kannada district. Botanical authentication was done by Dr. Krishna Kumar, Dept. of Applied Botany, Mangaluru University. Fruits were washed and fruit rind was separated from the pulp and was shade and air dried for a period of 3 weeks. The dried fruit rind was powdered using mixer grinder and taken for extraction.

2.2. Preparation of the extract

The powder which weighed 400g was taken for extraction in soxhlet apparatus using 95% ethanol[9] as a solvent. Temperature was maintained around 60-70^{0}C. Time duration of extraction was 10 days. The extract was concentrated in the rotavapour and subsequently in the water bath over a period of one day. The resultant brown coloured extract weighed 184.8g. The yield was 46.2% w/w. *Garcinia indica* ethanolic extract (GIEE) was dissolved in 0.1% sodium salt of carboxymethyl cellulose in distilled water[10] and administered to animals in various doses.

2.3. Animals

Male and female Wistar albino rats, aged 3- 4 months, weighing 150-200g were used in this study. Animals were housed under standard conditions in the animal house with temperature maintained around 24+/- 2^{0}C with 12: 12 hour light: dark cycle. The rats were divided into four groups with six animals in each group as follows.

Group I- 0.1% carboxymethylcellulose (10ml/kg)[9]

Group II- *Garcinia indica* ethanolic extract ($GIEE_2$)-200mg/kg[10]

Group III- *Garcinia indica* ethanolic extract ($GIEE_3$)-400mg/kg[10]

Group IV- Diazepam- 1mg/kg[11]

All the drugs were administered orally for a period of 14 days. On the 14th day, after one hour of drug administration, animals were tested for anxiolytic activity.

2.4. Elevated plus maze

Elevated plus maze consists of two open arms measuring 50 x 10 cm, two closed arms measuring 50 x 10 x 40 cm and a central platform. Each rat was placed in the central platform facing one of the closed arms and observed for 5 minutes as shown in Figure 1. Time spent in open and closed arms were noted[11]. Anxiolytic activity was expressed by increase in time spent in open arm.

Fig. 1: Elevated plus maze

2.5. Light dark arena

This test consists of a box of which 1/3 is dark compartment and 2/3 is a compartment illuminated by a light source. They are divided by a wall which has a gap for the movement of rat, as shown in Figure 2. Each rat was placed in the light compartment and observed for 5 minutes. Time spent in each compartments were noted.[11] Anxiolytic activity was expressed by increase in time spent in illuminated compartment.

Fig. 2: Light dark arena

2.6. Statistical analysis

Data was tabulated and analyzed using the statistical software, GraphPad InStat. Results were represented as Mean ± SEM (Standard Error of Mean). Statistical significance was interpreted using one way ANOVA (Analysis of Variance) followed by Tukey Krammer Test. Data were considered very highly significant when P value less than 0.001 was obtained.

3. Results

3.1. Elevated plus maze

GIEE showed dose dependent difference in the time spent in open and closed arms compared to the control group, although not significantly difference from diazepam. GIEE at the dose of 200mg/kg and 400mg/kg showed very highly significant increase in the time spent in open arm compared to the control group (P value< 0.001). GIEE at the dose of 200mg/kg and 400mg/kg showed very high significant decrease in the time spent in closed arm compared to the control (P value< 0.001).

3.2. Light dark arena

GIEE showed dose dependent difference in the time spent in light and dark compartments compared to the control group, although not significantly difference from diazepam. GIEE at the dose of 200mg/kg and 400mg/kg showed very high significant increase in the time spent in light arena compared to the control group (P value< 0.001).

Also, GIEE at the dose of 200mg/kg and 400mg/kg showed very high significant decrease in the time spent in dark arena compared to the control (P value< 0.001).

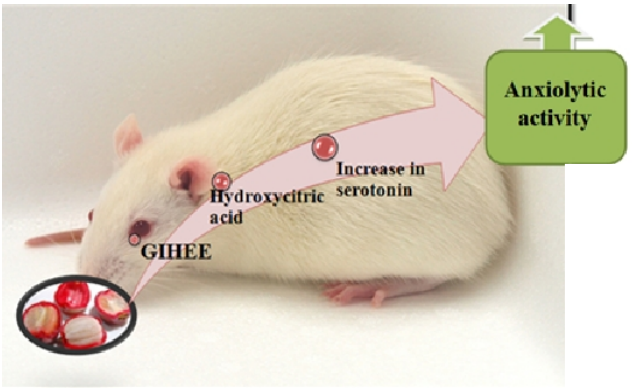

Fig. 3: Mechanism of action of GIEE

4. Discussion

Anxiety disorder is a chronic psychological condition which accounts for a major social and economic burden. It also interferes with the person's daily activities. It is known that GABAergic neurotransmission plays an important role in anxiety. There are also various literatures suggesting the role of monoamine neurotransmitters in the pathology of anxiety.[2,12] Serotonin, which is a monoamine neurotransmitter acts on about five lakh neurons in the central nervous system. It is also stated that serotonergic system is involved in the causation of anxiety disorder. SSRIs which increase the levels of serotonin are commonly used in various anxiety disorders. But they are available only for chronic anxiety conditions.[13] Benzodiazepines which act through $GABA_A$ receptors are effective in acute anxiety conditions but present with numerous problematic side effects like sedation and cognitive impairment[14] which necessitates the evaluation of a novel anxiolytic drug which is effective and well tolerated.

Various animal models are present for evaluating anti anxiety activity. Elevated plus maze and Light dark arena model were used for evaluating anxiolytic activity of GIEE. Elevated plus maze test is one of the popular tests used for evaluating a novel potential anti anxiety agent. Validity of this test is particularly very high i.e; rodents treated with anxiolytic drugs show increase in time spent in open arms whereas, rodents treated with anxiogenic drugs show decrease in time spent in open arms.[15] It is also a simple method for evaluating the anxiety behavior in rodents. Montgomery initially described an elevated maze in 'Y- shape' which was later modified by Handley and Mithani into a 'plus shape' maze containing two open and closed arms respectively.[16] GIEE in all three doses showed significant increase in time spent in open arms and decrease in time spent in closed arms compared to the control group.

Light dark arena is another model for evaluating the anxiolytic activity of an agent. When benzodiazepines were administered to rodents, they showed increase in

Table 1: Elevatedplus maze

S. No.	Group	Time spent in open arm (seconds)	Time spent in closed arm (seconds)
I	Control	15.6 ± 4.52	272.3 ± 5.05
II	$GIEE_1$	73.6 ± 10.27[a]	208.1 ± 10.41[a]
III	$GIEE_2$	83.8 ± 10.045[a]	201.5 ± 9.77[a]
IV	Diazepam	106.6 ± 11.42	175.1 ± 11.39

$GIEE_1$- *Garcinia indica* ethanolic extract 200mg/kg
$GIEE_2$- *Garcinia indica* ethanolic extract 400mg/kg
One way ANOVA followed by Tukey Krammer test
[a]P value< 0.001- very highly significant, compared to control

Table 2: Light dark arena

S. No.	Group	Time spent in light arena (seconds)	Time spent in dark arena (seconds)
I	Control	23.3 ± 4.31	260.8 ± 5.12
II	$GIEE_1$	73 ± 3.95[a]	209.5 ± 3.83[a]
III	$GIEE_2$	92.3 ± 6.09[a]	192.1 ± 6.04[a]
IV	Diazepam	106.5 ± 3.51	174.5 ± 3.78

$GIEE_1$- *Garcinia indica* ethanolic extract 200mg/kg
$GIEE_2$- *Garcinia indica* ethanolic extract 400mg/kg
One way ANOVA followed by Tukey Krammer test
[a]P value< 0.001- very highly significant, compared to control

exploratory behaviour between light arena and dark arena.[17] It is a known fact since ages that benzodiazepines act through modulation of $GABA_A$ receptors.[18] GIEE showed significant increase in time spent in light arena compared to control group indicating anti anxiety activity. Since modulation of $GABA_A$ receptor is involved in anti anxiety activity, this mechanism may be attributed to the anxiolytic activity demonstrated in GIEE treated rodents although there is no much objective evidence towards this. To find out whether GIEE possess any $GABA_A$ modulating property or not, diazepam has to be combined with GIEE and evaluated for anxiolytic activity.

It is also known that there is a definite correlation between serotonin and anxiety[2,12]and Hydroxycitric acid which is a constituent of *Garcinia indica* modulates serotonin levels, which is shown in Figure 3.

5. Conclusion

The study shows that *Garcinia indica* ethanolic (GIEE) extract has significant anxiolytic activity most probably due to serotonin modulating property.

6. Source of Funding

Nil.

7. Conflicts of Interest

None.

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