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ANTI-ANXIETY EFFECTS OF KRISHNATULSI (*OCIMUM TENUIFLORUM*) VIA IN-VIVO EVALUATION

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ABSTRACT

Persistent lifestyle-related disorders are the main cause of anxiety and mortality worldwide. Anxiety is a natural emotional response, but when it reaches a severe level, it can lead to the development of cardiovascular and psychiatric disorders. Moreover, According to WHO report in 2019, 1 in every 8 people or 950 people were suffering from psychological disturbances with stress and anxiety. Generalized anxiety disorder (GAD), panic disorder, phobias, and social anxiety disorders are all included in the broad range of anxiety disorders (Michaelides *et al.*, 2019). According to WHO report in 2019, 1 in every 8 people or 950 peoplewere suffering from psychological disturbances with stress and anxiety. Generalized anxiety disorder (GAD), panic disorder, phobias, and social anxiety disorders are all included in the broad range of anxiety disorders (Michaelides *et al.*, 2019). In the realm of medical treatment for anxiety disorders, there is a wide range of allopathic medications to choose from. Benzodiazepines, in particular, are frequently prescribed, but they come with a variety of systemic side effects.

KEYWORDS: Anxiety, Krishna Tulsi, Clonazepam, Swiss albino mice.

INTRODUCTION

Anxiety stands as a prominent symptom in numerous psychiatric conditions and frequently accompanies various medical and surgical illnesses. It represents a universal human emotion, closely intertwined with appropriate fear, likely serving adaptive psychobiological functions.[1] While anxiety is a natural emotional response, it can take on a pathological form, potentially triggering cardiovascular and psychiatric disorders when it becomes severe.[2] Although allopathic medicine offers numerous drugs for the treatment of anxiety disorders, they often come with systemic side effects or may exhibit diminished effectiveness with chronic uses.[3] Treatment for anxiety frequently combines therapies like cognitive-behavioural therapy, medication, lifestyle changes, and stress management approaches. Ayurvedic medicine has long emphasized the safety and reduced toxicity of many plant-based remedies compared to synthetic drugs. Medicinal plants are abundant sources of secondary metabolites, which hold significant potential as therapeutic agents. The key benefits attributed to the use of medicinal plants in treating various conditions include their safety profile, cost- effectiveness, efficacy, and ready accessibility.[4]

In Sanskrit, "Tulsi" translates to "one that is incomparable." Tulsi is known for its ability to promote overall health and well-being, exerting positive effects on both the body and the mind. *Ocimum tenuiflorum*, also known as Krishna Tulsi, holds a revered place in India as the most sacred herb. This plant belongs to the Lamiaceae family and boasts a multitude of medicinal properties that benefit human health.[5] Traditionally, different parts of the plant, including its leaves, flowers, and stems, have been utilized in the treatment of various ailments such as skin conditions, colds, coughs, fevers, vomiting, and swelling. Notably, *O. tenuiflorum* has been reported to exhibit a wide range of therapeutic properties, including anti-cancer, antimicrobial, antiseptic, antispasmodic, antifungal, antiviral, anti-inflammatory, analgesic, and immuno- stimulatory effects.[6] The primary chemical constituents found in *O. tenuiflorum* include eugenol, methyl cinnamate, camphor, and thymol. The objective of this study is to provide an updated review that highlights the scientifically validated medicinal activities of *O. tenuiflorum* against a variety of disorders.[7] *Ocimum tenuiflorum* consist of bioactive compounds known to give a soothing effect to the brain. In the experiment conducted, it was observed that eugenol which is chemically known as 4- allyl-2- methoxyphenol

and Ocimumosides influences the activity of neurotransmitters in the brain and shows the calming effect. Also, it is hydrophobic in nature. Therefore, eugenol is reported to be an anti-stress agentSince, anxiety disorders significantly affect our daily lives, it is important to explore alternative medicinal approaches for their treatment. Consequently, this study aimed to examine the potential anti-anxiety properties of an ethanolic extract derived from *O. tenuiflorum* leaves, and to compare its effectiveness with the established standard drug, Clonazepam.

MATERIALS AND METHODS

Drugs and Chemicals

The test substance, an ethanolic extract derived from *O. tenuiflorum* leaves, was prepared from the Pharmacognosy laboratory at Bengal School of Technology, West Bengal. This extract was administered at varying doses of 1.70 mg/kg, 4.30 mg/kg, and 8.45 mg/kg.[8] Clonazepam was obtained from ZOECIA HEALTHCARE in Haryana, India, and administered at a dose of 1 mg/kg.[9] Gum acacia, a dried exudate derived from *Acacia senegal* and certain other Acacia species, was employed as a white powder suspending agent.[10] It served as a control at a dose of 0.1 ml/10 g (1%) and was used as a vehicle to suspend both the standard drug (Clonazepam) and the test drug (*O. tenuiflorum* extract). These drugs and their respective vehicles were administered orally.

Animals

The study included healthy Swiss albino mice, both male and female, weighing between 20-40 grams and aged 3-4 months.[11] Pregnant, diseased animals, and those previously used in other experiments were excluded from the study. These mice had free access to both water and commercial food and were kept under standard laboratory conditions with natural light and dark cycles, at room temperature.

The experiments took place in the research laboratory of the Department of Pharmacology at Bengal School of Technology, conducted between 10:00 A.M. and 5:00 P.M. The experiments were carried out 30 minutes after the administration of the drug. A total of 60 animals (n = 60) were involved, divided into 10 groups, each consisting of six animals (**Table No. 1**). The Institutional Animal Ethics Committee approved the experimental study.

The animals were separated into 5 group, each containing of 6 animals, for both experimental models
Table -1

Groups (*n*=6)	Treatment
I	Control 1% gum acacia 10.0 ml/kg
II	Clonazepam 1.0 mg/kg
III	*O. tenuiflorum*- 1.70 mg/kg
IV	*O. tenuiflorum*- 4.30 mg/kg
V	*O. tenuiflorum*- 8.45 mg/kg

n*:** Number of animals in each group, ***O. tenuiflorum: *Ocimum tenuiflorum*

RESULT AND DISCUSSION

Light and dark exploration test

The experimental setup consisted of a pair of square enclosures, each measuring 50 cm × 50 cm × 50 cm, with a wooden divider between them. One of these enclosures was maintained in darkness, while the other was illuminated by a 7W/12V light bulb. Positioned in the middle of the wooden partition was a 6 cm × 6 cm opening, which could be opened or closed using a transparent plexiglass sliding door, allowing the mice to freely move between both compartments. Individual mice were then introduced into the center of the illuminated enclosure and observed for a duration of 5 minutes. Their time spent in each enclosure was recorded in seconds, along with the count of crossings made between the two compartments. Prior to placement in the illuminated enclosure, the mice received treatments involving *O. tenuiflorum* extract, clonazepam, or gum acacia, administered 30 minutes in advance.

Elevated plus maze test

The setup comprised a central platform measuring 10 cm × 10 cm, which was connected to two open arms measuring 50 cm × 10 cm each, as well as two closed arms measuring 50 cm × 40 cm × 10 cm in size. This maze was elevated 50 cm above the ground. Swiss albino mice, weighing between 20 and 40 grams, were subjected to treatments involving *O. tenuiflorum* extract, clonazepam, or gum acacia, administered 30 minutes before being individually placed at the center of the elevated plus maze, initially facing a closed arm. The mice were then observed for a period of 5 minutes, during which their time spent in both the open and closed arms was meticulously recorded in seconds. Additionally, the number of entries made into both the open and closed arms was tallied during the test, with an entry defined as having all four paws within the respective arm.

Statistical analysis

The analysis of the results was conducted through ANOVA, followed by a post-hoc test for further examination. A significance level of $P < 0.05$ was used to determine statistical significance.[14]

Light and dark exploration test

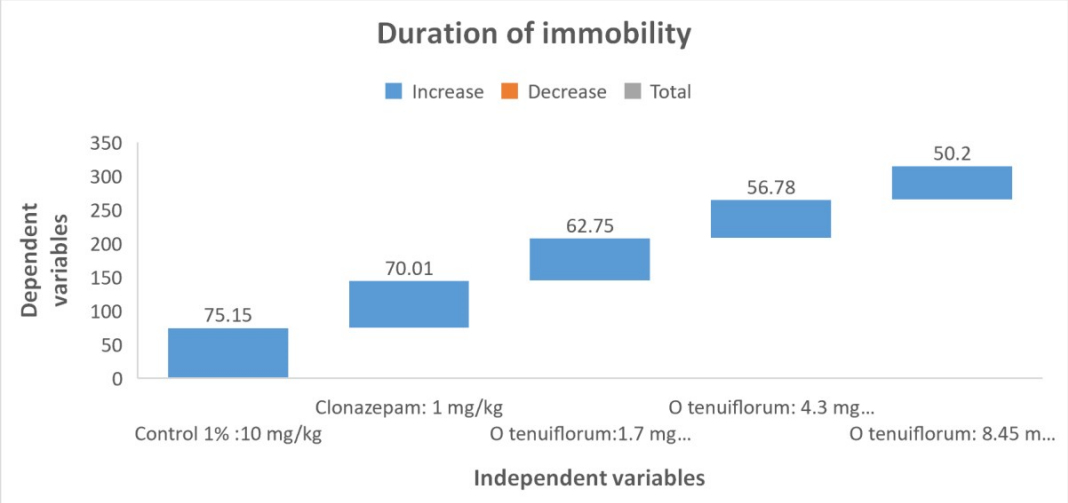
presents the findings, indicating that the rats treated with the standard drug, clonazepam, exhibited an extended duration spent in the illuminated area and a noticeable

increase in rearing behaviour. In contrast, rats treated with *O. tenuiflorum* demonstrated an augmentation in the time spent in the illuminated area at a dose of 1.70 mg/kg ($P < 0.05$) and a significant increase at doses of 4.30 and 8.45 mg/kg ($P < 0.01$). Furthermore, these *O. tenuiflorum*-treated rats displayed reduced immobility across all three dosage levels, as outlined in.

Effect of administration of O.tenniform on swis albino mice behavior in light and dark apparatus
Table-2

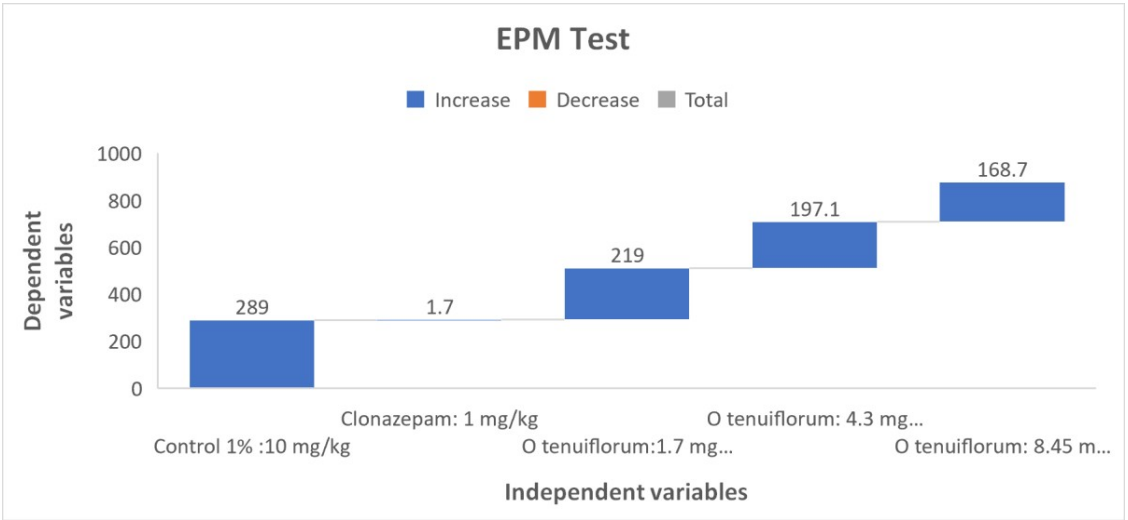
SL. No	Drug groups(*n*=6)	Number of bright chamber entries (s)	Time spent in bright chamber(s)	Number of rears in bright chamber(s)	Duration of immobility (s)
I	Control 1% gum acacia 10 ml/kg	1.3±0.22	13.6±0.80	2.5±0.40	75.15±4.0
II	Clonazepam 1.0mg/kg	4.5±0.44**	28.9±3.15**	3.98±0.25	70.01±2
III	*O. tenuiflorum*-1.70 mg/kg	1.78±0.35	26.70±3.02*	2.7±0.4	62.75±3.40*
IV	*O. tenuiflorum*-4.30 mg/kg	2±0.15	34.5±2.07**	2.7±0.89	56.78±3.04**
V	*O. tenuiflorum*-8.45 mg/kg	3±0.25	43.40±4.01**	5±0.89*	50.2±3.5**

The data is presented as mean values with standard error of the mean (SEM). Statistical analysis was performed using a one-way analysis of variance **(ANOVA)** followed by Tukey's post-hoc test, with significance levels indicated as *** for P<0.05** and **** for P<0.01**. : ***O. tenuiflorum:*** *Ocimum tenuiflorum*, **SEM:** Standard error mean.



The obtained results indicate that, *Ocimum tenuiflorum* has high efficiency in resisting anxiety-related disorders. Since, spending more time in the light chamber during a light and dark exploration test than the dark chamber could indicate lower anxiety for well-lit areas.

Observation was made that when *Ocimum tenuiflorum* was administered at higher doses (8.45mg/kg), it showed less duration of immobility as compared to the duration when it was administered at a low dose (1.7mg/kg).



Elevated Plus Maze proved to be an essential technique to observe the change in the behavioural pattern of the experimental mice. The locus coeruleus (LC) is a part of nor- epinephrine and is known for the fear response. Therefore, when nor- epinephrine gets activated and causes the over activation of ANS, it results in fear and anxiety. According to Light and dark exploration test, the statistical analysis of the obtained data showed the administration of *O tenuiflorum* to be much effective when compared to the control and clonazepam to control the anxiety disorders. However, the physiological responses observed was varied according to their dose strength. Therefore, it has been recorded that the therapeutic efficacy was much better with *O tenuiflorum* because overall stress management was seen during the experiment. The experimental beings were recorded much relaxed with the 8.45mg/kg dose of *O tenuiflorum* during bright light in chamber withhigh number of rears.

Elevated plus maze test

shows that rats treated with Clonazepam had more open arm entries and spent more time in open arms. They spent less time in closed arms. Experimental rodents (Swiss Albino mice) treated with *O. tenuiflorum* also had more open arm entries and spent more time in openarms, especially at 8.45 mg/kg dosage. They also reared more in open arms at this dosage but spent less time in closed arms at different doses (1.70, 4.30, and 8.45 mg/kg). These differencesare significant, as indicated.

Effect of administration of O.tenuiflorum on swiss albino mice on experimental rodent (Swiss albino mice) behavior in elevated plus maze

SL. No.	Drug Group (n=6)	Number of Open arms entries	Number of Total arm entries	Time spent in Open arms(S)	Time spent in closed arms(s)	Number of Rears in open arms(s)
I	Control 1% gum acacia 10 ml/kg	2.5±0.35	4.20±0.50	13.59±1.90	289.1±7.5	1.91±0.37
II	Clonazepam 1mg/kg	3.10±0.50*	6.10±0.50	81.5±2.80**	170.0±4.10**	1.50±0.30
III	*O. tenuiflorum*-1.70 mg/kg	1.60±0.27	4.60±0.42	44.6±3.35**	219.00±5.60**	3.7±0.50
IV	*O. tenuiflorum*-4.30 mg/kg	2.65±0.3	6.20±0.3	55.30±7.60**	197.10±6.10**	4.10±0.50
V	*O. tenuiflorum*-8.45 mg/kg	4.5±0.5**	7.0±0.50**	79.10±7.2**	168.70±3.42**	4.10±1.10*

The data is presented as mean values with standard error of the mean (SEM). Statistical analysis was performed using a one-way analysis of variance **(ANOVA)** followed by Tukey's post-hoc test, with significance levels indicated as *** for P<0.05** and **** for P<0.01**. : ***O. tenuiflorum:*** *Ocimum tenuiflorum*, **SEM:** Standard error mean.

In the present experimental data, it was revealed that the quantities of different drug groups were directly proportional to the therapeutic outcomes. In this study, *O tenuiflorum* when administered at 1.7mg/kg showed least time spent in open arms as compared to the 4.3 mg/kg dose. The highest efficacy was recorded at 8.45mg/kg dose of *O tenuiflorum* administered.

The direct correlation between dose and efficacy was depicted.

Cognitive-behavioural therapy (CBT), medication, or other therapies aimed at lowering anxiety and encouraging more adaptable behaviours are examples of therapeutic techniques. Anxiety or anxiety-related behaviours can be measured by a decrease in the amount of time spent in closed arms. Reduced time spent in the elevated plus maze's closed arms indicated that the experimental animal was more anxious than usual. Therefore, *O tenuiflorum* was observed to reduce the anxiousness with much better efficacy than clonazepam.

All the above data from light and dark exploration test are combined and shown in thisbelow Chart

The Elevated Plus Maze is the test based on experimental being's innate fear of open, exposed spaces (anxiety) and preference for secure, enclosed environments. Therefore, thedata depicted the effectiveness of *O tenuiflorum* in the management of anxiety disorder.

In the study, it was revealed that duration of immobility was high with the administration of control 1% gum acacia 10mg/kg. However, when *O. tenuiflorum* was administered the time spent in bright chamber was moderate. Also, the duration of immobility was much less when compared to the control 1%. Hence, it was concluded that *O. tenuiflorum* was observed to behighly effective against anxiety disorders.

CONCLUSION

The study's findings indicate that the leaf extract of *O. tenuiflorum* exhibits anxiolytic (anxiety- reducing) properties. This suggests that *O. tenuiflorum* could be a promising option for treatinganxiety disorders in clinical settings. However, more research is needed to understand how this plant extract works and identify the specific active compounds responsible for its effects.

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