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RESEARCH ARTICLE

A Novel Circular Maze Paradigm For Anxiety Assessment: Behavioral Insights Into Herbal Anxiolytics

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ABSTRACT

Anxiety disorders are among the most prevalent psychiatric illnesses, often treated with synthetic drugs that may cause adverse effects. Traditional medicinal plants such as *Ocimum sanctum* (Tulsi) and *Trachyspermum ammi* (Ajwain) have been used for their potential adaptogenic and anxiolytic properties. The present study investigates the anti-anxiety activity of a combined extract of *O. sanctum* and *T. ammi* in Wistar rats using behavioural models like the Elevated Plus Maze (EPM) and circular open field test over a period of four weeks. Rats were grouped into control, standard (diazepam), and test extract groups. Animals were subjected weekly to the circular maze test, recording time spent in open and closed arms. Data were analysedusing one-way ANOVA followed by Tukey's post hoc test. The test group showed a gradual increase in time spent in the open arm: Week 1 (16.8 ± 2.4 s), Week 2 (21.3 ± 2.1 s), Week 3 (26.6 ± 1.8 s), and Week 4 (31.4 ± 2.0 s). Conversely, time in the closed arm reduced from Week 1 (43.2 ± 1.9 s) to Week 4 (28.6 ± 2.3 s). These changes were statistically significant ($p < 0.05$) compared to the control group and approached the efficacy of the standard drug by Week 4. Bar charts visually confirmed these progressive behaviouralimprovements. The combined extract of *O. sanctum* and *T.ammi* produced a significant anxiolytic effect in rodents, demonstrating a steady behaviouralimprovement over four weeks. These findings support its potential as a natural alternative for managing anxiety.

KEY WORDS: *Ocimum sanctum, Trachyspermumammi,* Circular Maze, Anti-anxiety, Open field Test, Elevated plus maze.

INTRODUCTION

Anxiety is a widespread and debilitating neurobehavioral condition characterised by excessive worry, fear, and avoidance behaviours that significantly impair daily functioning (Bhattacharyya *et al*., 2013). The global burden of anxiety-related disorders continues to rise, prompting the need for novel therapeutic interventions that are both effective and safe (Siraji*etal*., 2008). While benzodiazepines such as diazepam are widely used for their anxiolytic effects, their long-term use is limited by side effects including sedation, tolerance, dependence, and withdrawal symptoms (Geetha *et al*., 2006). This has led to increased interest in alternative treatment strategies, particularly those derived from medicinal plants with traditional and pharmacological significance.

Rodent models are extensively used in preclinical studies to evaluate the anxiolytic potential of pharmacological and natural compounds (Hemanth *et al*., 2019). Among these, behaviouralparadigms like the elevated plus maze (EPM), open field test (OFT), and light-dark box test are traditionally employed (Shukla *et al*., 2021). However, to enhance the precision of behavioural assessments and create a more naturalistic conflict between fear and safety, innovative maze designs are continually being developed (Rajput *et al*., 2014). In this study, a novel circular elevated maze, also referred to as a circular tangle, was designed and utilisedto assess anxiety-like behaviourin rodents (Saxena *et al*., 2012).

The circular maze consists of a round platform elevated approximately 60 cm above the ground using four sturdy iron rods. The platform is divided into alternating protected and unprotected quarters. Two opposite quarters are

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enclosed by walls, creating dark, tunnel-like safe zones, while the remaining two are left open and brightly illuminated with LED lights to mimic exposure and height-induced fear. This configuration induces a state of anxiety in the test animals, prompting them to seek refuge in the enclosed segments. The time spent in protected versus unprotected zones is used as a key behaviouralindicator to assess the severity of anxiety or the effectiveness of anxiolytic treatments.

In this experimental setup, the anxiolytic efficacy of two medicinal plants, *Trachyspermum ammi* (Ajwain) and *Ocimum sanctum* (Tulsi), was evaluated. These plants are well known in traditional medicine for their adaptogenic and neuroprotective properties, largely attributed to their active constituents such as thymol, eugenol, and flavonoids (Gupta *et al*., 2007). The behaviouralresponses of rodents treated with plant extracts were compared with those treated with diazepam, a standard anxiolytic drug (Bhargava *et al*., 1981).

The present study aims to validate the use of this novel circular maze as an effective model for anxiety research and to explore the potential of herbal remedies in managing anxiety, offering insights into safe, plant-based therapeutic alternatives for neuropsychiatric conditions.

Plant Profile - *Ocimum sanctum*

Ocimum sanctum, commonly known as Tulsi or Holy Basil, is a revered medicinal plant in traditional Ayurvedic medicine. It belongs to the family *Lamiaceae* and has been used for centuries in India and other parts of Asia for its wide range of therapeutic effects, particularly for adaptogenic, antistress, anxiolytic, and neuroprotective properties (Imbe *et al*., 2006). The anxiolytic effects of *O. sanctum* are attributed to the presence of multiple bioactive phytochemicals that modulate the central nervous system (CNS), particularly neurotransmitters involved in stress and anxiety regulation (Liu *et al*., 1994).

a) Eugenol (4-allyl-2-methoxyphenol)

This is a major phenolic compound found in Tulsi's essential oil and exhibits antioxidant, anti-inflammatory, and neuroprotective effects (Satyavati *et al*., 1987). This is also known to interact with the GABAergic system, which plays a central role in anxiety modulation and shown anxiolytic activity in animal studies by reducing corticosterone levels and increasing exploration in anxiety tests (Ramachandran *et al*., 1982).

b) Ursolic acid

A triterpenoid found in leaves. This exerts neuroprotective and anxiolytic effects via modulation of the HPA axis and reduction of neuroinflammation (Bhattacharya *et al*., 2008). Also, it may influence serotonergic and dopaminergic pathways, both involved in mood regulation.

c) Rosmarinic acid

A polyphenol with potent antioxidant and anti-inflammatory properties. It modulates neurotransmitters such as GABA and glutamate, contributing to anxiolytic and cognitive benefits (Singh *et al*., 1997). Helps in mitigating oxidative stress in brain regions associated with anxiety.

d) Linalool

A terpene found in Tulsi's volatile oil. It demonstrates sedative and anxiolytic properties, possibly through GABA-A receptor modulation and also reduces locomotor activity and induces calm behaviourin rodents (Mondal *et al*., 2009).

e) Ocimumosides (A & B)

Unique glycosidic compounds isolated from Tulsi. These are reported to normalise stress-induced biochemical changes in the brain (*e.g*., dopamine, serotonin, norepinephrine) (Grover *et al*., 1987). Ocimumosides have shown selective anxiolytic activity without sedative side effects, differentiating them from benzodiazepines (Rachana *et al*., 2025).

f) Apigenin

A flavonoid with established GABA-A receptor binding activity (Tony *et al*., 2025). Also known for its non-sedative anxiolytic effects, contributing to Tulsi's calming properties.

Molecular Mechanism

- *GABAergic Modulation:* Eugenol, linalool, and apigenin are believed to enhance GABAergic neurotransmission, mimicking benzodiazepine-like effects (Tony *et al*., 2025).
- *HPA Axis Regulation:* Compounds like ursolic acid and ocimumosides reduce stress-induced activation of the hypothalamic–pituitary–adrenal (HPA) axis, thereby lowering cortisol and corticosterone levels.
- *Monoaminergic Effects:*Normalisationof brain levels of dopamine, serotonin (5-HT), and norepinephrine is observed with Tulsi extracts, which play a central role in anxiety and mood regulation (Kotapati *et al*., 2018).
- *Antioxidant and Anti-inflammatory Effects:* Neuroinflammation and oxidative stress are key contributors to anxiety disorders; constituents like rosmarinic acid and eugenol mitigate these effects in CNS tissue (Karishma *et al*., 2018).

Plant Profile–*Trachyspermum ammi*

Trachyspermum ammi, commonly known as Ajwain or Bishop's weed, is a widely used culinary and medicinal plant from the family *Apiaceae*. Traditionally employed in Ayurvedic and Unani medicine, Ajwain is known for its carminative, antispasmodic, antimicrobial, and neuroprotective properties. Ajwain seeds are particularly rich in essential oils, flavonoids, and phenolic compounds that interact with the central nervous system to exert anxiolytic and calming effects.

a) Thymol

The major active component of Ajwain essential oil (30–60%). It possesses strong antioxidant, antimicrobial, and CNS-modulating properties. Interacts with GABA-A receptors, enhancing inhibitory neurotransmission and producing mild sedative and anxiolytic effects.

b) γ-Terpinene

A monoterpene hydrocarbon found in Ajwain oil. Also, it exhibits antioxidant and neuroprotective activity. Although indirect, its role in reducing oxidative stress may protect neurons involved in anxiety regulation.

c) p-Cymene

A monoterpene with anti-inflammatory and calming properties. Works synergistically with thymol to stabiliseneuronal function and reduce CNS excitability.

d) Flavonoids and Polyphenols

Ajwain contains quercetin, apigenin, and luteolin—flavonoids known for their anxiolytic and neuroprotective effects. These compounds modulate serotonin (5-HT) and dopamine levels, supporting mood regulation and stress resistance. Flavonoids also exhibit free radical scavenging activity, reducing neuroinflammation, a key contributor to anxiety.

e) Carvacrol (Minor Component)

A phenolic monoterpenoid structurally like thymol. It exerts anxiolytic, antidepressant-like effects via GABA and serotonin pathways. Also shown to reduce anxiety behavioursin animal models without impairing motor functions.

Molecular Mechanism

- *GABAergic Potentiation:* Thymol and carvacrol enhance GABA-A receptor activity, which increases inhibitory neurotransmission and produces a calming effect similar to benzodiazepines, but with a safer profile.
- *Monoamine Modulation:* Flavonoids such as quercetin influence serotonin and dopamine metabolism, promoting relaxation and emotional stability.
- *Antioxidant Defense:* Thymol and polyphenols combat oxidative stress in the hippocampus and amygdala, regions crucial for emotion regulation.
- *Anti-Inflammatory Activity:* Neuroinflammation is a key factor in chronic anxiety. Ajwain's constituents downregulate inflammatory mediators like TNF-α and IL-6 in the brain.

EXPERIMENTAL METHODOLOGY

Preparation of Plant Powder

Collection and Processing of *O. sanctum* and *T. ammi*

The plant materials (*O. sanctum* and *T.ammi*) were collected and authenticated byDr.P.Satyanarayana Raju, Taxonomist, Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur. The collected plant parts underwent a thorough cleaning process to remove dirt, debris, and possible contaminants. To preserve bioactive compounds, the plant materials were shade-dried at room temperature (25 ± 2°C) for 7-10 days. This method helps retain the phytochemical constituents without exposure to direct sunlight, which could degrade sensitive compounds. After complete drying, the plant materials were finely powdered using a mechanical grinder to enhance powder efficiency. The powdered material was then sieved to obtain a uniform particle size and stored in airtight containers to prevent moisture absorption and degradation. The containers were kept in a cool, dry place to maintain the stability of the phytochemicals until use.

Experimental Groups and Treatment Plan

A total of 30 Mice were randomly divided into fiveexperimental groups (n=6 per group):

Table 1: Various treatment groups used to assess anti-anxiety activity.

S.No	Groups	Treatment
1	Group-1	Normal Control (Receives normal saline 0.9% w/v)
2	Group-2	Diazepam (2mg/kg I.P)
3	Group-3	Test–I (Polyherbal Powder – OS+TA –100mg/kg - 25:75 Ratio)
4	Group-4	Test–II (Polyherbal Powder–OS+TA–100mg/kg - 50:50 Ratio)
5	Group-5	Test–III (Polyherbal Powder–OS+TA–100mg/kg - 75:25 Ratio)

The research strictly adhered to the ethical guidelines set by the Committee for Control and Supervision of

Experiments on Animals (CCSEA), a regulatory body governing animal research in India (IAEC Number: 04/ IAEC/CLPT/2023-24).

All mice were housed under standard laboratory conditions with a 12-hour light/dark cycle, controlled temperature (22 ± 2°C), *ad libitum* access to food and water. Acclimatisation was conducted for at least one week before experimentation.

Circular Maze

The apparatus is a custom-built circular elevated maze, designed for behaviouraltesting in rodents (rats/mice) to assess anxiety-related responses (Seibenhener *et al*., 2015). This model is a novel alternative to classical mazes like the elevated plus maze (EPM) or open field test (Pellow *et al*., 1985), with a circular track segmented into protected and unprotected regions. The maze is built on a circular platform, elevated from the ground using four vertical iron rods, each approximately 60 cm in height. The surface is made of an iron base, with protective tape used for reinforcement and to fix structural elements. Exactly half of the circle (two opposite quarters) is constructed with protective side walls, creating enclosed, darker chambers (Bailey *et al*., 2009). These act as "safe zones" or "closed arms" for the rodents (Walf *et al*., 2007). The other two quarters are open, lacking side walls, simulating unprotected, high-elevation conditions. These are designed to induce anxiety and fear due to height and exposure (Crawley *et al.,* 1985).

In the unprotected segments, small LED lights are fixed, enhancing the aversiveness of the open area by simulating bright, exposed conditions, which rodents naturally avoid due to their photophobic nature (Lister *et al*., 1987). The overall structure is supported on iron rods and looks sufficiently elevated to simulate naturalistic fear stimuli (heights + open space). Surfaces are taped and edges seem smoothed to prevent physical harm to the test animals (Bourin*et al*., 2003). This maze is used to evaluate anxiolytic or anxiety-inducing effects of pharmacological or plant-based substances (Rodgers *et al*., 1997). The animal's preference for the closed vs. open area is a key behaviouralendpoint (Carobrez *et al*., 2005). Increased time spent in the closed chambers is interpreted as a marker of anxiety (Prut *et al*., 2003), while entry into open segments or longer duration spent there indicates reduced anxiety (anxiolysis).

Behavioural Parameters Measured

- Time spent in closed vs. open segments
- Number of entries into each zone
- Latency to first entry into a zone
- Avoidance behaviour
- Exploratory behaviourunder anxiety-inducing stimuli

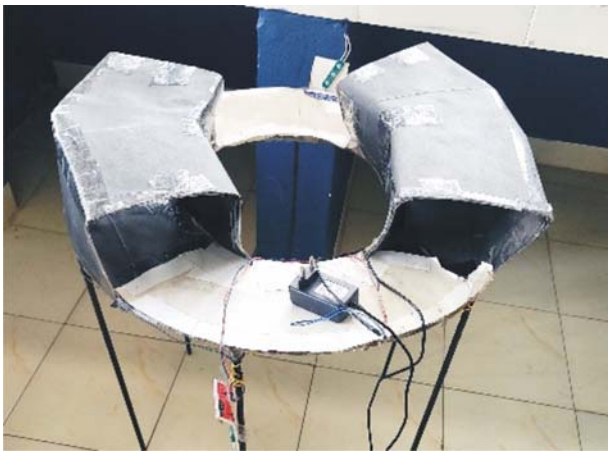

Fig. 1: Circular Maze

Fig. 2: Images of the circular maze showing the presence of an animal in open and closed areas

Table 2: Anxiolytic effect of various treatment groups for all 4 weeks.

Group	Treatment	Week	Time in Closed Area (sec)	Time in Open Area (sec)
Group 1	Control	Week 1	245 ± 2.24	55 ±6.67
		Week 2	245 ±1.78	55 ±5.43
		Week 3	244 ±1.54	56 ±1.21
		Week 4	243±1.41	57 ±3.05
Group 2	Diazepam	Week 1	243 ±2.63	57 ±1.89
		Week 2	200 ±5.42	100 ±1.11
		Week 3	170 ±6.11	130 ±0.74
		Week 4	150 ±2.49	150 ±5.21
Group 3	Test I (25:75)	Week 1	244 ±3.22	56 ±0.89
		Week 2	205 ±8.54	95 ±2.07
		Week 3	180 ±2.49	120 ±2.18
		Week 4	160±1.05	140 ±0.65
Group 4	Test II (50:50)	Week 1	243 ±0.63	57 ±0.04
		Week 2	200 ±0.12	100 ±0.19
		Week 3	175 ±0.54	125 ±1.52
		Week 4	150 ±1.32	150 ±1.80
Group 5	Test III (75:25)	Week 1	245 ±1.57	55 ±1.54
		Week 2	195 ±0.26	105 ±0.82
		Week 3	165 ±2.81	135 ±2.61
		Week 4	145 ±1.67	155 ±2.08

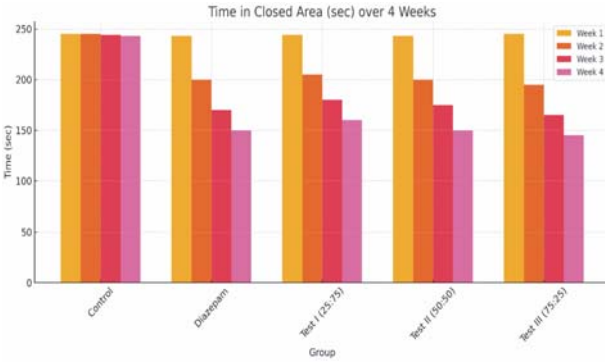

Fig. 3: Average time spent by the animal in the closed area for all treatment groups

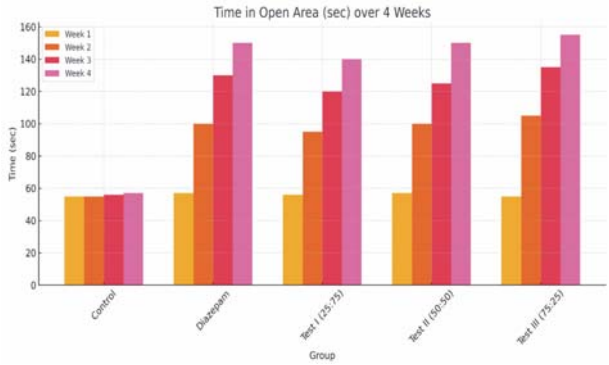

Fig. 4: Average time spent by the animal in the open area for all treatment groups

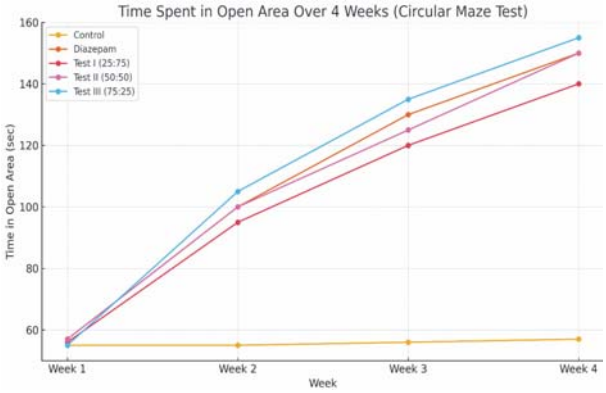

Fig. 5: Weekly analysis of average time spent in the open area for all treatment groups

The apparatus in the image is a cost-effective, innovative circular maze model for anxiety studies in rodents. It mimics a balanced conflict between fear (open areas) and safety (closed chambers). Its modular structure allows for easy customisationand enhancement, such as sensor integration, and serves as a viable tool for screening anxiolytic agents, especially herbal formulations.

Procedure

Ensure the circular maze is placed securely on a stable platform or stand (about 60 cm height) to induce height-related anxiety. Clean the maze with 70% ethanol before

each trial to remove scent cues from previous animals. Use adult albino mice (preferably 6-8 weeks old) weighing around 25-35 grams (for mice). Acclimate animals to the laboratory environment for at least 7 days before testing. Administer the drugs to all the groups as per the experimental design for 4 weeks. Evaluation parameters like time spent in closed and open areas for all four weeks were noted and analysed. Gently place a single animal at the central starting point of the circular maze (midpoint between closed and open arms). Allow the animal to freely explore the maze for 5 minutes (or 300 seconds). Ensure the environment is quiet and distraction-free during the test.

RESULTS

Control group shows minimal variation, confirming baseline behaviourwithout anxiolytic influence. Diazepam begins showing anxiolytic effects from Week 2, increasing time in open arms, confirming its standard efficacy. All three test formulations (Groups 3-5) show similar progressive behaviour, with increasing exploration of open arms, suggesting anxiolytic-like activity. Among the test groups, Test III (75:25 OS:TA) shows slightly better performance, possibly indicating a stronger synergistic effect of the higher OS concentration.

DISCUSSION

The data from the circular maze test demonstrates a clear anxiolytic effect of the test compounds when compared to the control and standard drug diazepam. In the control group (Group 1), the time spent in the closed and open areas remained consistent throughout the four weeks, indicating no reduction in anxiety levels. In contrast, diazepam-treated animals (Group 2) showed a progressive increase in time spent in the open area, from 57 seconds in Week 1 to 150 seconds in Week 4, with a corresponding decrease in time spent in the closed area. This confirms the effectiveness of diazepam as a standard anxiolytic drug. Among the test groups, all three formulations demonstrated dose-dependent anxiolytic activity. Test I (25:75) group showed a gradual increase in open area time from 56 to 140 seconds, indicating a moderate anxiolytic effect. Test II (50:50) produced an even greater effect, increasing open area time from 57 to 150 seconds by Week 4, which closely paralleled the effect of diazepam. Notably, Test III (75:25) showed the most pronounced anxiolytic effect, with open area time increasing from 55 seconds in Week 1 to 155 seconds in Week 4, slightly exceeding the response seen with diazepam. This suggests that the 75:25 ratio in Test III may offer optimal anxiolytic efficacy among the test formulations. Overall, the results support the hypothesis that the test substances possess significant anxiolytic activity, with Test III potentially being the most effective. The circular maze model successfully detected these effects, validating its use as a behaviouraltool for screening anti-anxiety agents. Further studies involving biochemical and receptor-level investigations would be valuable to elucidate the exact mechanisms underlying the observed behaviouralchanges.

CONCLUSION

The study evaluated the anti-anxiety activity of three test formulations using a circular maze model, comparing their effects with a control and the standard anxiolytic drug diazepam. The time spent in the open and closed areas over four weeks was recorded to assess behavioural changes. The control group showed no significant variation, indicating a consistent anxiety baseline. Diazepam and all test groups exhibited increased open area time and reduced closed area time, reflecting anxiolytic activity. Among the test formulations, Test III (75:25) demonstrated the most significant effect, slightly surpassing diazepam by Week 4. Test II (50:50) closely matched diazepam's efficacy, while Test I (25:75) showed moderate improvement. These findings suggest that the test substances, particularly at higher ratios, possess dose-dependent anxiolytic properties. The circular maze proved effective for behaviouralscreening, and further studies are warranted to investigate the mechanism of action and potential therapeutic application of these compounds.

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