RESEARCH PAPER

Evaluation of anxiolytic activity of pogostemon paniculatus (willd.) Benth using experimental animal models

Fareeha Qureshi[1], A. Venkateshwara Reddy[2], Praveena Chinthala[1*]

[1]Research scholar, Department of Pharmacy, Chaitanya Deemed to be University, Hanamkonda, Telangana, India

[1*]Department of Pharmacy, Chaitanya Deemed to be University, Hanamkonda, Telangana, India (for Praveena Chinthala)

[2]Anwarul Uloom College of Pharmacy, New Mallepally, Telangana, India

Corresponding author:

Praveena Chinthala,

Professor, Department of Pharmacy, Chaitanya Deemed to be University, Hanamkonda, Telangana, India.

E-mail : praveenamr18@gmail.com

Abstract

Anxiety disorders are among the most prevalent neuropsychiatric conditions and are often associated with cognitive impairment and altered behavioral responses. The present study aimed to evaluate the anxiolytic-like activity of *Pogostemon paniculatus* (PP) hydroalcoholic extract in scopolamine-induced anxiety and dementia-like conditions in rats. Fresh plant material of *Pogostemon paniculatus* was extracted using a Soxhlet apparatus with hydroalcohol, and the extract was administered orally at doses of 250 and 500 mg/kg. Male rats were divided into five groups: normal control, disease control (scopolamine 0.5 mg/kg, i.p.), standard control (diazepam 2 mg/kg, i.p.), and two treatment groups receiving PP at 250 and 500 mg/kg along with scopolamine. Anxiety-like behavior and locomotor activity were assessed using the Open Field Test (OFT) and Light–Dark Box (LDB) test. In the OFT, PP treatment significantly increased the time spent in the central zone and the number of central entries, while also modulating rearing and line-crossing behavior, indicating reduced anxiety without marked locomotor impairment. In the LDB test, PP produced a dose-dependent increase in time spent in the light compartment and the number of entries into the light box, comparable to the standard anxiolytic drug diazepam. Statistical analysis using one-way ANOVA followed by Tukey's test revealed significant differences among groups ($p < 0.05$). These findings suggest that *Pogostemon paniculatus* possesses significant anxiolytic-like activity and may serve as a potential natural therapeutic agent for the management of anxiety-related disorders, possibly through modulation of central nervous system pathways involved in anxiety.

Keywords: Anxiety, Anxiolytic activity, *Pogostemon paniculatus*, Scopolamine-induced model, Open Field Test, Light–Dark Box test, Diazepam, Rats

How to cite this article: Qureshi F, Reddy AV, Chinthala P. Evaluation of anxiolytic activity of pogostemon paniculatus (willd.) Benth using experimental animal models. Int J Drug Deliv Technol. 2026;16(11s): 404-410.

DOI: 10.25258/ijddt.16.11s.39

1. INTRODUCTION

Anxiety is a psychological condition characterized by persistent feelings of worry, fear, nervousness, or unease that may occur even without an immediate threat. It commonly affects both mental and physical health, presenting with symptoms such as restlessness, difficulty concentrating, sleep disturbances, rapid heartbeat, sweating, fatigue, and gastrointestinal discomfort. Anxiety can arise due to a combination of genetic predisposition, neurochemical imbalances involving serotonin, GABA, and norepinephrine, stressful life events, chronic illness, or environmental factors. While mild anxiety can be a normal response to stress, excessive or prolonged anxiety may interfere with daily activities, academic or occupational performance, and social interactions. Management typically involves lifestyle modifications, psychological therapies such as cognitive behavioral therapy, relaxation techniques including meditation and yoga, and pharmacological treatment, when necessary, under medical supervision [1].

While occasional anxiety is a normal and adaptive human emotion that aids in preparation and response to stress, an anxiety disorder is defined by fear or worry that is disproportionate to the situation, difficult to control, and significantly interferes with daily functioning. Anxiety disorders, which often begin in childhood or adolescence, are highly heterogeneous and include conditions such as Generalized Anxiety Disorder (GAD), Panic Disorder, Social Anxiety Disorder (SAD), and Specific Phobias. The high prevalence, chronicity, and comorbidity (often with depression) make anxiety disorders a critical public health concern, with substantial socioeconomic costs [2].

Clinical Manifestations and Classification

Anxiety disorders are classified based on the specific focus of the fear and worry. Key clinical types include:

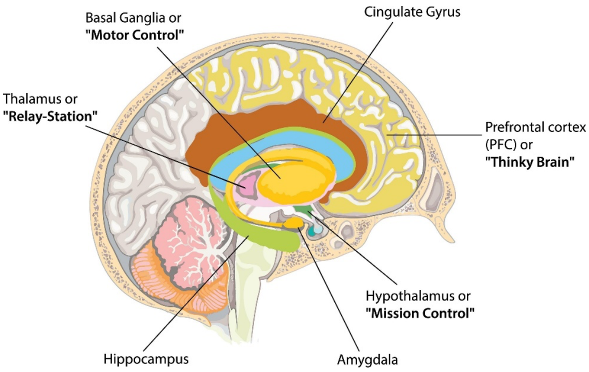
- **Generalized Anxiety Disorder (GAD):** Persistent, excessive, and unrealistic worry about everyday life events (e.g., job performance, health, finances).
- **Panic Disorder:** Recurrent, unexpected panic attacks—intense episodes of sudden fear accompanied by severe physical symptoms (e.g., palpitations, shortness of breath, chest pain, sense of impending doom).
- **Social Anxiety Disorder (SAD):** Intense fear and avoidance of social situations due to concerns about being judged, humiliated, or negatively evaluated by others.
- **Specific Phobias:** Excessive and persistent fear of a specific object, situation, or activity that is generally not harmful (e.g., fear of flying, heights, or certain animals).

Common symptoms across these disorders often involve a mix of psychological, physical, and behavioral elements:

- **Psychological/Cognitive:** Feeling restless, on edge, irritable, difficulty concentrating, uncontrollable obsessive thoughts, and a sense of impending danger or doom.
- **Physiological/Somatic:** Increased heart rate and palpitations, rapid breathing (hyperventilation), sweating, trembling, muscle tension, nausea or abdominal distress, and trouble sleeping.
- **Behavioral:** Avoidance of triggering situations or places [3-4].

Etiology and Pathophysiology

Anxiety disorders are not attributable to a single cause but result from a complex **biopsychosocial model** involving genetic, environmental, and neurobiological factors.



Dysfunction in specific neural circuits that regulate fear and emotional response is central to anxiety pathophysiology:

1. **Amygdala Hyperactivity:** The amygdala is the brain's "alarm center," central to processing fear and threat detection. In anxiety disorders, there is a heightened and exaggerated response of the amygdala to anxiety cues, contributing to excessive fear responses.
2. **Prefrontal Cortex (PFC) Dysregulation:** The PFC is responsible for executive functions, including the "top-down" cognitive control of emotional responses originating in the amygdala. Reduced or impaired inhibitory control from the PFC may lead to persistent and unregulated anxiety.
3. **Hippocampus:** This structure, involved in memory and context, may show reduced volume and activity, potentially impairing the ability to distinguish between safe and dangerous environments.

Neurotransmitter Imbalances:

- **Gamma-Aminobutyric Acid (GABA):** The primary inhibitory neurotransmitter. Dysfunction in the GABAergic system is linked to increased neuronal excitability and heightened anxiety.
- **Serotonin and Norepinephrine:** Systems involving these neurotransmitters are widely implicated in mood regulation and the "fight-or-flight" stress response, often showing dysregulation.
- **Choline:** Recent research suggests people with anxiety disorders show **lower choline levels** (an essential nutrient) in key brain regions like the prefrontal cortex, which may explain an intensified reaction to stress [5-6].

Genetic and Environmental Factors

- **Genetics:** Anxiety disorders tend to run in families, suggesting an inherited genetic vulnerability.
- **Environmental Stressors:** Traumatic events (e.g., abuse, neglect, severe loss) and chronic, ongoing stress (e.g., work stress, financial worry) can interact with genetic predisposition to trigger the onset of a disorder.
- **Medical Conditions:** Anxiety can sometimes be a symptom or consequence of underlying medical issues (e.g., thyroid conditions,

cardiovascular disease) or substance misuse/withdrawal.

Treatment and Management

Effective, evidence-based treatments exist, yet a significant portion of affected individuals remain untreated. Treatment generally involves a combination of psychotherapy and pharmacotherapy [7].

1. Psychological Interventions

- **Cognitive Behavioral Therapy (CBT):** Considered the gold standard, CBT helps individuals identify and modify maladaptive thought patterns and behaviors that maintain anxiety.
- **Exposure Therapy:** A core component of CBT, this involves creating a safe environment to gradually and systematically expose the individual to their feared objects, situations, or activities, helping them confront their fears and learn new, realistic associations. CBT has been shown to alter brain activity in children with anxiety, leading to a clinically significant drop in symptoms and improved brain functioning.
- **Stress Management and Mindfulness:** Techniques such as relaxation skills and mindfulness training are essential for reducing physical and cognitive symptoms of anxiety.

2. Pharmacotherapy

Medication is often used to provide symptom relief, particularly in conjunction with psychotherapy:

- **Antidepressants: Selective Serotonin Reuptake Inhibitors (SSRIs)** are commonly prescribed as a first-line treatment for chronic anxiety, even though they were originally developed for depression.
- **Benzodiazepines:** These are fast-acting anti-anxiety medications used for acute anxiety or short-term treatment due to the risk of dependence. They enhance the effects of GABA.
- **Beta-Blockers:** Occasionally used to manage the physical symptoms of anxiety (e.g., rapid heart rate, trembling), particularly for performance-related social anxiety.

3. Recent Therapeutic Advances

- **Novel Drug Targets:** Research continues to explore new targets, including compounds that interact with brain reward systems and immune cells in the brain (microglia), which have recently been implicated as "biological pedals" controlling anxiety levels.
- **Digital Interventions:** Technology-based CBT and remote interventions are being developed to increase access to care, with early results showing promise for widespread community-based approaches [8-9].

2. MATERIALS AND METHODS

The fresh *Pogostemon paniculatus* are collected and identified was purchased from local market in Andhra Pradesh, India, and authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, S.V University, Tirupati.

Extraction procedure

Coarsely powdered *Pogostemon paniculatus* were used for extraction with hydroalchol by using Soxhlet method for 6-8 hours. 50g of dried powder was weighed and 200mL of solvent hydroalchol is used for the extraction. The extracts were evaporated by using a rotary evaporator and dried at room temperature. The obtained crude extracts were weighed and stored at 4^{o}C for further analysis.

Experimental design:

The animals were divided into 5 groups (n = 6 in each group). Group I: Normal control received only vehicle, Group II: Disease control received scopolamine (0.5 mg/kg), i.p., Group III: standard control, received Diazepam 2 mg/kg i.p., + scopolamine 0.5 mg/kg i.p., Group IV: PP (250 mg/kg), p.o., + scopolamine (0.5 mg/kg) i.p., and Group V: PP (500 mg/kg), p.o. + scopolamine (0.5 mg/kg) i.p..

Table 1: Animal grouping and treatment

Group	Treatments
I	Control
II	Disease control (Scopolamine) - 0.5 mg/kg
III	Standard control (Diazepam) - 2 mg/kg
IV	Pogostemon paniculatus - 250 mg/kg
V	Pogostemon paniculatus - 500 mg/kg

Diazepam was used as a standard anxiolytic drug at a dose of 2 mg/kg, i.p. Scopolamine at a dose of 0.5 mg/kg, i.p., was used to produce Dementia. Drugs were dissolved in normal saline solution. The extracts were dissolved in DMSO solutions. Drugs were prepared daily before administration.

The control, standard drug, and Pogostemon paniculatus (PP) 250 and 500 mg/kg treated groups were given respective treatment once daily for 7 days, and 8 to 14 consecutive days for the Open field test (OFT) and Light-dark box test (LDB), respectively. Dementia was induced by administration of scopolamine (0.5 mg/kg; i.p.) after 45 min of drug administration for OFT, and LDB, respectively. Behavioural tests were performed 1 h after scopolamine challenge.

Behavioural assessments:

Two different models universally used for learning and memory (Anxiety) studies were done in the present study, i.e., the Open field test, and Light-dark box test in rats.

Open field test

The open-field test is a widely adopted paradigm to assess the animal's anxiety and general locomotion under the influence of test treatments, which could affect the performance of learning and memory [10]. The open areas tend to prove anxiogenic in rodents, but they simultaneously have an innate curiosity to explore these places. Before testing each animal, the open field apparatus (80 x 80 cm) was thoroughly cleaned with ethanol to avoid any lingering stimuli. On the experimental day, after one hour of treatments, each animal was individually exposed to the open field, and their behavior was examined for 5 min. The animal's anxiety was evaluated by monitoring its preference for open areas of the field. Other parameters, i.e., number of rearings and number of line crossings, were also considered and assessed via ANY-maze version 6.1, Stoelting Co. USA, to assess the influence of PP on the general locomotion of rats [11].

Light dark box testing

Testing on the light/dark test used a procedure described in the previous report [12]. Briefly, the light box apparatus consisted of two equal-sized compartments (27 cm × 23 cm × 27 cm), one light and one dark. The floors of each compartment were connected via a small opening (8.0 cm × 8.0 cm), enabling passage between the compartments. The box was elevated 70 cm above the floor and placed in indirect light (150 lx). Rats were kept in the experimental room for at least 1 h for adaptation before drug administration. Diazepam and PP were administered 30 min before the test. At the beginning of the five-minute test session, each rat was placed in the dark box for one minute. The cumulative time spent in the light box and the number of entries into the light box were then registered by monitoring the rat's movements on a monitor attached to a video camera system (LifeCam Studio Q2F00020). A light box visit was recorded when the rat moved at least half of its body into the light box. The observer was blinded to the treatment groups. After the removal of each animal, the apparatus was cleaned [13].

Statistical analysis:

Results were expressed as Mean ± SD. Statistical analysis was performed by one-way analysis of variance followed by post hoc Tukey's multiple range tests, using GraphPad Prism software version 8.0 (San Diego, CA, USA). Results were considered statistically significant when $p < 0.05$.

3. RESULTS

Effects of Pogostemon paniculatus in the Open Field Test

For behavioral tests, rats were divided into separate groups and administered orally with 250 and 500 mg/kg of PP in normal saline till the completion of the study. On the 29th day of the study, the animals were exposed to the open-field arena to evaluate the effect of PP on general locomotion and anxiety-like behavior. The one-way ANOVA revealed the significant overall inter-group variation for time spent in the centre of the field [$F (4, 10) = 28.58$, $P < 0.0001$] (Figure 3A & Table 2) and number of entries in the central zone [$F (4, 10) = 32.12$, $P < 0.0001$] (Figure 3B & Table 2). The post-hoc multiple comparison test revealed that animals of the control group preferred the central zone significantly less ($P < 0.0001$) as compared to diazepam-treated rats. Similarly, the rats treated with PP showed reduced anxiety-like behavior in a dose-dependent manner. The rats treated with 250 and 500 mg/kg of PP revealed reduced anxiety as compared to the control group, with $P < 0.05$ and $P < 0.001$, respectively. The number of line crossings (horizontal activity) and the number of rearings (vertical activity) were also monitored to assess the impact of PP on the animals' general locomotion. The animals of all treated groups significantly varied for the number of rearings [$F (4, 10) = 23.23$, $P < 0.0001$] and number of line crossings [$F (4, 10) = 53.03$, $P < 0.0001$]. The rats treated with 250 and 500 mg/kg of PP showed an increased number of rearings ($P < 0.05$) as compared to the control group (Figure 3C & Table 2). Likewise, the PP at 500 mg/kg led to increased line crossings ($P < 0.05$) as shown in Figure 3D and Table 2. However, the outcomes of all observed parameters were non-significant at the lowest used dose of PP (250 mg/kg).

Table 2: Effect of Pogostemon paniculatus treatment on scopolamine-induced dementia in behavioral study by the Open field test model in rats, showing time spent

in the centre zone, number of entries zone, number of rearing, and number of line crossing.

Group	Time spent in the center	Number of entries	Number of rearing	Number of line crossings
Control	6.00 ± 1.73	6.00 ± 2.00	7.33 ± 1.52	17.33 ± 1.52
Disease Control	2.66 ± 1.52	2.33 ± 1.15	19.00 ± 2.00	38.67 ± 2.51
Standard	18.33 ± 2.08	18.67 ± 2.51	5.33 ± 1.15	14.00 ± 2.00
PP 250 mg/kg	10.33 ± 2.51	11.33 ± 1.52	12.33 ± 2.08	24.67 ± 2.08
PP 500 mg/kg	13.67 ± 2.08	12.33 ± 2.08	9.33 ± 2.51	19.67 ± 3.05

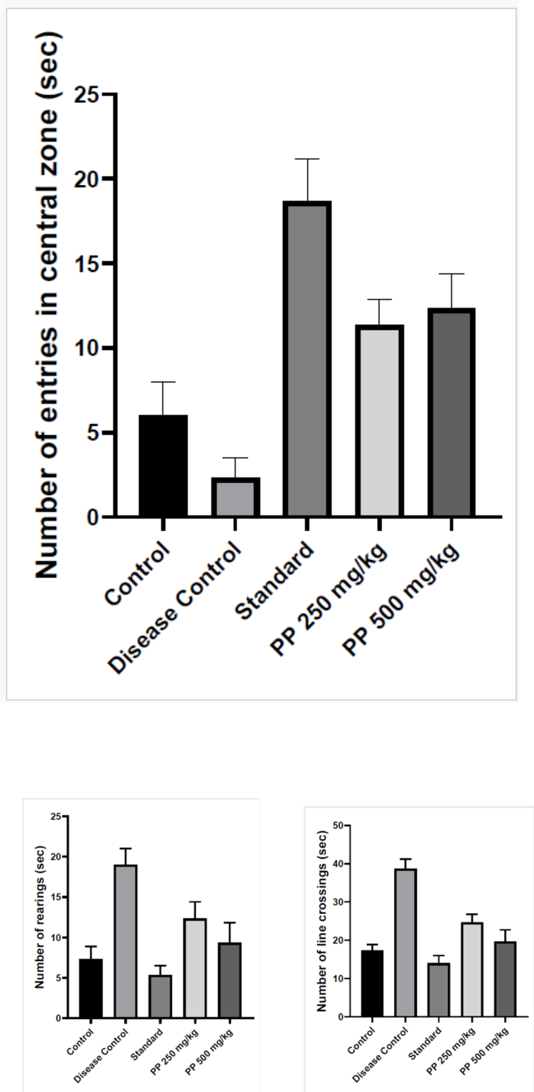


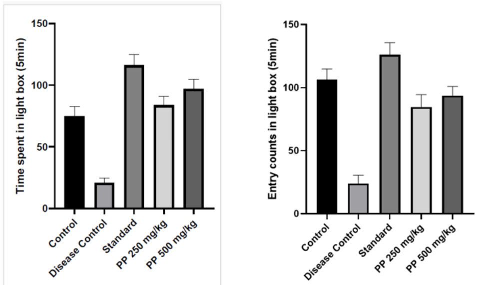
Figure 1: Evaluation of anxiolytic-like activity in rats treated with PP at doses of 250 and 500 mg/kg by the open field test. (A) The time spent in the centre zone, (B) number of centre zone entries, (C) number of rearings, and (D) number of line crossings were noted for 5 min and compared to control, in an open field test using diazepam as a positive control. Data are presented as Mean ± SD (n = 6). *** p < 0.001, **** p < 0.0001.

Effects of Pogostemon paniculatus (PP) in the light/dark test

We first examined anxiety-like behaviors and dose-related effects of PP in rats using the light/dark test. The light/dark test was used as a readily available model for detecting the anxiogenic properties of compounds. PP (250 and 500 mg/kg) significantly and dose dependently decreased the time rats spent in the light box (one-way ANOVA $F (4, 10) = 69.51$, $P < 0.0001$; Figure 2A & Table 3). PP also significantly decreased the number of entries into the light box (one-way ANOVA $F (4, 10) = 63.18$, $P < 0.0001$; Figure 2B & Table 3) when compared to the vehicle-treated group.

Table 3: Effect of Pogostemon paniculatus treatment on scopolamine-induced dementia in behavioral study by the Light-dark box test model in rats, showing time spent in the light box and entry counts in the light box.

Group	Time spent in the light box	Entry counts in the light box
Control	74.67 ± 8.02	106.3 ± 8.50
Disease Control	20.67 ± 4.04	24.00 ± 6.55
Standard	116.0 ± 9.00	126.0 ± 9.53
PP 250 mg/kg	83.67 ± 7.09	84.67 ± 9.71
PP 500 mg/kg	96.67 ± 8.02	93.67 ± 7.09



A B

Figure 2: Evaluation of anxiolytic-like activity in rats treated with PP at doses of 250 and 500 mg/kg by the light-dark box test. (A) The time spent in the light box, (B) Entry counts in the light box were noted for 5 min and compared to the control group, in a light-dark box test using diazepam as a positive control. Data are presented as Mean ± SD (n = 6). *** p < 0.001, **** p < 0.0001.

4. DISCUSSION

The present study evaluated the anxiolytic-like activity of the hydroalcoholic extract of *Pogostemon paniculatus* (PP) in scopolamine-induced anxiety and cognitive dysfunction in rats using validated behavioral models. Scopolamine is known to induce anxiety-like behavior by disrupting cholinergic neurotransmission and increasing oxidative stress in the central nervous system, thereby serving as a reliable experimental model for anxiety-associated dementia. In the current investigation, scopolamine-treated animals exhibited marked anxiety-like behavior, as evidenced by reduced exploration of the center zone in the open field test and decreased time spent in the light compartment in the light–dark box test.

Administration of PP at doses of 250 and 500 mg/kg significantly attenuated scopolamine-induced anxiety-like behavior in a dose-dependent manner. In the open field test, PP-treated rats showed a significant increase in time spent in the central zone and the number of central zone entries compared to the disease control group, indicating reduced anxiety levels. Importantly, the increase in exploratory behavior was not accompanied by excessive locomotor stimulation, suggesting that the anxiolytic effect of PP was not due to nonspecific motor activation. These findings are comparable to the effects observed with diazepam, a standard benzodiazepine anxiolytic, which further supports the anxiolytic potential of PP.

Similarly, in the light–dark box test, PP treatment significantly increased both the duration spent in the light compartment and the number of entries into the light box. Rodents naturally prefer dark environments; therefore, increased exploration of the light area is considered a robust indicator of anxiolytic activity. The observed behavioral changes following PP administration suggest a reduction in fear and avoidance behavior, reinforcing its anxiolytic-like effect. The higher dose (500 mg/kg) demonstrated a more pronounced effect, indicating a clear dose–response relationship.

The anxiolytic effects of *Pogostemon paniculatus* may be attributed to its phytochemical constituents, such as flavonoids, phenolic compounds, terpenoids, and alkaloids, which are known to exert neuroprotective, antioxidant, and GABAergic modulatory effects. Flavonoids, in particular, have been reported to interact with the benzodiazepine site of the GABAA_AA receptor, producing anxiolytic effects similar to standard anxiolytic drugs but with fewer side effects. Additionally, the antioxidant properties of PP may help counteract scopolamine-induced oxidative stress, thereby preserving neuronal function and emotional regulation.

Furthermore, the observed anxiolytic-like activity may involve modulation of multiple neurotransmitter systems, including GABAergic, serotonergic, and cholinergic pathways, which play a critical role in anxiety regulation. The ability of PP to reverse scopolamine-induced behavioral deficits suggests its potential to improve anxiety associated with cognitive impairment and neurodegenerative conditions.

5. CONCLUSION

The present study demonstrates that the hydroalcoholic extract of *Pogostemon paniculatus* exhibits significant anxiolytic-like activity in scopolamine-induced anxiety and dementia models in rats. Treatment with *Pogostemon paniculatus* at doses of 250 and 500 mg/kg effectively ameliorated anxiety-like behaviors, as evidenced by increased exploratory activity in the open field test and enhanced time spent in the light compartment and entry counts in the light–dark box test. The anxiolytic effects were dose dependent and comparable to those produced by the standard anxiolytic drug diazepam, without causing marked locomotor impairment.

The observed behavioral improvements suggest that *Pogostemon paniculatus* may exert its anxiolytic action through modulation of central neurotransmitter systems and by counteracting scopolamine-induced neurochemical and oxidative disturbances. These findings provide scientific support for the potential use of *Pogostemon paniculatus* as a natural therapeutic agent for the management of anxiety-related disorders, particularly those associated with cognitive dysfunction. However, further investigations focusing on phytochemical characterization, mechanistic pathways, and long-term safety studies are necessary to validate its clinical applicability and therapeutic efficacy.

REFERENCES

1. American Psychiatric Association. (2022). *Diagnostic and statistical manual of mental disorders* (5th ed., text rev.). Arlington, VA: American Psychiatric Publishing.
2. Baldwin, D. S., et al. (2013). Evidence-based pharmacological treatment of anxiety disorders, post-traumatic stress disorder and obsessive-compulsive disorder: A revision of the 2005 guidelines from the British Association for Psychopharmacology. *Journal of Psychopharmacology*, *27*(11), 1047–1079.
3. Charney, D. S. (2004). Neurobiological mechanisms of anxiety and fear: Implications for the treatment of anxiety disorders. *The Journal of Clinical Psychiatry*, *65*(Suppl 5), 3–14.

4. Craske, M. G., & Stein, M. B. (2016). Anxiety. *The Lancet*, *388*(10057), 3048–3059.
5. Foa, E. B., & Kozak, M. J. (1986). Emotional processing of fear: Exposure to corrective information. *Psychological Bulletin*, *99*(1), 20–35.
6. Haddad, A. D., et al. (2024). Brain choline levels linked to intensified anxiety in patients. *Nature Communications*, *15*(1), 183.
7. Hofmann, S. G., Asnaani, A., Vonk, I. J. J., Sawyer, A. T., & Fang, A. (2012). The efficacy of cognitive behavioral therapy: A review of meta-analyses. *Cognitive Therapy and Research*, *36*(5), 427–440.
8. Paulus, M. P., & Stein, M. B. (2006). An interactive neurobiological model of anxiety disorders. *The American Journal of Psychiatry*, *163*(10), 1640–1648.
9. Ressler, K. J. (2010). Genetics of anxiety and trauma-related disorders. *Biological Psychiatry*, *68*(10), 923–926.
10. Arika WM, Kibiti CM, Njagi JM, Ngugi MP. Effects of DCM Leaf Extract of Gnidia glauca (Fresen) on Locomotor Activity, Anxiety, and Exploration-Like Behaviors in High-Fat Diet-Induced Obese Rats. Behav Neurol. 2019;2019:7359235.
11. Sajjad Haider M, Ashraf W, Javaid S, Fawad Rasool M, Muhammad Abdur Rahman H, Saleem H, et al. Chemical characterization and evaluation of the neuroprotective potential of Indigofera sessiliflora through in-silico studies and behavioral tests in scopolamine-induced memory compromised rats. Saudi J Biol Sci. 2021 Aug;28(8):4384–98.
12. Saitoh A, Sugiyama A, Yamada M, Inagaki M, Oka JI, Nagase H, et al. The novel δ opioid receptor agonist KNT-127 produces distinct anxiolytic-like effects in rats without producing the adverse effects associated with benzodiazepines. Neuropharmacology. 2013 Apr;67:485–93.
13. Saitoh A, Makino Y, Hashimoto T, Yamada M, Gotoh L, Sugiyama A, et al. The voltage-gated sodium channel activator veratrine induces anxiogenic-like behaviors in rats. Behav Brain Res. 2015 Oct 1;292:316–22.