EVALUATING THE ANXIOLYTIC-LIKE PROPERTY OF METHANOL LEAF EXTRACTS OF *Ficus iteophylla* Miq. (MORACEAE) AND *Tamarindus indica* L. (FABACEAE) IN MICE.

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A DISSERTATION SUBMITTED TO THE DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DOCTOR OF PHARMACY (PHARM. D) DEGREE OF THE FACULTY OF PHARMACY, UNIVERSITY OF BENIN, BENIN CITY, NIGERIA.

NOVEMBER, 2025.

CERTIFIATION

This is to certify that this project work was successfully carried out by **Mr. Theophilus Uyiosa OGIEVA** with the matriculation number **PHA1908561** in the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Edo State, Nigeria, in partial fulfilment of the requirement for the award of the degree of doctor of pharmacy (Pharm. D) of the University of Benin, Benin City, Edo State, Nigeria.

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DEDICATION

I dedicate this work to my father, a man of faith, wisdom and joy. His love for God and family shaped the person I am today. His spirit lives on in me and in the hearts of all who loved him.

ACKNOWLEDGEMENTS

First and foremost, I express my deepest gratitude to the Almighty God for the gift of life, strength, sound health, and divine direction throughout this academic sojourn.

I gratefully acknowledge the unquantifiable contributions of my highly esteemed project supervisor, Professor Ray I. Ozolua, a rare gem and an academic father in the truest sense. Sir, your wealth of knowledge, meticulous guidance, unwavering patience, and fatherly counsel transformed this research from a mere idea into a reality. Not only did you supervise this work, you took me as one of your own children and invested in my intellectual and personal growth. I am forever grateful and sincerely pray that the same grace that has made you a blessing to many will continue to uplift you. May God bless you abundantly, sir. Amen.

My sincere appreciation also goes to the Head of Department, Dr. (Mrs.) A.P. Uchendu, for creating an enabling environment that allowed creativity to flourish. Ma, your encouragement and leadership have been instrumental in the successful completion of this project.

I remain immensely grateful to the Faculty of Pharmacy, University of Benin, for the prestigious Faculty Scholarship awarded to me for three consecutive academic sessions. This financial support did not only ease the burden on my family, it was a loud affirmation of hard work and excellence, and it also motivated me to keep raising the bar. I am proud to be a product of a Faculty that believes in and rewards merit.

I am grateful to every lecturer, academic staff, technical staff, and non-teaching staff of the Faculty of Pharmacy, University of Benin, who in diverse ways contributed to moulding us into

competent and responsible pharmacists. Your sacrifices, corrections, and words of encouragement did not go unnoticed. A special word of appreciation goes to Mrs. Emmar Elohor Obakpolor and Mr. Ibe Monday for their timely guidance and willingness to assist whenever I knocked on your door. Your support lightened the burden significantly.

To my wonderful project associates; Ogaga, Aniebiet, Mrs. Joy, and Simon, working with you all was a delight. Thank you for the teamwork, the late nights, the brainstorming sessions, and the camaraderie. May God bless and elevate each of you.

I extend my heartfelt thanks to my spiritual mentors and pillars of support, Professor E.E. Imarhiagbe and his dear wife. Thank you for checking on my well-being, offering prayers, wise counsel, and ensuring I stayed grounded in faith and focus. Your home was a refuge, and your encouragement a constant light. May God continue to bless your family.

Words cannot fully capture the depth of my gratitude to my precious family. To my ever gracious mother; Mrs. Vero Ogieva, you are my pillar, my prayer warrior, and the wind beneath my wings. You single-handedly filled every gap and showered me with love that knows no bounds. To my dear siblings; Goodluck, Morgan, Bassey, Matilda, Justice and Antonia, you are my first friends and my loudest cheerleaders. You celebrated my little wins, showed up for me in tangible ways, and reminded me who I am whenever I forgot. This degree is as much yours as it is mine. To the Omorodion family, my sincere thanks goes to each and every one of you. Special thanks to Mrs. Mercy Uhunwuosere, Mr. Eric Omorodion and Mr. Fredrick Omorodion, your generosity changed the course of this journey, I am eternally grateful for your belief in me. To my dear cousins; Mrs. Ade, Miss Modupe, Dr. Unity, Success, Kaylah, Favour, Happy, Sandis, Osazee, Osayomore and the rest of you who called, showed up for me in tangible ways, and offered unwavering support. You made me feel seen and loved. “Wahia-rú ẹ́sẹ́ kakabor”.

To my friends who have become family; Raphael, Iredia, Jeymar, Favour, Prosper, Eduwa, Dele, Efua, Eghosa, Ese, Great-Nelson, Kasim, Mildred, Naomi, Usman, Samuel, Victoria, Michelle, Winner, Siri, Fego, Ikechuckwu, Eze, Martin, Jeffery, the 560's, every members of the PCM FC, and the entire Pharmacitamols class. Your love and presence made the journey more memorable.

To my senior friends in the terrain; Dr. Moses, Mr. Zion, Dr. Nuella, Dr. Onyinye, Dr. Ikhide, Dr. Godwin, Mr. Stew, Dr. Amenze, Dr. Ohis, Dr. David, Dr. Eweka, Dr. Ebiuwa, Dr. Greg, Dr. Naomi, Dr. Agazuma, Dr. Oghenetejiri, Mr. Godwin and many others who looked out for me, shared materials, gave advice, corrected me when I was going off track, and always had my back, your kindness and mentorship meant the world. Thank you for showing me how it's done and for never letting me fall.

To my amazing junior colleagues in the ever therapeutic Faculty of Pharmacy and friends across other Faculties in the University of Benin and other schools; Minstrel, Karo, Oghosa, Oghohgo, Philip, Miracle, Anita, Esther, John, Prosper, Ose, Rejoice, Faridah, Michelle, Dominion, Dahlia, Praise, Oluchi, Hamisu, Obi, Ososeh, Success, Gloria, Princess, Osamagbe, Solace, Joan, Victory, Emery, Juddy, Benjamin, Eromosele, Olamide, Funmi, Emmanuel, Kizito, Ahmed, Precious, Samuel, Ovie, Frances, Gift, Valentina, members of Youth of Excellence UNIBEN/UBTH and every single one of you who sent voice notes, checked on me, prayed with me, or simply said "Theo, you can do this". Your good heart, and pure love reminded me every day why I must keep pushing and never give up. You are all part of this success story. I am proud of every one of you and I pray that everything you have sown in kindness will return to you in multiple folds.

Finally, I would like to extend my heartfelt gratitude to the exceptional digital platforms that transformed my research process into a seamless, efficient, and enjoyable experience;

ResearchRabbit, for effortlessly mapping and organizing literature collections and uncovering hidden connections that saved countless hours of searching. Grammarly, for meticulously proofreading my work so as to spot and eliminate grammatical errors to ensure professional clarity. Canva, for enabling the creation of stunning visuals, and presentations that elevated the overall quality of my project. These tools were indispensable allies, and this work truly bears their invaluable contributions.

To God alone, be all the glory!

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WHO – World Health Organization

NIMH – National Institute of Mental Health

APA – American Psychological Association

NHS – National Health Service

ANS – Autonomic Nervous System

IBS – Irritable Bowel Syndrome

DALYs – Disability-adjusted life years

CAMH – Centre for Addiction and Mental Health

5 HT – Serotonin

GABA – Gamma-Amino Butyric Acid

NE – Norepinephrine

DA – Dopamine

HPA – Hypothalamic-Pituitary-Adrenal axis

vmPFC – ventromedial Prefrontal Cortex

ACC – Anterior Cingulate Cortex

GAD – Generalized Anxiety Disorder

DSM-5 – Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition

OCD – Obsessive-Compulsive Disorder

PTSD – Post-Traumatic Stress Disorder

CRF - Corticotropin-Releasing Factor

IBS – Irritable Bowel Syndrome

GERD – Gastroesophageal Reflux Disease

COPD – Chronic Obstructive Pulmonary Disease

SSRIs – Selective Serotonin Re-uptake Inhibitors

ACTH – Adrenocorticotropic Hormone

NMDA – N-methyl-D-aspartate

CBT – Cognitive-Behavioral Therapy

SNRIs – Serotonin-Norepinephrine Reuptake Inhibitors

NET – Norepinephrine Transporters

SERT – Serotonin Transporter

VGCC – Voltage-Gated Calcium Channels

CNS – Central Nervous System

CBD – Cannabidiol

OFT - Open Field Test

HBT- Hole-Board Test

EPM – Elevated Plus Maze

EZM – Elevated Zero Maze

LDE – Light/Dark Test

SFC – Social Interaction Fear Conditioning

OECD – Organisation for Economic Co-operation and Development

ABSTRACT

Anxiety disorders are prevalent globally and the limitations in current treatments necessitate the exploration of ethnomedicinal plants. This study evaluated the putative anxiolytic-like potential of methanol leaf extracts of *Ficus iteophylla* (MEFI) and *Tamarindus indica* (METI) in mice, based on their traditional uses in Nigeria for neurobehavioural conditions.

Qualitative phytochemical screening and oral acute toxicity in mice were conducted on the extract. For each extract, mice were randomly allotted to groups (n=4): group 1 (negative control, given oral 1% Tween 80), groups 2, 3, 4 (extract-treated with doses of 100, 200, and 400 mg/kg p.o.), and group 5 (given 0.5 mg/kg of diazepam, i.p.). The animals were subjected to the hole-board test (HBT) and elevated plus maze (EPM). Groups of mice given 0.2 ml/day, 100, 200, and 400 mg/kg/day of the extract for 14 consecutive days. After the last dose on the 14th day, their brains were for the assay of superoxide dismutase (SOD), catalase (CAT), glutathione reductase (Gr), glutathione peroxidase (GPx) and malondialdehyde (MDA) levels.

Both extracts contained alkaloids, flavonoids, phenolic, saponins, tannins, and carbohydrates; terpenoids were present only in METI. The oral LD_{50} was estimated to be greater than 5000 mg/kg for both extract, with only transient mild sedation at high doses. In the hole-board test, both MEFI and METI significantly increased head-dip frequency at 200 mg/kg ($p < 0.05$ vs negative control), while 100 and 400 mg/kg doses showed non-significant numerical increases ($p > 0.05$); diazepam (0.5 mg/kg, i.p.) produced the greatest increase (*$p < 0.01$). In the elevated plus maze, open-arm entries and time spent in open arms showed inconsistent, non-dose-dependent changes with no significant differences from the negative control ($p > 0.05$). Antioxidant evaluations showed that both MEFI and METI modulated brain oxidative stress parameters in a dose-dependent manner. Treatment with MEFI and METI tended to increase

superoxide dismutase (SOD), catalase (CAT), glutathione reductase (Gr), and glutathione peroxidase (GPx) activities while reducing malondialdehyde (MDA) levels compared to the control group. However, these changes did not reach statistical significance ($p > 0.05$) across most doses.

MEFI and METI appear relatively safe but they failed to produce statistically significant anxiolytic-like effects in HBT and EPM models. Although the extracts showed a tendency to enhance antioxidant enzyme activities (SOD, CAT, Gr, GPx) and reduce lipid peroxidation (MDA), these effects were not statistically significant. These findings do not support the ethnomedicinal claims of anxiolytic activity for the methanol leaf extracts of *F. iteophylla* and *T. indica* in mice, suggesting that further investigation with different solvents, part of plant, or fractions may be necessary.

KEYWORDS: Anxiolytic-like activity, *Ficus iteophylla*, *Tamarindus indica*, Methanol leaf extract, Hole-board test, Elevated plus maze, Antioxidant enzymes, Phytochemical screening, Oral acute toxicity.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Overview of Anxiety

Everyone experiences moments of tension and worry. These feelings often arise before important events, during periods of uncertainty, or when facing genuine challenges. In moderation, they help individuals remain alert, plan ahead, and respond appropriately to threats. However, when this sense of unease occurs too frequently, becomes overwhelming, or manifests in situations that do not warrant it, it may indicate an underlying anxiety disorder. In such cases, the body's natural response to stress shifts from being protective to being disruptive, affecting thoughts, emotions, and physical well-being (Mayo Clinic, 2025).

Anxiety refers to a state of inner restlessness characterised by apprehension about possible future events. It is often accompanied by physical symptoms such as muscle tension, rapid heartbeat, sweating, and difficulty concentrating. According to Wikipedia (2025), it represents a combination of psychological and physiological changes that prepare the body for perceived danger. Unlike short-lived worry, anxiety tends to persist, influencing behaviour and interfering with social, occupational, and day-to-day functioning. It can range from mild uneasiness to severe fear that leads to avoidance of normal activities.

1.1.1 Standard definitions of Anxiety

Across psychological and medical literature, anxiety has been defined from multiple but overlapping perspectives.

The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) describes anxiety as an emotional state marked by excessive fear and apprehensive expectation about

future events that are difficult to control that happens more days than not for at least six months, centered on various aspects of life, such as work or school performance. This worry is accompanied by at least three of the following six symptoms, with some of these symptoms occurring on more days than not over the same six-month period; restlessness or feeling keyed up or on edge, being easily fatigued, difficulty concentrating or mind going blank, irritability, muscle tension, sleep disturbance, such as difficulty falling or staying asleep, or restless and unsatisfying sleep (Arnold, 2022).

The World Health Organization (WHO), defines anxiety disorders as characterised by excessive fear and worry, along with related behavioural disturbances and physical tension, which can significantly impair daily functioning. These reactions may be accompanied by physical signs of tension such as palpitations, sweating, trembling, and gastrointestinal discomfort, and the resulting distress interferes with daily social and occupational roles (World Health Organization, 2025).

According to the National Institute of Mental Health (NIMH, 2024), anxiety represents a heightened state of tension and unease involving worried thoughts and bodily changes such as increased heart rate, shortness of breath, and trembling. The NIMH further explains that when these experiences become frequent (occur in many situations), severe, and disproportionate to the situation, they constitute anxiety disorders that warrant professional evaluation and management.

The American Psychological Association (APA, 2018) defines anxiety as a state of apprehension or dread arising from the anticipation of danger or discomfort, often accompanied by increased alertness and physical tension. In contrast to fear, which usually results from an immediate external threat, anxiety stems from internalized or imagined concerns that persist and disrupt normal functioning.

Similarly, the National Health Service (NHS, 2024) describes anxiety as a feeling of unease, worry, or fear that may range from mild to severe. It notes that while temporary anxiety is a normal response to stress, persistent or exaggerated anxiety can become debilitating, affecting sleep, concentration, and overall quality of life.

Collectively, these authoritative perspectives present anxiety as both a natural human emotion and, when exaggerated or prolonged, a clinical condition. Despite variations in emphasis, all definitions converge on key features (excessive worry, physical arousal, and impairment of everyday functioning), underscoring anxiety's multidimensional nature and its relevance to both psychological and physiological health.

1.1.2 Historical perspective and development of Anxiety disorders

The understanding of anxiety has evolved over thousands of years, reflecting humanity's shifting view of the mind, the body, and the forces that influence emotional life.

In ancient civilizations, experiences resembling anxiety were often explained through spiritual or supernatural frameworks. In Mesopotamian and Egyptian medicine, emotional disturbances such as fear and restlessness were thought to arise from demonic possession or displeasure of the gods, and healing often involved rituals, incantations, or herbal remedies (Jackson, 1986). Similarly, ancient Greek physicians such as Hippocrates (460-370 BC) described anxiety-like symptoms as manifestations of bodily imbalance, especially involving the humors. He believed that disturbances in the balance of blood, black bile, yellow bile, and phlegm could lead to emotional agitation and melancholia (Rosen, 1968). This biological framing represented one of the earliest attempts to link mental distress to physiological causes rather than spiritual forces.

By the middle ages, anxiety and related emotional disorders were often viewed through a moral or religious lens. Emotional suffering was sometimes attributed to sin, moral weakness, or demonic influence. Despite this, traditional medicine in Europe and the Arab world continued to use herbal preparations such as valerian, chamomile, and passionflower to relieve nervous agitation, marking some of the earliest practical approaches to anxiety relief (Salim, 2014).

During the enlightenment period (17th-18th centuries), the rise of rationalism and empiricism encouraged physicians to study the mind scientifically. Emotional disturbances began to be recognized as natural human phenomena rather than signs of moral failing. By the 19th century, thinkers such as Robert Burton (in The Anatomy of Melancholy, 1621) and George Beard (who coined the term neurasthenia in 1869) described anxiety and nervous exhaustion as consequences of the pressures of modern civilization (Shorter, 1997). These early writings reflected the growing realization that emotional distress could stem from social, economic, and environmental change.

The late 19th and early 20th centuries marked a turning point with the emergence of psychology and psychiatry as scientific disciplines. Sigmund Freud (1856-1939) was among the first to conceptualize anxiety as a psychological conflict rather than a purely physical phenomenon. In his 1926 work Inhibitions, Symptoms and Anxiety, Freud described anxiety as a signal of internal tension arising from unresolved unconscious conflicts between instinctual drives and societal expectations (Freud, 1926). This psychodynamic perspective profoundly shaped early psychiatry and introduced the idea that anxiety could serve a protective or adaptive function.

As the 20th century progressed, new schools of thought emerged.

The behaviorists of the 1930s and 1940s, notably John B. Watson and B.F. Skinner, viewed anxiety as a learned response to environmental stimuli, shaped by conditioning and reinforcement (Watson and Rayner, 1920).

Later, cognitive theorists such as Aaron Beck in the 1960s and 1970s emphasized maladaptive thought patterns as central to the development of anxiety, leading to the birth of cognitive-behavioral therapy (CBT) (Beck, 1976).

These approaches shifted focus from unconscious conflict to conscious cognition, laying the groundwork for modern psychological treatments.

In the same period, advances in neuroscience and psychopharmacology transformed how anxiety was managed clinically.

Today, anxiety is understood as a multifactorial condition influenced by genetics, neurobiology, environment, and psychology. Research has identified key neurotransmitters such as gamma-aminobutyric acid (GABA), serotonin, and norepinephrine in its regulation. Modern theories integrate biological vulnerability with life stressors to explain how anxiety disorders develop and persist. This biopsychosocial understanding recognizes anxiety not as a weakness, but as a common, deeply human experience that becomes disordered when the mind's adaptive alarm system becomes overactive (Bandelow *et al*., 2017; Stein and Sareen, 2015).

1.1.3 Symptoms of Anxiety disorders

Symptoms of anxiety may manifest across multiple domains. (Wikipedia, 2025):

A. Cognitive

B. Emotional

C. Physiological

D. Behavioural

A. **Cognitive:** On the cognitive side, individuals often experience persistent and uncontrollable worry, racing thoughts, or a constant sense of dread about future events. Concentration becomes difficult, and decision-making may feel overwhelming due to intrusive, fear-based thinking.

B. **Emotional:** The emotional experience of anxiety typically includes feelings of tension, irritability, and uneasiness. People may report feeling on-edge or easily startled, even in non-threatening environments. This emotional state is frequently accompanied by a sense of detachment or unreality, known as de-realization or depersonalization, especially during intense anxiety episodes.

C. **Physiological:** Anxiety activates the autonomic nervous system (ANS), leading to a wide array of bodily sensations:

    i. **Neurological:** Symptoms can include headaches, tingling sensations (paresthesia), involuntary muscle twitches (fasciculation), dizziness, or a sensation of near fainting (presyncope).

    ii. **Digestive:** Individuals may experience abdominal discomfort, nausea, diarrhea, indigestion, dryness of the mouth, or a feeling of a lump in the throat (globus). Stress-related hormonal changes can affect bowel motility, leading to or aggravating symptoms similar to irritable bowel syndrome (IBS).

    iii. **Respiratory:** Breathing difficulties such as shortness of breath or excessive sighing are common during heightened anxiety states.

iv. **Cardiac:** Palpitations, rapid heartbeat (tachycardia), and chest tightness or pain are frequent cardiovascular responses to anxiety.

v. **Muscular:** Physical tension can manifest as fatigue, trembling, or involuntary muscle contractions (tetany).

vi. **Cutaneous:** Sweating and itchy skin may occur due to increased sympathetic activity.

vii. **Urogenital:** Frequent urination, a sudden urge to urinate, and painful intercourse (dyspareunia) can also appear as part of anxiety's physiological profile.

1.1.4 Classification of Anxiety disorders

Anxiety disorders, unlike other medical conditions such as diabetes or hypertension which typically present as discrete diagnostic entities with clear physiological markers, represent a spectrum of mental health conditions in which excessive, persistent worry, fear or nervousness overwhelm an individual's ability to function in daily life. Rather than being a fleeting reaction to a particular stressor, these disorders often persist without a clear trigger or continue long after the original challenge has passed.

The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) groups the following under anxiety disorders (APA, 2013; PsychCentral, 2021):

1. **Generalized anxiety disorder (GAD):** People with GAD experience ongoing and uncontrollable worry about everyday issues such as their health, finances, work, or relationships. The worry feels difficult to stop and often interferes with daily life.
2. **Panic disorder:** This involves sudden, repeated episodes of intense fear known as panic attacks. During these attacks, a person may feel chest pain, a racing heart, shortness of breath, or a sense of losing control.

3. **Agoraphobia:** This involves intense fear or avoidance of situations where escape might be difficult or help unavailable.
4. **Specific phobia:** This refers to marked fear of a particular object or situation (e.g., animals, heights).
5. **Social anxiety disorder (formerly social phobia):** This disorder centers on fear of negative evaluation or embarrassment in social interactions. This can make speaking, eating, or performing in public very distressing.
6. **Separation anxiety disorder:** This disorder causes intense distress when someone is apart from loved ones or safe environments. While common in children, adults can also experience it deeply and persistently.
7. **Selective mutism:** This is a condition occurs when a child who can speak normally remains silent in specific settings, like school or public places. They may talk freely at home but find it difficult or impossible to speak elsewhere. This pattern often disrupts learning, social interaction, and daily functioning.
8. **Substance-induced anxiety disorder:** Substance-induced anxiety disorder happens when drugs (e.g., corticosteroids, theophylline) or toxic substances trigger feelings of intense fear or panic. It can also occur during withdrawal after stopping certain substances
9. **Other specified anxiety or fear-related disorders:** Some anxiety problems do not fit exactly into a single category. Even so, they can still cause significant distress and interfere with daily living.

The DSM-5 also made key changes, e.g., panic disorder and agoraphobia are now distinct diagnoses rather than linked; separation anxiety disorder and selective mutism are included under

anxiety disorders for both children and adults; and obsessive-compulsive disorder (OCD) and post-traumatic stress disorder (PTSD) were removed from the anxiety disorders category.

The ICD-11 similarly recognises anxiety and fear-related disorders as a unified grouping, defined by excessive anxiety or fear and associated behavioural disturbances, leading to distress or impairment in personal, social, educational, occupational or other key areas of life.

Under ICD-11, traditional core diagnoses remain, such as GAD, specific phobia, panic disorder and social anxiety disorder, with some structural changes to improve clinical utility. For example, the earlier ICD-10 distinction between phobic anxiety disorders and other anxiety disorders was removed, favouring classification by focus of apprehension (i.e., what the person fears).

1.1.5 Health consequences of anxiety disorders

When anxiety shifts from an occasional, manageable reaction to a persistent disorder, the impact extends far beyond worries or sleepless nights. The body begins to bear the burden of constant alarm-mode activation, and in doing so, anxiety disorders can exact a wide range of health consequences, ranging from cardiovascular risk to digestive dysfunction, immune-system compromise, and musculoskeletal strain.

A. **Cardiovascular and autonomic effects:** Persistent anxiety keeps the sympathetic nervous system overactive, resulting in sustained elevation of stress hormones such as adrenaline and cortisol. This chronic physiological arousal promotes hypertension, endothelial dysfunction, and systemic inflammation, thereby significantly increasing the long-term risk of cardiovascular disease, coronary events, and cardiovascular-related hospitalization and mortality (Edmondson *et al*., 2013).

B. **Digestive and gastrointestinal consequences:** Anxiety profoundly impacts gastrointestinal function via the bidirectional brain-gut axis, where stress-induced release of corticotropin-releasing factor (CRF) and other hormones disrupts enteric nervous system signaling, alters gut motility, and impairs digestive enzyme release. This results in common symptoms like abdominal pain, nausea, diarrhea, constipation, and dyspepsia. Chronic anxiety is strongly associated with functional disorders such as irritable bowel syndrome (IBS) and gastroesophageal reflux disease (GERD), as persistent autonomic hyperactivity heightens visceral hypersensitivity and exacerbates mucosal inflammation (Konturek *et al*., 2011).

C. **Immune and endocrine implications:** While acute stress can transiently bolster immune surveillance through adaptive neuroendocrine signaling, chronic anxiety dysregulates the hypothalamic-pituitary-adrenal (HPA) axis, leading to sustained glucocorticoid excess that impairs immune cell proliferation, cytokine balance, and T-cell function, thereby fostering systemic inflammation and immunosuppression. Prolonged cortisol elevation compromises innate and adaptive immunity, heightening vulnerability to opportunistic infections, delaying tissue repair processes like wound healing, and predisposing individuals to metabolic derangements such as insulin resistance and visceral adiposity. Moreover, this chronic activation promotes pro-inflammatory cascades that may trigger autoimmune dysregulation and exacerbate risks for endocrine-metabolic disorders, including type 2 diabetes and thyroid autoimmunity (Chrousos, 2009).

D. **Respiratory and pulmonary effects:** Anxiety frequently disrupts breathing patterns, with individuals often experiencing shortness of breath, rapid or shallow breathing, and hyperventilation during episodes of worry or panic, which can exacerbate preexisting

respiratory conditions such as asthma or chronic obstructive pulmonary disease (COPD) (Meuret and Ritz, 2010).

E. **Musculoskeletal and neurological manifestations:** Muscle tension is one of the hallmark physical signs of anxiety. Chronic stress can lead to stiffness in the neck, shoulders, and back, as well as tension headaches and migraines (Advanced Psychiatry Associates, 2025). Continuous contraction of muscle fibers may also cause fatigue, tremors, and general weakness.

F. **Sleep and fatigue:** Anxiety disorders often cause difficulty falling or staying asleep due to persistent rumination or hypervigilance. Sleep deprivation then worsens irritability, concentration, and emotional regulation, perpetuating a vicious cycle of anxiety and exhaustion (McLeod, 2024). Poor sleep quality also contributes to metabolic dysregulation and increased cardiovascular risk.

1.1.6 Socioeconomic consequences of anxiety disorders

Anxiety disorders do not only affect emotional and physical wellbeing but also carry far-reaching social and economic costs that extend into homes, workplaces, and entire communities at large. Globally, they are among the most common mental health conditions, leading to significant disability and loss of productivity. Persistent anxiety can disrupt education, employment, and social relationships, while increasing the burden on healthcare systems and national economies.

From an educational standpoint, anxiety often begins early in life and interferes with learning and participation. Students with chronic anxiety experience difficulty concentrating, fear of public speaking, and test-related worry, which can result in poor academic performance or school avoidance (Bandelow *et al*., 2017). These early disruptions frequently continue into

adulthood, manifesting as reduced confidence and performance in the workplace. Many affected individuals struggle with decision-making, social interaction, and handling occupational stress, leading to decreased productivity, absenteeism, or even job loss. According to a World Health Organization fact sheet (2024), anxiety and depressive disorders are among the leading causes of work-related disability worldwide, contributing to an estimated $1 trillion in lost productivity each year (World Health Organization, 2024).

Financial strain is another critical dimension of the socioeconomic burden. Individuals with anxiety disorders incur significantly higher direct medical expenditures (approximately $1,658 more per person annually) due to increased healthcare utilization; including frequent hospital visits, medications, and inpatient care, often related to physical symptoms such as fatigue, chest pain, or dizziness (Shirneshan *et al*., 2013). In low- and middle-income countries such as Nigeria, these challenges are exacerbated by a considerable unmet need for mental health services (Gureje *et al*., 2006), a scarcity of mental health specialists, and inadequate community-level and primary care integration (Chu *et al*., 2022). The result is a cycle where financial hardship heightens anxiety, while untreated anxiety perpetuates economic vulnerability.

Socially, anxiety disorders can lead to withdrawal and isolation, disrupting interpersonal relationships and family life. The fear of embarrassment or criticism often discourages participation in community, cultural, or professional activities, reducing opportunities for social support and growth (National Institute of Mental Health, 2024). Within families, anxiety can strain relationships, impair parenting quality, and contribute to emotional fatigue among caregivers. These effects reverberate beyond the individual, impacting household stability and overall social cohesion. In societies where collective identity and cooperation are central, such

as many African communities, social withdrawal due to anxiety can weaken community engagement and productivity.

The global prevalence of anxiety means that healthcare systems face growing pressure to provide both psychological and medical care, even in contexts with limited resources.

Addressing anxiety is therefore not just a clinical priority, it is a public health and economic imperative, vital for strengthening both individual wellbeing and societal progress (Chisholm *et al*., 2016).

1.1.7 Epidemiology

Anxiety disorders are remarkably common, with research indicating that nearly 1 out of every 3 people is likely to experience such a condition at least once in their lifetime (Xiong, 2023).

The World Health Organization estimated that in 2019, about 301 million people were living with anxiety disorders globally, accounting for around 4.4% of the world's population (World Health Organization, 2025). Between 1990 and 2019, the absolute number of people with anxiety disorders rose from 194.92 million to 301.39 million, largely due to global population growth and demographic ageing (Yang *et al*., 2021). While the age-standardized prevalence has remained relatively stable, the burden in terms of disability-adjusted life years (DALYs) has grown from 18.66 million in 1990 to 28.68 million in 2019, highlighting anxiety disorders as a continuing public health priority (Yang *et al*., 2021). The COVID-19 pandemic has had a profound effect on anxiety prevalence. World Health Organization reported a 25% increase in the global prevalence of anxiety and depressive disorders in the first year of the pandemic (World Health Organization, 2022). This surge has been attributed to social isolation, fear of infection, economic uncertainty and disruption of mental health services. Pandemic-related

anxiety was particularly marked among young people and women, populations already at higher baseline risk. Women consistently show higher rates of anxiety disorders than men. Pooled global estimates indicate that women are approximately 1.66 times more likely to experience anxiety disorders (Javaid *et al*., 2023). Generally, anxiety often begins in adolescence or early adulthood. Community studies report prevalence rates as high as 8.3% in adolescents, underscoring its importance as an early-onset disorder (Merikangas *et al*., 2010). There is notable geographical variation, estimates are higher in high-income regions such as North America and Western Europe, often around 7-15.5%, compared with Africa and South-East Asia, where estimates range between 3.5-8.1% (Baxter *et al*., 2013). Differences likely reflect both real variation and disparities in diagnostic capacity and reporting.

In Nigeria, community surveys yield lower prevalence estimates in adult. For example, Gureje *et al*. (2006) report lifetime and 12-month prevalence rates of 5.7% and 4.1% respectively for anxiety disorders among Yoruba-speaking adults. However, studies among adolescents show much higher prevalence: Adewuya *et al*. (2007) found 15.0% 12-month prevalence among secondary school students (13-18 years old), with females affected more than males. During the COVID-19 lockdown, symptom surveys showed very high proportions of Nigerians reporting anxiety symptoms (Falade *et al*., 2022), though many of these were not clinically diagnosed disorders.

1.2 Pathophysiology of Anxiety disorders

Research indicates that there is no single explanation for why some individuals develop an anxiety disorder; instead, a variety of influences appear to play a role [Centre for Addiction and Mental Health (CAMH), 2024]. These include psychological predispositions, biological vulnerabilities, social context, and challenging life events. Examples of possible contributing

factors are: experiencing stressful or traumatic life events, having a family history of anxiety disorders, developmental issues in childhood, misuse of alcohol, medications or illegal substances, and having other medical or psychiatric conditions (CAMH, 2024).

The pathophysiology of anxiety disorders is complicated and multifactorial as it is influenced by the combination of biological, psychological, and social factors. The developments in neurobiology, neuroimaging and molecular psychiatry have assisted to understand how pathological anxiety can be caused by abnormalities in neural circuits, neurotransmitter systems and stress-related pathways. Modern research indicates that the key brain systems that are involved in processing fear, emotion, and stress responses become dysregulated; specifically the amygdala, hippocampus, and the prefrontal cortex (Hu and Stamoulis, 2024). Neuroimaging of the effects of these changes has been supported by some studies that indicate that the amygdala of patients with generalized anxiety and panic disorders is more reactive and the prefrontal inhibition is impaired. Neurochemically, the unbalance among various systems of neurotransmitters, which include serotonin (5 HT), gamma-aminobutyric acid (GABA), norepinephrine (NE) and dopamine (DA), plays a core role in the development and perpetuation of symptoms of anxiety. Alterations in GABAergic neurotransmission have been implicated in anxiety-related arousal and fear responses. Research by Tseilikman and his colleagues (2024), suggests that elevated serotonin levels, along with increased expression of serotonin transporter and 5-HT_{3A} receptors, contribute to anxiety-related behaviors, while dysregulation of noradrenergic activity is also involved in anxiety pathophysiology.

Along with neurotransmitter imbalance, there is also the prediction of neuroendocrine dysregulation, in particular, the hypothalamic-pituitary-adrenal (HPA) axis. Persistent activation

of the HPA axis leads to persistent discharge of cortisol that, in turn, changes the plasticity of neurons and may predispose the brain to the stressor in the future (Merkouris *et al*., 2025).

Recent studies have also highlighted the recent functions of oxidative stress and neuro-inflammation in developing anxiety. Psychological stress over the long term has been indicated to cause oxidative stress injury, disrupt mitochondrial activity, and promote neuro-inflammatory responses, which enhance anxiety-connected actions (Baghaei *et al*., 2023). It was proved by animal models that the antioxidant agents like resveratrol (3,5,4-trihydroxy-trans-stillbene) may alleviate anxiety-like symptoms with the help of oxidative stress reduction and balance in neurotransmitters (Tseilikman *et al*., 2024; Baghaei *et al*., 2023). Likewise, the neuro-inflammatory changes that are characterized by a rise in pro-inflammatory cytokines and microglial activity have been associated with the changes in the prefrontal and limbic structures, which is a biological proof of the relationship between the dysregulation of the immune system and anxiety disorders (Won and Kim, 2020).

1.2.1 Neuro-circuitry of anxiety

The core neural network implicated in anxiety (Figure 1.1) involves the amygdala, hippocampus, and prefrontal cortex (PFC).

A. **Amygdala:** The amygdala is central to fear perception and emotional salience in the brain. The amygdaloid complex also known as the amygdala consists of several neuronal cell bodies clumped together (nucleus), there are numerous nuclei within the amygdala, however, hyperactivity of the basal, lateral and central nuclei of the amygdala has been consistently associated with anxiety disorders (Etkin and Wager, 2007). This hyperactivity is due to an imbalance between the stimulatory inputs from the thalamus and sensory cortices and the inhibitory inputs from the hippocampus and prefrontal cortex, resulting in

stress-induced analgesia, freezing behaviors and facial expression of fear (via stimulation the trigeminal and facial nerve).

B. **Prefrontal cortex:** In contrast, the ventromedial prefrontal cortex (vmPFC) and anterior cingulate cortex (ACC) exert top-down inhibitory control over the amygdala. Functional imaging studies show reduced prefrontal cortex regulation in patients with generalized anxiety disorder (GAD) and social anxiety disorder (Etkin and Wager, 2007).

C. **Hippocampus:** The hippocampus is involved in contextual memory of fear and stress regulation. Reduced hippocampal volume and altered connectivity with the amygdala are common in chronic anxiety (Shin and Liberzon, 2010).

D. **Bed nucleus of the stria terminalis (BNST):** Emerging evidence suggests the BNST sustains anticipatory anxiety, complementing the amygdala's role in acute fear (Avery *et al.*, 2016; Lebow and Chen, 2016).

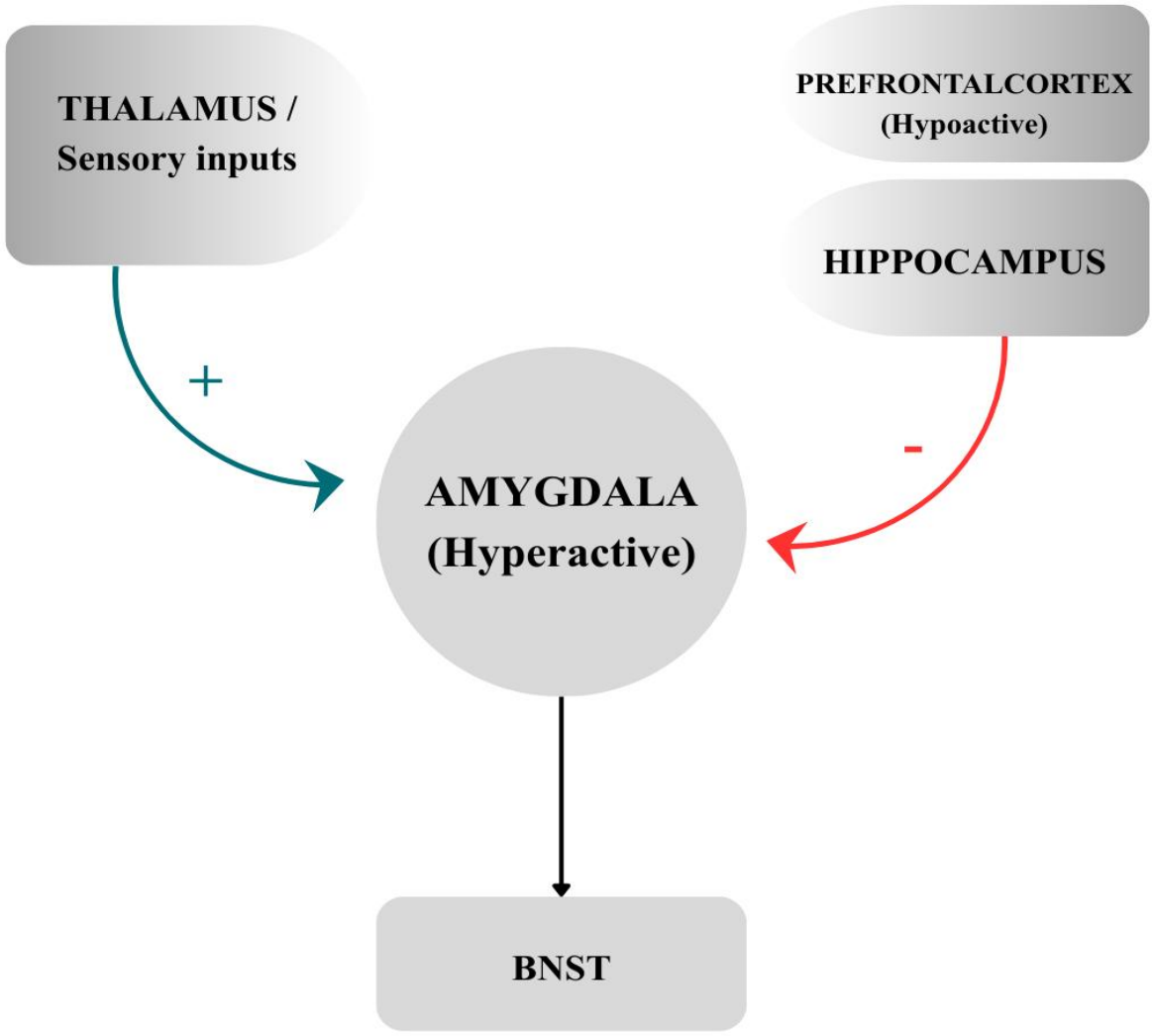

Figure 1.1: Schematic diagram that shows the neuro-circuitry of Anxiety Disorders. Showing the amygdala (hyperactive), prefrontal cortex (hypoactive), hippocampus, and BNST with their dysfunctional connections. This sketch was designed using Canva.

1.2.2 Neurotransmitter systems

A. **GABAergic system:** GABA is the major inhibitory neurotransmitter in the brain. Anxiety disorders are characterized by decreased GABAergic function, leading to hyper-excitability. Benzodiazepines, which enhance $GABA_A$ receptor activity, provide rapid anxiolytic effects and validate this pathway (Rudolph and Knoflach, 2011).

B. **Serotonergic system:** 5-HT modulates mood, fear, and stress responses. Polymorphisms in the serotonin transporter gene (5-HTTLPR) and altered 5-HT_{1A} receptor binding have been linked to heightened anxiety traits (Hariri and Holmes, 2006). Selective serotonin re-uptake inhibitors (SSRIs) remain first-line pharmacotherapy, further supporting serotonin's role.

C. **Noradrenergic system:** The locus coeruleus (blue spot), which releases NE, is hyper-responsive in anxiety. NE contributes to hypervigilance, autonomic arousal, and fear conditioning. The effect of central NE in the brain is paradoxical, i.e., it has both anxiogenic and anxiolytic effect depending on certain key factors (Goddard *et al.*, 2010).

D. **Dopaminergic and glutamatergic systems:** Dopaminergic and glutamatergic systems are the basic elements of the neurobiological basis of anxiety, which regulate motivation, affective state, and adaptation to stress. The DA neurotransmission particularly within the mesolimbic circuit which connects the ventral tegmental area (VTA) and the nucleus accumbens plays a central role in mediating the reward, salience and avoidance-related behavior. The interferences with such circuitry have been linked to increased fear learning, reduced motivations, and strong avoidance behavior, which are common features in anxiety disorders (Brandão *et al*., 2015). The disturbed dopaminergic signal is postulated to be the

cause of exaggerated threat-perception and impaired reinforcement learning that are the features of generalized and social anxiety disorders. At the same time, the glutamatergic system, which is the main network of excitatory neurotransmitters in the brain, has been pointed out as one of the core components to the pathophysiology of anxiety disorders. It is important to the fear conditioning process, extinction learning, synaptic plasticity, and affective processing through glutamatergic transmission through the N-methyl-D-aspartate (NMDA) receptors. It has been demonstrated that excessive activation of the NMDA receptors, which result in excitotoxicity in the circuit of the main performance structures (the amygdala, hippocampus, and medial prefrontal cortex) promotes the development of anxiety (Cortese and Phan, 2005). Early experimental research indicates that glutamatergic imbalances promote hyperexcitability within these pathways hence developing chronic anxious behavior (Masneuf *et al*., 2014).

1.2.3 Neuroendocrine and stress systems

The hypothalamic-pituitary-adrenal (HPA) axis mediates stress responses. Stress activates corticotrophin-releasing hormone (CRH) in the hypothalamus, stimulating adrenocorticotropic hormone (ACTH) release and subsequent cortisol secretion from adrenal glands. In anxiety disorders, hyperactivity of the HPA axis and impaired negative feedback are frequently observed (McEwen *et al*., 2016). Elevated cortisol disrupts hippocampal neurogenesis and potentiates amygdala activity, thereby perpetuating anxiety symptoms.

1.2.4 Genetic and epigenetic influences

A closer examination of anxiety disorders reveals that it has some familial and genetic element. According to the Centre of Addiction and Mental Health (CAMH), the research confirms that genetics has a say in the development of anxiety disorders. People tend to acquire an anxiety

disorder when they have a family member with such a disorder (CAMH, 2024). Moreover, the systematic reviews of the twin studies present estimates of heritability of different anxiety disorders making about ~30 to 50% (Shimada-Sugimoto *et al*., 2015). Practically this implies that the presence of a first degree relative (a parent or a sibling) with anxiety disorder highly risks an individual who has such a history in comparison with an individual without this medical history- although the genes cannot solely dictate the result. Gene-environment interactions explain why not all individuals with risk alleles develop anxiety, in other words genetics may load the gun but the environment pulls the trigger.

1.2.5 Oxidative stress and neuro-inflammation

Oxidative stress arises from an imbalance between the production of reactive oxygen species (ROS) and the capacity of endogenous antioxidant defense systems to neutralize them. This imbalance results in oxidative damage to lipids, proteins, and nucleic acids, processes that critically impair neuronal function and survival (Halliwell & Gutteridge, 2015). Recent findings link oxidative stress, i.e., the imbalance between reactive oxygen species (ROS) and antioxidant defenses, with anxiety disorders. Elevated levels of malondialdehyde (MDA), a lipid peroxidation marker, and reduced antioxidant enzymes [superoxide dismutase (SOD), catalase, glutathione peroxidase] have been detected in patients with GAD and panic disorder (Bouayed *et al*., 2009; Krolow *et al*., 2014).

The role of oxidative stress in anxiety is further underscored by studies demonstrating that antioxidant therapies attenuate anxiety-like behaviour in animal models. For example, administration of natural antioxidants such as flavonoids or phenolic acids reduces oxidative damage and restores behavioral stability in rodents subjected to stress paradigms (Bouayed *et al*., 2009). Similarly, pharmacological antioxidants have been reported to mitigate anxiety-like

behaviors by normalizing oxidative markers and improving neuronal resilience (Tseilikman *et al*., 2024; Baghaei *et al*., 2023). These findings are suggestive that the modulation of oxidative stress pathways represents a valid therapeutic strategy in anxiety management.

1.3 Drug Management of Anxiety Disorders

Pharmacological management of anxiety disorders aims to reduce excessive fear and worry, restore functional capacity, and improve overall quality of life of patients, while minimizing long term risks. Although psychotherapy (especially cognitive-behavioral therapy, CBT) is effective and often first-line, medications are widely used either alone or in combination, especially where symptoms are severe, persistent, or disabling (Bandelow *et al*., 2017). The role of self-care (e.g., deep breathing and relaxation techniques) in the management of anxiety disorders should not be undermined. Table 1.1 gives a brief overview of common pharmacotherapies for anxiety disorders, their mechanism of action and limitations.

1.3.1 First-line agents

These agents are generally recommended as initial therapy based on their proven efficacy, favorable safety profiles, and tolerability, either alone or alongside psychotherapy, with the goal of achieving symptom relief while minimizing adverse effects and dependency risks.

A. **Selective Serotonin Reuptake Inhibitors (SSRIs):** Example include fluoxetine, sertraline, escitalopram, paroxetine.

   Under normal physiology, after serotonin (5-hydroxytryptamine, 5-HT) is released into the synaptic cleft, it is rapidly taken back into the presynaptic terminal by serotonin transporter (SERT) for recycling. SSRIs (Figure 1.2) binds to and inhibit the serotonin transporter (SERT), thereby preventing presynaptic reuptake of serotonin and increasing synaptic 5-HT concentrations and prolonged activation of postsynaptic 5-HT receptors. Chronic

administration (2-6 weeks) leads to adaptive down-regulation of presynaptic auto-receptors and enhanced postsynaptic serotonergic neurotransmission, which accounts for their delayed but sustained anxiolytic and antidepressant effects (Katzung *et al.,* 2012). Early worsening of anxiety symptoms is common before improvement sets in; thus, adherence can be poor in real-world settings, especially in resource-limited contexts where patients expect rapid relief. Clinically, they are widely prescribed for GAD, panic disorder, social anxiety disorder. Patients on these medications may experience sexual dysfunction, weight gain, GI upset, sleep disturbances.

B. **Serotonin-Norepinephrine Reuptake Inhibitors (SNRIs):** They include venlafaxine, duloxetine.

SNRIs (Figure 1.3) inhibit both the serotonin (SERT) and norepinephrine (NET) transporters, thereby preventing presynaptic reuptake and increasing synaptic concentrations of 5-HT and NE. This dual mechanism enhances serotonergic and noradrenergic neurotransmission, accounting for their efficacy in generalized anxiety disorder and comorbid depression. As with SSRIs, therapeutic benefit requires adaptive receptor changes over several weeks (Katzung *et al.*, 2012). These agents are effective in both anxiety and comorbid depression, with evidence of superior efficacy in GAD compared to placebo (Strawn *et al*., 2018).

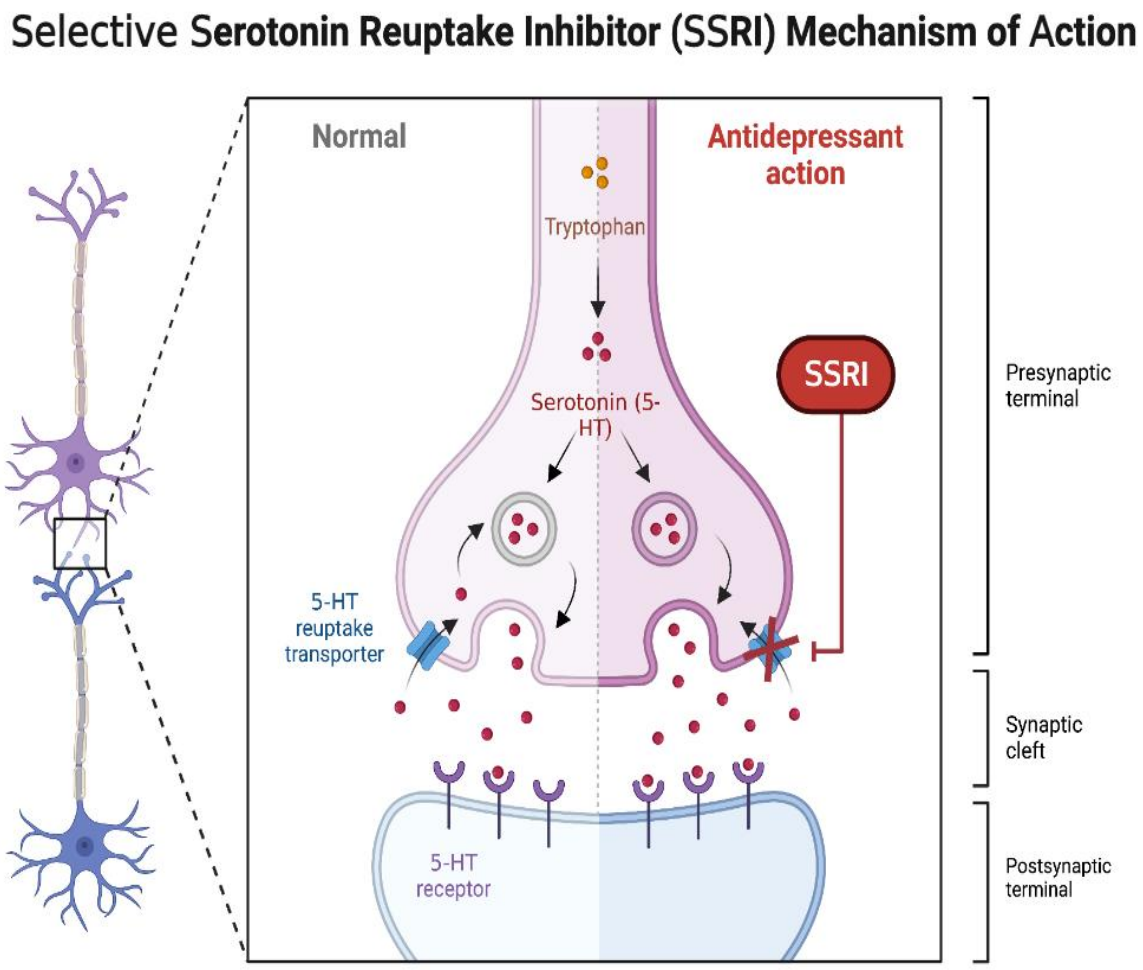

Figure 1.2: Mechanism of action of SSRIs. Available from https://www.biorender.com/template/mechanism-of-action-of-selective-serotonin-reuptake-inhibitors. Accessed 25th September, 2025.

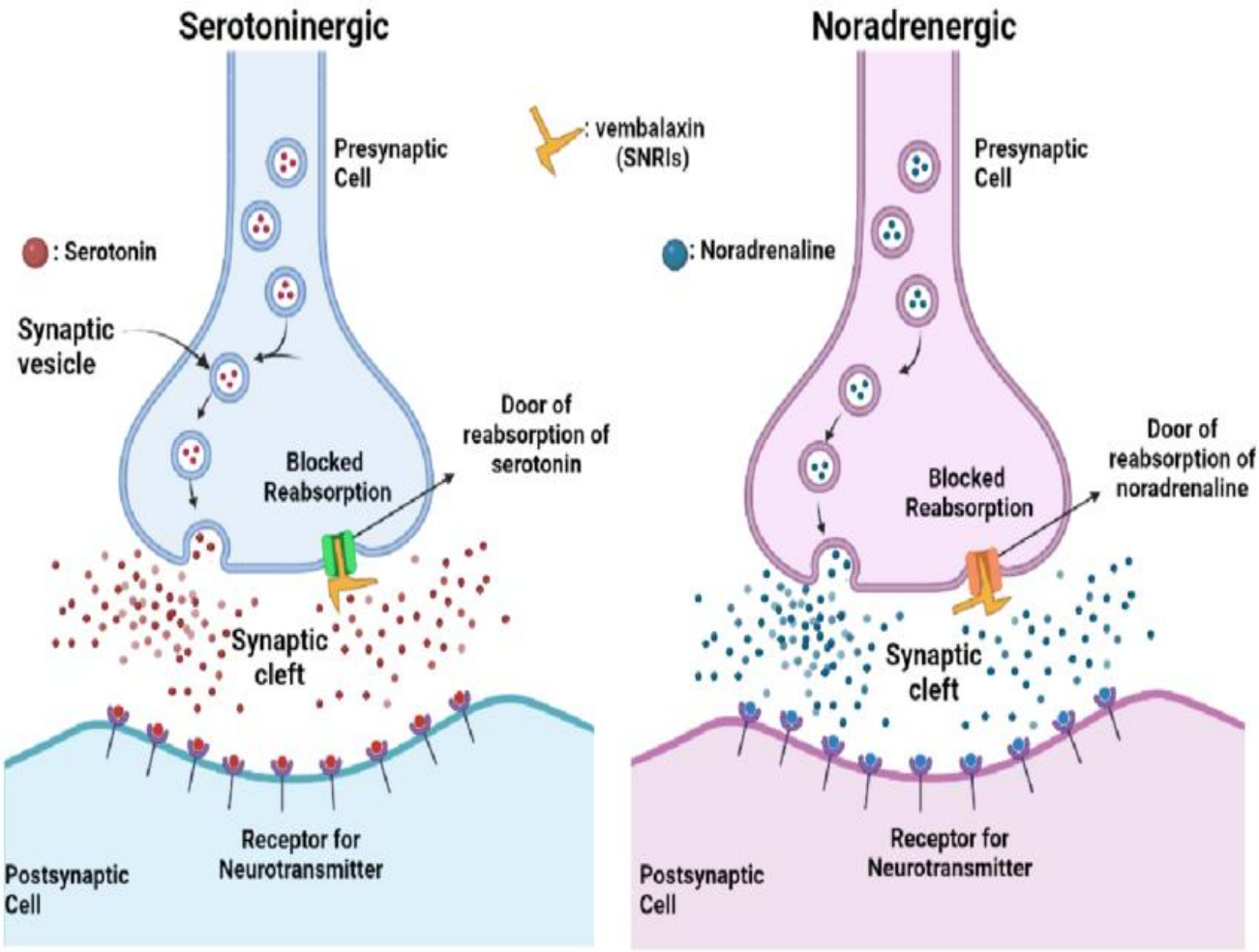

Figure 1.3: Mechanism of action of SNRIs. Available from https://www.researchgate.net/figure/SNRIs-mechanism-of-action_fig3_363492587. Accessed 25th September, 2025.

1.3.2 Second-line/adjunctive agents

When first-line treatments are insufficient, poorly tolerated, or contraindicated, second-line or adjunctive agents may be considered. These medications are typically used to enhance therapeutic response, target specific symptom clusters, or provide alternative options for patients who do not adequately respond to standard first-line therapies. Their use requires careful monitoring due to variable efficacy and potential side effects.

A. **Benzodiazepines:** Examples include diazepam, lorazepam, and alprazolam.

   Benzodiazepines (Figure 1.4) act as positive allosteric modulator of the $GABA_A$ receptor-chloride ion channel complex. By increasing the frequency of GABA-induced channel opening, they enhance inhibitory neurotransmission in limbic circuits such as the amygdala, producing rapid anxiolysis. However, they lack intrinsic activity in the absence of GABA, which explains their relative safety compared to barbiturates (Katzung *et al*., 2012). They are considered useful in acute anxiety, panic attacks, and for short-term bridging until SSRIs/SNRIs take effect due to their fast onset of action, although tolerance and dependence may develop with prolonged use. Thus, most guidelines recommend limiting use to 2-4 weeks maximum (Bandelow *et al*., 2017).

B. **Buspirone:** It is a partial agonist at serotonin $5\text{-}HT_{1A}$ receptors, exerting presynaptic inhibitory effects that reduce serotonergic firing and postsynaptic modulation in limbic regions. Unlike benzodiazepines, it does not interact with $GABA_A$ receptors, lacks sedative and dependence liabilities, and has a delayed anxiolytic onset, making it particularly suitable for generalized anxiety disorder (Katzung *et al*., 2012).

C. **Pregabalin:** It binds selectively to the α2δ subunit of presynaptic voltage-gated calcium channels (VGCC) in the Central Nervous System (CNS), reducing calcium entry and subsequent release of excitatory neurotransmitters such as glutamate and norepinephrine. This dampening of neuronal hyper-excitability accounts for its anxiolytic, anticonvulsant, and analgesic actions, although it does not act directly on GABA receptors despite being a GABA analogue (Katzung *et al*., 2012).

1.3.3 Emerging/off-label agents

A. **Atypical antipsychotics:** e.g., quetiapine, are sometimes used off-label for treatment-resistant GAD, but adverse effect burden is high (weight gain, metabolic syndrome).

B. **Cannabidiol (CBD):** Although gaining attention for anxiolytic effects, regulatory, dose-response, and safety issues remain unsettled.

1.3.4 Limitations of conventional drug therapy

1. **Delayed onset:** SSRIs/SNRIs take weeks before symptom improvement.
2. **Side-effect burden:** Sexual dysfunction, sedation, weight gain, GI issues reduce adherence.
3. **Dependence:** Benzodiazepines are effective but carry high abuse potential.
4. **Partial response:** Up to 40-50% of patients do not achieve full remission with first-line agents (Bandelow *et al*., 2017).
5. **Accessibility:** In low- and middle-income countries (like Nigeria), psychiatric medications are costly or unavailable, and specialist care is limited.
6. **Patient preference:** Many patients prefer natural or herbal therapies due to stigma, cost, or perceived safety.

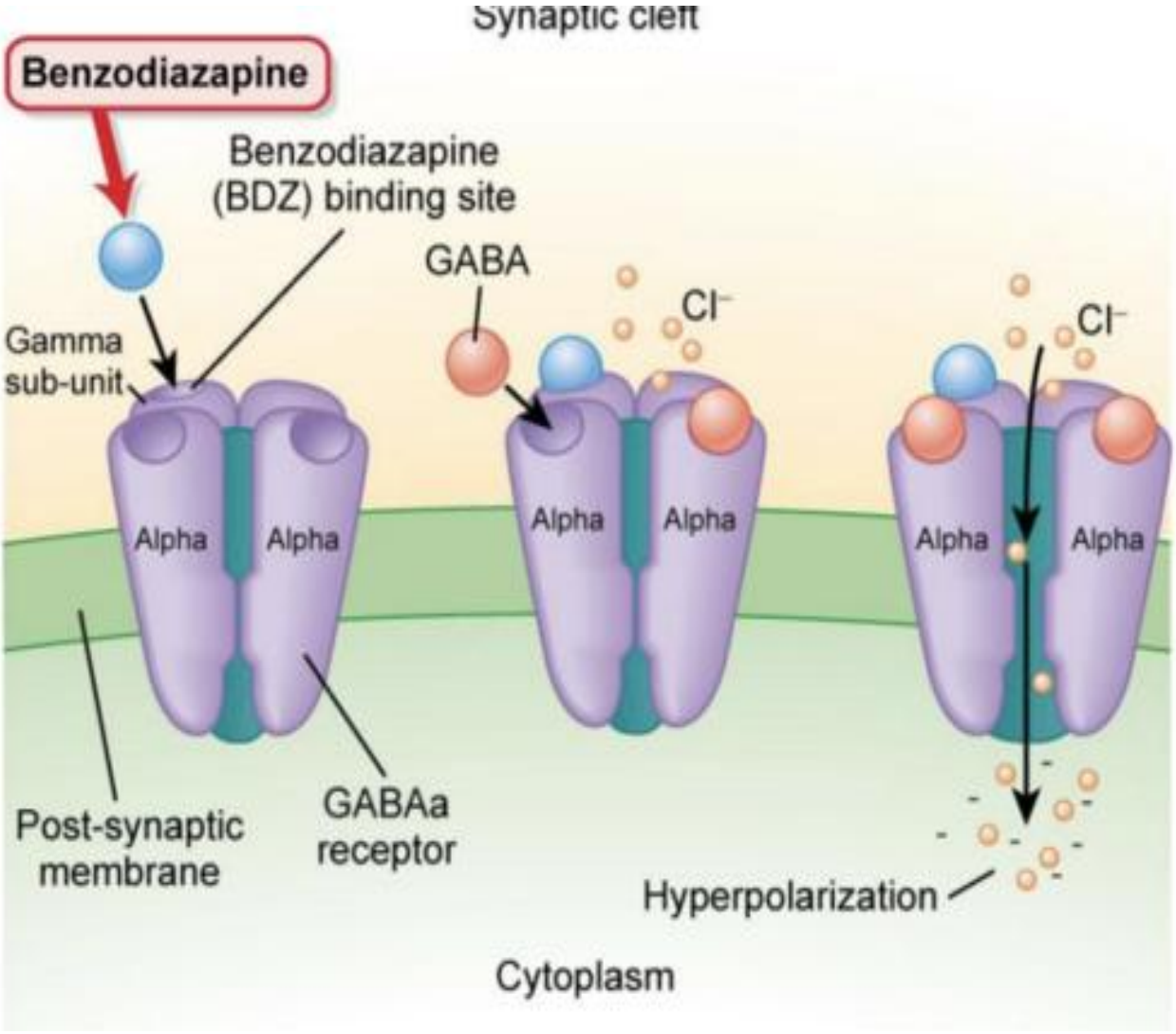

Figure 1.4: Mechanism of action of Benzodiazepines. Available from https://quizlet.com/671760315/the-benzodiazepines-and-non-benzodiazepine-sedatives-cns-stimulants-flash-cards/. Accessed 25th September, 2025.

Table 1.1: Summary of common pharmacotherapies for anxiety disorders.

Class	Examples	Mechanism	Limitations
SSRIs	Fluoxetine, sertraline	Block 5-HT reuptake	sexual dysfunction
SNRIs	Venlafaxine, duloxetine	Block 5-HT and NE reuptake	withdrawal
Benzodiazepines	Diazepam, lorazepam	GABA-A receptor positive modulators	Dependence, tolerance
Buspirone	-	5-HT_{1A} partial agonist	Delayed onset, not for acute anxiety
Pregabalin	-	Binds α2δ subunit, ↓ neurotransmitter release	misuse potential
Hydroxyzine	-	Antihistamine (H1 antagonist)	Sedation
Atypical antipsychotics (off-label)	Quetiapine	DA/5-HT antagonism	Weight gain, metabolic syndrome

1.4 Animal Models for Anti-Anxiety Studies

Anxiety in animals is not a unitary phenomenon. In the field of anxiety research, animal models are generally categorised into two primary categories. The first category involves conditioned responses to stressful situations, such as those caused by electric foot shock, the other another category involves ethological paradigms where spontaneous reactions, like flight, avoidance, and freezing, are observed in response to stressors that do not cause pain or discomfort (e.g. exposure to a novel, highly illuminated test apparatus or a predator). (Kumar *et al.*, 2013).

1.4.1 Importance of animal models in anxiety research

The complexity of anxiety disorders in humans necessitates the use of experimental animal models to explore underlying mechanisms and to evaluate potential anxiolytic agents. Since anxiety is inherently subjective, it cannot be measured directly in animals, instead, researchers rely on observable behavioral correlates that are validated as analogues of human anxiety.

Animal models of anxiety are indispensable in research for several reasons:

1. **Mechanistic Insights:** Animal models enable the study in more detail of the biological basis of anxiety-related states, such as neural circuits and neurotransmitter systems, endocrine axes (such as the HPA axis), changes in gene expression and epigenetics (Ren *et al*., 2024, Bartlett *et al*., 2017). Such studies would be significantly harder to experiment in human beings.
2. **Drug Discovery:** These models offer a testing platform on which to evaluate novel candidate anxiolytic compounds (and to test existing ones) in controlled system (Cryan and Sweeney, 2011).

3. **Ethical and practical necessity:** Early-stage evaluation of safety and efficacy in animals is essential before human clinical trials, given ethical restrictions in exposing humans to untested compounds (Cryan and Sweeney, 2011).
4. **Standardization:** The fact that most anxiety-behaviour assays (such as the elevated plus maze, open-field test, light/dark box) are highly characterised means that standard protocols used in animals improve reproducibility as well as enable cross-laboratory comparisons (Chen *et al*., 2024). Meta-analyses and reviews also show, however, that these tests can differ in what they capture and in sensitivity to drugs (Rosso *et al*., 2022).
5. **Translational relevance:** Despite existing inter-species variability, the animal models do have a predictive validity: the effect of the familiar anxiolytics on animals is often similar to its effect in humans. Therefore, these paradigms can be considered as a transition between simple neuroscience and treatment construction (Rosso *et al*., 2022).

1.4.2 Examples of models

To investigate the underlying neurobiological mechanisms and evaluate potential anxiolytic compounds, preclinical research relies heavily on animal models, particularly in rodents such as mice and rats (Campos *et al*., 2013). Many models are explicitly designed around a conflict between exploratory drive and avoidance of bright, open, novel, or elevated spaces (Lezak *et al.,* 2017). Rodents naturally exhibit thigmotaxis (preference for wall-hugging) and aversion to bright, exposed spaces, which mimic human avoidance in anxiety states (Rosso *et al*., 2022). The models to be discussed here [Open field test (OFT), hole-board test (HBT), elevated plus maze (EPM), elevated zero maze (EZM), light/dark test (LDT), four plates test, mirror chamber test, social interaction test, and social fear conditioning (SFC)] are ethologically valid, meaning that they draw on natural rodent behaviors without requiring extensive training, they assess

unconditioned (innate) or conditioned responses to stressors, providing insights into anxiety-like phenotypes (Campos *et al*., 2013). Each model's validity is supported by pharmacological sensitivity to known anxiolytics like benzodiazepines (e.g., diazepam), which typically increase exploration (Rosso *et al*., 2022).

A. **Open field test (OFT):**

The Open field test is one of the most widely used assays to measure anxiety-like behaviour, exploration, and locomotion across species, but its interpretation is sensitive to test conditions and specific behavioral readouts. The Open field test was first introduced by Calvin S. Hall in 1934 as a measure of emotionality, with defecation and thigmotaxis (wall-hugging) among the first indices (Hall, 1934). Modern work confirms it is now a staple assay for anxiety-like behavior and exploration, extended beyond rodents to multiple species (Larke, 2017). The test capitalizes on rodents' innate conflict between exploring a novel environment and avoiding open, brightly lit spaces, which evoke predation risk (Larke, 2017).

Rodents are placed in the center of a square (typically 38 × 38 cm to 72 × 72 cm for mice and rats respectively) enclosed by walls (30-50 cm high) to prevent escape (Walsh and Cummins, 1976). The arena is divided into a central zone (inner square) and peripheral zones (thigmotactic areas) (Seibenhener and Wooten, 2015). Testing occurs under moderate lighting for 5-30 min [Standard OFT duration is commonly 5-10 min (to balance novelty-induced anxiety with habituation), but ranges up to 15–30 min in some protocols (especially for locomotion/habituation studies)]. No prior habituation is needed, though pre-test handling reduces stress (Walsh and Cummins, 1976). For anxiety assessment, a single trial is common to capture novelty-induced responses (Lezak *et al*., 2017).

There are various behavioral parameters that are scored using the open field test: (i) number of entries into the central zone and duration of stay (ii) total distance traveled (locomotor activity), (iii) rearing (vertical exploration i.e., number of times the animal stands on hind legs (iv) grooming duration (self-directed behavior, often increased in anxiety), and (v) line crossings (grid divisions crossed, indicating activity).

Anxiogenesis is indicated by reduced central zone entries/time, increased thigmotaxis (peripheral preference), decreased rearing, and prolonged grooming (Walsh and Cummins, 1976). Anxiolytics increase central exploration without necessarily altering total locomotion (Seibenhener and Wooten, 2015).

B. **Hole-board test:**

The hole-board test apparatus was developed by Boissier and Simon in the early1960s as an open-field with floor holes, allowing quantification of head-dipping as a form of directed exploration, the hole-board test assesses exploratory behavior and anxiety of the rodent confronted with a new environment by exhibiting automatic stereotype head plunging or head dipping into holes, building on observations of rodents' neophilia (attraction to novelty) (Boissier and Simon, 1965; Casarrubea *et* al., 2023). It was refined in the 1970s to differentiate directed exploration (head dips) from general locomotion, making it a complementary tool to the OFT (Takeda *et al*., 1998).

The modified hole-board apparatus (Figure 1.5) is a square board white board (40 × 40 cm) with 16 evenly spaced holes (3 cm diameter) on the floor, elevated slightly above ground. The rodent is placed in the center (away from the observer) and allowed to explore for 5 minutes under dim lighting (Casarrubea *et al*., 2023). Behavior is video-recorded, no conditioning is required (Brown and Nemes, 2008). Key parameters

measured are: (i) number and duration of head dips (exploratory behavior), (ii) latency to first head dip, (iii) locomotor activity (line crossings or distance traveled), and (iv) rearing and grooming episodes (Takeda *et al*., 1998; Brown and Nemes, 2008). A head dip is when the mouse places its head into one of the holes to a minimum depth such that the ears are at same level with the floor of the apparatus (a new bout of head dipping is recorded if the animal raises its head fully out of the hole before resuming). Rearing is when the animal is stationary on its hind paws and raises its forepaws of the ground, extending its body vertically. Anxiety-like behaviour is shown by decreased head dips (avoidance of novelty) and increased latency, reflecting risk assessment (Takeda *et al*., 1998).

C. **Elevated plus maze (EPM):**

Elevated plus maze (EPM) remains a critical behavioural model of the use of measuring anxiety-like behaviour in rodents. The EPM is a four-arm maze with two open arms and two enclosed arms arranged in a plus shape and elevated above the floor (Komada *et al*., 2008).

An animal (a rodent) is placed on the central platform at the junction of the four arms, typically facing an open arm, and allowed to explore freely for about 5 min under low-light conditions (behavior is usually video-tracked) (Kraeuter, 2019). The EPM apparatus (Figure 1.6) capitalises on the innate conflict in rodents between exploration of a novel environment and avoidance of open, elevated spaces. The standard procedural parameters used in the case of mice usually include: arm dimensions approximated to about 25-30 by 5 cm, an elevation of about ~50 cm above the floor (Komada *et al*., 2008). Relevant parameters to be recorded includes; (i) time and entries in open vs. closed arms (percentage open-arm time/entries), (ii) latency to enter open arms, (iii) risk assessment

behaviors (e.g., stretch-attend postures, head dips), and (iv) total arm entries (locomotion control).

Figure 1.5: The modified hole-board apparatus. Available from https://www.youtube.com/watch?v=GROOCu6bLTM. Accessed 27th September, 2025.

Anxiety-like behaviour is indicated as less exploration of the open arm, which is measured using short open arm time or fewer open arm bio-occupancy, and gains in these measures indicate the presence of anxiolytic-like effects (Komada *et al*., 2008).

Notably, the EPM does not need an initial training due to the necessity of novelty that renders the method valid, and locomotor activity is usually regulated counting the total number of entries Anxiety-like behaviour is indicated as less exploration of the open arm, which is measured using short open arm time or fewer open arm bio-occupancy, and gains in these measures indicate the presence of anxiolytic-like effects (Komada *et al*., 2008).

The index of anxiety (open arm avoidance) is often calculated as:

100 – [(% Time in Open Arm + % entries into Open Arm)] / 2

If the anxiety index is at least 10 points < than the vehicle treated control group, the sample is anxiolytic, conversely, if anxiety index is at least 10 points > than the vehicle treated control group, then the sample is anxiogenic.

D. **Light/dark test:**

The light-dark test apparatus (figure 1.7) was developed by Crawley and Goodwin in 1980, it uses a two-compartment box; a small dark "safe" chamber (~1/3 volume) and a larger brightly lit chamber (~2/3), usually separated by a wall with a small doorway or tunnel (Serchov, 2016). This test exploits rodents' innate aversion to bright light (scotophobia) and preference for dark, enclosed spaces (Crawley and Goodwin, 1980). It was inspired by conflict paradigms and has

been widely adopted for screening anxiolytics (Bourin and Hascoët, 2003). The rodent starts in the light side and explores for 5-10 minutes (Bourin and Hascoët, 2003).

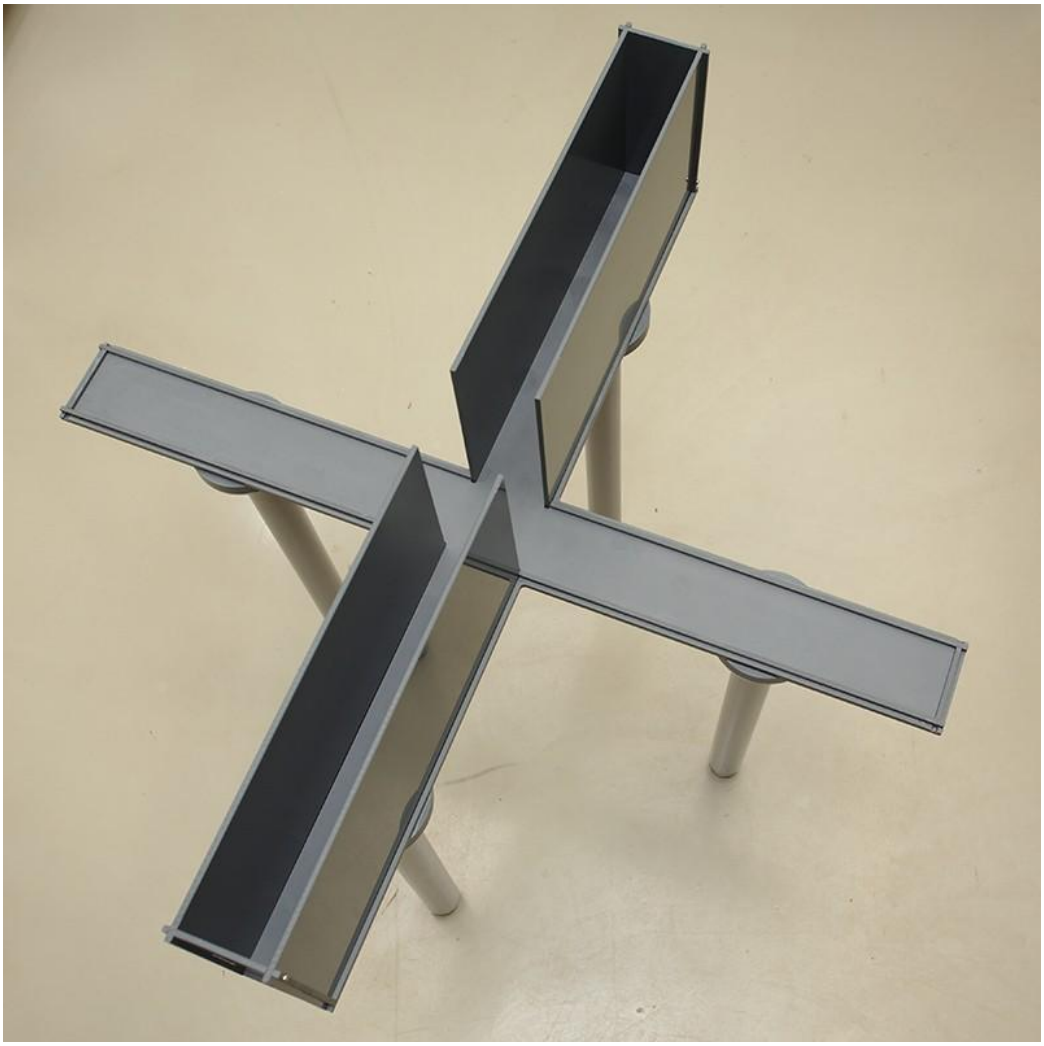

Figure 1.6: The Elevated plus maze apparatus. Available from https://www.izi.fraunhofer.de/en/central-facilities/center-for-experimental-medicine/preclinical-models/models/elevated-plus-maze.html. Accessed 27th September, 2025.

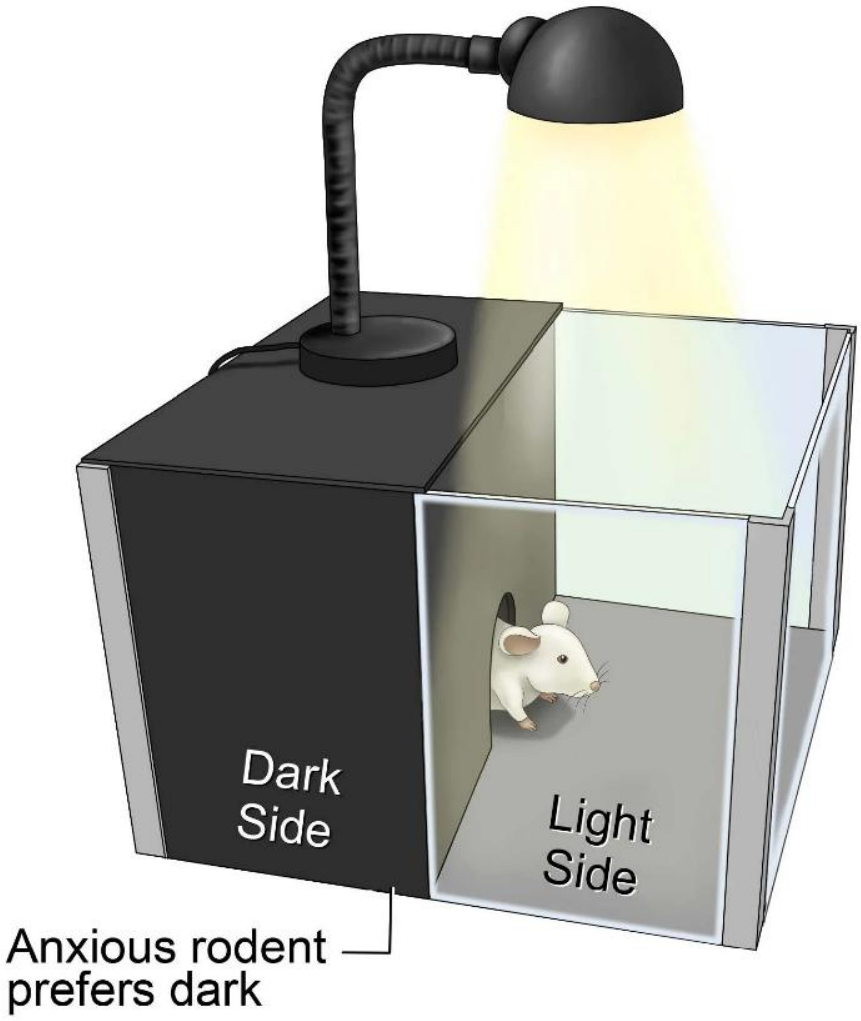

Figure 1.7: The light-dark box apparatus. Available from https://conductscience.com/assessing-anxiety-and-stress-the-light-dark-box-paradigm/. Accessed 27th September, 2025.

The time spent in light vs. dark compartment, the number of transitions between compartments and latency to enter dark compartment are measured.

Anxiety manifests as more time in dark and fewer transitions while anxiolytics increase light time/transitions (Bourin and Hascoët, 2003).

E. **Four plates test:**

The four-plate test apparatus (Figure 1.8) was introduced by Boissier and co-workers in 1968 and refined by Aron in 1971. This test uses mild electric footshocks to the animals thereby suppressing exploration, modeling conflict anxiety (Boissier *et al*., 1968). It was designed for rapid screening of tranquilizers (Ripoll *et al*., 2006). The arena has four metal plates (8 x 11 cm) separated from each other by a gap of 4mm, forming a floor, wired to deliver foot shocks (0.3-0.8 mA, 0.5 s) (Ripoll *et al*., 2006). Thirty min before the test, the animals are administered with the drug. At the beginning of the test, the animal is gently dropped onto each plate and is allowed to explore for 15 s. After this, every time the animal crosses from one plate to another the experimenter electrifies the whole floor for 0.5 s which evokes a clear flight response in the animal. The number of times the apparatus is electrified is counted each minute for 10 min. The number of shocks received during the first minute is taken as the parameter for evaluating anxiolytic activity.

Fewer crossings indicate anxiety (punishment avoidance); anxiolytics increase crossings (Boissier *et al*., 1968).

F. **Mirror chamber test:**

The mirror chamber (figure 1.9) was developed in the 1980s based on approach-avoidance to mirrors (rodents treat reflections as conspecifics), it was formalized by Toubas and team mates in 1990 for anxiety assessment. It draws on social and novelty conflicts (Toubas *et al*., 1990).

A chamber (30.5 × 30.5 × 30.5 cm) with mirrors on three walls is attached to a dark start box (Toubas *et al*., 1990). This model is primarily used in the assessment of anxiety level in rodents. It has been observed that animal species exhibit an approach-avoidance response upon placing of a mirror within their environment. Most animals are known to lack the ability to recognize themselves in the mirror. Thus, a chamber lined with mirrors will create a sense of anxiety and panic in the animals. The subject is allowed 30 min to acclimate to the testing arena. The subject is then placed on a corner of the mirrored chamber that is farthest from the exit, it is allowed to explore the apparatus for at least 5 min, during which observation of its behaviour is made. The following parameters are noted:

(i) Latency to enter mirror chamber, (ii) time spent in chamber, and (iii) number of entries and approaches (Toubas *et al*., 1990).

Delayed entry and reduced time indicate anxiety (avoidance of social reflection); anxiolytics decrease latency (Toubas *et al*., 1990).

Figure 1.8: The light-dark test apparatus. Available from https://www.panlab.com/en/products/four-plate-box-aron-test-panlab. Accessed 27th September, 2025.

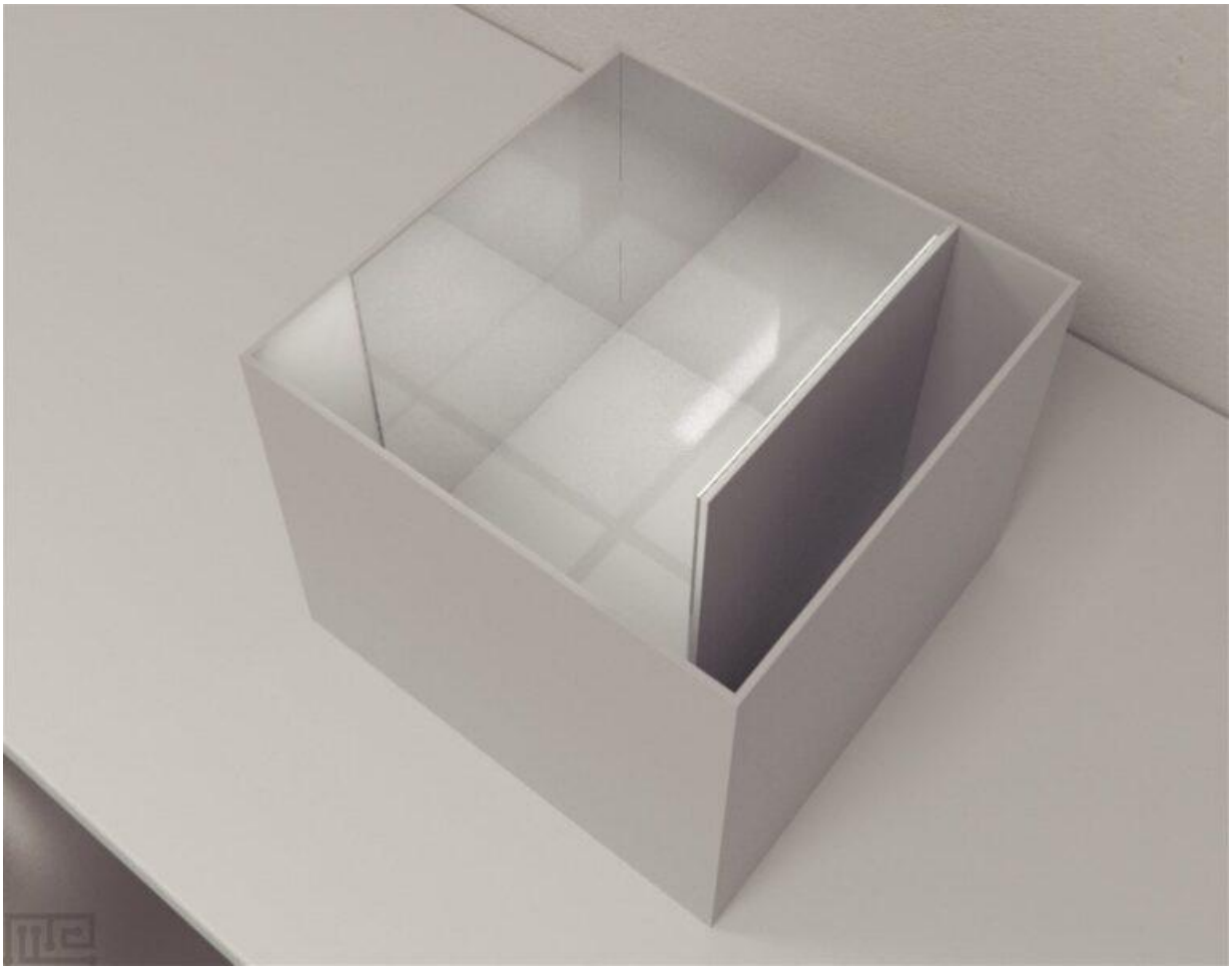

Figure 1.9: The mirror chamber apparatus. Available from https://maazeenineers. Com/portfolio/mirror-chamber-test/. Accessed 27th September, 2025.

G. **Social fear conditioning (SFC):**

SFC was introduced as a specific animal model of social anxiety disorder, designed to induce fear and avoidance of unfamiliar conspecifics without broad emotional or motor deficits. SFC uses operant conditioning with foot shocks paired with social stimuli (Toth *et al*., 2012).

The mice are first placed in a box containing unfriendly mice. Every time there is social contact during a period of 3 min, mice receive mild foot shocks (~2-5 shocks, 1 s, 0.7 mA) (Toth *et al*., 2012). The control mice are subjected to the same conditions, but do not receive the electric stimulation. Fifteen days later, the extinction of social fear memories over a short or long period of time is evaluated. This test consists of putting the mice in a similar box used during the first test day. Six mice are then placed in this box without their family members to evaluate social contact. The reduction of social contact and aversive responses to social stimuli, such as freezing, or hiding in the wood shavings of the box, indicates the SFC. In the third and sixteenth day, the evocation of fear extinction is evaluated. In this case, it is evaluated whether the stimulus repeated during fear extinction exposure leads to the complete reversal of social fear (Toth *et al*., 2012). In the study carried out by Toth *et al*, (2012), they showed that mice, which represented the social anxiety in SFC model, did not exhibit changes in other behavioral measures, such as the permanence time in the forced swim test model for depressive-like behavior. These

further support the use of the SFC model as a valid model of social anxiety (Toth *et al*., 2012).

1.4.3 Drugs used for induction of anxiety-like behavior

Pharmacological anxiogens are useful because they produce rapid, reproducible anxiety-related behaviours and allow mechanistic probing (they target a receptor or transmitter system). They are widely used to (i) validate test systems (produce anxiogenesis that is reversible by known anxiolytics), (ii) probe mechanism of action, and (iii) reveal whether a candidate compound blocks acute neurotransmitter-driven anxiety

A. **Yohimbine (α_2-adrenoceptor antagonist):** Yohimbine is a plant alkaloid originally derived from *Pausinystalia yohimbe* bark (Nowacka *et al*., 2024). It is used as a prototypical α_2-adrenoceptor (presynaptic receptors) antagonist, it removes negative feedback on NE release, raising synaptic NE and sympathetic activity. This noradrenergic surge leads to central hyperarousal, sympathetic activation, tachycardia and anxiety-like behavior. In rodents, acute doses reliably reduce open-arm exploration in the elevated plus maze and increase avoidance in anxiety assays. Many researchers continues to confirm its anxiogenic profile and validate it as a stress-provoking tool for both behavioural and pharmacological investigations (Nowacka *et al*., 2024; Sheng *et al*., 2015)
B. **Corticotropin-Releasing Factor (CRF/CRH):** CRF is a key neuropeptide coordinating stress responses via the HPA axis and acting in stress-related brain regions (e.g., amygdala, BNST). Rodent models using intracerebroventricular or intra-amygdalar administration of CRF reliably produce anxiety-like behaviours (reduced social interaction, increased avoidance, elevated startle). The model has excellent face and

construct validity with human anxiety disorders and continues to be refined in recent studies (Maita *et al*., 2022).

1.5 Plants with anxiolytic property

A growing body of evidence highlights the therapeutic potential of various medicinal plants in the management of anxiety disorders. These botanicals act through diverse mechanisms that help restore neurochemical balance. Many have been validated in animal models and, in some cases, human clinical studies, showing comparable efficacy to conventional anxiolytics but with fewer side effects. Table 1.2 below presents plants with demonstrated anxiolytic-like activity, indicating the part used and corresponding references for each.

Table 1.2: Summary of plants with anxiolytic-like properties

Plant(Part used)	Evidence for anxiolytic-like effects	Reference
Piper aduncum (Leaf essential oil)	Recent work in mice reports anxiolytic-like and antioxidant effects of *P. aduncum* essential oil. Discussion links effects to bioactive terpenes such as linalool, nerolidol, β-caryophyllene.	Alvarado-García *et al*., 2025
Alpinia zerumbet (Leaves/extracts)	Hydro-ethanol leaf extract showed anxiolytic-like effects in the LDE and antidepressant-like activity in mice, alongside strong antioxidant properties.	Júnior *et al*., 2013
	Reviews describe *A. zerumbet* as having antidepressant and anxiolytic potential in humans with low reported toxicity.	Ferreira *et al*., 2024
Passiflora incarnata (Leaf extracts)	Commonly known as Passionflower. A GABA-phytomedicine review identifies passionflower as one of 10 plants with in-vitro GABA modulation, rodent anxiolytic-like effects, and human clinical trials showing anxiolysis.	Savage *et al*., 2018
Lavandula angustifolia (Flower/aerial parts)	Commonly known as lavender. A 2022 clinical-focus review notes lavender among plants that have consistent clinical evidence for relieving mild anxiety, depression and stress, generally with good safety	Kenda *et al*., 2022

Centella asiatica (Whole plant)	The GABA-modulating phytomedicine review lists *C. asiatica* among plants with preclinical GABAergic activity and human studies showing clinical anxiolytic effects	Savage *et al*., 2018
Bacopa monnieri (Entire plant)	Reviews of herbal psychotropics and pharmacogenomics discuss Bacopa spp among plants with potential anxiolytic and antidepressant activity, acting via neurotransmitter modulation and neuroprotective/antioxidant mechanisms	Sahoo and Brijesh, 2019

1.6 The Plant *Ficus iteophylla* Miq.

1.6.1 Taxonomy

Kingdom: Plantae

Class: Angiosperms

Order: Rosales

Family: Moraceae

Genus: Ficus

Species: *F. iteophylla* Miq

1.6.2 Common Names/Vernacular Names:

The plant is known across different region by different name, (Orwa *et al*., 2009; PROTA, 2012):

The common name in English is African fig.

Kenya: Omuhoro (Kamba); Kiumo (Karagwe); Mshasha, Mtenza, Mtoma, Munyan-wonyo (Kikuyu)

Nigeria: Odan (Yoruba), Chediya (Hausa).

1.6.3 Geographical distribution and morphological description

F. iteophylla is a hemi-epiphytic fig tree native to tropical Africa, with a broad distribution in West and Central Africa (including Burkina Faso, Nigeria, Cameroon, and Gabon) and typical fig-like morphological traits, including aerial roots and axillary figs (Sawadogo *et al*., 2024).

It is a medium-sized tree, occasionally epiphytic at early stages, with smooth pale bark and rounded crown. The fruits (syconia) occur in leaf axils or on older wood, often in pairs, and measure about 5-10 mm in diameter. It occurs naturally in open woodland and riverine forests, tolerating a range of ecological conditions (Raji and Downs, 2021).

The leaves of *F. iteophylla* (Figure 1.10) display features typical of the Moraceae family but also bear unique traits that aid identification. Viz;

(i) Arrangement: Leaves are simple, alternate, and often borne on short petioles.

(ii) Lamina: Typically ovate to elliptic, sometimes broadly lanceolate.

(iii) Size: Moderate in size, measuring approximately 6-15 cm in length and 3-7 cm in width.

(iv) Apex: Usually acute to acuminate, with a pointed tip.

(v) Base: Cuneate to rounded, sometimes slightly asymmetric at the petiole junction.

(vi) Margin: Entire (smooth), occasionally slightly undulate.

(vii) Texture: Coriaceous (leathery) and firm; surface is glabrous (smooth) above, with faint pubescence beneath.

(viii) Venation: Prominent pinnate venation, with a distinct midrib and lateral veins arching toward the margin.

(ix) Color: Dark green and glossy on the upper surface; paler, duller green on the underside.

(xi) Odor/Taste: When crushed, the leaves exude a milky latex, characteristic of Ficus species; taste is slightly bitter.

1.6.4 Ethnomedicinal uses

F. iteophylla has a long history of use in African ethnomedicine, where various parts of the plant (particularly the leaves, bark, and roots) are employed to manage a wide range of ailments. Traditionally, decoctions or macerations of the leaves have been administered for the treatment of inflammatory conditions, wounds, and gastrointestinal disturbances, such as diarrhea and dysentery. The bark extract is used as an analgesic and to alleviate fever, while root preparations are sometimes prescribed for respiratory ailments and as general tonics (Shi *et al*., 2018).

In several West African communities, *F. iteophylla* serves an important role in managing infections and reproductive disorders. Studies have documented its use for treating gonorrhea, menstrual irregularities, and infertility, suggesting possible hormonal or antimicrobial effects (Hasnat *et al*., 2024; Shi *et al*., 2018). Moreover, leaf and bark extracts are applied topically for the treatment of skin infections, ulcers, and wound healing-activities supported by studies showing antibacterial and antioxidant properties of the plant's secondary metabolites (Singh and Sharma, 2023).

1.6.5 Phytochemical constituents

Phytochemical investigations of *F. iteophylla* have revealed that the plant is particularly rich in secondary metabolites, many of which are associated with pharmacological activity. Preliminary

screenings of leaf and bark extracts have consistently identified the presence secondary metabolites such as flavonoids, saponins, alkaloids, tannins, phenolic acids, etc., (Hasnat *et al*., 2024). These classes of compounds are not merely structural constituents of the plant but possess biological activity relevant to anxiety and related disorders.

Among these, flavonoids such as quercetin, kaempferol, and rutin, commonly reported in Ficus species, are known for their antioxidant, anti-inflammatory, neuroprotective (Dahan *et al*., 2025; Singh and Sharma, 2023; Shim *et al*., 2022). Phenolic compounds enhance free radical scavenging capacity and mitigate oxidative stress (Salehi *et al*., 2021), a process strongly implicated in the pathophysiology of anxiety.

Tannins and saponins, also present in *F. iteophylla*, contribute additional pharmacological actions. Tannins are associated with antimicrobial and wound-healing activities (Hasnat *et al*., 2024), supporting the ethnomedicinal applications of the plant. Saponins have been reported to exhibit immunomodulatory and antistress activity (Singh and Sharma, 2023). In addition to that, the presence of alkaloids provides potential for neuromodulatory effects, as many alkaloids are known to interact with neurotransmitter systems, influencing mood and anxiety (Singh and Sharma, 2023; Shim *et al*., 2022).

1.6.6 Previous pharmacological studies

Research on *F. iteophylla* and related Ficus species has revealed a wide array of biological activities. Although relatively underexplored compared to other species within the Ficus genus*, F. iteophylla* has demonstrated a range of pharmacological activities that support its ethnomedicinal applications and suggest potential central nervous system effects. Studies on the methanol leaf extract revealed significant antimicrobial and anti-inflammatory properties, consistent with its traditional use in the treatment of infections, fever, and inflammatory disorders (Salehi *et al*.,

2021). These activities are largely attributed to the plant's rich complement of flavonoids, phenolic compounds, and saponins, which are known to modulate inflammatory pathways and oxidative stress. Importantly, extracts of *F. iteophylla* have also demonstrated antioxidant activity, reflected in their ability to scavenge free radicals and enhance endogenous antioxidant defenses (Mukhtar *et al.,* 2020). This is particularly relevant in the context of anxiety, where oxidative stress and reactive oxygen species contribute to neuronal dysfunction and heightened amygdala excitability. Thus, the antioxidant potential of the plant provides a plausible mechanistic basis for its putative anxiolytic action.

1.6.7 Previous toxicological studies

Toxicological evaluations of *F. iteophylla* have generally demonstrated a favourable safety profile, articularly in acute settings. In a study carried out by Coker, (2014), no mortality or signs of toxicity were observed in mice at doses up to 5000 mg/kg body weight in an acute oral toxicity study, with no changes noted in skin, fur, eyes, motility, respiration or behaviour (including tremors, convulsions, salivation, diarrhea, or lethargy). This suggests that the extract is relatively non-toxic by the oral route, with a high LD_{50} value, which aligns with the World Health Organization's criteria for preliminary safety in herbal medicines. Further observations from the same study showed no gross pathological changes in vital organs such as the liver, kidney, and heart, and serum biochemical markers of hepatic and renal function remained within normal ranges, minor hematological changes were observed at doses of 100-200 mg/kg, including rises in red blood cell count and mean corpuscular hemoglobin, but these were not indicative of adverse effects and did not persist at higher doses. Thus, these findings indicate an absence of overt hepatotoxic or nephrotoxic effects at therapeutic and supra-therapeutic doses.

Considering the long-standing use of *F. iteophylla* in traditional medicine for fever, wound healing, and inflammatory conditions without documented reports of toxicity also suggests low risk to humans when used appropriately. Nonetheless, the absence of detailed chronic, reproductive, and genotoxicity studies represents a gap in current knowledge and underscores the need for cautious extrapolation to long-term human use.

Figure 1.10: *F. iteophylla* showing the leaves in its natural habitat around the premises of the Faculty of Engineering, University of Benin, Benin City, Edo State, Nigeria. (6.40348o N, 5.61385o E).

1.7 The Plant *Tamarindus indica* L.

1.7.1 Taxonomy

Kingdom: Plantae

Class: Angiosperms

Order: Fabales

Family: Fabaceae

Genus: Tamarindus

Species: *T. indica*

1.7.2 Common Names:

The plant is known across different region by different name, (Orwa *et al*., 2009; PROTA, 2012):

It is commonly called Tamarind, Indian date, Tamarind tree in English

Nigeria: Tsaniya (Hausa); Ajagbon (Yoruba); Icheku oyibo (Igbo).

1.7.3 Geographical distribution and morphological description

T. indica is believed to have originated in tropical Africa, where it still grows wild across the savanna and semi-arid regions of countries such as Sudan, Nigeria, and Ethiopia (Figure 1.11). Over centuries, it spread through trade and cultivation to South and Southeast Asia, where it became naturalized and widely grown, particularly in India, Thailand, and the Philippines (Orwa *et al.*, 2009). The species thrives in dry to semi-humid tropical climates, commonly found along riverbanks, farmlands, and open woodlands, and tolerates a wide range of soil conditions (Orwa *et al*., 2009). *T. indica* is a medium to large, long-lived tree reaching 12-25 m in height, with a

dense, spreading crown and greyish-brown fissured bark. The flowers are yellowish with red veins and fragrant. It produces curved, brown pods containing sticky, acidic pulp and hard, glossy seeds (Orwa *et al*., 2009).

The leaves of *T. indica* (Figure 1.12) are highly distinctive, being compound and pinnate, a key taxonomic feature of the Fabaceae family.

(i) Arrangement: Paripinnate (even-pinnate) leaves, arranged alternately on slender branches.

(ii) Shape of leaflets: Oblong to obovate, sometimes narrowly elliptic.

(iii) Size: Leaflets measure 1-2.5 cm long and about 0.5 cm wide; entire leaf can be 7.5-15 cm in length.

(iv) Number of leaflets: Each compound leaf typically bears 10-20 pairs of opposite leaflets, neatly aligned.

(v) Apex: Rounded or obtuse, occasionally slightly retuse (notched).

(vi) Base: Rounded to subcordate (heart-shaped) at the junction with the rachis.

(vii) Margin: Entire (smooth, non-serrated).

(viii) Texture: Thin, delicate, and papery in texture compared to Ficus leaves.

(ix) Venation: Each leaflet shows fine pinnate venation, with the midrib clearly visible but lateral veins faint.

(x) Color: Bright green when young, turning to a deeper green with age; paler beneath.

(xi) Petiole/Rachis: Each compound leaf arises from a slender rachis, with a short petiole of 2-4 cm.

(xii) Odor/Taste: When crushed, the leaflets emit a faint acidic or sour odor due to organic acids.

The compound, evenly pinnate arrangement and the presence of numerous small oblong leaflets make *T. indica* leaves morphologically unmistakable

1.7.4 Ethnomedicinal uses

The plant is widely valued not just for its nutritional benefits but also for its broad ethnopharmacological applications, several of which indicate potential central nervous system (CNS) effects that warrant further exploration for anxiolytic properties (Siddiq *et al.*, 2014). In traditional medicinal practices throughout Africa and Asia, *T. indica* has been utilised as a multifaceted therapeutic agent. Its leaves, bark, fruit pulp, and seeds are incorporated into diverse formulations such as decoctions, infusions, and poultices. The leaves and pulp are frequently used to address fever, gastrointestinal ailments, jaundice, and inflammatory disorders, whereas the bark features prominently in various indigenous healing systems and oral traditions (De Caluwé *et al*., 2010; Bhadoriya *et al*., 2011). Descriptions in folk medicine highlight *T. indica* as a remedy that fosters calmness and alleviates agitation or "heat of the head" (an expression used in folk terminology to denote restlessness, mental stress, and nervous exhaustion). Such applications are evident in Ayurvedic medicine, where tamarind-based preparations are recommended for balancing pitta dosha (a state associated with irritability, agitation, and restlessness).

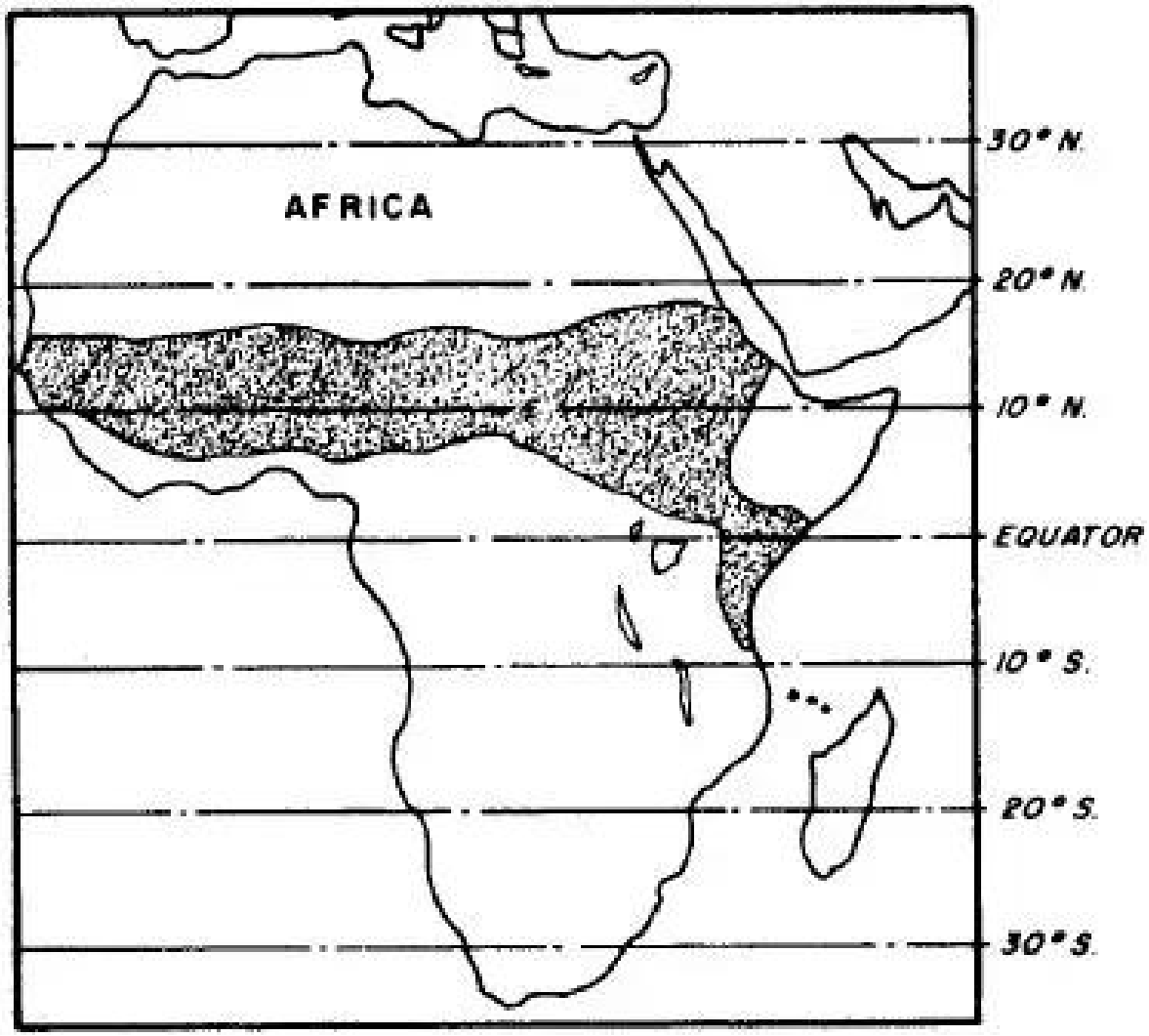

Figure 1.11: Geographical distribution of *T. indica* in Africa (shaded area represent approximate native range of tamarind). Available from https://www.researchgate.net/figure/Geographical-distribution-of-T-indica-in-Africa-Shaded-area-represent-approximate_fig1_3314.

Figure 1.12: *T. indica* showing the leaves in its natural habitat around the premises of the Faculty of Engineering, University of Benin, Benin City, Edo State, Nigeria. (6.40162o N, 5.61488o E).

1.7.5 Phytochemical constituents

Phytochemical investigations have revealed that *T. indica* exhibits a remarkably diverse chemical profile, comprising polyphenols, flavonoids, alkaloids, terpenoids, tannins, saponins, and glycosides (De Caluwé *et al*., 2010; Bhadoriya *et al*., 2011). Flavonoids are particularly abundant, with compounds such as catechin, epicatechin, procyanidins (e.g., procyanidin B2), and apigenin featuring prominently. These molecules are well-recognised for their antioxidant, anti-inflammatory, and neuroprotective effects, properties that are particularly relevant in the context of anxiety research, given their ability to modulate oxidative stress pathways and interact with GABAergic and serotonergic neurotransmission, two key systems involved in anxiolysis.

In addition to flavonoids, *T. indica* contains a range of phenolic acids, notably caffeic acid and ferulic acid, which further enhance its free radical scavenging capacity. These compounds have been implicated in mood regulation and neuroprotection, reinforcing the therapeutic plausibility of the plant in neuropsychiatric conditions (Razali *et al*., 2015). The alkaloids and saponins identified in *T. indica* also carry pharmacological importance, as they contribute to the plant's overall bioactivity, with related metabolites in other species demonstrating central nervous system effects, including potential anxiolytic-like properties (Siddiq *et al*., 2014).

The plant is also rich in organic acids, particularly tartaric acid, which is responsible for the fruit’s characteristic sour taste. Beyond sensory appeal, these organic acids contribute to the plant’s antioxidant potential, complementing the polyphenolic fraction and strengthening its protective effects against oxidative stress (De Caluwé *et al*., 2010).

1.7.6 Previous pharmacological studies

Multiple pharmacological actions of *T. indica* have been scientifically reported, many of which align with its prominent ethnomedicinal applications. Leaf and fruit pulp extracts have demonstrated significant anti-inflammatory, analgesic, and antipyretic properties, supporting their traditional use in treating fever, pain, and inflammatory conditions (Komakech *et al*., 2019; Kaladhar e*t al*., 2013). These effects are attributed to high levels of flavonoids, phenolic acids, and other polyphenols that inhibit prostaglandin synthesis and modulate inflammatory responses (De Caluwé *et al*., 2010).

In addition to peripheral effects, activities of particular relevance to neuropsychopharmacology have been observed in leaf and pulp extracts; these include reduced reactive oxygen species-induced lipid peroxidation in both in vitro and in vivo models, alongside enhanced activities of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase (Lim *et al*., 2013; Razali *et al*., 2015). Given the established role of oxidative stress in the pathophysiology of anxiety disorders, through mechanisms involving amygdala hyperexcitability and dysfunctional prefrontal regulation, the antioxidant properties of *T. indica* offer a plausible mechanistic basis for protecting neural integrity and modulating anxiety-related circuitry.

Moreover, neurobehavioural studies have revealed central nervous system effects in extracts of the plant, including decreased locomotor activity and evidence of anxiolytic-like activity in behavioural models such as the elevated plus-maze (increased open-arm exploration) and potentiation of barbiturate-induced sleep (Siddiq *et al*., 2014; Vuyyala *et al*., 2022). These findings suggest interactions with GABAergic and serotonergic pathways, akin to established anxiolytics.

The convergence of anti-inflammatory, antioxidant, and neurotransmitter-modulating effects provides robust mechanistic support for the potential anxiolytic-like activity of *T. indica*, warranting further investigation.

1.7.7 Previous toxicological studies

Toxicological studies of *T. indica* suggest that the plant is generally safe for human and animal use at therapeutic doses. Acute toxicity tests in rodents have shown no mortality at doses up to 5000 mg/kg p.o., indicating a high LD_{50} value and broad safety margin (Bhadoriya *et al*., 2011). No gross pathological changes were reported in the liver, kidney, or heart, and biochemical markers of organ function remained within normal ranges.

Chronic use studies are less extensive, but long-term consumption of tamarind fruit and pulp in humans as food adds to its safety credibility. However, some studies have reported mild gastrointestinal disturbances with very high doses, likely attributable to the fruit's acidic components (De Caluwé *et al*., 2010).

1.8 Rationale for the Study

Anxiety disorders rank among the leading contributors to global disability, with research indicating that nearly 1 out of every 3 people is likely to experience such a condition at least once in their lifetime (Bandelow and Michaelis, 2015). Although established pharmacotherapies such as benzodiazepines, SSRIs, and SNRIs demonstrate clinical efficacy, they are associated with notable limitations.

Benzodiazepines are hampered by tolerance, dependence and cognitive side effects, while SSRIs and SNRIs act slowly and can produce early-phase worsening of anxiety or intolerable adverse reactions (Bandelow *et al*., 2017; Katzung *et al.,* 2012). These limitations are compounded in

low- and middle-income settings by restricted access to psychiatric care, high treatment costs, and poor adherence (Saxena *et al*., 2007). There is thus an urgent need for safer, more accessible, and mechanistically diverse anxiolytic agents.

Emerging neurobiological understanding frames anxiety as a disorder of circuit dysregulation and cellular vulnerability, with oxidative stress and neuroinflammation emerging as critical drivers. Oxidative damage in limbic circuits impairs synaptic plasticity, disturbs monoaminergic signalling, and sustains hyperexcitability changes directly linked to anxiety phenotypes (Bouayed *et al*., 2009; Salim, 2014). Oxidative damage in limbic regions disrupts synaptic plasticity, monoaminergic signalling, and sustains hyperexcitability linked to anxiety phenotypes. Therapeutic strategies that combine neurotransmitter modulation with antioxidant and anti-inflammatory properties thus hold promise for anxiolysis.

Within this context, *F. iteophylla* and *T. indica* are compelling candidates. Both plants are integral to, and deeply rooted in ethnomedicinal practice across Nigeria and West Africa, traditionally employed in conditions described as restlessness, heat of the head, insomnia, and agitation which are folkloric descriptors that overlap strikingly with anxiety symptomatology (Abdulmalik *et al*., 2011; De Caluwé *et al*., 2010).

- *F. iteophylla* has been shown to contain abundant flavonoids, tannins, alkaloids, and saponins. Its extracts display antioxidant, antimicrobial, and anti-inflammatory activities, and related Ficus species have demonstrated CNS-depressant and sedative-like properties in rodents (Abdulmalik *et al*., 2011). Yet, its specific potential to alleviate anxiety remains underexplored, with limited evidence tying its phytochemical profile to anxiolytic outcomes.

- *T. indica*, a monotypic genus species widely consumed as both food and medicine, is equally rich in flavonoids (catechin, epicatechin, apigenin), polyphenols, and organic acids with potent antioxidant and neuroprotective properties (De Caluwé *et al*., 2010; Bhadoriya *et al*., 2011). Preliminary behavioural studies indicate CNS effects, including anxiolytic-like activity in models such as the elevated plus-maze and potentiation of barbiturate-induced sleep (Siddiq *et al*., 2014; Vuyyala *et al*., 2022), but systematic evaluation of its leaf extracts in validated anxiety models especially with mechanistic correlation to oxidative stress modulation is lacking.

The overlap of phytochemical richness, ethnomedicinal claims, and incomplete pharmacological evaluation places both plants at the frontier of rational anxiolytic discovery. Their dual potential to scavenge free radicals and modulate neurotransmission makes them uniquely suited for screening in experimental anxiety models.

Therefore, this study is designed to systematically evaluate the anxiolytic-like properties of *F. iteophylla* and *T. indica* leaf extracts, determine their safety profiles, and evaluate their antioxidant mechanisms. Establishing such evidence will either validate or refute their traditional use, and may identify novel, affordable, and mechanistically grounded phytotherapeutic agents for anxiety.

1.9 Hypotheses

1.9.1 Null hypothesis (H_0)

F. iteophylla and *T. indica* leaf extracts have no significant anxiolytic-like effects in mice when compared with their sham-treated counterparts.

1.9.2 Alternate hypothesis (H_1)

F. iteophylla and *T. indica* leaf extracts exert significant anxiolytic-like effects in mice when compared with their sham-treated counterparts.

1.10 **Aim and Objectives** of the Study

1.10.1 Aim

The aim of the study was to evaluate the anxiolytic-like activity of the methanol leaf extracts of *F. iteophylla* and *T. indica* in mice.

1.10.2 Objectives

a) To determine the classes of phytoconstituents in the extracts.

b) To determine the acute oral toxicity (LD_{50}) of the extracts.

c) To evaluate the anxiolytic-like effects of the extracts

d) To evaluate the brain antioxidant potentials of the extracts

CHAPTER TWO
MATERIALS AND METHODS

2.1 Materials

2.1.1 Plant source and identification

Fresh leaves of *F. iteophylla* and *T. indica* were collected from the premises of the Faculty of Engineering, University of Benin, Benin City, Edo State, Nigeria, during the month of June 2025. The geographical coordinates of the collection location were obtained from Google Maps were: 6.40348° N, 5.61385° E (*F. iteophylla*) and 6.40162° N, 5.61488° E (*T. indica*). Each plant specimen was taxonomically identified and authenticated by Dr. H. A. Akinibosun, a taxonomist in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria. Voucher specimens were deposited in the departmental herbarium with identification numbers UNIBEN/2025/FI-02 (*F. iteophylla*) and UNIBEN/2025/TI-01 (*T. indica*).

2.1.2 Preparation, Extraction and Concentration

The leaves of both *F. iteophylla* and *T. indica* were thoroughly washed with distilled water and dried at 60°C for one week. The leaves were further dried in an oven at 30°C after which they were ground into fine powder using a British milling machine.

Extraction was carried out for 48 h using 99.8% methanol. For *F. iteophylla*, 300 g of dried powdered leaves was soaked in 1800 ml of methanol. Similarly, 300 g of *T. indica* leaf powder was soaked in 2100 ml of methanol in a separate maceration bottle. The contents of each bottle were shaken vigorously at 0, 24, and 48 h. The mixture was filtered using a clean sieving cloth and the filtrates were evaporated to dryness in a Genlab Oven (Genlab Ltd., Widnes, Cheshire,

United Kingdom) at 40°C for two weeks. The percentage yield of each extract was calculated to be to be 7.5% (MEFI) and 18.73% (METI), using the formula:

$$\text{Percentage yield (\%)} = (\text{Weight of Extract} / \text{Weight of Powdered Material}) \times 100$$

The resulting dried extracts were stored in airtight containers and kept in a fridge at 4°C until further use.

2.1.3 Drugs and Chemicals

The following drugs and chemicals were used:

1. Diazepam (5 mg/kg) – Roche Pharmaceuticals, Germany
2. Methanol (analytical grade) - BDH Chemicals Ltd., England
3. Tween-80 - Merck Chemicals, Germany

All reagents were of analytical grade and freshly prepared on the day of the experiment. Extracts were suspended in 1% Tween 80 solution before administration. Biochemical assays were conducted using a UV-Visible spectrophotometer (Model 721 Visible Spectrophotometer manufactured by Searchtech Instruments, England.) and refrigerated centrifuge (Hettich Universal II manufactured by Andreas Hettich GmbH & Co. KG).

All chemicals and reagents were obtained from reputable sources.

2.1.4 Experimental animals

Seventy-five (75) healthy adult albino mice (*Mus musculus*) of either sex, weighing between 20-30 g, were procured from an accredited animal facility in Ibadan, Nigeria and transported to the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Edo State, Nigeria. The animals were housed in well-ventilated

cages containing clean wood shavings as bedding (changed daily) and maintained under natural conditions of temperature, humidity, and light. They had access to standard rodent pellets and clean tap water *ad libitum.* The animals were allowed a 14-day acclimatization period. The animals were carefully monitored throughout this period to ascertain their well-being and proper adaptation.

All experimental procedures were conducted in strict compliance with internationally accepted guidelines for care and use of laboratory animals (National Research Council, 2011). Ethical clearance for the study was sought from the Faculty of Pharmacy Ethical Committee on Animal Use, University of Benin, Benin City, Edo State, Nigeria.

2.2 Methods

2.2.1 Phytochemical Screening

Standard phytochemical procedures were employed to qualitatively determine the presence of secondary metabolites in the plants samples, as described by Evans (2002).

The powdered leaf samples (2 g each) were weighed and extracted with 20 ml of distilled water. The resulting mixture was gently heated on a water bath for 10 min, allowed to cool to room temperature, and then filtered through Whatman No. 1 filter paper. The obtained filtrate served as the working solution for the subsequent qualitative phytochemical screening.

A. **Test for alkaloids (Mayer's test)**

   The filtrate (2 ml) was transferred into a clean test tube. Two drops of Mayer's reagent was added and the mixture was gently shaken to ensure proper mixing. The formation of a creamy white precipitate indicated the presence of alkaloids.

B. **Test for flavonoids (Shinoda's test)**

The filtrate (2 ml) was mixed with a small piece of magnesium ribbon, followed by the careful addition of 3-5 drops of concentrated hydrochloric acid. The development of a pink, red, or orange coloration confirmed the presence of flavonoids. In some cases, slight heating for 1-2 min enhances color development.

C. **Test for phenolic compounds (ferric chloride test)**

The filtrate (2 ml) was treated with 3-5 drops of freshly prepared 1% ferric chloride ($FeCl_3$) solution. By mixing 2 drops of 5% ferric chloride solution with 5 ml of distilled water, a blank test was conducted. The appearance of a deep blue, green, or black coloration indicated the presence of phenolic compounds.

D. **Test for terpenoids (Salkowski's test)**

The filtrate (2 ml) was mixed with 2 ml of chloroform in a test tube. Then, 3 ml of concentrated sulfuric acid (H_2SO_4) were carefully added (drop by drop) along the inner wall of the test tube (inclined at 45^o) to form a separate layer. The appearance of a reddish-brown coloration at the interface between the two layers signified the presence of terpenoids.

E. **Test for saponins (Frothing test)**

The filtrate (2 ml) was measured into a test tube and shaken vigorously for about 15 min, the mixture was then allowed to stand undisturbed for 10 min. The formation of a stable, persistent froth of at least 1 cm high indicated the presence of saponins. If necessary, a few drops of olive oil may be added; formation of an emulsion further confirms the presence of saponins.

F. **Test for tannins (Ferric chloride test)**

The filtrate (2 ml) was treated with a few drops of 1% ferric chloride solution and gently mixed. The development of a blue-black or green precipitate confirms the presence of tannins.

G. **Test for carbohydrates (Molisch's test)**

The filtrate (2 ml) was treated with 2-3 drops of Molisch's reagent (α-naphthol in ethanol). Then, 2 mL of concentrated sulfuric acid was carefully added down the side of the test tube. Formation of a purple ring at the interface indicates the presence of carbohydrates.

2.2.2 Oral acute toxicity

The acute oral toxicity study was conducted according to the method described by Lorke (1983). A total of thirty-two (32) healthy adult mice were used for the experiment. The study was performed in two phases, and prior to dosing, all animals were acclimatised to laboratory conditions for 3 hours.

A. **Phase I**

In the first phase, twelve (12) mice were randomly divided into four groups of three animals each (n=3). Three groups received *F. iteophylla* leaf extract at single oral doses of 10, 100, and 1000 mg/kg body weight, respectively, while the fourth group received 0.2 ml of Tween-80 (10 ml/kg, 1%v/v p.o.) and served as the vehicle control.

All animals were closely observed for the first 2 hours and then periodically for 24 hours post-administration. Parameters monitored included alertness, sedation, diarrhea, convulsions, and mortality, using the Tween-80 control group as reference. No mortality or signs of acute toxicity were recorded in any group during this phase.

B. **Phase II**

Since no deaths occurred in Phase I, the study proceeded to Phase II using four (4) new mice. These were divided into four groups of one animal each (n=1). Three animals received single oral doses of 1600, 2900, and 5000 mg/kg body weight of *F.iteophylla* leaf extract, respectively, while the fourth animal received 0.2 ml of Tween-80 (p.o.) as control.

Observations for behavioural changes and signs of toxicity were made at 1, 2, 4, 8, and 24 hours post-treatment, using the control animal as reference. No mortality was recorded up to the highest dose of 5000 mg/kg.

The same procedure described above for *F. iteophylla* leaf extract was adopted identically for *T. indica* leaf extract using a separate set of sixteen (16) mice (12 animals forming four groups of n=3 for Phase I and 4 animals forming four groups of n=1 for Phase II). No mortality was observed for *T. indica* either.

From the collected data [i.e., the dose at which no animals died (a) and the dose at which all animals died (b)], the LD_{50} is calculated using the geometric mean:

$$LD_{50} = \sqrt{(a \times b)}$$

Where a = highest non-lethal dose and b = lowest lethal dose.

2.2.3 Evaluation of Anxiolytic-like activity

After the two-week acclimatization period, forty (40) healthy adult mice with no visible signs of illness, distress, or physical deformity were randomly assigned to five distinct experimental groups (n = 4), each consisting of 2 males and 2 females as follows:

- **Group 1 (Negative Control):** Treated with 0.2 ml of Tween 80 (10 ml/kg, 1% v/v).
- **Group 2:** Treated with methanol leaf extract of *Ficus iteophylla* (MEFI) at 100 mg/kg.
- **Group 3:** Treated with MEFI at 200 mg/kg.

- **Group 4:** Treated with MEFI at 400 mg/kg.
- **Group 5:** Treated with diazepam at 0.5 mg/kg.

Suspensions of MEFI were prepared at concentrations of 15.7 mg/ml (Group 2), 37.8 mg/ml (Group 3), and 58.8 mg/ml (Group 4) using Tween 80.

The same experimental grouping was used for methanol leaf extract of *T. indica*. Suspensions of the extract (METI) were prepared at concentrations of 11.0 mg/ml (Group 2), 28.9 mg/ml (Group 3), and 66.8 mg/mL (Group 4).

Treatments were by the oral route using an oro-gastric tube except for the diazepam (0.5 mg/kg) group which was administered intraperitoneal (i.p.).

Behavioral trials were conducted 30 min after treatment (to allow sufficient absorption and onset of pharmacological activity of both the extracts and diazepam), under controlled environmental conditions in a quiet room with consistent lighting to minimize external interference. Tests were carried out between 9:00 a.m. and 5:00 p.m. to minimize circadian variability. The anxiolytic-like activity was evaluated using the hole-board test and elevated plus maze.

A. **Hole-board test (model I)**

The hole-board test was used to evaluate exploratory behavior and anxiety in mice as described by Takeda *et al.* (1998) and more recently validated in similar protocols by Casarrubea *et al*. (2023) The apparatus consists of a wooden square board (40 cm x 40 cm) suspended 25 cm above the ground, with 16 evenly spaced holes, each 3 cm in diameter.

Each mouse was placed gently at the center of the board, away from the observer and allowed to explore freely for 5 min under dim lighting. The number of head dips (when

the mouse inserts its head into a hole to the level of the ears and withdraws it completely) were counted and recorded.

The apparatus was cleaned thoroughly between trials with swab (cotton wool soaked in 70% ethanol) to eliminate the scent cues that might affect subsequent observations. All behavioral recordings were carried out with the observer blind to the treatment the mice had received to prevent bias.

B. **Elevated plus maze (model II)**

The elevated plus maze test is used to evaluate anxiety-related behavior in mice as described by Kraeuter (2019). This method has been widely adopted and remains a standard paradigm in contemporary behavioral neuroscience. The apparatus consists of two open arms (30 cm x 5 cm) and two closed arms of the same dimensions, with 15 cm high opaque walls, arranged opposite to each other to form a plus shape. The maze was elevated 50 cm above the floor to induce a mild aversive stimulus associated with height.

Each mouse was placed gently at the center of the maze, away from the observer, facing one of the open arms, and allowed to explore freely for 5 min under dim lighting. The number of entries into and the time spent in the open and closed arms were recorded. An entry is defined as all four paws entering an arm.

The apparatus was cleaned thoroughly between trials with cotton wool soaked in 70% ethanol to eliminate scent cues that could influence subsequent observations. All behavioral recordings were conducted with the observer blind to the treatment groups to prevent bias.

2.2.4 Antioxidant evaluations

Groups of mice given 0.2 ml/day, 100, 200, and 400 mg/kg/day of the extract for 14 consecutive days. After the last dose on the 14th day, their brains were carefully excised, weighed, suspended in 1 ml of ice-cold normal saline in EDTA bottles, which were then homogenized by crushing and placing in 4 ml normal saline (0.9% w/v NaCl), and later centrifuged at 3000 rpm for 15 min, and stored in the refrigerator for subsequent biochemical analysis.

Activities of antioxidants [superoxide dismutase (SOD), catalase (CAT)], Glutathione reductase (Gr), glutathione peroxidase (GPx), and malondialdehyde (MDA) concentration were analysed using colourimetric methods.

A. **Determination of superoxide dismutase (SOD) activity**

The assay of SOD activity was carried out according to the method described by Misra and Fridovich (1972), based on the inhibition of adrenaline auto-oxidation to adrenochrome. The assay was performed in two stages. For the reference tube, 0.2 ml of distilled water was mixed with 2.5 ml of carbonate buffer, after which 0.3 ml of freshly prepared adrenaline solution was added and rapidly mixed. For the test samples, 2.5 ml of carbonate buffer was first added to each tube, followed by 80 µl of the sample and 120 µl of adrenaline solution. The mixtures were quickly agitated, and absorbance was recorded at 420 nm at 30-120 s intervals using a UV-visible spectrophotometer (Model 721 Visible Spectrophotometer manufactured by Searchtech Instruments, England.). Distilled water served as the blank to calibrate the instrument.

B. **Determination of catalase activity**

The catalase (CAT) activity was determined using the colorimetric method described by Sinha (1972). This method is based on the enzymatic decomposition of hydrogen peroxide (H_2O_2),

followed by the reaction of residual H_2O_2 with potassium permanganate in acidic medium to produce a purple colour that is measured spectrophotometrically.

Distilled water was measured into the blank test tubes while 0.5 ml of the sample was measured into labelled test tubes. A quantity (2.5 ml) of 30 mM hydrogen peroxide was added into both the sample and blank test tubes. After exactly 3 min, the reaction was terminated by adding 1 ml of 6 M H_2SO_4, followed immediately by 3.5 ml of 0.01 M potassium permanganate. Absorbance was read within 30-60 s at 480 nm using a UV-visible spectrophotometer (Model T80+ UV/VIS, PG Instruments Ltd, Leicestershire, UK). A spectrophotometric standard was prepared by adding 3.4 ml of 0.01 M potassium permanganate to a mixture of 5.5 ml of 0.05 M phosphate buffer (pH 7.0) and 1.0 ml of 6 M sulphuric acid solution. The spectrophotometer was zeroed using distilled water.

C. **Determination of glutathione reductase (Gr) activity**

To determine glutathione reductase (Gr) levels, 0.5 ml of 5% trichloroacetic acid (TCA) was added to each test tube, followed by 1.5 ml of the sample. The mixture was allowed to stand briefly, during which a precipitate formed, and then centrifuged for 10 min. After centrifugation, 0.5 ml of Ellman's reagent was added to a new set of clean test tubes, followed by 1.5 ml of phosphate buffer. Subsequently, 2.5 ml of the supernatant from the centrifuged sample was added to the same tubes. The absorbance of each mixture was measured at 416 nm using a UV-visible spectrophotometer (Model 721 Visible Spectrophotometer manufactured by Searchtech Instruments, England.). A standard reference solution, prepared in the same way but without the sample, served as the control for comparison.

D. **Determination of glutathione peroxidase (GPx) activity**

The activity of glutathione peroxidase (GPx) was determined using the colorimetric method originally described by Rotruck *et al.* (1973). This indirect assay quantifies the enzyme-catalysed oxidation of reduced glutathione (GSH) by hydrogen peroxide (H_2O_2); residual GSH is subsequently measured by its reaction with 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB), yielding a yellow chromophore with absorbance read at 450 nm.

Test tubes were prepared with 0.2 M sodium phosphate buffer (pH 7.0) as follows: 1,500 µl for sample tubes, 1,900 µl for control tubes, 1,900 µL for standard tubes, and 2,000 µl for the blank. Subsequently, 100 µl of tissue homogenate was added to the sample and control tubes, while 200 µl of 20 mM reduced glutathione (GSH) solution was added to the sample and standard tubes. The enzymatic reaction was initiated by adding 200 µl of 1.25 mM H_2O_2 to the sample and standard tubes. All tubes were incubated at 37 °C for 10 min.

The reaction was terminated by adding 0.5 ml of 8 % trichloroacetic acid (TCA), followed by centrifugation at 3,000 rpm for 15 min. In a new set of labelled tubes (sample, standard, and blank), 1 ml of the resulting supernatant was mixed with 3 ml of working reagent (0.4 M phosphate buffer pH 7.0 containing 0.4 mM DTNB). After incubation for 30 min at room temperature, absorbance was read at 450 nm using a UV-visible spectrophotometer (Model 721 Visible Spectrophotometer manufactured by Searchtech Instruments, England.).

E. **Determination of malondialdehyde (MDA) concentration**

The concentration of malondialdehyde (MDA), as an index of lipid peroxidation, was determined using the thiobarbituric acid reactive substances (TBARS) colorimetric method originally described by Satoh (1978).

The tissue homogenate (0.6 ml) was mixed with 3 ml of a prepared reagent containing 15% trichloroacetic acid (TCA), 0.375% thiobarbituric acid (TBA), and 0.25 N hydrochloric acid (HCl) mixed in a 1:1:1 ratio (v/v). The mixture was thoroughly vortexed and heated in a boiling water bath for 15 min to allow colour development. After cooling to room temperature, the tubes were centrifuged at 1,000 rpm for 10 min. The absorbance of the supernatant was measured at 535 nm against a reagent blank using a UV-visible spectrophotometer (Model 721 Visible Spectrophotometer manufactured by Searchtech Instruments, England).

2.2.5 Data presentation and Statistical analysis

All data are expressed as mean ± standard error of the mean (SEM), with 'n' as the number of mice in an experimental group. Statistical analysis was performed using One-way analysis of variance (ANOVA) followed by Tukey's post hoc test (GraphPad Prism version 6.0 software). $P < 0.05$ was taken to be statistically significant.

CHAPTER THREE

RESULTS

3.1 Phytochemical Screening

The study revealed that the methanol leaf extracts of *F. iteophyll*a and *T. indica* contained some secondary metabolites as shown in Table 3.1. Both plant extracts revealed the presence of several major classes of secondary metabolites. A key difference was observed in the terpenoid content, as terpenoids were not detectable at the tested assay condition *F. iteophylla*. All other tested phytochemicals were present in both species.

3.2 Oral Acute Toxicity

Oral administration of MEFI and METI up to 5000 mg/kg did not produce any toxic effects in mice. No mortality was observed and both extracts were found to be safe at the given doses. The clinical observations and dose-dependent responses are presented in Table 3.2a and 3.2b for MEFI and METI, respectively. Mild sedation was observed in a single animal at 1600 mg/kg and persisted at the higher doses of 2900 mg/kg and 5000 mg/kg. However, this effect was transient, resolving spontaneously without the need for intervention. Notably, no instances of diarrhea, excessive salivation, or convulsions were recorded at any dose level.

Table 3.1: Phytochemical screening of methanol leaf extracts of *F. iteopphylla* (MEFI) and *T. indica* (METI).

TEST	MEFI	METI
Alkaloids	+	+
Flavonoids	+	+
Phenolic	+	+
Terpenoids	-	+
Saponins	+	+
Tannins	+	+
Carbohydrates	+	+

KEY:

+ = present

– = absent

Table 3.2a: Oral acute toxicity of methanol leaf extract of *F. iteophylla* (MEFI) in mice

Phase	Dose (mg/kg, p.o.)	Symptoms			
		Sedation	Diarrhea	Convulsion	Mortality
I	10	0/3	0/3	0/3	0/3
	100	0/3	0/3	0/3	0/3
	1000	0/3	0/3	0/3	0/3
II	1600	1/1	0/1	0/1	0/1
	2900	1/1	1/1	0/1	0/1
	5000	1/1	1/1	0/1	0/1

Table 3.2b: Oral acute toxicity of methanol leaf extract of *T. indica* (METI) in mice

Phase	Dose (mg/kg, p.o.)	Clinical sign			
		Sedation	Diarrhea	Convulsion	Mortality
I	10	0/3	0/3	0/3	0/3
	100	0/3	0/3	0/3	0/3
	1000	0/3	0/3	0/3	0/3
II	1600	1/1	0/1	0/1	0/1
	2900	1/1	1/1	0/1	0/1
	5000	1/1	1/1	0/1	0/1

LD_{50} > 5000 mg/kg

KEY:

Data are presented as number of animals exhibiting the sign / total number of animals tested at each dose level.

p.o. = *per os* (oral route)

Phase I = range-finding

Phase II = confirmatory phase

3.3 Anxiolytic-like effect of the extracts

3.3.1 Effect of MEFI on HBT

The effect of MEFI (100, 200, and 400 mg/kg, p.o.) on exploratory behaviour in the hole-board test are shown in Figure 3.1. Each mouse was placed individually in the hole-board apparatus and the number of head pokes were noted. With the doses of 100 mg/kg and 400 mg/kg p.o, of MEFI, there was no significant increase in number of head pokes when compared with control group (10 ml/kg of 1% Tween 80), although numerical increases were produced ($p > 0.05$). The extract at 200 mg/kg significantly increased the number of head-dips ($p < 0.05$. The reference standard (diazepam, 0.5 mg/kg, i.p.)-treated group showed significant increase in exploratory activity ($P < 0.01$ vs control).

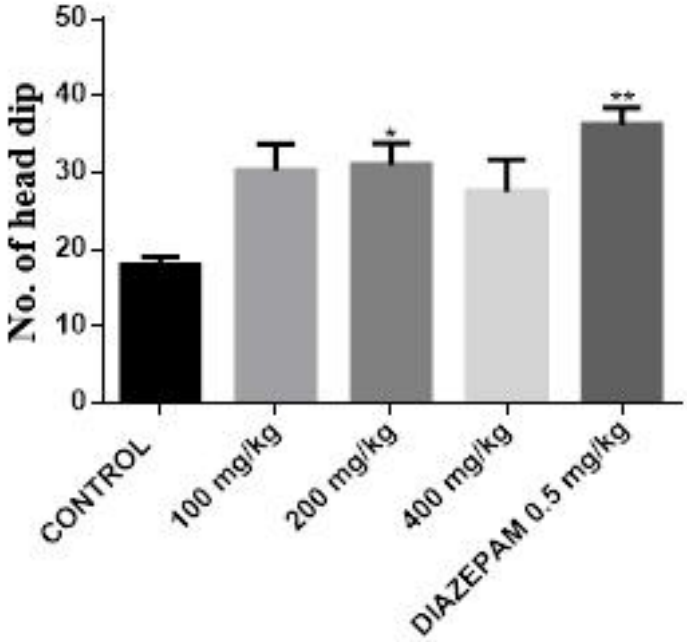

Figure 3.1: Effect of MEFI on the number of head dips by mice in the hole-board. *P<0.05; **P<0.01 versus Control (10 ml/kg, p.o., 1% Tween 80). n=4.

3.3.2 Effect of METI on HBT

The result (Figure 3.2) shows that, compared to the negative control (Tween 80, 10 ml/kg p.o.), METI produced a dose-dependent increase in the number of head dips at 100 mg/kg and 200 mg/kg, with the peak exploratory activity observed at 200 mg/kg ($p < 0.05$ vs negative control), indicating anxiolytic-like effect. This highest head-dip count at 200 mg/kg was comparable to that produced by diazepam (0.5 mg/kg i.p.; $p < 0.05$ vs negative control). The 400 mg/kg dose produced a lower head-dip count than the 200 mg/kg dose but remained higher than the negative control, though the difference was not statistically significant ($p > 0.05$).

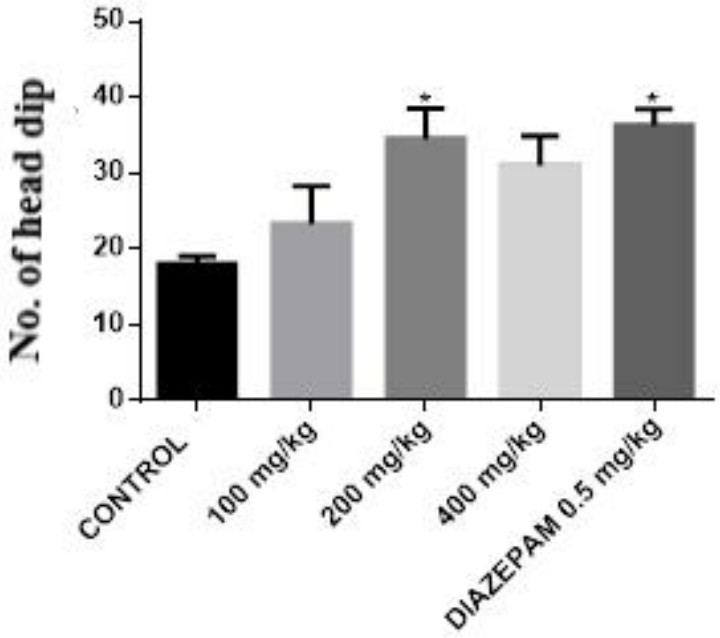

Figure 3.2: Effect of METI on the number of head dips by mice in the hole-board. *P<0.05 versus Control (10 ml/kg, p.o., 1% Tween 80). n=4.

3.3.3 Effect of MEFI on number of open-arm entry in the EPM

The effects of MEFI (100, 200, and 400 mg/kg, p.o.) on the number of open-arm entries in the elevated plus maze are shown in Figure 3.3.

Compared to the negative control (Tween 80, 10 ml/kg p.o.), MEFI produced a dose-dependent increase in the number of open-arm entries up to 200 mg/kg, followed by a mild reduction at 400 mg/kg. The 100 mg/kg dose caused a slight numerical increase above the negative control level. The 200 mg/kg dose produced the highest increase among the extract-treated groups, whereas the 400 mg/kg dose remained higher than the 100 mg/kg dose but lower than the 200 mg/kg dose. None of the doses reached statistical significance ($P > 0.05$ versus negative control). The standard anxiolytic diazepam (0.5 mg/kg, i.p.) produced the highest number of open-arm entries ($P < 0.01$ versus negative control), significantly exceeding all doses of the extract.

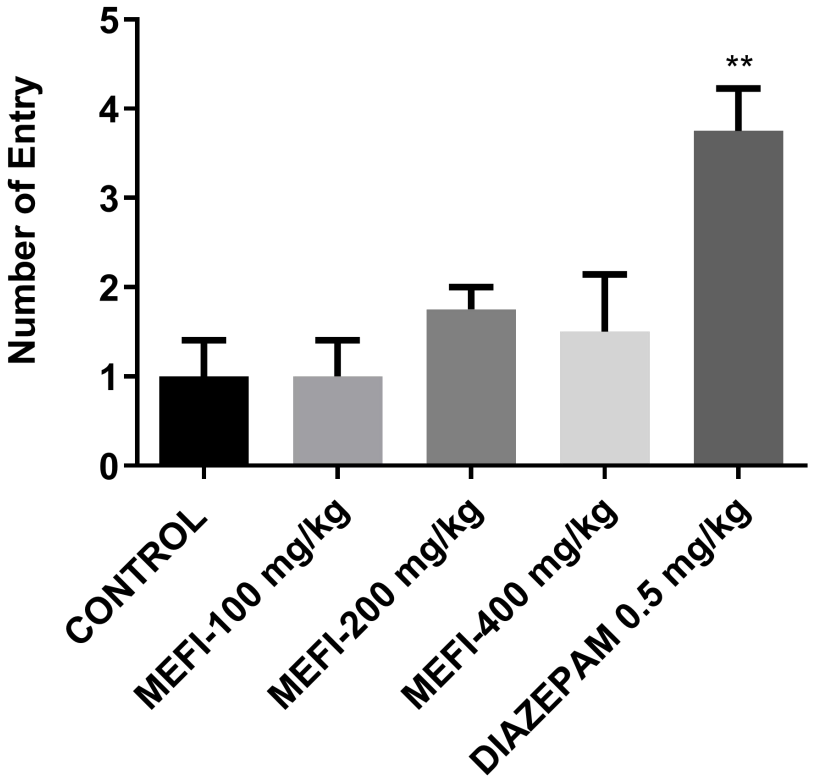

Figure 3.3: Effect of MEFI on the number of open-arm entries by mice in the elevated plus maze. **p<0.01 versus Control (10 ml/kg, p.o., 1% Tween 80). n=4.

3.3.4 Effect of METI on number of open-arm entry in the EPM

The METI (100, 200, and 400 mg/kg, p.o.) on the number of open-arm entries in the elevated plus maze are shown in Figure 3.4. Compared to the negative control (Tween 80, 10 ml/kg p.o.), METI produced a dose-dependent increase in the number of open-arm entries up to 200 mg/kg, followed by a slight reduction at 400 mg/kg. The 100 mg/kg dose caused a modest numerical increase above the negative control. The 200 mg/kg dose produced the highest number of open-arm entries among the extract-treated groups, whereas the 400 mg/kg dose remained higher than the 100 mg/kg dose but lower than the 200 mg/kg dose. None of the doses reached statistical significance ($p > 0.05$ versus negative control). The standard anxiolytic diazepam (0.5 mg/kg, i.p.) produced the highest number of open-arm entries ($p < 0.01$ versus negative control), significantly surpassing all doses of the extract.

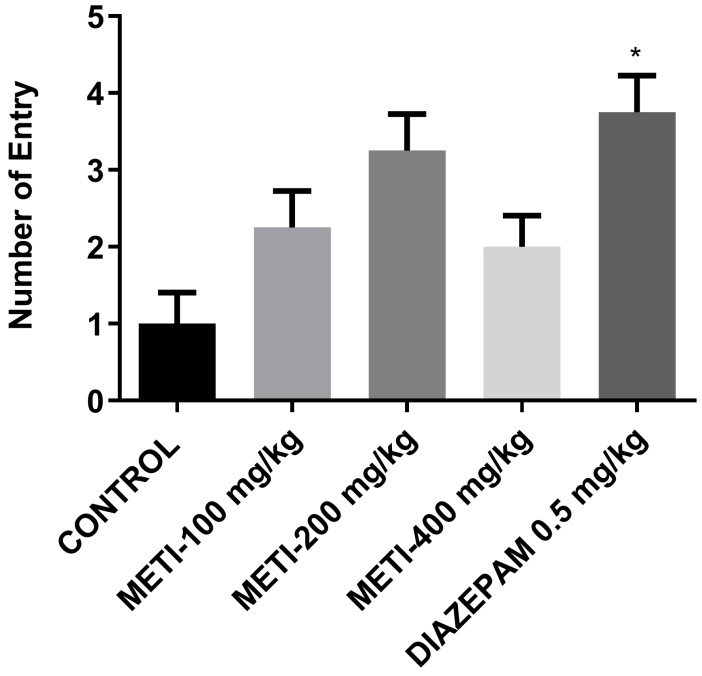

Figure 3.4: Effect of METI on the number of open-arm entries by mice in the elevated plus maze. *p<0.01 versus Control (10 ml/kg, p.o., 1% Tween 80). n=4.

3.3.5 Effect of MEFI on duration of stay in open arms in the EPM

The effects of MEFI (100, 200, and 400 mg/kg, p.o.) on the time spent in the open arms of the elevated plus maze are shown in Figure 3.5. Compared to the negative control (Tween 80, 10 ml/kg p.o.), MEFI increased the time spent in the open arms at 100 mg/kg and 400 mg/kg, whereas the 200 mg/kg dose showed reduced duration compared to both 100 mg/kg and 400 mg/kg. The 100 mg/kg dose produced a modest numerical increase above the negative control. The 400 mg/kg dose produced a further numerical increase, but the 200 mg/kg dose remained below the 100 mg/kg and 400 mg/kg values. None of the doses reached statistical significance ($p > 0.05$ versus negative control). The standard anxiolytic diazepam (0.5 mg/kg, i.p.) produced the longest time spent in the open arms ($p < 0.01$ versus negative control), far exceeding all doses of the extract.

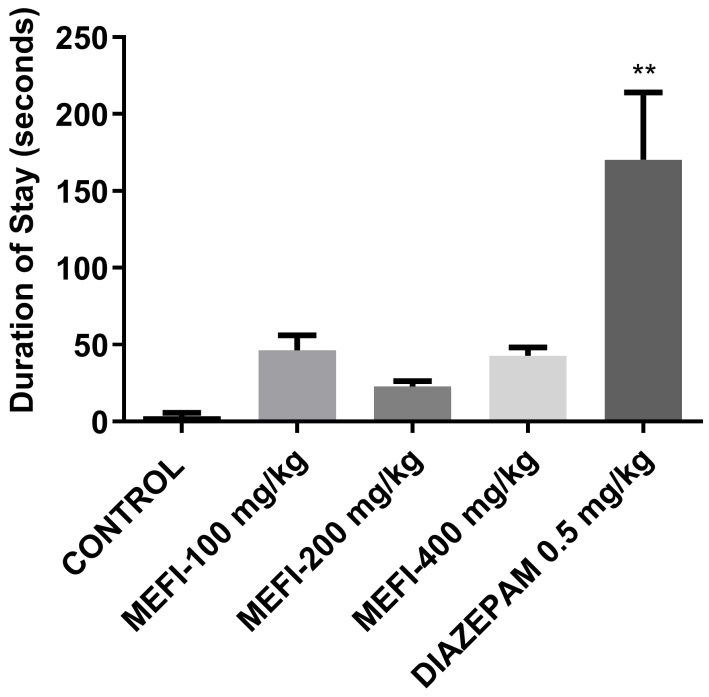

Figure 3.5: Effect of MEFI on duration of stay in open arms (seconds) by mice in the elevated plus maze. **p<0.01 versus Control (10 ml/kg, p.o., 1% Tween 80). n=4.

3.3.6 Effect of METI on duration of stay in open arms in the EPM

The effects of METI (100, 200, and 400 mg/kg, p.o.) on the time spent in the open arms of the elevated plus maze are shown in Figure 3.6.

Compared to the negative control (Tween 80, 10 ml/kg p.o.), METI increased the time spent in the open arms at 100 mg/kg and 400 mg/kg, whereas the 200 mg/kg dose showed reduced duration compared to both 100 mg/kg and 400 mg/kg. The 100 mg/kg dose produced a modest numerical increase above the negative control. The 400 mg/kg dose produced a further numerical increase, but the 200 mg/kg dose remained below the 100 mg/kg and 400 mg/kg values. None of the doses reached statistical significance ($p > 0.05$ versus negative control). The standard anxiolytic diazepam (0.5 mg/kg, i.p.) produced the longest time spent in the open arms ($p < 0.01$ versus negative control), far exceeding all doses of the extract.

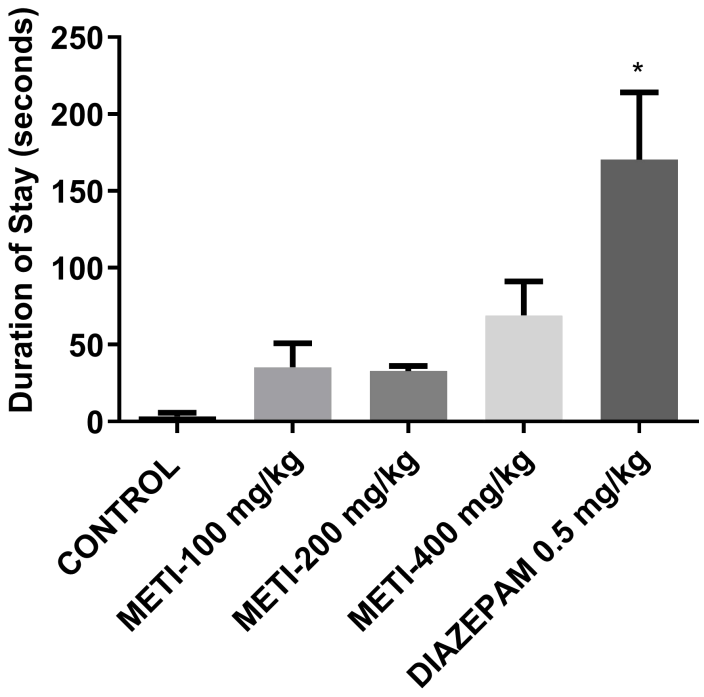

Figure 3.6: Effect of METI on duration of stay in open arms (seconds) by mice in the elevated plus maze. *p<0.01 versus Control (10 ml/kg, p.o., 1% Tween 80). n=4.

3.4 Antioxidant evaluation

The antioxidant properties of MEFI and METI were evaluated using the brain tissue of the animals. The antioxidant parameters measured include; Superoxide dismutase (SOD), Catalase (CAT), Glutathione reductase (Gr), Glutathione peroxidase (GPx), and Malondialdehyde (MDA). Table 3.4a shows the results for MEFI Table 3.4b shows the results for METI.

Table 3.3a: Antioxidant properties of MEFI

ANTIOXIDANT PARAMETER	Gr ($x10^{-4}$)	SOD (U/mg protein $x10^{-2}$)	MDA (nmol/mg protein $x10^{-4}$)	CAT µmol/min/mg protein × 10^{-4}	GPx nmol/min/mg protein × 10^{-4}
Control	7.23±1.72	5.03±0.52	2.91±0.74	8.97±1.41	0.79±0.28
100ng/kg	9.62±2.22	3.43±0.15	3.97±0.61	7.00±0.54	0.47±0.04
200mg/kg	10.85±1.29	5.79±0.95	3.76±0.64	11.51±5.61	0.43±0.21
400mg/kg	8.93±0.71	5.02±0.12	4.47±0.19	4.33±0.93	0.14±0.02
Imipramine	7.55±0.56	5.58±1.80	3.26±0.45	13.52±5.66	0.65±0.31

Values are not significantly different from control. n=4.

Table 3.3b: Antioxidant properties of METI

ANTIOXIDANT PARAMETER	Gr ($x10^{-4}$)	SOD (U/mg protein $x10^{-2}$)	MDA (nmol/mg protein $x10^{-4}$)	CAT µmol/min/mg protein × 10^{-4}	GPx nmol/min/mg protein × 10^{-4}
Control	7.23±1.72	5.03±0.52	2.91±0.74	8.97±1.41	0.79±0.28
100ng/kg	7.01±1.23	5.74±0.75	3.05±0.89	8.79±5.55	0.31±0.07
200mg/kg	9.52±1.08	4.02±0.32	2.99±0.50	12.85±1.73	0.40±0.22
400mg/kg	10.16±1.33	3.68±0.94	3.98±0.65	9.87±4.83	0.38±0.16
Imipramine	7.55±0.56	5.58±1.80	3.26±0.45	13.52±5.66	0.65±0.31

KEY: Gr= Glutathione reductase; SOD= Superoxide dismutase; MDA= Malondialdehyde; CAT= Catalase; GPx= Glutathione peroxidase

CHAPTER FOUR

DISCUSSION

4.1 Phytochemical Constituents and Acute Safety profile

The qualitative phytochemical screening of MEFI and METI revealed the presence of several secondary metabolites, including alkaloids, flavonoids, phenolic compounds, saponins, tannins, and carbohydrates in both extracts, with terpenoids detected exclusively in METI. This phytochemical profile strongly supports earlier reports on both plants and reinforces their ethnomedicinal relevance.

The detection of flavonoids, phenolics, tannins, saponins, and alkaloids in MEFI aligns closely with the findings of Hasnat *et al*. (2024), who reported similar constituents in leaf and bark extracts of *F. iteophylla*, emphasizing flavonoids and phenolics as key players in contributing to antioxidant, anti-inflammatory, and neuroprotective effects (Ahmadu *et al*., 2010). Similarly, Abdulmalik *et al*. (2011) identified flavonoids, steroids, tannins, and saponins in ethanol extracts, corroborates the results obtained in this research and its reported bioactivities and provides a biochemical basis for its traditional use in conditions associated with inflammation and stress.

In METI, the observed phytochemical constituents are consistent with extensive literature describing *T. indica* leaves as chemically diverse and rich in polyphenols, flavonoids, and terpenoids (Bhadoriya *et al*., 2011; Komakech *et al*., 2019). Bhadoriya *et al*. (2011) confirmed alkaloids, saponins, tannins, flavonoids, and terpenoids in leaf extracts, attributing them to anti-inflammatory and analgesic effects. Likewise, Razali *et al*. (2015) noted high polyphenol and

flavonoid (such as catechin, epicatechin, procyanidins, and apigenin) content, associated with antioxidant and neuroprotective functions.

The exclusive presence of terpenoids in METI may stem from species-specific ecological adaptations, as *T. indica* often inhabits arid environments where such compounds aid in resilience to drought and other stressors (Ebifa-Othieno *et al*., 2020). Alkaloids and saponins, shared by both extracts, probably originate from nitrogen-based metabolic routes and function as defensive agents, whereas tannins and phenolics offer astringent properties and antioxidant capabilities, common in Moraceae (Ficus) and Fabaceae (Tamarindus) families (Orwa *et al*., 2009).

However, the absence of terpenoids in MEFI contrasts with some broader reviews on the Ficus genus, where terpenoids (including triterpenoids) are frequently reported in leaf extracts across various species, contributing to antimicrobial and other bioactivities (Madrigal-Santillán *et al*., 2024; Cruz *et al*., 2022). This discrepancy could arise from methodological differences in extraction (e.g., solvent polarity or processing conditions affecting volatile terpenoids) or environmental variables influencing biosynthesis, as terpenoids are sensitive to such factors (Evans, 2002). Therefore, the lack of terpenoids in MEFI does not necessarily negate their presence in trace amounts but highlights phytochemical variability influenced by ecological and methodological factors. No notable contrasts were identified for METI's phytochemical profile or for the shared compounds in both extracts, which consistently align with the referenced literature.

Regarding acute safety, MEFI and METI using Lorke's method revealed LD_{50} values exceeding 5000 mg/kg body weight for both, with no mortality observed across doses up to this threshold and only transient mild sedation at higher levels (1600–5000 mg/kg) (Abdulmalik *et al*., 2011).

This classifies the extracts as practically non-toxic (class 5) according to OECD Guideline 425 and criteria for herbal medicines, indicating a broad therapeutic window suitable for oral administration (OECD, 2022). The absence of severe clinical signs, such as diarrhea or convulsions, underscores minimal acute disruption to gastrointestinal or central nervous system, likely attributable to the protective effects of phenolic compounds and flavonoids that mitigate oxidative stress and inflammation even at supra-therapeutic doses (Ahmadu *et al*., 2010). Supporting evidence for MEFI includes Abdulmalik *et al*. (2011), who reported an intraperitoneal LD50 of 3807.8 mg/kg for ethanol leaf extracts, suggesting route-dependent variability but confirming overall safety, as oral exposure (mimicking traditional use) proves less toxic due to first-pass metabolism and lower bioavailability of potentially irritant saponins. The mild sedation observed at higher doses of MEFI (1600-5000 mg/kg) may be attributed to CNS modulation by flavonoids and alkaloids, which are abundant in Ficus species and are known to interact with GABAergic pathways, potentially inducing transient CNS depression or sedative effects at supratherapeutic levels in rodent models (Savage *et al*., 2018). While direct evidence specifically linking *F. iteophylla* leaf extracts to sedation is limited, similar mild sedative-hypnotic activity has been reported in related Ficus species, such as methanol extracts of *F. exasperata*, where flavonoids and other secondary metabolites were implicated in prolonging sleep duration and reducing locomotor activity in mice (Mikail *et al*., 2019). Importantly, the sedation in the present study was transient, fully reversible, and occurred without convulsions, mortality, or other severe signs, reinforcing the extract's favourable acute safety profile and aligning with previous toxicity evaluations of *F. iteophylla* extracts that reported no lethal effects or major behavioural abnormalities up to 5000 mg/kg (Abdulmalik *et al*., 2011). Similarly, METI's LD_{50} >5000 mg/kg corroborates extensive literature on *T. indica* leaf extracts, showing

no toxicity up to 5000 mg/kg orally; for example, Chigurupati *et al*. (2022) reported safe ethanolic leaf extracts in rats, with no hematological or biochemical changes. Bhadoriya *et al*. (2011) similarly noted non-toxicity in rodents, linking it to the plant's culinary safety. Contradictorily, some reports on high-dose seed coat extracts suggest mild nitric oxide modulation without acute harm but potential subchronic concerns (Komutarin *et al*., 2004), which underscores dose-dependency. However, while the acute toxicity data are reassuring, it is important to note that they do not preclude the possibility of adverse effects following prolonged or repeated exposure. As highlighted by Sookying *et al*. (2022), sub-chronic, chronic, and genotoxicity studies remain necessary to fully characterise the long-term safety of these extracts. Nonetheless, the present findings provide strong evidence supporting the traditional oral use of *F. iteophylla* and *T. indica* leaves in Nigerian ethnomedicine.

4.2 Anxiolytic property

The behavioural assessments using the HBT and EPM revealed partial anxiolytic-like activity of MEFI and METI, limited to the hole-board test.

In the HBT, both extracts produced dose-dependent increases in head-dip frequency compared to the negative control (Tween 80, 10 ml/kg p.o.). The peak exploratory activity was observed at 200 mg/kg ($p<0.05$ versus negative control for both MEFI and METI), indicating anxiolytic-like effect at this dose. This maximum head-dip count was comparable to that of diazepam (0.5 mg/kg i.p.; $p<0.01$ versus negative control). The 100 mg/kg and 400 mg/kg doses produced numerical increases that did not reach statistical significance ($p>0.05$). The biphasic pattern (peak at 200 mg/kg and mild decline at 400 mg/kg) is consistent with a transition from anxiolysis to mild sedation at higher doses, a phenomenon reported for GABAergic modulators (Takeda *et al*., 1998; Casarrubea *et al*., 2023). The significant increase in head-dips aligns with the known

role of secondary metabolites like flavonoids, saponins, which are present in both extracts and frequently modulate GABAergic or serotonergic pathways to promote exploratory behavior in the HBT (Savage *et al*., 2018).

In the EPM, neither extract produced statistically significant anxiolytic-like effects. Compared to the negative control. The number of open-arm entries showed a dose-dependent numerical increase up to 200 mg/kg followed by a mild reduction at 400 mg/kg, but none of the doses differed significantly from the negative control ($p>0.05$). Time spent in open arms exhibited a non-monotonic pattern, with modest numerical elevations at 100 mg/kg and 400 mg/kg but a dip at 200 mg/kg, again, no dose reached statistical significance ($p>0.05$ versus negative control). Diazepam markedly increased both parameters ($p<0.01$ versus negative control), confirming assay sensitivity and validity. The observed dissociation between open-arm entries (highest at 200 mg/kg) and time spent (dip at 200 mg/kg) may reflect mild locomotor stimulation at the peak effective dose in the HBT, leading to more frequent but shorter open-arm visits without altering overall anxiety state (Rodgers and Dalvi, 1997). Closed-arm parameters provided no evidence of anxiolysis. Closed-arm entries and time spent in closed arms showed variable numerical changes across doses, but none differed significantly from the negative control ($p>0.05$).

The limited anxiolytic-like activity observed here contrasts with some reports on related Ficus and Tamarindus species. For instance, methanol extracts of other Ficus leaves (e.g., *F. exasperata, F. hispida*) have shown more consistent anxiolytic effects in both HBT and EPM, often attributed to flavonoids enhancing GABAergic transmission (Mikail *et al*., 2019; Sivaraman *et al*., 2012). Similarly, *T. indica* flower or other extracts have demonstrated anxiolytic potential in EPM and related tests, sometimes with significant open-arm exploration at moderate doses (Vuyyala *et al*., 2022). However, these positive findings often involve different

plant parts (e.g., flowers, bark), extraction solvents, or longer/chronic dosing regimens, which may explain the weaker effects in the present study.

4.3 Antioxidant property

The antioxidant evaluation conducted on brain tissue homogenates from mice treated orally with MEFI or METI at doses of 100, 200, and 400 mg/kg/day for 14 consecutive days revealed no statistically significant alterations in any of the measured parameters compared to the control group ($p > 0.05$). Specifically, activities of superoxide dismutase (SOD), catalase (CAT), glutathione reductase (Gr), and glutathione peroxidase (GPx), together with malondialdehyde (MDA) levels as an index of lipid peroxidation, remained comparable across all treatment groups for both the extracts and the control ($p > 0.05$). These results indicate that, under the conditions of the present study (healthy, unstressed mice with normal baseline antioxidant status and a relatively short treatment duration) the extracts did not elicit detectable modulation of brain oxidative stress markers.

This contrasts with several reports on *F. iteophylla* and *T. indica*, where various extracts (often from leaves, bark, or pulp) have demonstrated robust antioxidant and neuroprotective properties, particularly when administered in models involving oxidative insult or prolonged treatment.

For example, hydromethanolic stem bark extract of *F. sycomorus* significantly lowered MDA levels and elevated glutathione content in the whole brain and temporal lobe of rats subjected to unpredictable chronic mild stress (Foyet *et al*., 2017). Likewise, ethanolic leaf extract of *F. hispida* (200 mg/kg/day, 4weeks) mitigated (amyloid beta) Aβ 25-35-induced oxidative damage in mouse hippocampus, restoring SOD, GPx and Gr activity (Sivaraman *et al*., 2012).

Usman *et* al. (2022) reported that ethanol fruit pulp extract of *T. indica* (200-400mg/kg/day, 28 days) demonstrated neuroprotective and antioxidant effects in chemically-induced neuronal injury models, significantly enhancing antioxidant enzyme activities, reducing lipid peroxidation, and modulating pro-inflammatory cytokines and acetylcholinesterase activity in rats subjected to acrylonitrile-induced oxidative damage, supporting its potential role in attenuating oxidative stress and neuronal injury.

The key factors accounting for the lack of effect in this study can be explained by several methodological differences from the contrasting literature:

i. Short duration of administration (14 days): Most studies reporting clear central effects used extended administration periods (≥28 days), allowing sufficient time for blood-brain barrier penetration, enzyme induction, and accumulation of bioactive polyphenols, flavonoids, tannins, and other constituents.
ii. Use of healthy, non-stressed animals with baseline-normal antioxidant status: The animals in this study were healthy and unstressed, maintaining physiological baseline levels of antioxidant enzymes and low MDA. Positive antioxidant outcomes are far more pronounced in models with induced oxidative imbalance (e.g., chronic stress, amyloid-β infusion), where depleted enzymes and elevated lipid peroxidation create a detectable therapeutic window.
iii. The inherent challenge of polyphenol penetration into brain tissue: Many polyphenolic compounds in methanol leaf extracts exhibit limited blood-brain barrier crossing and rapid peripheral metabolism, often requiring chronic dosing, stress-induced permeability changes, or higher effective cerebral concentrations to influence central antioxidant systems (Razali *et al*., 2015).

The mild, non-significant numerical trends observed (e.g., slight increases in SOD, CAT, Gr, and GPx activities and reductions in MDA at higher doses) are consistent with patterns reported for polyphenol-rich extracts in non-stressed models, where effects remain subtle without an oxidative burden (Razali *et al*., 2015). Thus, these observations do not refute the well-documented in vitro and peripheral antioxidant capacity of both *F. iteophylla* and *T. indica* leaves, but underscore that, significant central neuroprotection likely requires optimised conditions, viz; longer treatment, stress/toxin models, or purified fractions, to become evident.

CHAPTER FIVE

CONCLUSION

5.1 Conclusion

The main focus of this study was to evaluate the putative anxiolytic-like potential of MEFI and METI in mice using the HBT and EPM, alongside assessments of phytochemical composition, oral acute toxicity, and brain antioxidant parameters. Qualitative screening revealed the presence of alkaloids, flavonoids, phenolics, saponins, tannins, and carbohydrates in both extracts, with terpenoids detected exclusively in METI. The oral LD_{50} was greater than 5000 mg/kg for both extracts, indicating relative safety, with only transient mild sedation at high doses. In the HBT, both extracts significantly increased head-dip frequency at 200 mg/kg ($p < 0.05$ vs negative control), suggesting mild anxiolytic-like effects, while 100 and 400 mg/kg doses showed non-significant numerical increases. In the EPM, open-arm entries and time spent in open arms exhibited inconsistent, non-dose-dependent changes with no significant differences from the negative control ($p > 0.05$). Antioxidant evaluations indicated that both MEFI and METI tended to increase superoxide dismutase (SOD), catalase (CAT), glutathione reductase (Gr), and glutathione peroxidase (GPx) activities while reducing malondialdehyde (MDA) levels in a dose-dependent manner, but these changes did not reach statistical significance ($p > 0.05$) across all doses. This research suggests that while MEFI and METI are relatively safe and show mild exploratory enhancement in the HBT, they do not produce robust anxiolytic-like effects in the

tested models. While MEFI and METI possess phytochemical profiles supportive of antioxidant activity, the present 14-day regimen in healthy mice did not produce statistically significant changes in brain oxidative stress parameters, thus, highlight the importance of experimental design in detecting central effects and suggest that further studies incorporating chronic administration, oxidative challenge models, or targeted fractions are warranted to fully explore any potential neuroprotective contributions relevant to ethnomedicinal anxiolytic claims.

Bye and large, the findings do not strongly support the ethnomedicinal claims for anxiety management at the doses evaluated.

5.2 Contribution to knowledge

This study has contributed several key insights to the existing body of knowledge on the neuropharmacological potential of MEFI and METI, particularly in the context of putative anxiolytic-like effects and related biochemical properties in mice. The findings build upon and extend prior ethnomedicinal claims in Nigeria while addressing gaps in experimental validation for these specific leaf methanol extracts.

1. MEFI and METI can mildly enhance exploratory behavior in the hole-board test but lack consistent anxiolytic-like effects in the elevated plus maze.
2. It has shown that these extracts possess a tendency to modulate brain oxidative stress parameters, though without statistical significance, and can be considered relatively safe for oral use based on acute toxicity profiles.

These findings add to the growing evidence base on Nigerian medicinal plants and encourage targeted follow-up research for potential development as complementary agents in neurobehavioral health.

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APPENDIX

The various doses (of the extracts and diazepam) administered was calculated using this formula:

Dose to administer (ml) = Weight of the animal (kg) x Dose (mg/kg) / Concentration of the stock solution (mg/ml)

A volume of 0.2 ml of Tween-80 (10 ml/kg, 1% v/v) was administered to all negative control group.

Picture showing the refrigerated centrifuge used in the course of this study (Hettich Universal II manufactured by Andreas Hettich GmbH & Co. KG).