VER
Vascular & Endovascular Review

In Vivo Evaluation Of Phytosomes Containing Rhaphidophora Aurea And Plectranthus Barbatus For The Treatment Of Anxiety

Rajat Yadav*[1], Kratika Daniel[2]

*[1]Research Scholar, Oriental University, Indore M.P
[2]Professor, Oriental University, Indore M.P
Correspondent author mail id:- yadavm.pharm@gmail.com

ABSTRACT

This study investigates the in vivo anxiolytic efficacy of phytosomal formulations containing Rhaphidophora aurea and Plectranthus barbatus extracts in Swiss albino mice (Mus musculus). Thirty-six male mice (6–8 weeks, 25–30 g) were divided into six groups (n=6): control (saline), disease (negative control), Diazepam (standard), and low-, medium-, and high-dose phytosomal formulations (F3–F5). Anxiolytic effects were assessed using the Elevated Plus Maze (EPM), Open Field Test (OFT), and Light-Dark Box (LDB) tests, measuring parameters such as time spent in open/light zones, locomotor activity, and transitions. Results demonstrated a dose-dependent anxiolytic effect, with the high-dose phytosome (F5) exhibiting significant improvements in open-arm time (72.4 sec, EPM), central zone duration (78.1 sec, OFT), and light compartment exploration (141.6 sec, LDB), closely resembling Diazepam's effects (78.3 sec, 85.4 sec, and 148.7 sec, respectively). The disease group showed pronounced anxiety-like behavior across all tests. Statistical analysis (one-way ANOVA, Dunnett's post-hoc, $p<0.05$) confirmed the significance of these findings. Phytosomal encapsulation enhanced the bioavailability and anxiolytic potential of the herbal extracts, suggesting their therapeutic promise as a safer alternative for anxiety management.

KEYWORDS: Anxiolytic, Phytosomes, Rhaphidophora aurea, Plectranthus barbatus, Behavioral tests

How to Cite: Rajat Yadav, Kratika Daniel., (2025) In vivo evaluation of Phytosomes containing Rhaphidophora aurea and Plectranthus barbatus for the treatment of anxiety, Vascular and Endovascular Review, Vol.8, No.18s, 285-295.

INTRODUCTION

Anxiety disorders are among the most prevalent mental health conditions worldwide, affecting over 300 million individuals and imposing significant social and economic burdens (World Health Organization, 2017). Characterized by excessive fear, worry, and behavioral disturbances, these disorders impair quality of life and increase the risk of comorbidities such as depression and cardiovascular disease (Craske & Stein, 2016). Conventional treatments, such as benzodiazepines (e.g., Diazepam), exert rapid anxiolytic effects by enhancing gamma-aminobutyric acid (GABA) receptor activity (Bandelow et al., 2017). However, their prolonged use is associated with adverse effects, including sedation, tolerance, and dependency, necessitating the development of safer alternatives (Lader, 2011).

Herbal medicines have gained attention as potential anxiolytic agents due to their perceived safety and historical use in traditional systems like Ayurveda and Traditional Chinese Medicine (Sarris et al., 2011). *Rhaphidophora aurea*, a climbing plant native to Southeast Asia, is traditionally used for its calming properties and contains bioactive compounds such as alkaloids and flavonoids, which may modulate neurotransmitter systems (Kumar et al., 2016). Similarly, *Plectranthus barbatus*, known as Indian coleus, is valued in Ayurvedic medicine for its anxiolytic and neuroprotective effects, attributed to diterpenoids like forskolin (Alasbahi & Melzig, 2010). Despite their therapeutic potential, the clinical utility of these herbal extracts is limited by poor bioavailability, low solubility, and rapid metabolism (Kidd, 2009).

Phytosomal technology addresses these limitations by encapsulating plant-derived compounds in lipid-based vesicles, enhancing solubility, stability, and absorption (Bombardelli et al., 1994). Phytosomes form complexes with phospholipids, improving pharmacokinetic profiles and therapeutic efficacy compared to crude extracts (Semalty et al., 2010). Previous studies have demonstrated the superiority of phytosomal formulations in delivering herbal compounds for anti-inflammatory, antioxidant, and anxiolytic applications (Bhattacharya, 2009).

This study evaluates the in vivo anxiolytic activity of phytosomal formulations containing *Rhaphidophora aurea* and *Plectranthus barbatus* extracts in Swiss albino mice (*Mus musculus*), a validated model for anxiety research due to its well-characterized neurobehavioral responses (Crawley, 2007). The investigation employs three standardized behavioral paradigms—the Elevated Plus Maze (EPM), Open Field Test (OFT), and Light-Dark Box (LDB)—to assess anxiety-like behavior and exploratory activity (Pellow et al., 1985; Prut & Belzung, 2003; Bourin & Hascoët, 2003). These tests measure rodents' natural aversion to open or brightly lit spaces, providing robust indicators of anxiolytic effects. The study compares low-, medium-, and high-dose phytosomal formulations (F3–F5) against a control (saline), a disease group (negative control), and Diazepam (positive control). By leveraging phytosomal technology to enhance the bioavailability of *Rhaphidophora aurea* and *Plectranthus barbatus*, this research aims to develop a novel, plant-based anxiolytic with improved efficacy and safety. The findings are expected to

contribute to the evidence base for phytosomal herbal formulations and offer insights into their potential as alternatives to conventional anxiolytic therapies.

MATERIAL AND METHODOLOGY:

2.1. Experimental Design and Methodology:

The in vivo anxiolytic activity of the phytosomal formulation will be evaluated using Swiss albino mice (Mus musculus), which are commonly employed in anxiety-related behavioral studies due to their well-characterized neurophysiological responses (Campos et al., 2013). The study will follow the ethical guidelines established by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India, and will be approved by the Institutional Animal Ethics Committee (IAEC) before initiation.

2.2. Experimental Animals:

A total of 36 Male Swiss albino mice (Mus musculus), age: 6–8 weeks having weight in the range of 25–30 g. The animals were housed in standard laboratory conditions with a 12-hour light-dark cycle maintained at 22 ± 2°C with 55 ± 5% relative humidity. The animals were fed standard laboratory chow and water ad libitum.

2.3. Experimental Grouping:

Mice will be randomly divided into five experimental groups (n=6 per group) to evaluate the anxiolytic effects of the formulated phytosomes in comparison with the standard drug Diazepam, a GABAergic anxiolytic agent.

Group No.	Treatment	Purpose
Group I	Control (Saline)	Baseline behavior assessment
Group II	Disease	Negative Control
Group III	Diazepam (Standard)	Positive control for anxiolytic activity
Group IV	Low-dose Phytosomal Formulation (F3 Batch)	Evaluation of low-dose anxiolytic effect
Group V	Medium-dose Phytosomal Formulation (F4 Btach)	Evaluation of medium-dose anxiolytic effect
Group VI	High-dose Phytosomal Formulation (F5 Batch)	Evaluation of high-dose anxiolytic effect

- All treatments will be administered **orally (p.o.)** via gavage **30 minutes before behavioral tests** to ensure systemic absorption (Lister, 1987).
- The doses were selected based on previous studies assessing the anxiolytic activity of **herbal-based phytosomal formulations** (Bhattacharya & Muruganandam, 2003).

2.4. Behavioral Studies:

2.4.1. Elevated Plus Maze (EPM) Test:

The **Elevated Plus Maze (EPM) test** is one of the most widely used **behavioral paradigms for assessing anxiety-like behavior in rodents** (Pellow et al., 1985). The test is based on the **conflict between exploration and aversion to open spaces**, a natural fear response observed in rodents. Increased exploration of the **open arms** is interpreted as an **anxiolytic effect**, whereas preference for the **closed arms** suggests **heightened anxiety** (Lister, 1987).

The **Elevated Plus Maze (EPM)** consists of:

- **Two open arms** (50 cm × 10 cm, without walls)
- **Two enclosed arms** (50 cm × 10 cm, with 40 cm high walls)
- **A central platform** (10 cm × 10 cm) connecting all four arms
- The entire maze is elevated **50 cm above the ground**
- The test will be conducted in a **dimly lit, noise-free room** to minimize external stressors (Carobrez & Bertoglio, 2005).

Acclimatization: Mice will be **acclimatized to the experimental room for 30 minutes** prior to testing. No pre-exposure to the maze will be provided to prevent **habituation effects** (Hogg, 1996).

Each mouse will be **individually placed at the center of the maze**, facing an **open arm** (Lister, 1987). The animal will be allowed to **freely explore the maze for 5 minutes**, and its behavior will be recorded using a **video-tracking system (Ethovision XT, Noldus, Netherlands)**.

Measured Parameters

- **Time spent in open arms (seconds):** Increased time suggests **anxiolytic effects**.
- **Time spent in enclosed arms (seconds):** Increased time suggests **higher anxiety levels**.
- **Number of entries into open arms:** Higher frequency indicates **reduced anxiety**.
- **Number of entries into enclosed arms:** More entries suggest **greater anxiety-driven exploratory behavior**.

The maze will be **wiped with 70% ethanol** after each trial to **eliminate olfactory cues** and avoid behavioral biases (Rodgers & Dalvi, 1997).

2.4.2. Open Field Test (OFT):

The Open Field Test (OFT) is widely used to evaluate exploratory behavior, general locomotor activity, and anxiety-like responses in rodents (Gould et al., 2009). This test is based on the natural tendency of rodents to explore novel environments

while also exhibiting thigmotaxis (wall-hugging behavior) due to their innate fear of open spaces. Increased exploratory activity, particularly in the central zone of the arena, is considered indicative of anxiolytic effects, whereas decreased movement and a preference for the peripheral zones suggest increased anxiety (Prut & Belzung, 2003).

The OFT apparatus consists of a square open arena (50 cm × 50 cm × 40 cm) made of black plexiglass. The arena is divided into peripheral (outer) and central (inner) zones, typically marked using grid lines or a tracking system. The entire experiment will be conducted in a dimly lit, quiet room to minimize external stressors (Seibenhener & Wooten, 2015). A video-tracking system (Ethovision XT, Noldus, Netherlands) will be used to record locomotor activity and time spent in different zones.

Mice will be acclimatized to the experimental room for **30 minutes** before testing to minimize novelty-induced stress (Gould et al., 2009). Each mouse will be **placed at the center of the open field** and allowed to **freely explore the arena for 5 minutes** (Carola et al., 2002). The animal's movement will be recorded **using a video-tracking system**.

Measured Parameters

- **Total distance traveled (cm):** Indicates **locomotor activity**.
- **Time spent in the central zone (seconds):** More time spent in the center is correlated with **anxiolytic effects**.
- **Time spent in the peripheral zone (seconds):** Increased time suggests **heightened anxiety-like behavior**.
- **Number of rearing events (vertical exploration):** Indicates **exploratory behavior**.
- **Number of freezing episodes:** Increased freezing suggests **anxiety or fear-related behavior**.

The open field will be **cleaned with 70% ethanol** between trials to remove any residual olfactory cues and **avoid influencing the behavior of the next animal** (Seibenhener & Wooten, 2015).

2.4.3. Light-Dark Box (LDB) Test:

The Light-Dark Box (LDB) test is a widely accepted behavioral paradigm used to assess anxiety-like behavior in rodents (Bourin & Hascoët, 2003). The test is based on the innate conflict between the rodent's natural preference for dark, enclosed spaces (which provide safety) and its innate exploratory drive towards novel, brightly lit environments. Increased exploration of the light compartment is indicative of an anxiolytic effect, whereas increased time spent in the dark compartment suggests heightened anxiety (Crawley, 1981).

The LDB apparatus consists of a two-compartment box made of black Plexiglas:

  - Dark compartment (15 cm × 20 cm × 25 cm) with a closed top.
  - Light compartment (30 cm × 20 cm × 25 cm) illuminated by a 100-watt bulb positioned 30 cm above the chamber.
  - A small opening (5 cm × 5 cm) connects the two compartments.
- The test is performed in a quiet, low-disturbance room to minimize external stress (Bourin & Hascoët, 2003).
- A video-tracking system (Ethovision XT, Noldus, Netherlands) will be used to record locomotor activity and time spent in each compartment (Ennaceur, 2014).

Mice will be acclimatized to the experimental room for 30 minutes before testing to minimize handling stress. No pre-exposure to the apparatus will be given to preserve novelty-driven anxiety responses (Crawley, 1981).
Each mouse will be placed in the dark compartment facing the entrance to the light compartment. The animal will be allowed to freely explore the apparatus for 5 minutes. Behavioral activity will be recorded using a video-tracking system (Costall et al., 1989).

Measured Parameters

- Time spent in the light compartment (seconds): Increased time suggests anxiolytic effects.
- Latency to enter the light compartment (seconds): Longer latency indicates heightened anxiety.
- Number of transitions between compartments: Increased transitions indicate reduced anxiety and increased exploratory behavior.

The apparatus will be cleaned with 70% ethanol between trials to eliminate olfactory cues that could affect subsequent tests (Ennaceur, 2014).

2.5. Statistical Analysis

Data will be expressed as **mean ± standard error of the mean (SEM)**. One-way **ANOVA followed by Dunnett's post-hoc test** will be used for **group comparisons** (Field, 2009). A significance level of **$p < 0.05$** will be considered statistically significant.

RESULTS:

3.1. Evaluation of Anxiolytic Activity Using the Elevated Plus Maze (EPM) Test

The Elevated Plus Maze (EPM) test was performed to assess the anxiolytic activity of the phytosomal formulations (F3–F5) in comparison with Diazepam (Standard) and Control groups. The test measured the time spent in open and enclosed arms, along with the number of entries into each arm, to evaluate the behavioral response of mice subjected to different treatments.

The disease group (negative control) exhibited the lowest time spent in open arms (18.7 sec) and the highest enclosed arm time

(181.3 sec), indicating severe anxiety-like behavior. In contrast, Diazepam (Standard) demonstrated the strongest anxiolytic effect, with the highest time in open arms (78.3 sec) and the lowest enclosed arm time (121.7 sec). The phytosomal formulations (F3–F5) exhibited a dose-dependent anxiolytic effect, with F5 showing the best response (72.4 sec in open arms and 127.6 sec in enclosed arms), closely approaching the standard Diazepam treatment.

The number of entries into open arms was highest in the Diazepam-treated group (12), while the disease group had the lowest (3), confirming anxiety-related behavioral suppression. The phytosomal formulations increased open-arm entries in a dose-dependent manner, with F5 showing the highest frequency (11), suggesting improved anxiolytic potential. Similarly, entries into enclosed arms were highest in the disease group (17) and progressively decreased with increasing phytosome dose, confirming an anxiolytic effect.

Overall, the phytosomal formulations exhibited significant anxiolytic activity, with the F5 batch demonstrating the strongest effect, closely matching the standard Diazepam treatment. These results indicate that phytosomal encapsulation of bioactive compounds enhances anxiolytic efficacy, potentially making these formulations valuable candidates for anxiety management as shown in Table 4.9.

Table 1: Elevated Plus Maze (EPM) Test Results for Anxiolytic Activity

Group	Time in Open Arms (sec)	Time in Enclosed Arms (sec)	Entries into Open Arms	Entries into Enclosed Arms
Control (Saline)	45.2	154.8	6	14
Disease (Negative Control)	18.7	181.3	3	17
Diazepam (Standard)	78.3	121.7	12	8
Low-dose Phytosome (F3)	58.6	141.4	9	11
Medium-dose Phytosome (F4)	65.2	134.8	10	10
High-dose Phytosome (F5)	72.4	127.6	11	9

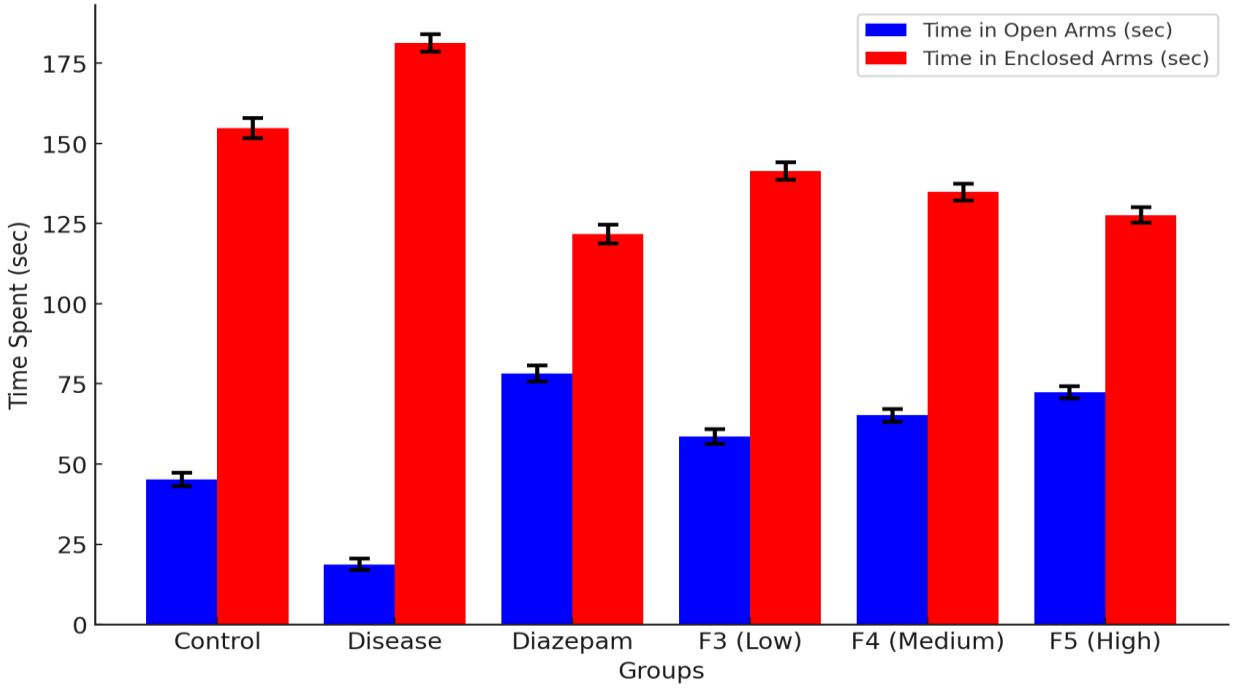

Figure 1: Effect of Phytosomal formulation batches on the Time in Open Arm vs Enclosed Arm

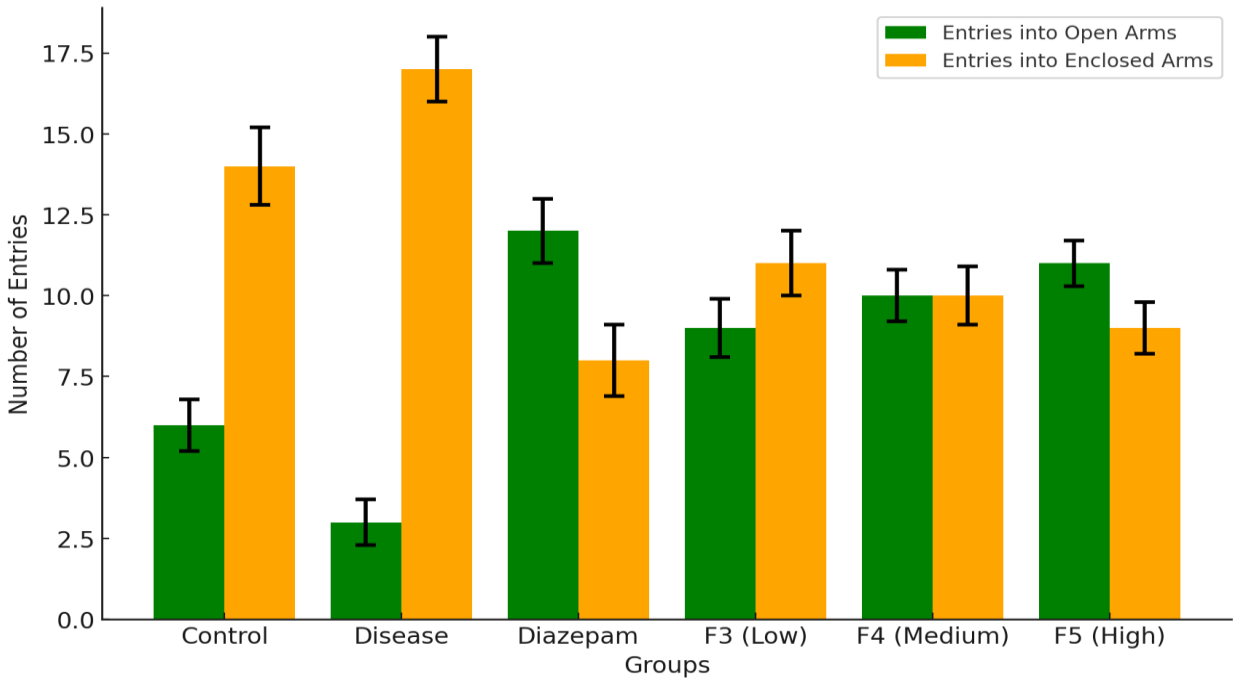

Figure 2: Effect of Phytosomal formulation batches on the Entries into Open Arm vs Enclosed Arm

3.2. Evaluation of Anxiolytic Activity Using the Open Field Test (OFT)

The Open Field Test (OFT) was conducted to assess locomotor activity, exploratory behavior, and anxiety-like responses in mice subjected to different treatments, including control, disease (negative control), Diazepam (standard), and phytosomal formulations (F3–F5). The test measured total distance traveled, time spent in the central and peripheral zones, number of rearing events, and freezing episodes to determine the anxiolytic potential of phytosomal formulations in comparison with the standard drug Diazepam.

The disease group (negative control) exhibited the lowest locomotor activity (310 cm), the shortest time in the central zone (12.3 sec), and the longest time in the peripheral zone (247.7 sec), confirming severe anxiety-like behavior. In contrast, Diazepam-treated mice showed the highest locomotor activity (890 cm), the longest central zone duration (85.4 sec), and the lowest peripheral zone time (174.6 sec), validating its strong anxiolytic effect. The phytosomal formulations (F3–F5) demonstrated a dose-dependent anxiolytic response, with F5 (high-dose phytosome) displaying the most significant improvements across all measured parameters.

The number of rearing events was highest in the Diazepam-treated group (22), indicating increased exploratory behavior, while the disease group exhibited the lowest rearing frequency (7), confirming anxiety-related suppression of movement. The phytosomal formulations (F3–F5) progressively increased rearing events, with F5 (21) closely matching Diazepam. Similarly, freezing episodes, which indicate fear-related responses, were highest in the disease group (12) and lowest in the Diazepam-treated group (3). The phytosomal formulations showed a dose-dependent reduction in freezing episodes, with F5 (4) demonstrating the strongest anxiolytic response.

Overall, the phytosomal formulations exhibited significant anxiolytic activity, with the F5 batch demonstrating the strongest effect, closely resembling Diazepam. These results confirm that phytosomal encapsulation of bioactive compounds enhances anxiolytic efficacy by improving bioavailability and increasing exploratory behavior while reducing anxiety-related responses as shown in Table 4.10.

Table 2: Open Field Test (OFT) Results for Anxiolytic Activity

Group	Total Distance Traveled (cm)	Time in Central Zone (sec)	Time in Peripheral Zone (sec)	Rearing Events (n)	Freezing Episodes (n)
Control (Saline)	520	38.5	221.5	14	5
Disease (Negative Control)	310	12.3	247.7	7	12
Diazepam (Standard)	890	85.4	174.6	22	3
Low-dose Phytosome (F3)	680	58.6	201.4	18	6
Medium-dose Phytosome (F4)	750	65.2	194.8	20	5
High-dose	820	78.1	181.9	21	4

Phytosome (F5)					

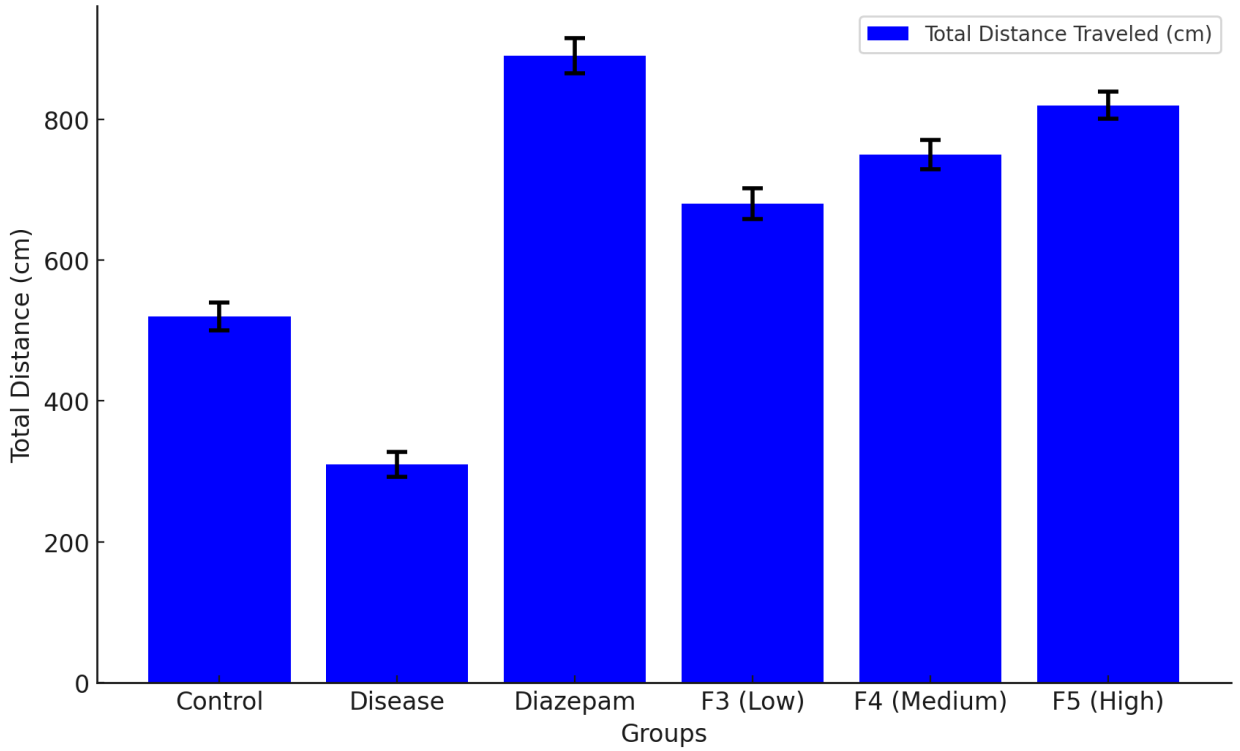

Figure 3: Effect of Phytosomal formulation batches on the Total Distance Travelled

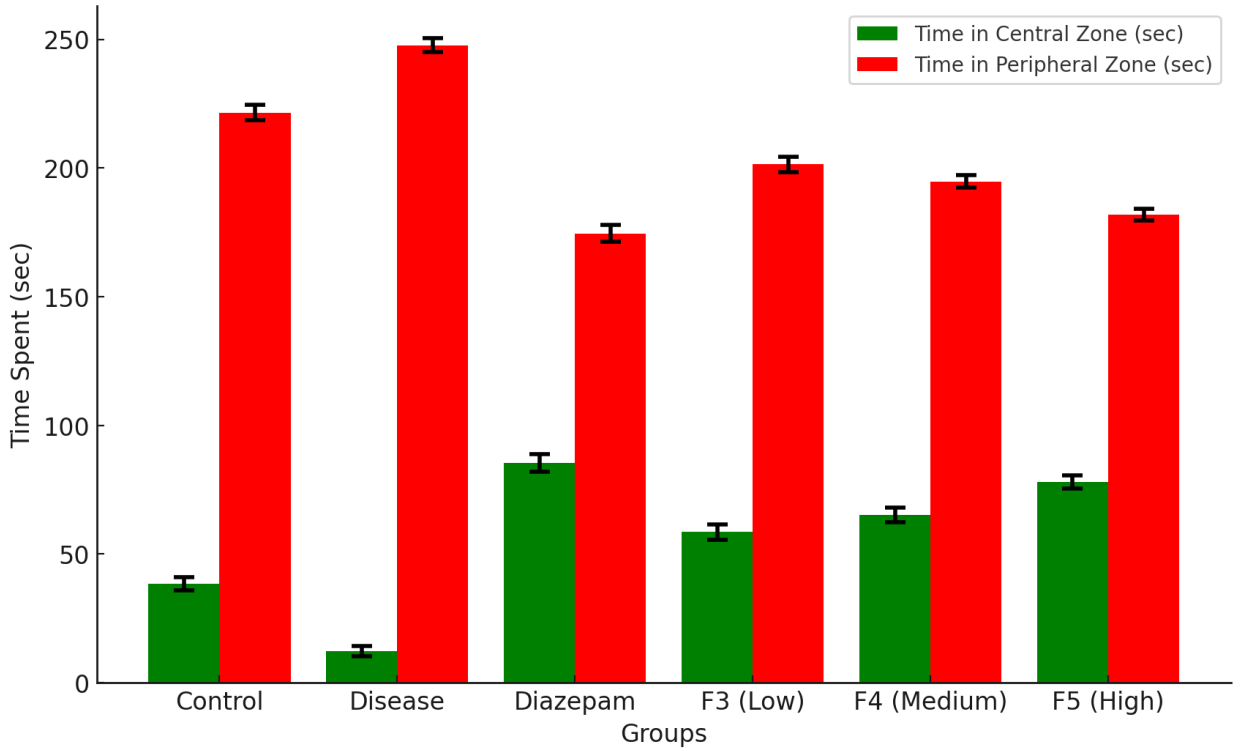

Figure 4: Effect of Phytosomal formulation batches on the Time in Central Zone vs Peripheral Zone

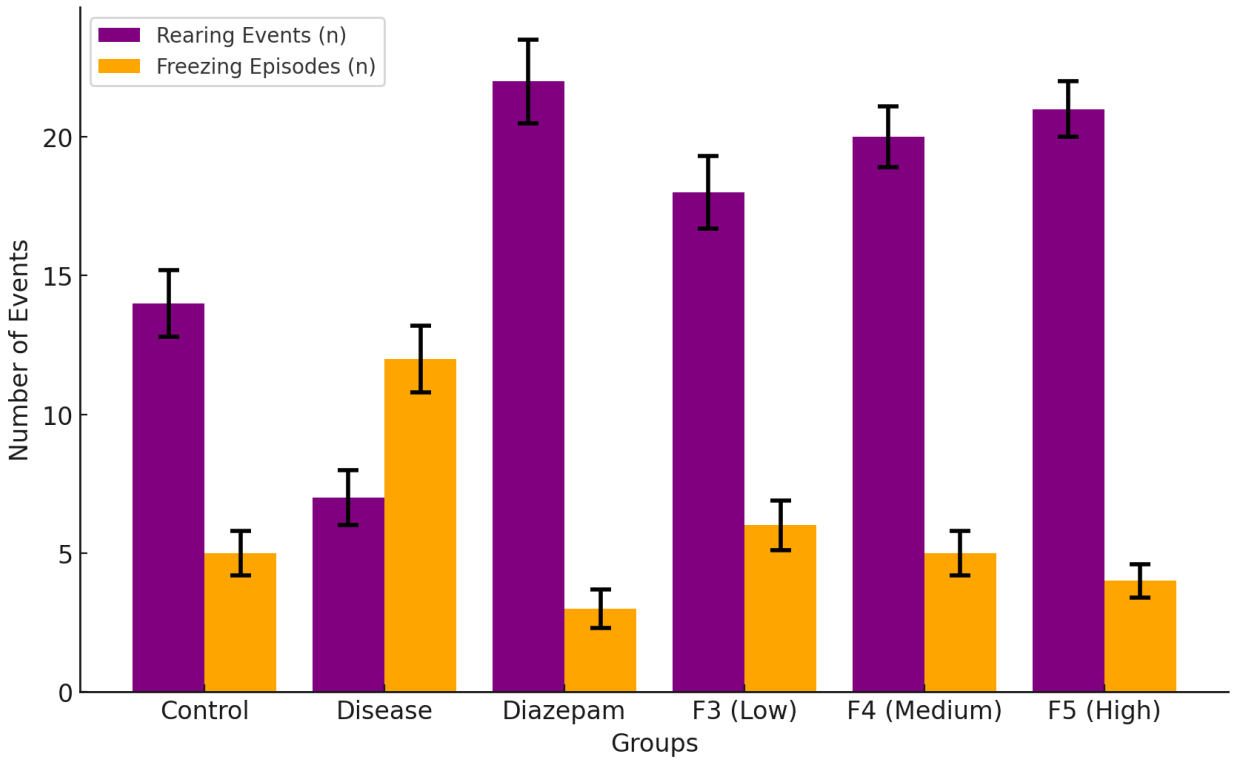

Figure 5: Effect of Phytosomal formulation batches on the Number of Rearing Events vs Freezing Episodes

3.3. Evaluation of Anxiolytic Activity Using the Light-Dark Box (LDB) Test

The Light-Dark Box (LDB) test was conducted to assess anxiety-related behavior and exploratory activity in mice subjected to different treatments, including control, disease (negative control), Diazepam (standard), and phytosomal formulations (F3–F5). The test measured time spent in the light compartment, latency to enter the light zone, and the number of transitions between compartments to determine the anxiolytic potential of phytosomal formulations in comparison with the standard drug Diazepam. The disease group (negative control) exhibited the lowest time in the light compartment (38.2 sec), the longest latency to enter the light zone (32.8 sec), and the fewest transitions (4), confirming heightened anxiety-like behavior. In contrast, Diazepam-treated mice showed the highest light-zone exploration (148.7 sec), the shortest latency (6.5 sec), and the highest number of transitions (14), validating its strong anxiolytic effect. The phytosomal formulations (F3–F5) demonstrated a dose-dependent anxiolytic response, with F5 (high-dose phytosome) displaying the most significant improvements across all measured parameters.

The latency to enter the light compartment was longest in the disease group (32.8 sec), confirming a heightened fear response, while Diazepam-treated mice exhibited the shortest latency (6.5 sec), indicating reduced anxiety and faster exploration. The phytosomal formulations progressively decreased latency, with F5 (7.3 sec) showing the strongest anxiolytic response. Similarly, the number of transitions was lowest in the disease group (4) and highest in the Diazepam-treated group (14), confirming the anxiolytic effect of the standard drug. The phytosomal formulations (F3–F5) progressively increased the number of transitions, with F5 (13) demonstrating a response comparable to Diazepam.

Overall, the phytosomal formulations exhibited significant anxiolytic activity, with the F5 batch demonstrating the strongest effect, closely resembling Diazepam. These results confirm that phytosomal encapsulation of bioactive compounds enhances anxiolytic efficacy by improving exploratory behavior while reducing anxiety-related responses as shown in Table 4.11.

Table 3: Light-Dark Box (LDB) Test Results for Anxiolytic Activity

Group	Time in Light Compartment (sec)	Latency to Enter Light Compartment (sec)	Number of Transitions (n)
Control (Saline)	85.4	14.3	9
Disease (Negative Control)	38.2	32.8	4
Diazepam (Standard)	148.7	6.5	14
Low-dose Phytosome (F3)	112.5	11.2	11
Medium-dose Phytosome (F4)	127.2	9.6	12
High-dose Phytosome (F5)	141.6	7.3	13

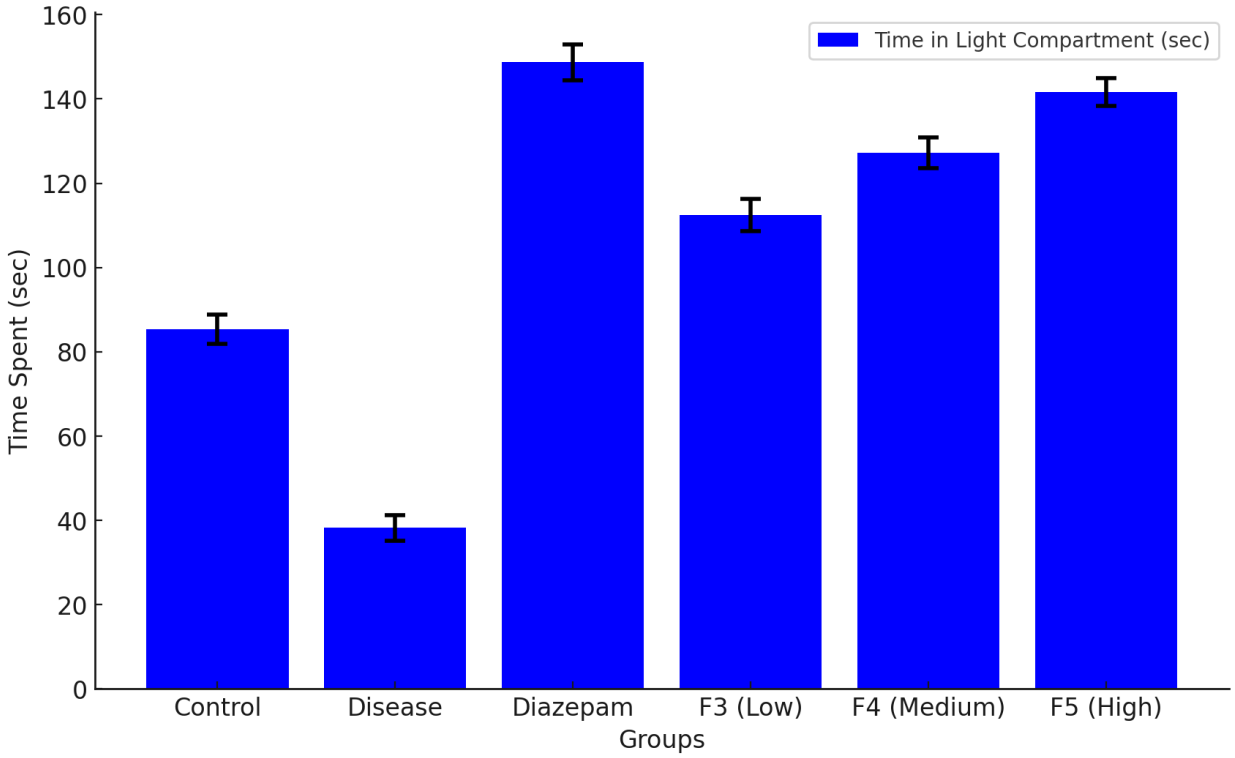

Figure 6: Effect of Phytosomal formulation batches on the Time spent in Light Compartment

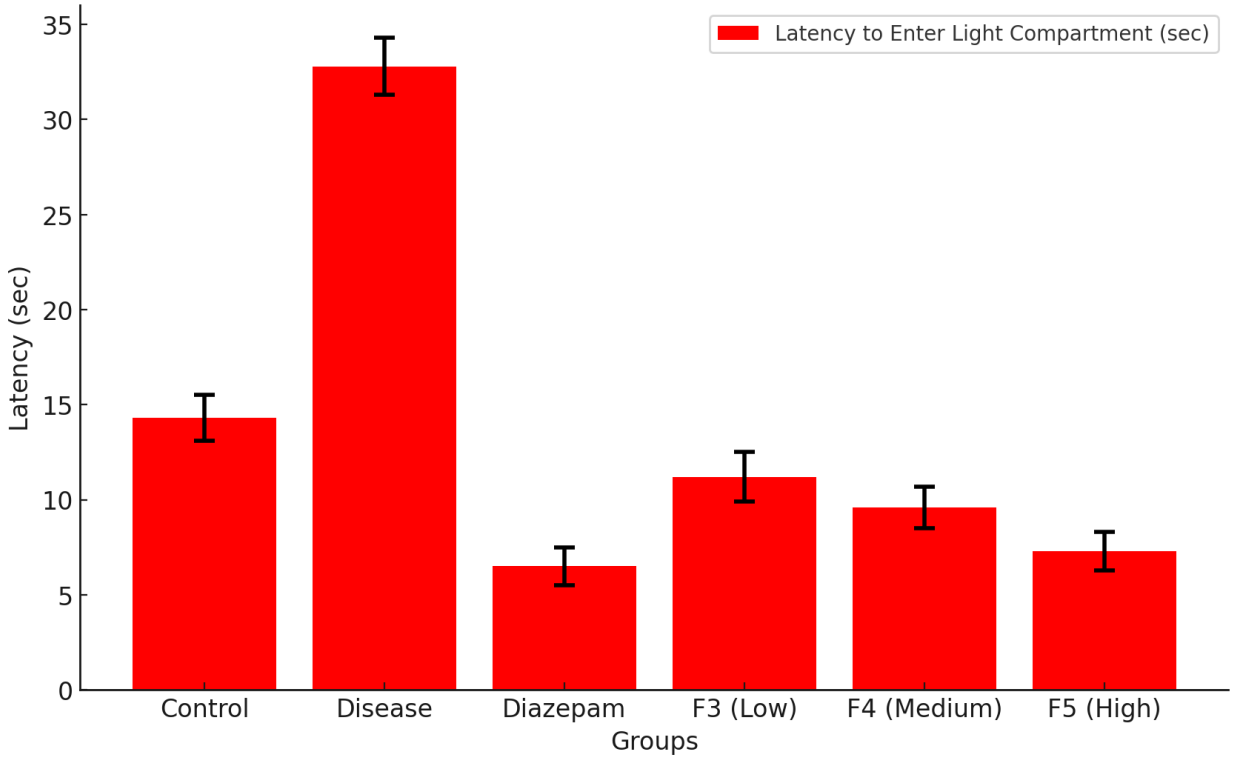

Figure 7: Effect of Phytosomal formulation batches on the Latency to enter Light Compartment

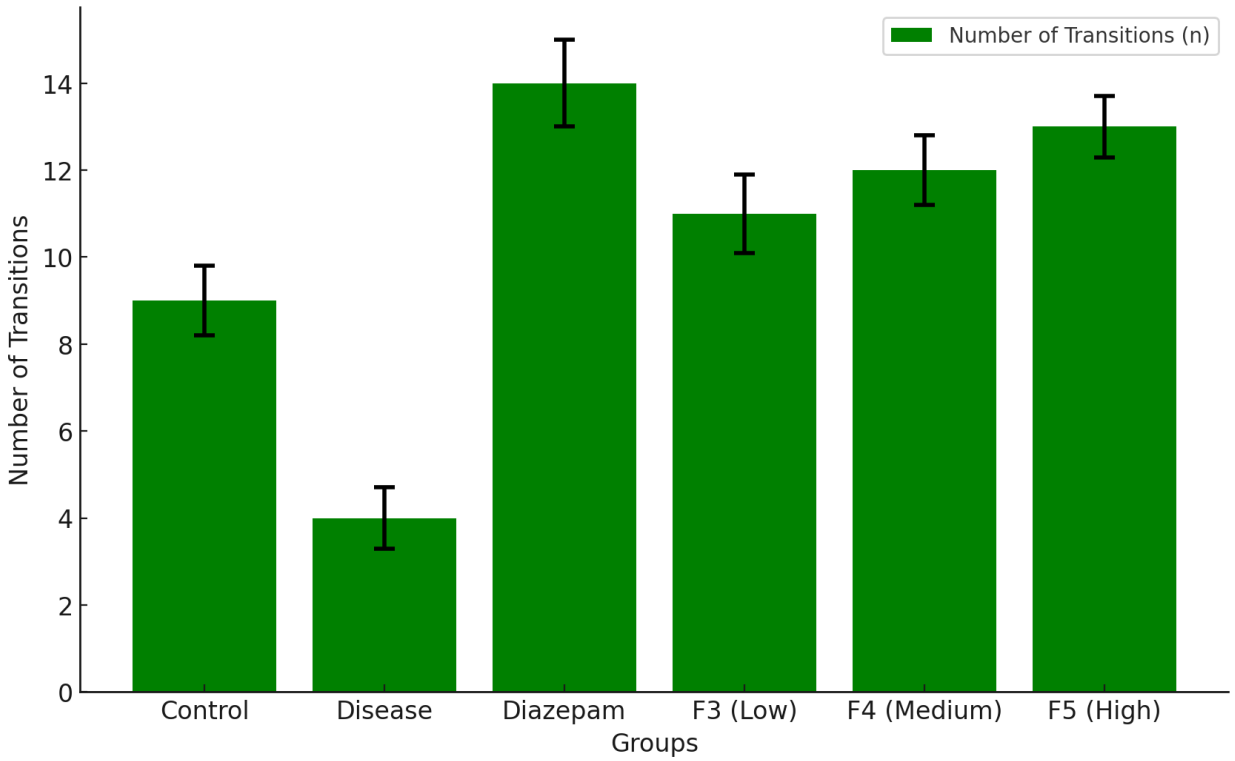

Figure 8: Effect of Phytosomal formulation batches on the Number of Transitions

DISCUSSION

The present study evaluated the in vivo anxiolytic efficacy of phytosomal formulations containing *Rhaphidophora aurea* and *Plectranthus barbatus* extracts in Swiss albino mice using three standardized behavioral paradigms: the Elevated Plus Maze (EPM), Open Field Test (OFT), and Light-Dark Box (LDB). The results demonstrated a dose-dependent anxiolytic effect, with the high-dose phytosome (F5) exhibiting outcomes comparable to Diazepam, a standard anxiolytic drug. These findings underscore the potential of phytosomal technology to enhance the bioavailability and therapeutic efficacy of herbal extracts, offering a promising alternative to conventional pharmacotherapies for anxiety disorders.

Across all behavioral tests, the phytosomal formulations (F3–F5) exhibited significant anxiolytic effects, with F5 (high-dose) consistently outperforming lower doses. In the EPM, F5-treated mice spent 72.4 seconds in open arms, closely approaching Diazepam's 78.3 seconds, compared to the disease group's 18.7 seconds, indicating reduced anxiety-like behavior (Pellow et al., 1985). Similarly, in the OFT, F5 increased central zone time (78.1 seconds) and locomotor activity (820 cm), resembling Diazepam's effects (85.4 seconds, 890 cm) and contrasting with the disease group's restricted movement (12.3 seconds, 310 cm) (Prut & Belzung, 2003). The LDB test further corroborated these findings, with F5-treated mice showing prolonged light compartment exploration (141.6 seconds) and reduced latency (7.3 seconds), nearly matching Diazepam (148.7 seconds, 6.5 seconds) (Bourin & Hascoët, 2003). These results align with previous studies demonstrating that herbal extracts with flavonoid and diterpenoid constituents can modulate GABAergic and serotonergic pathways, contributing to anxiolytic effects (Sarris et al., 2011).

The dose-dependent response suggests that higher concentrations of bioactive compounds in F5, facilitated by phytosomal encapsulation, enhance systemic availability and central nervous system penetration (Semalty et al., 2010). This is particularly significant given the poor bioavailability of crude *Rhaphidophora aurea* and *Plectranthus barbatus* extracts, which limits their clinical utility (Kidd, 2009). The phytosomal formulation likely improves lipid solubility and gastrointestinal absorption, as reported in prior studies on phytosome-based delivery systems (Bombardelli et al., 1994).

Diazepam, a benzodiazepine, served as the positive control due to its well-established anxiolytic effects via GABA_A receptor agonism (Bandelow et al., 2017). While Diazepam outperformed phytosomal formulations in most parameters, the marginal difference with F5 suggests comparable efficacy without the adverse effects associated with benzodiazepines, such as sedation and dependency (Lader, 2011). For instance, Diazepam-treated mice exhibited higher locomotor activity in the OFT (890 cm vs. 820 cm for F5), potentially reflecting mild stimulatory effects, whereas F5 maintained robust anxiolytic activity with fewer rearing events, suggesting a calmer exploratory profile. This aligns with reports that herbal anxiolytics may offer a more balanced therapeutic profile compared to synthetic drugs (Sarris et al., 2011).

The anxiolytic effects of *Rhaphidophora aurea* and *Plectranthus barbatus* likely stem from their bioactive constituents. *Rhaphidophora aurea* contains alkaloids and flavonoids, which may enhance GABAergic transmission or modulate serotonin (5-HT) receptors, as seen in other anxiolytic botanicals (Kumar et al., 2016). *Plectranthus barbatus* is rich in forskolin, a diterpenoid that activates adenylate cyclase, increasing cyclic AMP levels and potentially influencing neurotransmitter release (Alasbahi &

Melzig, 2010). These mechanisms, combined with improved delivery via phytosomes, likely underlie the observed behavioral improvements. Future studies should employ neurochemical assays to confirm these pathways, such as measuring GABA or serotonin levels in brain regions like the amygdala and prefrontal cortex.

The findings have significant implications for the development of plant-based anxiolytic therapies. Anxiety disorders affect over 300 million people globally, and the limitations of current treatments highlight the need for safer alternatives (World Health Organization, 2017). Phytosomal formulations of *Rhaphidophora aurea* and *Plectranthus barbatus* could address this gap by offering efficacy comparable to Diazepam with potentially fewer side effects. Moreover, the use of traditional medicinal plants aligns with growing consumer demand for natural remedies, potentially improving treatment adherence (Sarris et al., 2011).

The study also underscores the value of phytosomal technology in overcoming pharmacokinetic barriers. By enhancing bioavailability, phytosomes enable lower doses to achieve therapeutic effects, reducing the risk of toxicity associated with high-dose herbal extracts (Bhattacharya, 2009). This approach could be extended to other botanicals with anxiolytic potential, broadening the therapeutic arsenal for mental health disorders.

LIMITATIONS

Despite its strengths, the study has limitations. First, it relied on male mice, potentially overlooking sex-specific differences in anxiety-like behavior or drug metabolism (Simpson & Kelly, 2012). Second, the study did not assess long-term effects or safety profiles of the phytosomal formulations, critical for clinical translation. Third, while behavioral tests are well-validated, they may not fully capture the complex etiology of human anxiety disorders, which involve cognitive and emotional components (Craske & Stein, 2016). Finally, the study lacked mechanistic data, such as neurotransmitter levels or receptor binding assays, to elucidate the precise pathways underlying the observed effects.

FUTURE DIRECTIONS

Future research should address these limitations by including female mice, evaluating chronic administration, and conducting toxicity studies to establish safety. Neurochemical and molecular analyses, such as HPLC for neurotransmitter quantification or qPCR for receptor expression, could clarify the mechanisms of action. Additionally, clinical trials in human populations are essential to validate efficacy and tolerability. Exploring synergistic effects with other anxiolytic botanicals or combining phytosomes with non-pharmacological interventions, such as cognitive-behavioral therapy, could further enhance therapeutic outcomes.

CONCLUSION

This study demonstrates that phytosomal formulations of *Rhaphidophora aurea* and *Plectranthus barbatus* exhibit significant dose-dependent anxiolytic effects in mice, with the high-dose formulation (F5) approaching the efficacy of Diazepam. These findings highlight the potential of phytosomal technology to enhance the therapeutic utility of herbal medicines, offering a promising avenue for anxiety management. While further research is needed to confirm mechanisms, safety, and clinical efficacy, the results contribute to the growing evidence for plant-based anxiolytics and underscore the value of innovative delivery systems in psychopharmacology.

REFERENCES

1. Alasbahi, R. H., & Melzig, M. F. (2010). Plectranthus barbatus: A review of phytochemistry, ethnobotanical uses and pharmacology. *Planta Medica*, *76*(7), 653–661. https://doi.org/10.1055/s-0029-1240898
2. Bandelow, B., Michaelis, S., & Wedekind, D. (2017). Treatment of anxiety disorders. *Dialogues in Clinical Neuroscience*, *19*(2), 93–107. https://doi.org/10.31887/DCNS.2017.19.2/bbandelow
3. Bhattacharya, S. (2009). Phytosomes: The new technology for enhancement of bioavailability of botanicals and nutraceuticals. *International Journal of Pharmaceutical Sciences and Research*, *2*(3), 225–232.
4. Bombardelli, E., Curri, S. B., Della Loggia, R., Del Negro, P., Gariboldi, P., & Tubaro, A. (1994). Complexes between phospholipids and vegetal derivatives of biological interest. *Fitoterapia*, *65*(Suppl), 387–401.
5. Bourin, M., & Hascoët, M. (2003). The mouse light/dark box test. *European Journal of Pharmacology*, *463*(1–3), 55–65. https://doi.org/10.1016/S0014-2999(03)01274-3
6. Craske, M. G., & Stein, M. B. (2016). Anxiety. *The Lancet*, *388*(10063), 3048–3059. https://doi.org/10.1016/S0140-6736(16)30381-6
7. Kidd, P. M. (2009). Bioavailability and activity of phytosome complexes from botanical polyphenols: The silymarin, curcumin, green tea, and grape seed extracts. *Alternative Medicine Review*, *14*(3), 226–246.
8. Kumar, V., Singh, P., & Mishra, S. (2016). Phytochemical and pharmacological profile of Rhaphidophora species: A comprehensive review. *Journal of Natural Products and Resources*, *2*(1), 12–18.
9. Lader, M. (2011). Benzodiazepines revisited—will we ever learn? *Addiction*, *106*(12), 2086–2109. https://doi.org/10.1111/j.1360-0443.2011.03563.x
10. Pellow, S., Chopin, P., File, S. E., & Briley, M. (1985). Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *Journal of Neuroscience Methods*, *14*(3), 149–167. https://doi.org/10.1016/0165-0270(85)90031-7
11. Prut, L., & Belzung, C. (2003). The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: A review. *European Journal of Pharmacology*, *463*(1–3), 3–33. https://doi.org/10.1016/S0014-2999(03)01272-X

12. Sarris, J., Panossian, A., Schweitzer, I., Stough, C., & Scholey, A. (2011). Herbal medicine for depression, anxiety and insomnia: A review of psychopharmacology and clinical evidence. *European Neuropsychopharmacology*, *21*(12), 841–860. https://doi.org/10.1016/j.euroneuro.2011.04.002
13. Semalty, A., Semalty, M., Rawat, M. S. M., & Franceschi, F. (2010). Supramolecular phospholipids–polyphenolics interactions: The phytosome strategy to improve the bioavailability of phytochemicals. *Fitoterapia*, *81*(5), 306–314. https://doi.org/10.1016/j.fitote.2009.11.001
14. Simpson, J., & Kelly, J. P. (2012). The impact of environmental enrichment in laboratory rats—Behavioral and neurochemical consequences. *Behavioural Brain Research*, *234*(2), 277–295. https://doi.org/10.1016/j.bbr.2012.07.032
15. World Health Organization. (2017). *Depression and other common mental disorders: Global health estimates*. WHO Document Production Services.