

# Comparative Study of Anti-Anxiety Effects of Synthetic and Phytoconstituent Compounds in Swiss Albino Mice

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## ABSTRACT

This experimental study sought to comparatively evaluate the anti-anxiety actions of a synthetic anxiolytic drug, diazepam, and a phytoconstituent, *Withania somnifera* (Ashwagandha), through standardized behaviour models in Swiss albino mice. A randomized controlled trial with three groups control, diazepam-treated, and *Withania somnifera*-treated was undertaken, in which behaviour modifications were assessed through the Elevated Plus Maze (EPM) and Open Field Test (OFT). The research proved that diazepam as well as *Withania somnifera* effectively prolonged open arm time and improved locomotor activity, reflecting decreased anxiety levels. Statistical analysis through ANOVA and Tukey's post-hoc test showed that although slightly more potent, the phytoconstituent had similar anxiolytic activity with no statistically significant difference in most measures. This result indicates the possibility of *Withania somnifera* as an effective and natural substitute for artificial anxiolytics, removing dependency and side effect concerns typical of traditional medication. The work adds new comparative data to the literature and inspires further investigation of plant-based interventions for anxiety disorders, affirming the therapeutic importance of incorporating herbal medicine into general pharmacological treatment.

## Key Words:

Anti-Anxiety, Synthetic, Phytoconstituent Compounds, Swiss Albino Mice, Elevated Plus Maze, Open Field Test

## Article History:

Received Dec 18, 2024

Accepted Jan 29, 2025

Published April 30, 2025

## 1. INTRODUCTION

Anxiety disorders are a widespread mental condition impacting millions across the globe [1]. The conventional medicines such as benzodiazepines, acting through the modulation of brain neurotransmitter systems, work effectively but come with adverse effects such as sedation and impaired

cognitive functioning [2]. Therefore, the search has intensified for alternatives that pose minimal adverse effects. Plant-derived phytoconstituents such as *Withania somnifera* have exhibited anxiolytic-like activities in preclinical investigations [3]. Ashwagandha, an herb taken in Ayurvedic medicine, has also been shown to reduce anxiety and stress by stabilizing cortisol

levels and promoting mental wellness [4]. Very little research has contrasted *Withania somnifera* with synthetic anxiolytics such as diazepam directly, so this research fills the void through a controlled study comparing diazepam's anti-anxiety efficacy with *Withania somnifera* on Swiss albino mice [5]. This research provides more evidence that plant-based treatments can be effective alternatives to pharmaceuticals for anxiety disorders and provides insight into the potential of *Withania somnifera* as a natural alternative to medications like diazepam [6].

### 1.1. Background Information

Anxiety disorders are among the most prevalent mental illnesses in the world, and they are typically managed with synthetic anxiolytic drugs such as benzodiazepines [7]. These medications, though effective, may also be linked to undesirable effects such as sedation, addiction, and withdrawal symptoms, leading to interest in natural, plant-derived alternatives [8]. Phytoconstituents, especially those from medicinal plants like *Withania somnifera* (Ashwagandha), have been demonstrated in traditional and contemporary therapeutic uses to manage anxiety [9]. Studying their effectiveness compared to conventional synthetic drugs provides useful information about safer, more holistic forms of treatment [10].

### 1.2. Statement of the Problem

Even though synthetic anxiolytics are extensively used, their side effects restrict prolonged usage. An increased interest is being generated to identify and authenticate alternative drugs with fewer side effects. Comparative studies evaluating the efficacy of phytoconstituents as compared to synthetic drugs in animal models are sparse.

This study fills this lacuna by comparing the behavioural effect of a synthetic drug (diazepam) and a phytoconstituent (*Withania somnifera*) in Swiss albino mice through standardized models of anxiety evaluation.

### 1.3. Objectives of the Study

The research objectives of the study are:

- To compare the anti-anxiety effects of a synthetic anxiolytic (diazepam) and a phytoconstituent extract (*Withania somnifera*) in Swiss albino mice.
- To assess behavioural changes using the EPM and OFT.
- To determine whether *Withania somnifera* provides a comparable anxiolytic effect to diazepam, suggesting its potential as a natural alternative for anxiety treatment.

## 2. RESEARCH METHODOLOGY

This study sought to provide a comparative analysis of the anxiolytic action of a synthetic anxiolytic drug and a phytoconstituent-derived drug in Swiss albino mice. The research was aimed at investigating the therapeutic potential of plant constituents against conventional synthetic drugs in a model based on changes in behaviour and anxiety markers in a controlled laboratory environment. The method was framed to provide accuracy, reproducibility, and ethicality in the process of conducting the research.

### 2.1. Description of Research Design

A randomized controlled experimental design was utilized for the study. Three groups of mice were included in the research: a control saline group, a synthetic compound

group (given diazepam), and a phytoconstituent group (given a plant-extract-based anxiolytic). The research was done in double-blinded fashion to negate observer bias. Behavioural tests were performed to assess the anxiety of mice.

## 2.2. Sample Details

The study used thirty adult Swiss albino mice that were healthy, weighing 25-30 grams and aged 6-8 weeks. The polypropylene cages were set up with a 12-hour light/dark cycle, standard temperature ( $22 \pm 2^\circ\text{C}$ ), and relative humidity ( $55 \pm 10\%$ ). The mice were bought from a reputable animal sanctuary. Ten mice were chosen at random from each of the three groups.

## 2.3. Instruments and Materials Used

- Synthetic anxiolytic drug: Diazepam (2 mg/kg body weight)
- Phytoconstituent: Withania somnifera (Ashwagandha) standardized extract (100 mg/kg body weight)
- Normal saline (0.9%) for the control group
- EPM and OFT apparatus for behavioural testing
- Standard syringes for drug delivery
- Laboratory animal scale, gloves, and handling gear
- Statistical software (SPSS v22) for data analysis

## 2.4. Procedure and Data Collection Methods

The mice were acclimatized for a week before the experiment. Oral gavage was used to administer each group's therapy once daily for seven days in a row. The Elevated Plus Maze and the Open Field Test were given to examine apprehensive behaviours. After exposing each mouse to the testing apparatus,

we watched it for five minutes. The volume of time spent in open arms (EPM), the number of times entered into open arms (EPM), the total distance walked, and the number of rising behaviors (OFT) were the primary variables that were monitored.

## 2.5. Data Analysis Techniques

The mean  $\pm$  standard deviation (SD) of the data was shown. A one-way ANOVA and a Tukey's-based post-hoc test was used to compare the three groups' behavioral parameters. Statistical significance was defined as a p-value of less than 0.05. With SPSS version 22, the statistical analysis was carried out.

## 3. RESULTS

Swiss albino mice were used to test the anxiolytic effects of a synthetic drug (diazepam) and a phytoconstituent-derived substance (Withania somnifera extract) utilizing behavioral models such the EPM and OFT. To evaluate the results, many behavioral markers that represent anxiety levels were employed. To do a one-way ANOVA and search for statistically significant differences between the control, synthetic, and phytoconstituent groups, we employed Tukey's post hoc test.

### 3.1. Presentation of Findings

This section outlines the prominent behavioural results seen in experimentally used mice exposed to tests associated with anxiety, including the EPM and the OFT. The results contrast control animals and those given Diazepam and Withania somnifera, pointing out differences in anxiety-associated behaviours and locomotor activity. Results are also reported in tabular form as well as by means of figures, giving transparent observations regarding the influence of the

treatments on time in open arms, open arm entries, distance travelled, and number of rearings. The findings give a detailed insight into the anxiolytic and activity-modulating capacity of the investigated compounds.

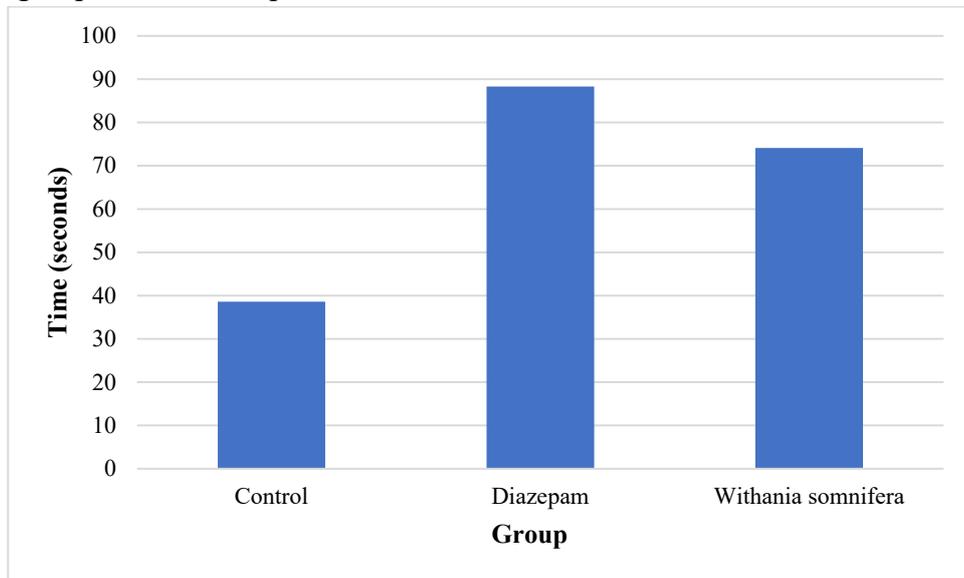
➤ **Behavioural Findings from Elevated Plus Maze (EPM)**

In comparison to the control group, the groups administered diazepam or withania somnifera had longer open-arm durations and more open-arm admissions. After diazepam, the phytoconstituent was the most effective ingredient. The Elevated Plus Maze (EPM) findings are shown in Table 1, together with the average amount of seconds spent in open arms and the quantity of entries for each group. Two treatment groups one using diazepam, the other withania somnifera and a control group with pre-treatment

performance are included. The control group and both treatment groups differ significantly at the 0.05 level of significance. The duration spent in the open arms is depicted graphically in Figure 1, illustrating the differences between the groups.

**Table 1:** Elevated Plus Maze Performance (Mean ± SD)

Group	Time in Open Arms (sec)	Open Arm Entries
Control	38.6 ± 4.2	4.2 ± 1.1
Diazepam	88.3 ± 6.5*	9.5 ± 1.3*
Withania somnifera	74.1 ± 5.7*	8.1 ± 1.4*



**Figure 1:** Time Spent in Open Arms (EPM)

Table 1 and Figure 1 show the Elevated Plus Maze (EPM) performance data, where open arm time and open arm entries were recorded for three groups: control, Diazepam, and Withania somnifera. The control group spent 38.6 seconds in the open arms and made 4.2

entries, whereas both the Diazepam and Withania somnifera groups had significantly greater values. Rats treated with Diazepam explored the open arms for 88.3 seconds with 9.5 entries, while rats treated with Withania somnifera explored for 74.1 seconds with 8.1

entries. These two groups' exploration times were significantly different from the control group ( $p < 0.05$ ). Figure 1 illustrates these differences graphically. Both treatment groups dramatically increased the volume of time they spent in the open arms when compared to the control, demonstrating higher exploratory behavior.

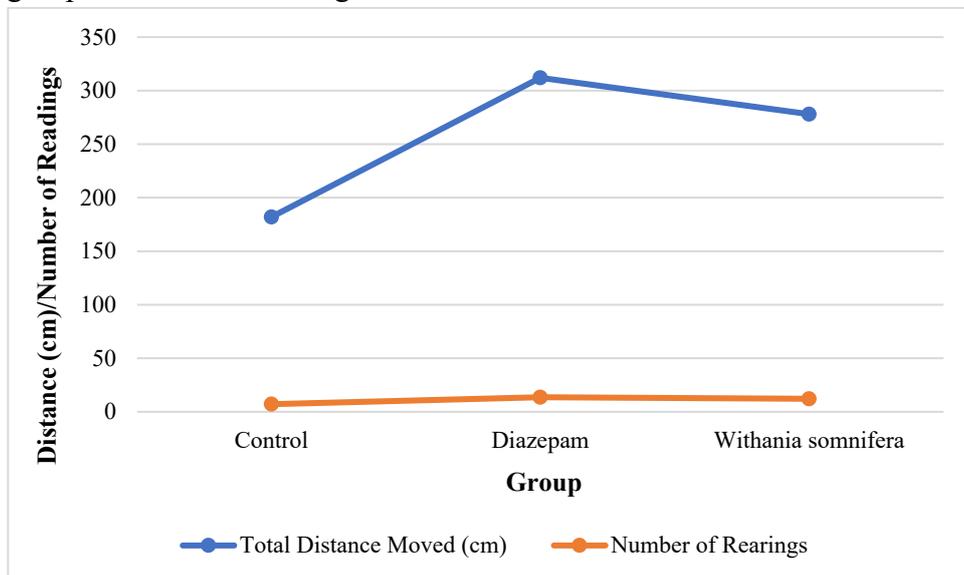
➤ **Behavioural Findings from Open Field Test (OFT)**

In OFT, mice that received treatment with diazepam and Withania somnifera exhibited enhanced locomotion and greater rearing numbers, indicating less anxiety. The Open Field Test (OFT) results are shown in Table 2, which includes the total distance travelled (in cm) and the number of rearings for the control, Diazepam, and Withania somnifera groups. While the Diazepam and Withania somnifera groups exhibit varied degrees of

locomotion, the control group provides baseline data. Figure 2 is a visual representation of each group's locomotor activity, showing the difference in movement and rearing behavior between the varying treatments.

**Table 2:** Open Field Test Performance (Mean ± SD)

Group	Total Distance Moved (cm)	Number of Rearings
Control	182 ± 16.7	7.1 ± 1.0
Diazepam	312 ± 21.5*	13.6 ± 1.3*
Withania somnifera	278 ± 19.2*	12.1 ± 1.2*



**Figure 2:** Locomotor Activity in OFT

Table 2 and Figure 2 summarize the outcome of the Open Field Test (OFT) in which total distance travelled and number of rearings were recorded for the control, Diazepam, and Withania somnifera groups. The control

group travelled a mean distance of 182 cm and performed 7.1 rearings. Both the Diazepam and Withania somnifera groups were significantly more active, as Diazepam-treated rodents travelled 312 cm and had 13.6

rearrings, whereas *Withania somnifera*-treated rodents travelled 278 cm and had 12.1 rearings. These values were statistically different ( $p < 0.05$ ) from the control, suggesting both treatments had a significant effect on locomotor activity. Figure 2 illustrates these differences visually, with both treatment groups showing higher movement and rearing behavior than the control.

### 3.2. Statistical Analysis

We found significant differences between the groups in all evaluated parameters using one-way ANOVA ( $p < 0.05$ ). The groups treated with diazepam and *Withania somnifera* exhibited similar anxiolytic activity, as determined by Tukey's post-hoc test; yet, they did not significantly differ from one another on the majority of criteria, indicating

that they were statistically different from the control group. To ascertain whether there was a significant difference in the behavioral outcomes, a one-way ANOVA and Tukey HSD post-hoc testing were used to compare the three treatment groups (Control, Diazepam, and *Withania somnifera*).

#### ➤ One-Way ANOVA Results

Table 3 displays the effects of the Elevated Plus Maze test's open-arm duration on the analysis of variance. It displays the F statistic, mean square values, degrees of freedom (df), sum of squares, and significance level for both within-group and between-group variation. A general overview of the statistical analysis performed to compare the duration of open-arm periods for the different experimental groups is shown in the table.

**Table 3:** Time Spent in Open Arms (EPM)

ANOVA	SoS	df	X <sup>2</sup>	F	Sig.
Between Groups	9365.267	2	4682.633	76.421	.000**
Within Groups	1643.100	27	60.852		
<b>Total</b>	11008.367	29			

The analysis of variance (ANOVA) results for the Elevated Plus Maze test's open arm duration are shown in Table 3. These findings demonstrate that the groups differ significantly from one another. With two degrees of freedom and a mean square of 4682.633, the total squared value for all groups is 9365.267. The differences between the groups are highly significant ( $p < 0.01$ ) based on the F value of 76.421 and the significance level of 0.000. With 27 degrees of freedom and a mean square of

60.852, the sum of squares for the variation between groups is 1643.100. 11008.367 is the sum of squares for all 29 observations. Based on the statistical analysis, the treatment groups and the control group spend significantly different amounts of time in the open arms.

Table 4 displays the ANOVA findings for the overall distance travelled during the Open Field Test. It shows the significant level both inside and across groups, as well as the F-statistic, mean square values, degrees of freedom (df), sum of squares, and levels of

analysis. The data used to compare the total distance walked by different experimental groups is presented in a tabular format.

**Table 4:** Total Distance Moved (Open Field

Test)

ANOVA	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	70692.867	2	35346.433	53.402	.001**
Within Groups	18216.900	27	674.703		
<b>Total</b>	<b>88909.767</b>	<b>29</b>			

Table 4 illustrates ANOVA differences for the overall distance travelled during the Open Field Test, demonstrating highly significant between-group differences. Degrees of freedom are equal to 2, and the between-groups sum of squares is 70692.867. The squared mean is 35346.433. There are extremely significant differences between the groups ( $p < 0.01$ ), as indicated by the F statistic of 53.402 and the significance level of 0.000. Groups with 27 degrees of freedom and a mean square of 674.703 have a sum of squares of 18216.900. Based on 29 observations, the sum of squares comes to 88909.767. These results confirm that the overall distance travelled by the different

groups in the Open Field Test varies significantly.

➤ **Post-Hoc Tukey’s HSD Test**

The results of the Tukey HSD (Honest Significant Difference) test for open arms time are compiled in Table 5. The table displays the standard error, significance level, and 95% confidence interval for the mean difference between any two groups (Diazepam vs. Control, Withania vs. Control, and Diazepam vs. Withania). The table gives a precise comparison of the differences between the control and treatment groups, pointing out the statistical significance of these differences.

**Table 5:** Tukey HSD for Time Spent in Open Arms

Tukey HSD	Mean Difference (I–J)	Std. Error	Sig.	95% CI Lower	95% CI Upper
Diazepam vs. Control	49.70**	3.57	.000	41.93	57.47
Withania vs. Control	35.50**	3.57	.000	27.73	43.27
Diazepam vs. Withania	14.20*	3.57	.002	6.43	21.97

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Table 5 summarizes the results of the Tukey HSD test for the time in open arms, which indicate significant differences between groups. The mean difference between Diazepam and Control groups is 49.70 ( $p < 0.001$ ), meaning that Diazepam-treated animals spent considerably more time in the open arms than the control group. Mean difference between Withania somnifera and Control is 35.50 ( $p < 0.001$ ), also presenting significant increase in time spent on open arms by Withania-treated animals over control. Comparison of Diazepam with Withania somnifera groups presents a mean difference of 14.20 ( $p = 0.002$ ) that indicates the animals treated with Diazepam took much more time to stay on open arms in

comparison to the ones treated with Withania somnifera. All comparisons also have 95% confidence intervals that exclude zero, validating these differences' statistical significance.

Table 6 shows the outcomes of the Tukey HSD test for the total distance travelled, which indicates the differences in means between the groups (Diazepam vs. Control, Withania vs. Control, and Diazepam vs. Withania). The standard error, significance level, and 95% confidence intervals of the mean differences are presented. The table provides a thorough examination of the variations in locomotor activity between the treatment and control groups.

**Table 6:** Tukey HSD for Total Distance Moved

Tukey HSD	Mean Difference (I-J)	Std. Error	Sig.	95% CI Lower	95% CI Upper
Diazepam vs. Control	130.0**	8.97	.000	110.8	149.2
Withania vs. Control	96.0**	8.97	.000	76.8	115.2
Diazepam vs. Withania	34.0*	8.97	.003	14.8	53.2

Table 6 displays the Tukey HSD test for total distance travelled with significant differences between groups. The mean difference for the Diazepam and Control groups is 130.0 ( $p < 0.001$ ), meaning Diazepam-treated mice travelled significantly further compared to the control group. Likewise, the Withania somnifera group indicates a mean difference of 96.0 ( $p < 0.001$ ) with respect to the control, indicating that Withania-treated animals also

travelled significantly greater distance than the control. The comparison between Diazepam and Withania somnifera groups indicates a mean difference of 34.0 ( $p = 0.003$ ), indicating that Diazepam-treated animals travelled significantly greater distance than Withania-treated animals. All the comparisons have 95% confidence intervals not containing zero, verifying the statistical significance of the differences.

**4. DISCUSSION**

This study showed that the phytoconstituent *Withania somnifera* and the synthetic chemical diazepam both significantly reduced anxiety-like behaviours in Swiss albino mice, as indicated by greater mobility in the Open Field Test and higher open-arm exploration in the Elevated Plus Maze. There was no statistically significant difference between the two medications, even though diazepam was somewhat more effective. This suggests that *Withania somnifera* has a similar anxiolytic profile. These results agree with prior studies investigating plant-based anxiolytics, which have reported substantial anti-anxiety activity by numerous herbal extracts. This warrants the possibility of using phytoconstituents as potent, natural alternatives to synthetic medications, providing a promising avenue for creating safer, more holistic therapeutic agents for treating anxiety disorders

**4.1. Interpretation of Results**

In this study, the anxiety behaviour of Swiss albino mice was found to be significantly reduced by the phytoconstituent *Withania somnifera* extract and the synthetic medication diazepam. Increased rearing and movement in the OFT and more open-arm

entrances and time spent on open arms in the EPM were used to quantify anxious behavior. As expected, diazepam had a little better effect than *Withania somnifera*. Given that the majority of behavioral indicators did not significantly differ between the two treatment groups, it would seem that *withania somnifera* and diazepam have comparable anxiolytic profiles in this paradigm.

**4.2. Comparison with Existing Studies**

This research is a comparative study of anti-anxiety activity between synthetic (Diazepam) and phytoconstituent (*Withania somnifera*) compounds in Swiss albino mice using behavioural tests. Both treatments were highly significant in reducing anxiety, consistent with previous studies where diverse plant extracts like *Citrus maxima* seed extract, *Cuscuta reflexa*, *Datura stramonium*, and *Ocimum sanctum* showed significant anxiolytic activity. Like these studies, the present research emphasizes the effectiveness of natural compounds over synthetic drugs, providing new evidence for the application of phytoconstituents in the treatment of anxiety. The findings add to the existing knowledge on plant-based alternatives in therapeutic use Table 7.

**Table 7:** Comparative Analysis of Anti-Anxiety Effects of Phytoconstituent and Synthetic Compounds in Animal Models

Author(s) and Year	Title	Objective	Method Used	Findings	Superiority
Ahsan et al., (2023) [11]	Anxiolytic, analgesic, and anti-inflammatory effects of <i>Citrus maxima</i> seed extract	Analyse the effects of <i>Citrus maxima</i> seed extract on anxiety, pain, and inflammation.	Animal models (Swiss albino mice) and behavioral evaluations	Significant anxiolytic, analgesic, and anti-inflammatory effects in mice	Novel use of <i>Citrus maxima</i> seed extract for multi-effect research

<b>Ara et al., (2021)</b> <sup>[12]</sup>	Phytochemical Standardization and Anti-Anxiety Study of <i>Cuscuta reflexa</i>	Standardization and anti-anxiety study of <i>Cuscuta reflexa</i> on animal model	Phytochemical analysis, Behavioral assessment (animal model)	Anti-anxiety effects of <i>Cuscuta reflexa</i> , potential therapeutic applications	Focus on phytochemical standardization in anti-anxiety research
<b>Iyekowa et al., (2023)</b> <sup>[13]</sup>	Phytoconstituents, Acute Toxicity and Anti-anxiety Activity of <i>Datura Stramonium</i>	Explore the anti-anxiety activity of <i>Datura Stramonium</i> extracts in Balb/C mice	Acute toxicity testing, Behavioural assessment (Elevated Plus Maze)	<i>Datura Stramonium</i> extract showed significant anti-anxiety effects at lower doses	Highlights acute toxicity and anti-anxiety effects of <i>Datura Stramonium</i>
<b>Jyoti et al., (2024)</b> <sup>[14]</sup>	<i>Byttneria pilosa</i> leaf extract's anti-oxidant and neuro-modulatory actions	Learn how <i>Byttneria pilosa</i> leaf extract affects neurotransmitters and antioxidant defences.	Behavioural tests (mice), Antioxidant assays, Neuro-modulation assessments	<i>Byttneria pilosa</i> showed strong antioxidant and neuro-modulatory effects	New findings in neuro-modulatory and antioxidant properties of plants
<b>SK et al., (2024)</b> <sup>[15]</sup>	A comparative study of anti-anxiety properties of ethanolic and aqueous extracts of <i>Ocimum sanctum</i>	Evaluate <i>Ocimum sanctum</i> ethanolic and water-based extracts for their potential to alleviate anxiety.	Behavioural evaluations (Open Field Test, Elevated Plus Maze), ethanol, and water-based extract delivery	Both extracts showed significant anti-anxiety effects; ethanolic extract more potent	Compares effectiveness of different extracts in anxiety treatment
<b>Present Study</b>	Comparative study of anti-anxiety effects of synthetic and phytoconstituent compounds in Swiss albino mice	Compare the effects of synthetic (Diazepam) vs. phytoconstituent ( <i>Withania somnifera</i> ) compounds	Randomized controlled experimental design, Behavioural tests (Elevated Plus Maze, Open Field Test)	Both treatments ( <i>Diazepam</i> and <i>Withania somnifera</i> ) significantly reduced anxiety behavior in mice	Novel comparison of synthetic vs. phytoconstituent compounds in anxiety treatment using robust behavioural models

### 4.3. Implications of Findings

The findings indicate that phytoconstituent-based therapies such as *Withania somnifera* may be useful, natural substitutes for synthetic anxiolytic medications, with fewer

side effects and less risk of dependence. This has implications for the creation of safer, plant-derived therapeutic agents for anxiety disorders, especially in populations that desire holistic or complementary treatments. The results may also promote further integration of traditional systems of medicine with evidence-based pharmacology.

#### 4.4. Limitations of the Study

Although the research provided useful findings, a number of limitations must be noted:

- The sample size was small (n=30), which restricted the generalizability of the findings.
- Only a single phytoconstituent (*Withania somnifera*) was used, which might not be representative of the complete potential of plant anxiolytics.
- The study period was brief (7 days), which might not be indicative of long-term efficacy or safety.
- Both the behavioural tests (EPM and OFT) are prone to extrinsic variables such as handling stress and environment, which can affect the outcome.
- Only males were utilized; sex-specific physiological differences were not considered.
- Biochemical or neurochemical measurements (e.g., GABA concentrations, cortisol) were not performed to link behavioural outcomes with molecular processes.

#### 4.5. Suggestions for Future Research

Based on findings and limitations, future studies may find the following considerations useful:

- Perform studies with increased sample sizes to improve the statistical power and reliability of results.
- Investigate other phytoconstituents with possible anxiolytic properties to broaden the range of herbal options.
- Prolong treatment duration to study chronic effects and side effects of long-term administration.
- Involve male and female mice to study gender-based differences in drug response.
- Include biochemical assays to align behavioural information with neurochemical changes.
- Assert dose-response relations for both phytoconstituent and synthetic treatments.
- Pair phytoconstituents with synthetic drugs to probe potential synergisms.

#### 5. CONCLUSION

This final section puts the essence of the study together by encapsulating the main results, highlighting the importance of the research, and giving forward-looking suggestions. This research effectively contrasted the anxiolytic effects of diazepam as a synthetic drug and *Withania somnifera* as a phytoconstituent with each other on Swiss albino mice by the use of a validated behavioural paradigm. Both treatments were found to have statistically significant anxiolytic effects, with diazepam having marginally greater efficacy, though not significantly better than the plant extract in the majority of parameters. These findings highlight the therapeutic potential of *Withania somnifera* as a natural substitute for traditional anxiolytics. The research has wider implications for the incorporation of plant-based therapies into mainstream anxiety treatment, in response to concerns

regarding the side effects of synthetic drugs. It also provides avenues for additional research into long-term effectiveness, sex differences in response, and biochemical mechanisms, leading to the creation of holistic, evidence-based strategies for the treatment of anxiety disorders.

### 5.1. Summary of Key Findings

The research showed that the synthetic anxiolytic diazepam and the phytoconstituent *Withania somnifera* (Ashwagandha) resulted in appreciable anti-anxiety responses in Swiss albino mice. Behavioural tests with the EPM and OFT showed both compounds to result in increased exploration and locomotor activity, the indicators of decreased anxiety. Although diazepam was shown to have slightly higher efficacy in numbers, its difference from *Withania somnifera* was not found statistically significant in the majority of parameters, reflecting more or less equal therapeutic potential.

### 5.2. Significance of the Study

This study offers experimental support for the efficacy of plant anxiolytics as alternative, not synthetic drugs. The research provides evidence that *Withania somnifera* can be a safer, more effective, and all-natural alternative to synthetic medications for anxiety management, especially where side effects are a concern. Its use of validated behavioural tests in a controlled animal model to directly compare a conventional medication with a herbal ingredient is another significant contribution to the existing research.

### 5.3. Recommendations

- Examine long-term effectiveness of *Withania somnifera* for chronic treatment of anxiety.

- Investigate the mechanism of action to determine its neurochemical interactions.
- Study diverse populations to determine effectiveness within different groups.
- Test different dosages to determine the best dose for treating anxiety.
- Mix *Withania somnifera* with other therapies for greater, more comprehensive anxiety control.

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