EAS Journal of Pharmacy and Pharmacology
Abbreviated Key Title: EAS J Pharm Pharmacol
ISSN: 2663-0990 (Print) & ISSN: 2663-6719 (Online)
Published By East African Scholars Publisher, Kenya

OPEN ACCESS

Volume-7 | Issue-6 | Nov-Dec- 2025 | DOI: https:/doi.org/10.36349/easjpp.2025.v07i06.004

Original Research Article

Comparative Behavioral Study of Withania Somnifera and Piper Longum in Experimental Models of Depression

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Article History
Received: 01.11.2025
Accepted: 22.12.2025
Published: 30.12.2025

Journal homepage:
https://www.easpublisher.com

Quick Response Code

Abstract: The study aimed to evaluate and compare the in vitro monoamine oxidase (MAO) inhibitory properties of both plant extracts, assess their antidepressant-like effects in both acute and chronic models using the Forced Swim Test (FST) and Tail Suspension Test (TST), examine their influence on reserpine-induced depressive symptoms such as ptosis, catatonia, and sedation, and explore whether Withania somnifera enhances the efficacy of the standard antidepressant imipramine. Methodologically, the study utilized established behavioral assays and biochemical evaluations in animal models to assess efficacy and safety. The results revealed that Piper longum, through its active compound piperine, functions as a potent, reversible, and selective MAO inhibitor, offering rapid antidepressant effects and a favorable safety profile. In contrast, Withania somnifera demonstrated a gradual yet sustained antidepressant action, mediated by modulation of multiple neurobiological systems, and showed enhanced effects when combined with imipramine. Both plants were well-tolerated, and their distinct mechanisms suggest potential for targeted or complementary use in managing various forms of depression.

Keywords: Depression, Herbal medicine, Behavioral model, Antidepressant activity.

1. INTRODUCTION

Depression is a chronic mental illness that is helplessly associated with persistent sadness, lack of interest, fatigue, and mental impairment and is a problem of millions of people around the world due to the weaknesses and side effects of pharmaceutical antidepressants. Research has identified the potential of these plants as therapeutic agents, and indicates a broad range of benefits, including the adjustment of oxidative stress, neurotransmitter homeostasis, and inflammatory processes [1] highlighted the antioxidant capacity of plant-derived compounds, which is essential given that the importance of oxidative stress is essential in the pathophysiology of depression. On the same note [2], have shown the successful in vitro growth of Piper longum, which has the potential to advance the production of bioactive.

Traditional medicinal systems describe Ashwagandha (Withania somnifera) and Pippali (Piper longum) as rejuvenating and nervine tonics. Previous studies suggest that Ashwagandha exhibits adaptogenic and anxiolytic properties, while Piper longum has CNS-stimulating and neuroprotective effects. However, direct comparative behavioral evaluation in standardized depression models remains limited.

2. RESEARCH OBJECTIVES

- To evaluate and compare the in vitro monoamine oxidase (MAO) inhibitory potential of Piper longum and Withania somnifera extracts.
- To assess the acute and chronic antidepressant-like effects of Piper longum and Withania somnifera in established behavioral models (FST and TST).
- To investigate the effects of Piper longum and Withania somnifera extracts on reserpine-induced depression symptoms (ptosis, catatonia, and sedation).

3.MATERIALS AND METHODS

The outlines different systematic experiments that were used in comparing two medicinal plants; Piper longum and Withania somniferous in their antidepressant effect. The chapter describes the procedures involved in collection of plant material and its authentication, extraction procedures of both plants, qualitative and

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quantitative screening of phytochemicals and isolation of bioactive compounds and characterization of the same by use of spectroscopic methods [3]. It also talks about animal models of preclinical screens, in vitro assays i.e. MAO inhibition, in vivo behavioral tests: Forced Swim Test, Tail Suspension Test. chapter also includes the preparation of the test doses, and the layout of the experiment which includes 6 groups of animals whose methods are used to conduct the biochemical assays as well as ethical considerations.

3.1 Plant Material and Extraction

Roots of Withania somnifera and fruits of Piper longum were authenticated and dried. Hydroalcoholic extraction (ethanol) was performed using Soxhlet apparatus. Extracts were concentrated and stored [4].

3.2 Experimental Animals

Adult Wistar rats (180–220 g) were used. Animals were maintained under standard laboratory conditions. Study protocol was approved by Institutional Animal Ethics Committee (IAEC).

Experimental Design

- Group I: Control (Normal saline, 0.01 mL/g)
- Group II: Standard (Imipramine, 15 mg/kg, i.p.)
- Group III: T1-AGG (Withania somniferous, 20 mg/kg)
- Group IV: T2-AGG (Withania somniferous, 40 mg/kg)
- Group V: T3-AGG + Imipramine (10 mg/kg each)
- Group VI: Piper longum (200 mg/kg) (10)

4. VIVO MODELS BEHAVIOR MODELS

4.1 The Forced Swimming Test (FST)

It is also called the Porsolt Swim Test, was developed by Roger Porsolt to assess depressive-like behavior in rodents [5].

Procedure:
1. Animal: Rat or mouse
2. Apparatus: Transparent cylinder filled with water (23–25°C), deep enough to prevent touching the bottom
3. Pre-test (Day 1): 15 minutes swimming
4. Test (Day 2, 24 hrs later): 5–6 minutes swimming
5. Observation: Record time spent immobile, swimming, and climbing(6)

4.2 Tail Suspension Test (TST)

The Tail Suspension Test (TST) is a behavioral test used in mice to evaluate depressive-like behavior and screen antidepressant drugs [7].

Procedure:
1. Animal: Mouse (commonly used; not suitable for rats)
2. Setup: Mouse is suspended by the tail using adhesive tape, Hung 50–60 cm above the floor

Test conducted in a quiet environment [8]
3. Duration: 5–6 minutes total
4. Observation: Measure time spent immobile (no active escape movements)
Main Outcome: Increased immobility time = depressive-like behavior
Reduced immobility after antidepressant treatment = antidepressant-like effect
After the test, the mouse is gently removed and returned to its cage.

4.3 Open Field Test

The Open Field Test (OFT) is a behavioral test used in rodents to assess locomotor activity, exploration, and anxiety-like behavior.

Procedure:
1. Animal: Rat or mouse
2. Apparatus: Square or circular open arena, Marked with grid lines (center and peripheral zones) Brightly lit
3. Procedure: Place the animal in the center of the arena Allow free exploration for 5–10 minutes, Record behavior [9]
4. Parameters Measured: Total distance traveled (locomotor activity), Time spent in center vs. periphery (anxiety level), Number of line crossings, Rearing and grooming behavior
Interpretation: More center time & movement → lower anxiety
Less movement & staying near walls (thigmotaxis) → higher anxiety

5. RESULT AND DISCUSSION

Antidepressant Activity in Behavioral Models

Table 5.1: Percentage Reduction in Immobility Time in FST and TST for Different Treatments

Treatment	FST (% reduction)	TST (% reduction)
Piperine (10 mg/kg)	55.3	57.6
T2-AGG (40 mg/kg) - Chronic study	49.4	43
Imipramine (15 mg/kg) - Chronic study	67.4	57.9

Percentage reduction in FST immobility time & TST.by different groups in comparison with control animals are presented in table and slight differences were observed indicating the relative antidepressant-like action in the different groups of drugs tested Chronic treatment by imipramine (15 mg/kg) as an anti-depressant also had the most effective role since it can reduce 67.4% of immobility time in FST, & decrease immobility time in TST by 57.9 %, which again is in full agreement with the well-known anti-depressant effects of this drug. Piperine at dose of 10 mg/kg elicited similar antidepressant-like activity, and decreased immobility time by 55.3% in the FST and 57.6% in the TST, indicating a well- balanced and potent effect in both behavioural models. Chronic treatment of T2-AGG (40 mg/kg) leads to 49.4% (FST) and 43% (TST) reductions which are moderate and slightly lesser than that of piperine and imipramine. From a statistical point of view, these results indicate that all three treatments resulted in significant improvement in these behaviors (not shown), & that imipramine had the greatest effect.

Table 5.2: Effect of Withania somnifera on Immobility Time in FST and TST (Chronic Studies)

Groups (n=6 in each)	Doses	FST (Chronic)	TST (Chronic)
I Control-NS	00.01ml/gm	195.5±5.22	190.5±5.22
II Imipramine	15mg/kg	82.2±1.4*	95.5±1.32*
III T1: AGG	20mg/kg	135.5±4.34**	132.3±4.31**
IV T2: AGG	40mg/kg	112.7±4.20**	116.3±5.20*
V T3: AGG & Imipramine	10mg/kg each	98.3±1.37**	99.6±1.21**

These findings support the antidepressant activity of W. somnifera." Withania somnifera extracts, especially in higher doses significantly inhibited the symptoms induced by reserpine like ptosis, catatonia and sedation

Table 5.3: Percentage Reduction in Immobility Time in FST and TST for Different Treatments

Treatment	Dose (mg/kg)	FST (% Reduction)		TST (% Reduction)	
		Acute	Chronic	Acute	Chronic
Piperine	5	32.4	–	35.1	–
	10	45.3	–	47.6	–
T1-AGG	20	5.8	30.7	4.9	30.6
T2-AGG	40	8.3	46.5	8.3	39.4
Imipramine	15	15.3	60.9	11.0	51.9
T3-AGG + Imipramine	10 mg/kg each	19.9	53.3	11.9	49.8
Fluoxetine	10	47.8	–	49.3	–

OFT: Open Field Test (OFT) was conducted to assess whether reduction particular antidepressant was cause of immobility shown in FST & TST. Effect or general increase in locomotor activity [10].

Table 5.4: Effect of Treatments on Locomotor Activity in OFT

Treatment	Dose (mg/kg)	Number of Squares Crossed
Control	-	103 ± 9
Piperine	3	97 ± 7
	5	93 ± 6
	10	91 ± 5
T1-AGG	20	95 ± 7
T2-AGG	40	93 ± 6
Imipramine	15	90 ± 5
T3-AGG + Imipramine	10 each	91 ± 6
Fluoxetine	10	87 ± 4

Neither piperine nor Withania somnifera extracts significantly altered locomotor activity in the OFT, indicating that reduction in immobility observed in FST & TST was due to specific antidepressant-like effects rather than general increase in locomotor activity

6. COMPARATIVE ANALYSIS

The difference between treatment groups and the effect of Piper longum and Withania somnifera were compared with the standard antidepressants after comparing each as a comparative [11-12].

Table 6.1: Comparative Analysis of Antidepressant Efficacy (% Reduction in Immobility Time in Chronic FST)

Treatment	Dose (mg/kg)	% Reduction	Relative Efficacy (% of Imipramine)
Imipramine	15	57.9	100
T2-AGG	40	42.4	73.2
T3-AGG + Imipramine		49.7	85.8
Piperine (Acute)	10	45.3	78.2

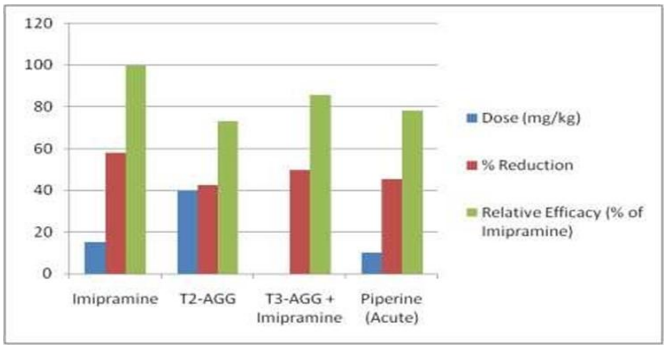

Fig 6.1: Comparative Analysis of Antidepressant Efficacy

7. CONCLUSION

The potential of two centuries-old medicinal herbs ashwagandha (Withania somnifera) and Piper longum (long pepper) as antidepressants was evaluated and compared in this study. In order to assess the antidepressant properties it employed comprehensive methodology that involved phytochemical, bioactive component isolation, in vitro enzyme inhibition studies and in vivo behavioral experimentation.

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Cite This Article: Raginee Kori, Jitender Kumar Malik, S.B. Sahu, Deepak Jhariya (2025). Comparative Behavioral Study of Withania Somnifera and Piper Longum in Experimental Models of Depression. *EAS J Pharm Pharmacol,* 7(6), 126-129.