Global Academic Journal of Pharmacy and Drug Research

Available online at https://www.gajrc.com
DOI: https://doi.org/10.36348/gajpdr.2025.v07i03.002

OPEN ACCESS

ISSN (P): 2706-9044
ISSN (O): 2707-255X

Original Research Article

Effect of Standardized Withanolide-Rich Extract of *Withania somnifera* and *Piper longum* on Reserpine-Induced Depression

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Article History
Received: 21.06.2025
Accepted: 12.08.2025
Published: 30.08.2025

Abstract: Depression is a serious neuropsychiatric disorder associated with disturbances in monoaminergic neurotransmission, oxidative stress, neuroinflammation, and impaired neuroplasticity. Reserpine-induced depression is a widely accepted experimental model that mimics monoamine depletion and depressive-like behavior in animals. Withania somnifera (Ashwagandha) is an important medicinal plant known for its adaptogenic, antioxidant, and neuroprotective properties. The present study aims to evaluate the antidepressant potential of a standardized withanolide-rich extract of Withania somnifera in a reserpine-induced depression model. *Piper longum*, or long pepper, also exhibited antidepressant properties that were mainly achieved by the mechanism of modulation, especially of serotonin and dopamine. The findings may provide scientific evidence supporting the use of Withania somnifera as a natural therapeutic agent for depression.

Keywords: *Withania somnifera, Piper longum* Ashwagandha, Withanolides, Reserpine, Depression, Antidepressant Activity, Oxidative Stress.

INTRODUCTION

Depression has become widespread and crippling mental illness which, as per estimates given by World Health Organization indicate that approximately 280 million individuals are affected all over the world. Reserpine-induced depression is one of the most established pharmacological models used to study depressive disorders. Reserpine irreversibly blocks vesicular monoamine transporter-2 (VMAT-2), resulting in depletion of serotonin, dopamine, and norepinephrine from neuronal terminals[1]. This monoamine depletion produces behavioral and biochemical alterations resembling depressive symptoms.

Withania somnifera (Ashwagandha) is a medicinal plant. Its major bioactive constituents, known as withanolides, exhibit antioxidant, anti-inflammatory, adaptogenic, and neuroprotective activities. Recent evidence suggests that Ashwagandha may improve mood, reduce stress, and modulate neurotransmitter function. *Piper longum*, or long pepper, also exhibited antidepressant properties that were mainly achieved by the mechanism of modulation, especially of serotonin and dopamine. *Piper longum*. These results are consistent with conventional knowledge but most importantly, they put these herbs in a modern scientific context that may allow their incorporation into complementary and alternative medicine plans of depression[2].

Specific Objectives

1. To prepare and standardize a withanolide-rich extract of Withania somnifera.

2. To induce depression experimentally using reserpine and establish a validated animal model of depression.
3. To evaluate antidepressant activity of the extract using behavioral tests such as: Forced Swim Test (FST)
4. To assess the effect of the extract on locomotor activity and depression-associated behavioral changes.
5. To determine the effect of the extract on oxidative stress[3]

Depression and Monoamine Hypothesis

The monoamine hypothesis proposes that depression is associated with reduced levels of serotonin, norepinephrine, and dopamine in the brain. Reserpine became historically important because its monoamine-depleting action contributed to the development of this theory. reserpine-induced behavioral changes remain useful for experimental antidepressant screening.

Scope of study

The present study is focused on the comparative evaluation of the antidepressant potential of *Piper longum and Withania somniferous* extracts through a series of in vitro and in vivo experimental models. It specifically examines their ability to inhibit monoamine oxidase (MAO) activity, assesses their acute and chronic antidepressant-like effects using behavioral models such as the Forced Swim Test (FST) and Tail Suspension Test (TST), & investigates their efficacy in alleviating reserpine-induced depressive symptoms, including ptosis, catatonia, and sedation[4].

MATERIALS AND METHODS

The methodology describes the procedures involved in collection of plant material and its authentication, extraction procedures of both plants, qualitative and quantitative screening of phytochemicals and isolation of bioactive compounds and characterization of the same by use of spectroscopic methods[5].

Extraction Method for *Withania sonmifera*

Fresh roots of *Withania sonmifera* (500 g) were processed as follows:

- **Cleaning:** Roots were washed thoroughly with tap water followed by distilled water
- **Drying:** Washed roots were shade-dried for 7 days
- **Grinding:** Dried roots were pulverized using mechanical grinder
- **Soxhlet extraction:** Powdered material was extracted with methanol using Soxhlet apparatus for 48 hours at 60-65°C
- **Filtration:** Extract was filtered through Whatman No. 1 filter paper
- **Concentration:** Filtrate was concentrated under reduced pressure at 40°C [6]
- **Fractionation:** The concentrated extract was successively fractionated with:
  - Petroleum ether
  - Chloroform
  - Ethyl acetate

Extraction Method for *Piper longum*

Dried fruits of *Piper longum* (450 g) were powdered using a mechanical grinder. The powder (100 g) was extracted using cold maceration method with the following protocol:

1. **Maceration:** Plant material was soaked in 95% ethanol (1:4 w/v ratio) at room temperature for 72 hours
2. **Extraction cycles:** Four extraction cycles were performed
3. **Filtration:** Each extract was filtered through Whatman No. 1 filter paper
4. **Concentration:** Combined filtrates were concentrated under reduced pressure at 40°C using rotary evaporator (Buchi Rotavapor R-200, Switzerland)
5. **Yield:** Approximately 45 g of dark brown residue was obtained

Fractionation: The dried extract was successively fractionated using liquid-liquid partitioning with solvents of increasing polarity: Hexane, Dichloromethane (CH_2Cl_2), Ethyl acetate (EtOAc), n-Butanol (BuOH) [7]

Phytochemical Constituents: *Withania somniferous* contains more than 80 bioactive compounds, with withanolides (steroidal lactones) being the characteristic and most pharmacologically significant constituents. The withanolides include Withaferin A, which is the most extensively studied compound, along with Withanolide A, B, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, and S. Other related compounds such as withasomnine, withasomniferols A, B, and C, withasomidienone, withastramonolide, withanone, 27-deoxywithaferin A, and 17-hydroxy withaferin A have also been identified. *Piper longum* is a source of a wide variety of bioactive phytochemicals, the most typical of which co Other important alkaloids and amides include piperlongumine, piperlonguminine, pipernonaline, piperundecalidine, piperderidine, retrofractamide A, and pellitorine. The plant also contains volatile oils (1–2.5%) such as caryophyllene, piperonal, zingiberene, germacrene D, limonene, bisabolene, and sylvestrene. Lignans like sesamin, pulviatilol, fargesin, and eudesmin have also been reported institute alkaloids and amides [8].

Experimental Animal Models:

Tail Suspension Test (TST): The Tail Suspension Test is a behavioral test used to evaluate

antidepressant activity in Rats. In this method, a rat is suspended upside down by its tail using adhesive tape fixed about 1 cm from the tip of the tail. The mouse is then hung on a horizontal bar or stand at a height so that it cannot touch any surface. Initially, the animal shows vigorous movements, but after some time it becomes immobile. The total duration of the test is usually 6 minutes. During the last 4 minutes, the immobility time is recorded. Immobility means the animal remains motionless except for slight movements required for breathing. A decrease in immobility time indicates antidepressant-like activity of the test drug [9].

Forced Swim Test (FST): The Forced Swim Test is a commonly used behavioral test to evaluate antidepressant activity in rodents, usually mice or rats. In this method, the animal is placed individually in a transparent cylindrical container filled with water up to a level where the animal cannot touch the bottom or escape. The water temperature is usually maintained at around 25 ± 1°C to avoid hypothermia. Initially, the animal shows vigorous escape-directed movements such as swimming and climbing. After some time, it adopts a posture of immobility, floating with minimal movements to keep its head above water. The total test duration is generally 6 minutes, and the immobility time is recorded during the last 4 minutes. A decrease in immobility time indicates antidepressant-like activity of the test drug [10].

Induction of Depression-like State: Reserpine-Induced Depression Model is the application of reserpine at a dosage of 4mg/kg to induce depletion of monoamine neurotransmitters (especially serotonin, norepinephrine, and dopamine). This drainage simulates the symptom of depression in human beings, which results in various behavioral modifications in animals. The typical effects caused by reserpine are ptosis (eyelid drooping), catatonia (muscular inflexibility and immobility), and sedation (loss of responsiveness). These symptoms are strictly observed since they are indicators of neurochemical variations with depression conditions [11].

Treatment Protocol: After induction of the depression-like condition using reserpine, test substances are administered to determine the capacity of the substance to reverse the symptoms. The efficacy of the treatments will be determined at certain times (1, 2, 3, and 4 hours of the administration). This enables the determination of any changes in the cases of depression-like symptoms as time increases and the presence and persistence of the therapeutic effects of the test substances [12].

Assessment Parameters: The evaluation of the depression-like symptoms is conducted through the measure of a series of behaviors. Ptosis is rated on a 0-3 scale where 0 indicates no ptosis whereas 3 indicates total eyelid cover. The Catatonia condition is determined through muscular rigidity and immobility as the degree of immobility and lack of voluntary movement. Sedation is assessed through decreased responsiveness of the rat to external stimuli e.g. touch or noise. The blinded observers make all observations in order to limit bias and measure the treatment effects objectively.

Such behavioral paradigms will give a thorough evaluation of the antidepressant-like properties of the plant extracts, and this will set the effectiveness of the extracts in acute and long-term treatment paradigms of antidepressant-like substances [13].

Effect of AGG on Reserpine-Induced Ptosis, Catatonia, and Sedation

Current study was aimed to investigate therapeutic potential of *Withania sonmifera* here known as Ashwagandha to modulate reserpine-reserpine induced depression like behavior in experimental models of animals. Reserpine is drug used in laboratory to induce depressive states in animals. Its pharmacological action is controlled by emptying of pre-synaptic vesicles in brain of monoamines neurotransmitters; dopamine, serotonin and noradrenaline. In recent decades, there has been expanding interest in the putative antidepressant effects of Ashwagandha mainly because of its effects on stress response systems like hypothalamic- pituitary-adrenal (HPA) axis & monoamines pathways. Its bioactive constituents like withanolides are assumed to exert its therapeutic effects by increasing activity of neurotransmitters, decreasing oxidative burden, & by its action on neuroplasticity. Based on these perspectives effect of *Withania sonmifera* in reserpine model of depressive animals were verified in the present investigation, to evaluate if a treatment of *Withania sonmifera* may reverse the reserpine induced depressive like behavior in experimental models [14]. For this a controlled experimental study was done in which animals were pre-treated with reserpine to induce depression and then given fixed duration of treatment with Ashwagandha extract. Subsequently, behavioral tests such as open field test (to measure locomotion and exploratory activity), forced swim test (to measure despair behaviour), & sucrose preference test (to measure anhedonia) were performed. Results of the study: It was observed from study that animals treated with Ashwagandha revealed notably greater improvement in behavioral indicators in comparison to rats treated with reserpine alone. Specifically, compared to the reserpine group, ashwagandha therapy resulted in significant increase in spontaneous locomotor activity, decrease in the length of immobility during

the forced swim test, & preference for sucrose. These data show that Ashwagandha could have involvement in therapy of depression, possibly through stability of monoamines as well as the attenuation of stress resilience [15-16].

RESULT AND DISCUSSION:

The mean and standard error (SEM) of the ptosis antagonism score caused by reserpine over time in a long-term study

Groups (n=6 in each)	Doses	Ptosis (mean ± SEM)			
		1hr	2hr	3hr	4hr
I	Control-NS	0.01	2.0±0.00	3.2±0.32	3.0±0.25
II	Imipramine	15mg/kg	1.2±0.100*	1.2±0.10*	1.3±0.21*
III	T1--AGG	20.0mg/kg	2.4±0.13	2.2±0.16*	2.6±0.21
IV	T2-AGG	40.0mg/kg	2.1±0.11	1.4±0.12*	1.6±0.30*
V	T3-AGG + Imipramine	10.0mg/kg each	1.3±0.23*	1.7±0.20*	1.93±0.25*

Ashwagandha ghrutha (AGG) group differed considerably from control group ($p < 0.05$).

The mean and standard error (SEM) of the antagonistic effects of reserpine-induced catatonia over time in a long-term study

Groups (n=6 in each)	Doses	Catatonia (mean ± SEM)			
		1hr	2hr	3hr	4hr
I	Control-NS	0.01	2.8±0.160	2.8±0.16	3.2±0.05
II	Imipramine	15mg/kg	1.3±0.12*	1.2±0.63*	1.4±0.93*
III	T1--AGG	20.0 mg/kg	1.6±0.21*	1.9±0.25*	2.3±0.34*
IV	T2-AGG	40 mg/kg	1.3±0.23*	1.5±0.24*	1.9±0.21*
V	T3-AGG + Imipramine	10 mg/kg each	1.1±0.11*	1.2±0.34*	1.5±0.23*

*AGG- Ashwagandha ghrutha, *: significantly different from control group ($p < 0.05$).*

Mean and standard error (SEM) of reserpine-induced sedation antagonism with time in long-term research

Groups (n=6 in each)	Doses	Sedation (mean± SEM)			
		1hr	2hr	3hr	4hr
I	Control-NS	0.01ml/gm	1.8±0.46	2.6±0.21	3.3±0.24
II	Imipramine	15mg/kg	0.1±0.34*	0.3±0.12*	1.5±0.10*
III	T1--AGG	20mg/kg	0.9±0.41	0.9±0.22*	1.9±0.40*
IV	T2-AGG	40mg/kg	0.8±0.40	0.7±0.41*	1.9±22*
V	T3-AGG + Imipramine	10 mg/kg each	0.3±0.20*	0.3±0.12*	1.7±0.10*

AGG- Ashwagandha ghrutha,: significantly different from control group ($p < 0.05$)*

Catatonia scores over time by treatment group

Mean and standard error (SEM) of reserpine-induced sedation antagonism with time in a long-term research

Groups (n=6 in each)	Doses	Sedation (mean± SEM)			
		1hr	2hr	3hr	4hr
I	Control-NS	0.01ml/gm	1.8±0.46	2.6±0.21	3.3±0.24
II	Imipramine	15mg/kg	0.1±0.34*	0.3±0.12*	1.5±0.10*
III	T1--AGG	20mg/kg	0.9±0.41	0.9±0.22*	1.9±0.40*
IV	T2-AGG	40mg/kg	0.8±0.40	0.7±0.41*	1.9±22*
V	T3-AGG + Imipramine	10 mg/kg each	0.3±0.20*	0.3±0.12*	1.7±0.10*

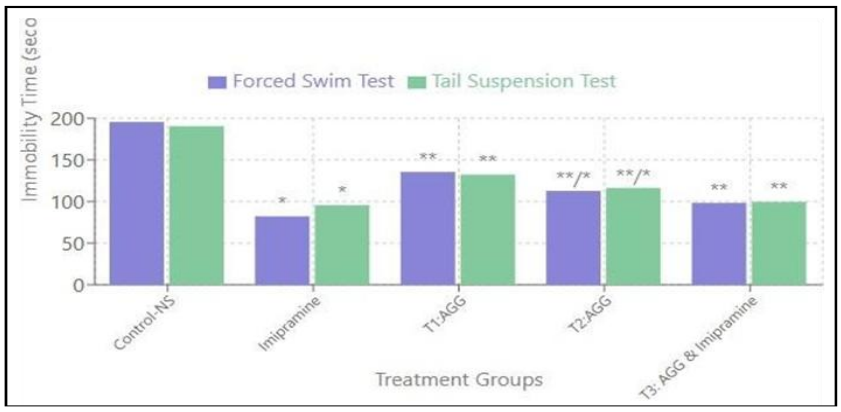
AGG- Ashwagandha ghrutha,: significantly different from control group ($p < 0.05$).*

Effect of *Withania sonmifera* on Immobility Time in FST and TST

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**

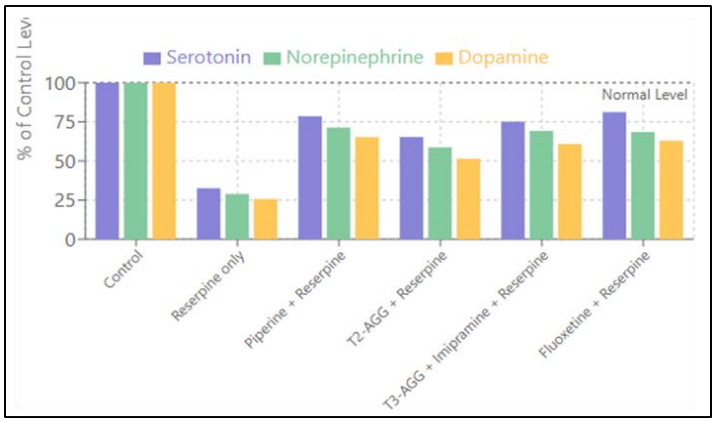
These findings support the antidepressant activity of *Withania sonmifera* extracts, especially in higher doses significantly inhibited the symptoms induced by reserpine like ptosis, catatonia and sedation as observed.



Effect of *Withania sonmifera* on Reserpine-Induced Symptoms

Effect of Treatments on Brain Neurotransmitter Levels in Reserpine-Induced Depression Model

Treatment	Dose (mg/kg)	% of Control Levels		
		Serotonin	Norepinephrine	Dopamine
Control	-	100	100	100
Reserpine only	4	32.6	28.9	25.7
Piperine + Reserpine	10 + 4	78.6	71.3	65.2
T2-AGG + Reserpine	40 + 4	65.3	58.7	51.4
T3-AGG + Imipramine + Reserpine	10 each + 4	75.1	69.2	60.8
Fluoxetine + Reserpine	10 + 4	81.2	68.5	62.9



Effect of Treatments on Brain Neurotransmitter Levels in Reserpine- Induced Depression Model

Reserpine-Induced Symptoms

In the reserving-induced depression model, both piperine and *Withania sonmifera* extracts significantly antagonized ptosis, catatonia, and sedation.

Effect of Treatments on Reserpine-Induced Symptoms (% Reduction from Control at 3 Hours)

Treatment	Dose (mg/kg)	% Reduction from Control		
		Ptosis	Catatonia	Sedation
Piperine	10	46.2	52.3	43.5
T2-AGG	40	53.3	45.7	40.2
T3-AGG + Imipramine	10 each	58.1	55.3	52.7

These results suggest that both piperine and *Withania sonmifera* extracts can reverse the biochemical and behavioral changes induced by reserpine, which is consistent with their antidepressant-like effects observed in FST & TST.

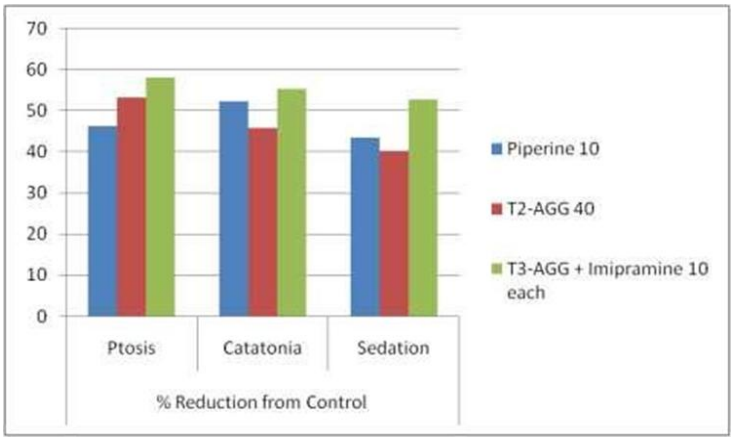


Fig. Effect of Treatments on Reserpine-Induced Symptoms

DISCUSSION

This comparative study of *Piper longum* and *Withania sonmifera* for antidepressant activity has yielded several important findings that contribute to our understanding of potential of these traditional medicinal plants as natural antidepressants. Both plants showed notable effects similar to those of antidepressants in well-established animal models of depression, The different profiles of *Piper longum* and *Withania sonmifera* suggest that they might be suited for different clinical scenarios. The rapid onset of action and potent MAO inhibition by piperine might be beneficial in acute depression requiring rapid intervention, while the gradual but sustained effects of *Withania sonmifera* might be more suitable for chronic depression requiring long-term management. The favorable safety profiles of both plants at the tested doses suggest that they may be well-tolerated alternatives or adjuncts to conventional antidepressant medications, which often cause significant side effects that limit patient adherence. It is important to note that although both plants showed promising antidepressant effects in Clinical research and animal models are required to verify their safety & effectiveness in humans.

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