Evaluating Withaferin A: Neuropsychopharmacological Impact through Pentobarbitone-Induced Sleep, Forced Swim Test, and Spontaneous Locomotor Activity Modulation in Mice

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Abstract

Withaferin A, a bioactive compound from Withania somnifera, was evaluated for its neuroprotective and neuropsychopharmacological effects. The study investigated its ability to mitigate oxidative stress in SH-SY5Y neuronal cells and its impact on sleep, depressive-like behaviour, and locomotion in Swiss albino mice. In the in vitro assay, Withaferin A significantly reduced intracellular reactive oxygen species (ROS) levels induced by hydrogen peroxide in a dose-dependent manner. In vivo, Withaferin A demonstrated mild sedative effects in the pentobarbitone-induced sleeping time assay, with a dose-dependent but non-significant increase in sleep duration. In the forced swim test, Withaferin A exhibited antidepressant-like effects, significantly reducing immobility time at higher doses (10 and 20 mg/kg). The spontaneous locomotor activity test revealed sedative properties at 20 mg/kg, with a significant reduction in photocell counts. These findings highlight Withaferin A's potential as a neuroprotective and mood-modulating agent, capable of reducing oxidative stress and improving behavioral outcomes. The dose-dependent effects observed warrant further investigation into its mechanisms of action and therapeutic applications in neurodegenerative and neuropsychiatric disorders.

Keywords: *Withaferin A, Behavioural pharmacology, Neuropsychopharmacology, Pentobarbitone-induced sleeping time, Forced Swim Test (FST), Spontaneous Locomotor Activity (SMA), Sedative, Antidepressant-like effects, Withania somnifera*

1. INTRODUCTION

Neurodegenerative and psychiatric disorders represent a significant burden on global health, affecting millions of individuals and contributing to reduced quality of life, high healthcare costs, and mortality. These disorders are often linked to oxidative stress, which results from an imbalance between the production of reactive oxygen species (ROS) and the body's ability to counteract their harmful effects through antioxidant defenses. Oxidative stress is a key factor in the pathogenesis of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis, as well as mood disorders like depression and anxiety. The search for therapeutic agents to combat these disorders has intensified, with natural compounds gaining attention for their safety profiles and multifaceted pharmacological properties (Frackowiak, 2004; Hesdorffer, 2016; Lassen, Ingvar, & Skinhøj, 1978; Tarazi & Schetz, 2005; Tyler, 2012).

Withaferin A, a bioactive compound isolated from Withania somnifera (commonly known as ashwagandha), has emerged as a promising candidate for addressing neurodegenerative and

neuropsychiatric conditions. Traditionally used in Ayurveda for its adaptogenic properties, Withania somnifera has been extensively studied for its neuroprotective, anti-inflammatory, and antioxidant effects (L.-X. Chen, He, & Qiu, 2011; Sultana et al., 2021). Withaferin A, one of its primary active constituents, has shown potent biological activities, including the ability to reduce oxidative stress, enhance neuronal survival, and modulate neurotransmitter systems. These properties suggest its potential to alleviate the cellular and molecular disturbances underlying neurodegeneration and psychiatric disorders (Hernandez-Hernandez et al., 2024; Hirakawa et al., 2024; Ho et al., 2024; Hortua Triana, Márquez-Nogueras, Fazli, Quinn, & Moreno, 2024; Iepsen et al., 2024; Jæger, Charwat, Wall, Healy, & Tveito, 2024; Sultana et al., 2021).

Oxidative stress exacerbates neuronal damage by promoting mitochondrial dysfunction, lipid peroxidation, and DNA damage. This leads to impaired cellular functions and ultimately cell death, driving the progression of neurodegenerative diseases. The reduction of ROS levels and enhancement of antioxidant defenses are therefore critical therapeutic strategies for protecting neuronal integrity. Withaferin A's strong antioxidant properties make it a suitable candidate for countering oxidative stress and mitigating its harmful effects on neuronal cells (Elliott & Ram, 2011; Longhena et al., 2021; Russell, 1988; Tarazi & Schetz, 2005).

In addition to its neuroprotective effects, Withaferin A has shown potential in modulating mood and behavior. Neurochemical imbalances, particularly disruptions in serotonin, dopamine, and norepinephrine pathways, are common in mood disorders such as depression. Traditional pharmacological treatments for depression and anxiety, including selective serotonin reuptake inhibitors (SSRIs) and benzodiazepines, often come with significant side effects and limited efficacy in some patients. Natural compounds like Withaferin A, which can influence neurotransmitter systems and exhibit mood-stabilizing effects, offer an alternative or adjunctive therapeutic option (Kutzsche et al., 2024; Lankford, Maddala, Jablonski, & Rao, 2024; Li et al., 2024; Liao, Hwang, Liu, & Lai, 2024; Lima et al., 2024; McKeever & Hamilton, 2018).

The evaluation of Withaferin A's effects on neuropsychiatric and neurodegenerative conditions requires robust experimental models that mimic key pathological and behavioral features (Y. Chen et al., 2023; Dutta, Nagendra, Raizada, Bhattacharya, & Sharma, 2023; Forlenza, Schamberger, & Buckingham, 2023; Hsu, Tsai, & Hsu, 2023; Levine, 2023; Wu et al., 2022). In this study, in vitro and in vivo methodologies were employed to assess its neuroprotective and neuropsychopharmacological properties. The in vitro evaluation was performed using SH-SY5Y neuronal cells, a widely used model for studying oxidative stress and neuroprotection. By measuring intracellular ROS levels under oxidative stress induced by hydrogen peroxide (H_2O_2), the study aimed to determine Withaferin A's ability to scavenge free radicals and protect neuronal cells (Wardas, Schneider, Klugbauer, Flockerzi, & Beck, 2023; Wei, Su, & Gao, 2023; Welsh, Bose, & Sahhar, 2023; Wickline et al., 2023; Wołek, Matusiak, Machoczek, Partyka, & Zasda, 2023; Zhao et al., 2023).

In vivo studies were conducted using Swiss albino mice to evaluate Withaferin A's effects on behavior and neurochemical modulation. The pentobarbitone-induced sleeping time assay was employed to assess its sedative potential, while the forced swim test (FST), a widely used model for antidepressant activity, was used to evaluate its effects on depressive-like behavior (Padmapriyadarsini et al., 2024; Wang et al., 2024; Wu, Gong, & Hu, 2024). The spontaneous locomotor activity (SMA) test was performed to examine its impact on locomotion and potential sedative effects. These behavioral assays provide critical insights into Withaferin A's ability to influence mood, sedation, and motor activity, which are relevant for managing neuropsychiatric disorders (Wardas et al., 2023; Wei et al., 2023; Welsh et al., 2023; Wickline et al., 2023; Wołek et al., 2023; Zhao et al., 2023). This study aims to bridge the gap in understanding Withaferin A's multifaceted pharmacological properties by integrating biochemical and behavioral approaches. By evaluating its antioxidative and behavioral effects, this research seeks to elucidate Withaferin A's therapeutic potential in managing oxidative stress-induced neurodegeneration and mood disorders. The findings are expected to contribute to the growing body of evidence supporting the use of natural compounds as safe and effective alternatives or adjuncts to conventional pharmacological treatments for neurological and psychiatric conditions.

2. MATERIAL AND METHODS

Drugs, Chemicals and Reagents

The study employed a selection of carefully sourced drugs and chemicals to ensure reliable and accurate results. Apomorphine Hydrochloride (Himedia, Mumbai, India) was used for its dopaminergic effects in neuropharmacological studies. Chlorpromazine Hydrochloride and Diazepam (Sigma Aldrich, Mumbai, India) served as standard antipsychotic and anxiolytic agents, respectively, in behavioral tests. Haloperidol (Himedia, Mumbai, India) was included as another neuroleptic agent. Pentobarbitone Sodium (Sigma Aldrich) was used for its sedative effects in inducing sleep in animal models, while

Pentylenetetrazol (Himedia) helped assess seizure susceptibility. Withaferin A, sourced from Angel Herbs in Mandi District, Himachal Pradesh, was a key test compound in the study. Additionally, Tween 80 (LobaChem, India) was used as an emulsifying agent to facilitate proper drug dispersion. These substances provided a robust framework for evaluating neuropsychopharmacological effects in the experimental models.

Animals

For this investigation, albino Swiss mice (weighing 22–25 grams, either female) were selected as experimental subjects. These animals were sourced from the Agricultural University at Mannuthy, Thrissur, Kerala. Prior to the commencement of the study, the mice were provided with ad libitum access to food and water, ensuring they were well-nourished. They were housed in groups of six to eight in polypropylene cages under a controlled environment with a natural light-dark cycle, allowing them to acclimatize to their surroundings. This acclimatization period was essential to minimize stress and ensure reliable results during the experiments. The animals were maintained under standard laboratory conditions, with ambient temperature and a quiet, evenly lit room designated for all experimental observations. Each observation session was conducted between 9:00 AM and 5:00 PM to maintain consistency. Each animal was used only once during the study to prevent overlapping influences from repeated testing. All experimental protocols were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) in strict compliance with the guidelines of the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), New Delhi, India. This ensured that all procedures adhered to ethical standards for the humane treatment and care of the animals throughout the study (Pereira, Veeraraghavan, Ghosh, & Gandhi, 2004).

Neuroprotective activity: Withaferin A decreases intracellular reactive oxygen species level

The neuroprotective activity of Withaferin A was evaluated by assessing its ability to decrease intracellular reactive oxygen species (ROS) levels in SH-SY5Y neuronal cells (Jia & Misra, 2007). The SH-SY5Y cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin, and 1% L-glutamine, and incubated at 37°C in a humidified atmosphere with 5% CO_2. For the experiment, cells were seeded in 96-well plates at a density of 1 × 10^4 cells per well and allowed to adhere overnight. The cells were divided into four groups: an untreated control group, a negative control group treated with hydrogen peroxide (H_2O_2, 100 µM) to induce ROS, and test groups pretreated with Withaferin A at concentrations of 5, 10, and 20 µM for 2 hours, followed by exposure to H_2O_2 for 1 hour. To measure ROS levels, the cells were incubated with 10 µM dichlorodihydrofluorescein diacetate (DCFH-DA) for 30 minutes at 37°C in the dark. After incubation, the cells were washed with phosphate-buffered saline (PBS) to remove excess dye, and the fluorescence intensity was measured using a microplate reader at an excitation/emission wavelength of 485/530 nm. The fluorescence data were normalized to the control group and expressed as a percentage of ROS levels. All experiments were performed in triplicate, and the results were presented as mean ± standard deviation (SD). Statistical analysis was conducted using one-way ANOVA followed by Tukey's post hoc test, with $p < 0.05$ considered statistically significant. This method enabled a robust evaluation of Withaferin A's neuroprotective potential, demonstrating its ability to mitigate oxidative stress by reducing intracellular ROS levels in SH-SY5Y neuronal cells.

Pentobarbitone induced sleeping time

Pentobarbitone (PB) (45 mg/kg; i.p.) was administered to the control group. Thirty minutes before to receiving an injection of pentobarbitone, the other three groups were given three doses of Withaferin A (i.p., 5, 10, and 20 mg/kg) (Siemens, Kalant, Khanna, Marshman, & Ho, 1974; Tsuji, Isobe, & Kawasaki, 1996).The pentobarbitone-induced sleeping time assay was conducted to evaluate the sedative effects of Withaferin A in albino Swiss mice. The experimental design consisted of four groups, with each group comprising six animals (n=6). The treatments and dosages administered are detailed in Table 1.

Experimental Procedure

Swiss albino mice (22–25 g, female) were sourced from the Agricultural University, Mannuthy, Thrissur, Kerala, and acclimatized under standard laboratory conditions for seven days prior to the experiment. The animals were fasted overnight, with free access to water, before the test.

Treatment Groups:

The mice were divided into four groups as per the experimental design:

- **Group A**: *Control group, received Pentobarbitone Sodium at 45 mg/kg intraperitoneally (i.p.).*
- **Group B**: *Received Withaferin A at 5 mg/kg (i.p.) followed by Pentobarbitone Sodium at 45 mg/kg (i.p.).*
- **Group C**: *Received Withaferin A at 10 mg/kg (i.p.) followed by Pentobarbitone Sodium at 45 mg/kg (i.p.).*

- **Group D**: *Received Withaferin A at 20 mg/kg (i.p.) followed by Pentobarbitone Sodium at 45 mg/kg (i.p.).*

Withaferin A was administered intraperitoneally 30 minutes prior to the injection of Pentobarbitone Sodium. Following the administration of Pentobarbitone Sodium, the mice were observed for the onset of sleep (loss of righting reflex) and total sleep duration (time from onset to regaining of righting reflex). Sleep onset and duration were recorded in minutes for each animal in all groups. The mean sleeping time (± standard deviation) was calculated for each group. Results were analyzed to evaluate the dose-dependent effects of Withaferin A on pentobarbitone-induced sleeping time. All experimental procedures were approved by the Institutional Animal Ethical Committee (IAEC) and conducted in accordance with CPCSEA guidelines to ensure the humane treatment of animals. This methodology allowed for the systematic evaluation of the potential sedative effects of Withaferin A in conjunction with Pentobarbitone Sodium.

Table 1. Experimental design table for pentobarbitone induced sleeping time

Group	Treatment Description	Dosage
Group – A	Control, received Pentobarbitone Sodium	45 mg/kg; i.p.
Group – B	Test, received Withaferin A + Pentobarbitone Sodium	Withaferin A: 5 mg/kg; i.p. + Pentobarbitone Sodium: 45 mg/kg; i.p.
Group – C	Test, received Withaferin A + Pentobarbitone Sodium	Withaferin A: 10 mg/kg; i.p. + Pentobarbitone Sodium: 45 mg/kg; i.p.
Group – D	Test, received Withaferin A + Pentobarbitone Sodium	Withaferin A: 20 mg/kg; i.p. + Pentobarbitone Sodium: 45 mg/kg; i.p.

Forced swim test

The behavioural despair swim test, also known as the forced swim test, has been used to assess behaviour like depression. All of the main kinds of antidepressant medications can be detected by this test (Can et al.,2012; Yankelevitch-Yahav, Franko, Huly,& Doron, 2015).The forced swim test (FST) was performed to evaluate the potential antidepressant-like effects of Withaferin A in albino Swiss mice. The experimental design included four groups, with six animals (n=6) in each group, as detailed in Table 2.

Experimental Procedure

Swiss albino mice (22–25 g, female) were sourced from the Agricultural University, Mannuthy, Thrissur, Kerala, and acclimatized under standard laboratory conditions for seven days before the experiment. Animals were fasted overnight with access to water prior to the test.

Treatment Groups:

Mice were divided into four groups:

- **Group A**: *Control group, received 1% aqueous solution of Tween 80 at a dose of 10 ml/kg intraperitoneally (i.p.).*
- **Group B**: *Test group, received Withaferin A at 5 mg/kg (i.p.).*
- **Group C**: *Test group, received Withaferin A at 10 mg/kg (i.p.).*
- **Group D**: *Test group, received Withaferin A at 20 mg/kg (i.p.).*

Treatments were administered intraperitoneally 30 minutes before the test to allow adequate systemic absorption. Each mouse was placed individually in a transparent cylindrical tank (25 cm height, 10 cm diameter) filled with water (23–25°C) to a depth of 15 cm to prevent the animal from touching the bottom. The test duration was six minutes, during which the mice's behavior was observed and recorded. The total immobility time during the last four minutes of the test was recorded. Immobility was defined as the lack of movement except for the minimal effort required to keep the animal's head above water. The mean immobility time (± standard deviation) for each group was calculated. Results were analyzed to determine the dose-dependent effects of Withaferin A on depressive-like behavior. All procedures were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) and conducted in compliance with CPCSEA guidelines to ensure animal welfare during the experiments. This methodology facilitated the assessment of the antidepressant-like properties of Withaferin A through systematic observation of immobility in the forced swim test.

Table 2. Experimental design table for the forced swim test

Group	Treatment Description	Dosage
Group – A	Control, received 1% aqueous solution of Tween 80	10 ml/kg; i.p.
Group – B	Test, received Withaferin A	5 mg/kg; i.p.
Group – C	Test, received Withaferin A	10 mg/kg; i.p.
Group – D	Test, received Withaferin A	20 mg/kg; i.p.

Spontaneous Locomotor Activity (SMA) using Actophotomer

A photocell activity cage was used to measure spontaneous locomotor activity. This method was used to measure the degree of depression (Gosavi, Kamdi, Kalambe, & Bohra, 2020a, 2020b). The spontaneous locomotor activity (SMA) test was conducted to evaluate the effects of Withaferin A on locomotor behavior in albino Swiss mice. The experimental design included four groups, each consisting of six animals (n=6), as shown in Table 3.

Experimental Procedure

Swiss albino mice (22–25 g, female) were sourced from the Agricultural University, Mannuthy, Thrissur, Kerala, and acclimatized under standard laboratory conditions for seven days before the test. Mice were fasted overnight with free access to water prior to the experiment.

Treatment Groups:

Mice were divided into four groups:

- **Group A**: *Standard group, received Chlorpromazine at a dose of 3 mg/kg intraperitoneally (i.p.).*
- **Group B**: *Test group, received Withaferin A at 5 mg/kg (i.p.).*
- **Group C**: *Test group, received Withaferin A at 10 mg/kg (i.p.).*
- **Group D**: *Test group, received Withaferin A at 20 mg/kg (i.p.).*

All treatments were administered intraperitoneally 30 minutes prior to the locomotor activity test. After the treatment period, each mouse was placed individually in an actophotometer (digital photoactometer) for a duration of five minutes. The apparatus measured the total locomotor activity in terms of the number of beam crossings (photocell counts) by the mouse. The total locomotor activity (beam counts) for each mouse was recorded, and the mean locomotor activity (± standard deviation) for each group was calculated. Data were analyzed to determine the dose-dependent effects of Withaferin A on spontaneous locomotor activity. All experimental protocols were approved by the Institutional Animal Ethical Committee (IAEC) and conducted in compliance with the guidelines of the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), ensuring humane treatment and care of the animals throughout the study. This methodology allowed for the systematic evaluation of the potential sedative or stimulant effects of Withaferin A on spontaneous locomotor activity in mice.

Table 3. Experimental design table for spontaneous locomotor activity

Group	Treatment Description	Dosage
Group – A	Standard, received Chlorpromazine	3 mg/kg; i.p.
Group – B	Test, received Withaferin A	5 mg/kg; i.p.
Group – C	Test, received Withaferin A	10 mg/kg; i.p.
Group – D	Test, received Withaferin A	20 mg/kg; i.p.

Statistical analysis

All experimental data were expressed as mean ± standard deviation (SD). Statistical comparisons between groups were performed using one-way analysis of variance (ANOVA) followed by post hoc Tukey's multiple comparison test to identify significant differences among the treatment groups. A significance level of $p < 0.05$ was considered statistically significant. The analysis was conducted using GraphPad Prism (version 8) software. The results were presented in tabular and graphical formats to facilitate interpretation. This approach ensured the robust evaluation of the dose-dependent effects of Withaferin A across various behavioural paradigms.

3. RESULTS AND DISCUSSION

In vitro Neuroprotective activity: Impact on intracellular reactive oxygen species level

The data in Table 7 illustrate the impact of Withaferin A on intracellular reactive oxygen species (ROS) levels in SH-SY5Y neuronal cells under oxidative stress induced by hydrogen peroxide (H_2O_2). The control group exhibited a baseline ROS level of 100, while exposure to H_2O_2 (500 µM) significantly elevated ROS levels to 276 ± 8.65, demonstrating pronounced oxidative stress. Pretreatment with Withaferin A revealed a dose-dependent reduction in ROS levels. At lower concentrations of 5 and 10 µg/ml, ROS levels were slightly reduced to 275 ± 7.26 and 235 ± 7.42, respectively. Higher concentrations of 20, 40, and 80 µg/ml showed marked reductions in ROS, with levels decreasing to 217 ± 6.27, 157 ± 5.14, and 153 ± 5.16, respectively.

These results indicate that Withaferin A effectively mitigates oxidative stress, particularly at higher concentrations, by significantly reducing ROS levels induced by H_2O_2. The data suggest that Withaferin A exhibits potent antioxidative properties, likely through its ability to scavenge free radicals and enhance cellular antioxidant defenses. This finding underscores its potential role as a neuroprotective agent in

managing oxidative stress-associated neurodegenerative diseases. Further studies should explore its underlying mechanisms and evaluate its therapeutic efficacy in vivo.

Table 4. Impact of Withaferin A in intracellular reactive oxygen species level.

Control	**H_2O_2**	**Withaferin A Concentration (µg/ml)**				
	500 µm	5	10	20	40	80
100	276±8.65	275±7.26	235±7.42	217±6.27	157±5.14	153±5.16

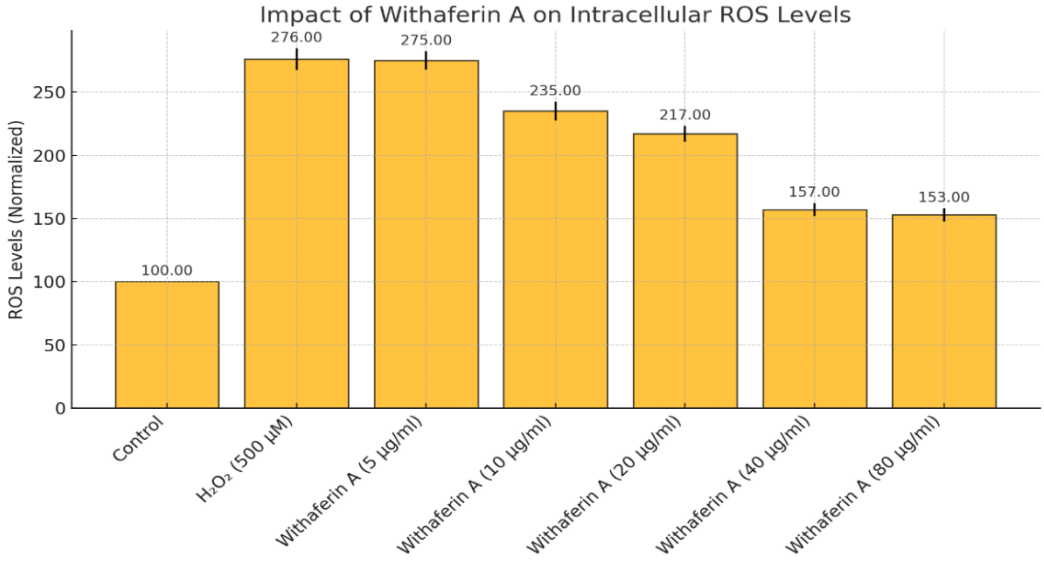

Figure 1. Impact of Withaferin A in intracellular reactive oxygen species level

Pentobarbitone - induced sleeping time

The effect of Withaferin A on pentobarbitone-induced sleeping time in mice is summarized in Table 5. The control group, which received only pentobarbitone sodium (40 mg/kg, i.p.), exhibited a mean sleep duration of 78.76 ± 2.10 minutes. Administration of Withaferin A at 5, 10, and 20 mg/kg did not produce statistically significant changes in sleep duration compared to the control group, as indicated by the "ns" (non-significant) label in the table. However, a dose-dependent increase in the sleeping time was observed, with durations of 79.82 ± 2.18, 81.58 ± 2.13, and 86.73 ± 1.97 minutes for 5, 10, and 20 mg/kg doses, respectively. These findings suggest that while Withaferin A at higher doses exhibits a trend toward prolonging pentobarbitone-induced sleeping time, the changes were not statistically significant under the conditions of this study. This may indicate a mild sedative-like effect of Withaferin A, possibly enhancing the action of pentobarbitone at higher doses, although further studies with larger sample sizes or alternative experimental designs may be necessary to confirm this trend and elucidate the underlying mechanisms.

Table 5. Effect of Withaferin A on Pentobarbitone - induced sleeping time in mice

Treatment (mg/kg,i.p)	**Duration of action (Min.)**
Control PB (40 mg/kg, i.p)	78.76 ± 2.10
Withaferin A (5)	79.82 ± 2.18 **ns**
Withaferin A (10)	81.58 ± 2.13 **ns**
Withaferin A (20)	86.73 ± 1.97 **ns**

Values are mean ± SEM of 6 animals in each group, ns (One way ANOVA followed by Dunnett's test as compared with Pentobarbitone treated group)

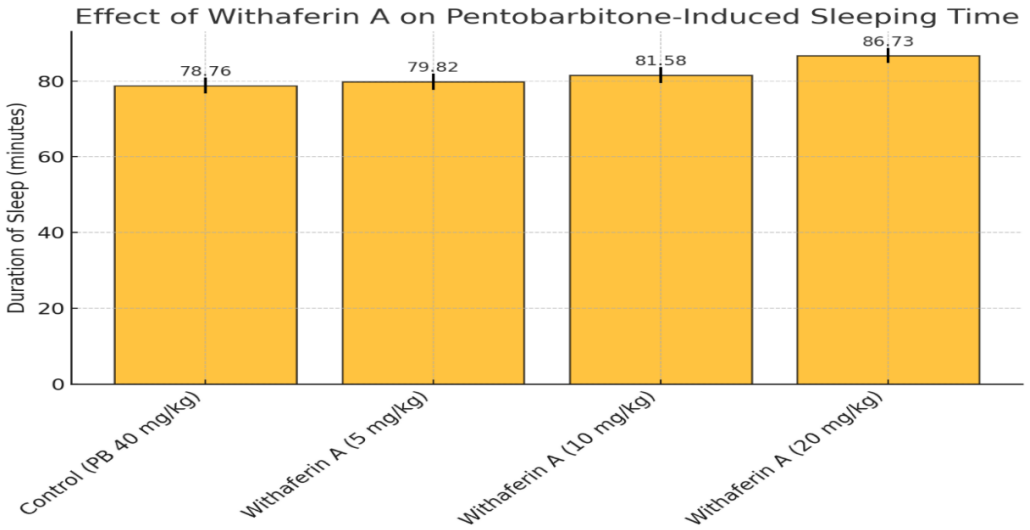

Figure 2. Effect of Withaferin A on Pentobarbitone - induced sleeping time in mice

Forced swim test

The results of the forced swim test (FST) evaluating the effect of Withaferin A on immobility duration in mice are presented in Table 6. The control group exhibited an immobility duration of 155.95 ± 6.37 seconds, indicating baseline depressive-like behavior. Withaferin A at a dose of 5 mg/kg showed a slight, non-significant increase in immobility duration (170.81 ± 6.05 seconds), suggesting no notable antidepressant-like effect at this dose. However, Withaferin A at 10 mg/kg significantly reduced immobility to 112.75 ± 5.62 seconds, and at 20 mg/kg, the duration further decreased to 99.99 ± 4.98 seconds, with the latter reaching statistical significance ($p < 0.05$). These findings suggest a dose-dependent antidepressant-like effect of Withaferin A, particularly at higher doses (10 and 20 mg/kg). The reduction in immobility time implies that Withaferin A potentially enhances coping behavior in response to stress in the FST. This effect may be mediated by its modulation of neurochemical pathways involved in mood regulation. Further studies are needed to elucidate the underlying mechanisms and confirm its therapeutic potential in mood disorders.

Table 6. Effect of Withaferin A on Force Swim Test in mice

Treatment (mg/kg, i.p)	**Duration of Immobility (seconds)**
Control	155.95 ± 6.37
Withaferin A (5)	170.81 ± 6.05
Withaferin A (10)	112.75 ± 5.62
Withaferin A (20)	99.99 ± 4.98*

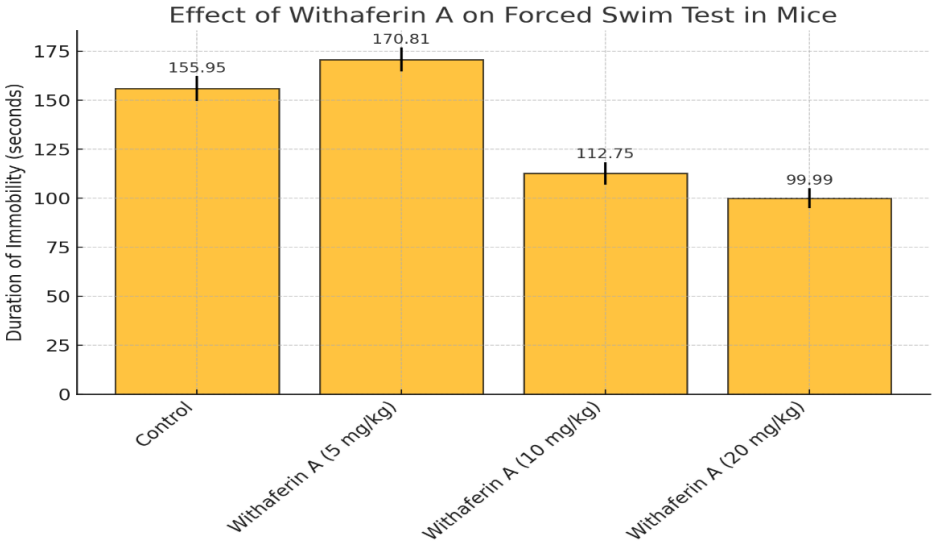

Figure 3. Effect of Withaferin A on Force Swim Test in mice

Spontaneous Locomotor Activity (SMA) using Actophotometer

The results of the spontaneous locomotor activity (SMA) test, measured using an actophotometer, are summarized in Table 7. The control treatment with chlorpromazine (3 mg/kg) significantly reduced photocell counts from 543.76 ± 17.90 to 418.94 ± 16.22 ($p < 0.001$), confirming its sedative effect. Withaferin A at 5 mg/kg caused a minor reduction in locomotor activity from 541.59 ± 17.87 to 502.38 ± 17.32, which was not statistically significant. Similarly, the 10 mg/kg dose showed negligible change, with counts decreasing from 504.67 ± 18.94 to 496.67 ± 14.79. However, Withaferin A at 20 mg/kg significantly reduced photocell counts from 540.92 ± 14.86 to 434.48 ± 15.91 ($p < 0.001$), indicating a dose-dependent sedative-like effect at higher concentrations. These findings suggest that Withaferin A may have mild sedative properties, particularly at higher doses, as demonstrated by the reduced spontaneous locomotor activity. The lack of significant changes at lower doses indicates a threshold effect for its sedative action. Further investigations are needed to explore the underlying mechanisms of this dose-dependent behaviour.

Table 7. Effect of Withaferin A on Spontaneous Locomotor Activity (SMA) using Actophotometer

Treatment (mg/kg,i.p)	**Photocell count**	
	Before administration	**After administration**
Chlorpromazine (3 mg/kg)	543.76 ±17.90	418.94±16.22***
Withaferin A (5)	541.59± 17.87	502.38±17.32
Withaferin A (10)	504.67± 18.94	496.67±14.79
Withaferin A (20)	540.92±14.86	434.48±15.91***

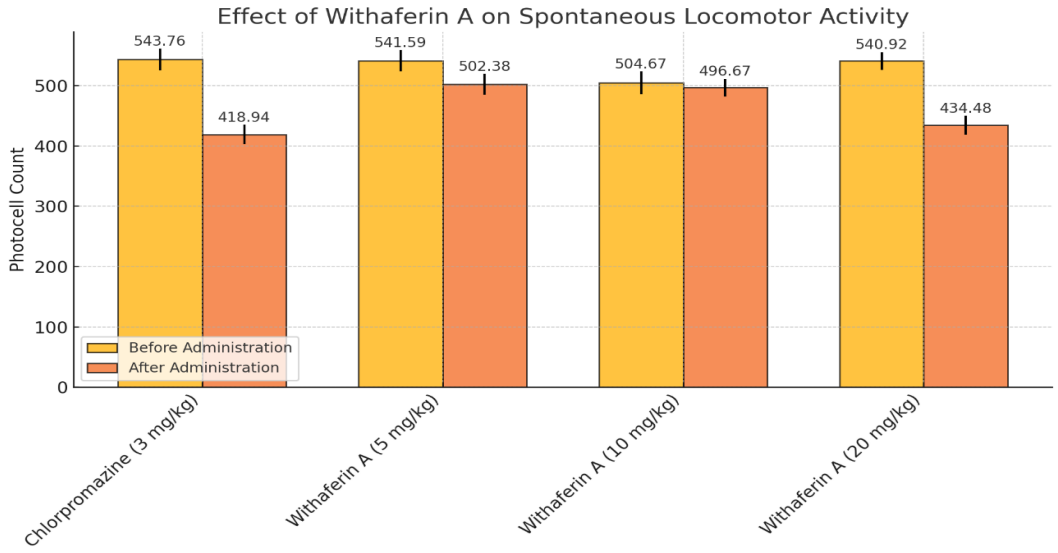

Figure 4. Effect of Withaferin A on Spontaneous Locomotor Activity (SMA)

4. CONCLUSIONS

The study demonstrates the promising neuroprotective and neuropsychopharmacological properties of Withaferin A. In vitro, it significantly reduced intracellular ROS levels in SH-SY5Y neuronal cells, emphasizing its antioxidative potential. In vivo, Withaferin A showed mild sedative effects in the pentobarbitone-induced sleeping time assay and significant antidepressant-like activity in the forced swim test, reducing immobility time at higher doses. Additionally, its sedative effects were evident in the spontaneous locomotor activity test at 20 mg/kg, indicating a dose-dependent threshold for behavioral modulation. These findings suggest that Withaferin A can mitigate oxidative stress, enhance coping behaviours, and modulate locomotor activity, supporting its potential use in managing oxidative stress-associated neurodegeneration and mood disorders. Further research is needed to elucidate its mechanisms of action, optimize dosing regimens, and evaluate its efficacy in clinical settings.

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