RESEARCH PAPER

Antistress Biochemical And Histopathological Exploration Of Phytogenic Nanoformulation

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Abstract

Depression is one of the most common mental health disorders affecting millions worldwide, often leading to serious emotional and physical distress. Traditional plant-based remedies have gained attention as safer alternatives to conventional medications. In this study, we investigated the anti-depressant potential of biosynthesized silver nanoparticles (Ag-NPs) derived from *Cissampelos pareira* leaf extracts. Using a green synthesis method, Ag-NPs were prepared and characterized. Their effects were evaluated in a rodent model of depression using behavioral tests (Force Swim Test), biochemical markers of oxidative stress, and brain histology. The results demonstrated that *Cissampelos pareira*-derived Ag-NPs significantly reduced depression-like behaviors, lowered oxidative stress levels, and showed protective effects on brain tissues. These findings suggest that green-synthesized Ag-NPs from *Cissampelos pareira* may represent a promising natural therapeutic approach for managing depression.

Keywords: *Depression, Cissampelos pareira, Silver Nanoparticles, Green Synthesis, Force Swim Test, Oxidative Stress.*

How to cite this article: Devi D, Barman M, Thakur P, Sharma N, Singh D, Chakraborty T. Antistress Biochemical and Histopathological Exploration of Phytogenic Nanoformulation. Int J Drug Deliv Technol. 2026;16(12s): 177-185. DOI: 10.25258/ijddt.16.12s.19

Introduction

Depression is a common and debilitating mental health disorder characterized by persistent sadness, lack of interest or pleasure, disturbed sleep or appetite, and cognitive impairments [1]. It affects over 280 million people worldwide and is a leading cause of disability according to the World Health Organization [2]. While conventional antidepressant drugs such as selective serotonin reuptake inhibitors (SSRIs) and tricyclic antidepressants (TCAs) are widely used, they are often associated with delayed onset of action, side effects, and limited efficacy in a significant portion of patients [3]. These limitations have driven a growing interest in alternative therapeutic strategies, particularly those derived from natural products and nanotechnology-based interventions [4]. *Cissampelos pareira*, commonly known as velvetleaf or "Patha" in traditional Ayurvedic medicine, has long been used for its diverse pharmacological properties [5]. The phytoconstituents present in *C. pareira*—such as alkaloids, flavonoids, and tannins serve as natural reducing and capping agents in nanoparticle synthesis, potentially enhancing the biological efficacy of the synthesized AgNPs [6]. Among various nanoparticles, silver nanoparticles (AgNPs) have attracted significant attention due to their unique physicochemical properties and bioactivity [7]. Recent studies have suggested that extracts from this plant possess antioxidant and anti-inflammatory properties, which may help in reducing depression [8]. With advancements in nanotechnology, green synthesis using plant extracts has emerged as an eco-friendly method to produce nanoparticles [9]. Silver nanoparticles (Ag-NPs) are known for their antioxidant, antimicrobial, and potential neuroprotective effects [10]. Thus, combining *Cissampelos pareira* extracts with silver nanoparticles could offer a novel approach depression treatment [11].

Material and Methods

Plant Material Collection and Extraction

Leaves of *Cissampelos pareira* were collected, dried, and processed to prepare extracts. Phytochemical screening confirmed the presence of key bioactive compounds such as flavonoids and phenolic compounds. *Cissampelos pareira* extract leaves were processed for soxhlet extraction procedure and then extract was found to contain flavones and identify its total ash content by TLC.

Duration of Experimental Protocol

Duration of experimental protocol was of 8 days. In which 7 days of Force Swim Test and 3 days of treatment protocol and after that the animals were sacrificed.

Dosage of *Cissampelos pareira*

Cissampelos pareira extract was administered in the doses i.e. (50mg/kg, 100mg /kg & 200mg/kg) from 4th day to 7th day.

Duration of Experimental Protocol/ Animal Grouping

There were six groups in the present study and each group was comprised of five animals to obtain accurate and precise data from these groups of animals, each animal was subjected to experimental studies and tests for TBARS, GSH and other histological examination.

Green Synthesis of Silver Nanoparticles

Silver nanoparticles were synthesized using the plant extract as a reducing and stabilizing agent. The synthesis conditions included controlled concentration, temperature, and stirring speed to ensure uniform particle formation. Recent advancements in nanotechnology have highlighted the potential of green synthesis methods for producing silver nanoparticles (Ag-NPs), which offer a safer and more sustainable alternative to conventional chemical processes. One promising approach involves the use of plant-based materials rich in natural reducing agents. Among these, *Cissampelos pareira*, known for its medicinal properties, has emerged as a valuable source for the eco-friendly synthesis of (Ag-NPs). Extracts from this plant contain various bioactive compounds — including phenolics, flavonoids, and terpenoids — that not only facilitate the formation of nanoparticles but also enhance their biological functionality.

Table No. 01: Variables used for Green Synthesis of Silver Nano particles

Sr. no.	*Cissampelos pareira* Extract conc. (mg/ml)	Conc. Of AgNO3 solution	Temperature (°C)	Stirring speed (rpm)
1.	6	1mMAgNO3	35	400
2.	8		40	500
3.	10		50	600
4.	12		60	700
5.	14		70	800

Animal Model and Experimental Design

Rodents were used as the experimental model to evaluate depression-related behaviors. The animals were divided into groups: Normal group, control group, standard drug group and Ag-NP-treated groups.

Table. No. 02: Experimental protocol in which animals were often separated into six groups, with five animals per group.

Groups	Pharmacological Interventions	Dose(mg/kg)	Route (p.o.)	No. of animals
Group 1	Normal control Group	Water	Oral	5
Group 2	Disease Control Group	FST	-	5
Group 3	Test group-I	50	Oral	5
Group 4	Test group-II	100	Oral	5
Group 5	Test group-III	200	Oral	5
Group 6	Standard Group (Imipramine)	10	Oral	5

Behavioral Assessment

FST In this test, mice are placed in a cylindrical container (10 cm in diameter, 25 cm in height) filled with 19 cm of water maintained at 23-25 °C. The mice were gently placed in the water allowed to swim randomly for 6 minutes. During the measurement only the head was above the water, while the body was floating in the Water as show in **Fig No. 1.**

Measurement of Locomotors movement

A computerised actophotometer was used to measure locomotor activity for 5 minutes. Mice were placed individually in a transparent plastic cage and allowed to

acclimate to the observation chamber for 2 minutes. Each animal was observed using a digital photo acto photometer in a square closed arena equipped with infrared light sensitive photocells, and locomotion was measured in total counts per 5 minutes per animal. The apparatus was placed in testing room that was darkened, light and sound attenuated and ventilated.

Assessment of Rotarod test

Rotarod test, although simple, is used for the motor coordination impairment, grip strength, neurotoxicity and ataxic effects of the compound on the animals. Standard rotarod apparatus consisting of a rotating rod of diameter 3cm was used. Animals were trained to remain at the rod rotating at12 rpm. The cut- off time in the training was 3 minutes and the animals were gently back on the rod if they fail to remain on it for 3 minutes. The cut-off time in the testing phase was 1 minute and the animals were tested to remain at the rod rotating at 18 rpm to test their motor coordination post kindling. The time to first fall was recorded for analysis purposes.

Figure 1: Behavioural Estimations using Force Swim Test Maize.

Biochemical Analysis

Brain tissues were analyzed for oxidative stress markers, including glutathione (GSH) levels and Thio barbituric acid-reactive substances (TBARS). Depression was experimentally induced in mice using the Force Swim Test (FST) model to assess oxidative stress by measuring TBARS (Thio barbituric Acid Reactive Substances) levels in brain tissue. TBARS, a key indicator of lipid peroxidation, was significantly elevated in the depression-induced group compared to the control, reflecting increased oxidative stress. Treatment with the standard antidepressant drug imipramine (10mg/kg) significantly reduced TBARS levels, indicating its antioxidant and neuroprotective effects. Similarly, administration of *Cissampelos pareira* extract at varying doses (50, 100, and 200 mg/kg) produced a dose-dependent reduction in TBARS levels. TBARS readings were similar to those of the imipramine-treated group, indicating that the 200 mg/kg dose was especially effective. *Cissampelos pareira* may be used therapeutically to treat depression disorders because of its strong antioxidant activity and potential to provide neuroprotection against oxidative damage brought on by anxiety.

Glutathione (GSH), a significant natural antioxidant, is essential for preventing oxidative damage to brain tissue. In this study, mice were given the Force Swim Test (FST) model to elicit depression. This led to a large drop in brain GSH levels, suggesting that depression is related with increased oxidative stress. GSH levels were markedly restored after treatment with the common antidepressant medication imipramine (10 mg/kg), indicating its neuroprotective and antioxidant properties. Likewise, GSH levels increased in a dose-dependent manner when 50, 100, and 200 mg/kg of *Cissampelos pareira* extract were given. The greatest rise was seen at 200 mg/kg, which almost brought GSH levels back to normal. *Cissampelos pareira* potent antioxidant properties can successfully lower oxidative stress by raising GSH levels in the brain. Consequently, providing neuroprotection in situations where depression is present.

Histopathological Examination

Brain sections were examined under a microscope to assess structural changes and neuronal protection. The histological analysis of the brains in the various groups will probably show clear alterations associated with depression and the results of therapy. Brain tissue from the normal group should show no symptoms of inflammation or degeneration and a healthy neuronal architecture. As a result of depression-induced oxidative stress and neuroinflammation, the raised plus maze disease group may exhibit gliosis, increased apoptosis, and neuronal damage. Imipramine treatment (10mg/kg) is anticipated to promote neuroprotection by lowering glial activity and maintaining neuronal structures. While the 200 mg/kg dose should exhibit stronger neuroprotective effects, as compared to Imipramine, with restored neuronal integrity, reduced apoptosis, and decreased neuroinflammation, the 100 mg/kg dose display partial protection in the *Cissampelos pareira* groups with some reduction in neuronal damage and gliosis. The findings imply that *Cissampelos pareira* has antidepressant and neuroprotective effects that are dose-dependent and may be mediated by its antioxidant qualities.

Estimation of TBARs:

Using the will's method and thiobarbituric acid-reactive compounds, the malondialdehyde content- a gauge of lipid peroxidation-was ascertained. 0.5 ml of postmitochondrial supernatant and 0.5 ml of Tris HCL were incubated for two hours at 37°C. After incubation, 1 ml of 10% trichloroacetic acid was added, and the

mixture was centrifuged at 1000g for 10 minutes. The tubes were immersed in boiling water for 10. minutes after being filled with 1 ml of supernatant and 1 ml of 0.67% Thio barbituric acid. After cooling, 1ml of double- distilled water was added, and absorbance at 532 nm was calculated. Thio barbituric acid-reactive compounds were measured using the extinction coefficient of 1.56 * 105M/cm and expressed as nm of malondialdehyde per mg of protein of Thio barbituric acid. The amount of malondialdehyde protein in the brain was estimated using the biuret method and expressed as nm of malondialdehyde per mg of protein.

Estimation of Glutathione:

The amount of reduced glutathione in the brain was calculated using Ellman's method. To calculated the reduced (GSH) level, the tissue homogenate was taken and dissolved in 0.1 M phosphate buffer, pH 7.4. The homogenate was combined with an equal amount of 20% trichloroacetic acid that contained 1mM EDTA in order to precipitate the tissue proteins. The mixture stood for 5 minutes before being centrifuged. A new set of tubes was then filled with the supernatant (200 microliters), and 1.8 ml of Ellman's (0.5-dithio bis-2 nitrobenzoic acid) (0.1m M), which was made in 0.3 M phosphate buffer with 1% sodium citrate, was added. When all test tubes had accumulated 2 ml. following the completion of the complete reaction, the solution' 412nm wavelengths were measured in comparison to a blank. The absorbance results were compared using a standard curve built using a recognized GSH standard curve.

Statistical Analysis

The observations were statistically analysed using InStat3, and all results were presented as the mean ± SEM. A one-way analysis of variance (ANOVA) was conducted, followed by Tukey's multiple comparison test, with $p < 0.05$ considered statistically significant.

Results

Qualitative Analysis of the Phytochemicals of *Cissampelos pareira* extract

The preliminary phytochemical investigation revealed that Cissampelos pareira are enriched with various secondary metabolites such as Alkaloids, Flavonoids, Terpenoid, Phenols and Tannin.

Table no. 3: Phytochemical Screening of Hydroethanolic Extract of Leaves of *Cissampelos pareira*

Sr. No.	Extract Constituents	Indication/Results
1.	Alkaloids	+
2.	Terpenoids	+
3.	Carbohydrates	+
4.	Phenolic	+
5.	Tannins	+
6.	Proteins	-
7.	Saponins	-
8.	Steroids	-
9.	Glycosides	-

'+' indicates positive means presence and '-'indicates negative means absent

Behavioral Outcomes FST In this test, mice are placed in a cylindrical container (10 cm in diameter, 25 cm in height) filled with 19 cm of water maintained at 23-25 °C. The mice were gently placed in the water allowed to swim randomly for 6 minutes.

Green Synthesis of C. pareira –AgNP The Cissampelos pareira silver nanoparticles [C. pareira - AgNPs] were formed within an hour of the reaction marked with prominent change of colour from yellow to dark

Reddish brown indicating that silver has been reduced as depicted in Fig No. 9 The blackening of the previously white reaction mixture may be attributable of the surface Plasmon resonance of the silver nanoparticles, which is regarded as the key Characteristic of nanoparticles production. Most silver-synthesized nanoparticles exhibited the color change as pale yellow to dark reddish brown.

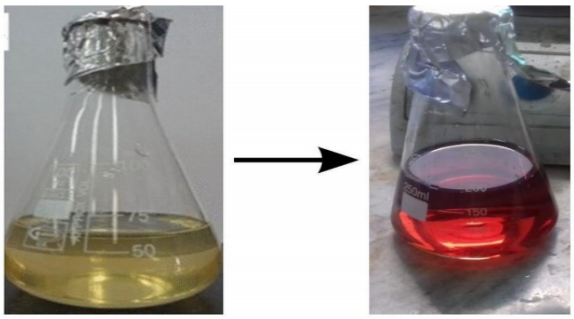


Figure 2: Green Synthesis of *Cissampelos pareira* leaves Extract AgNPs.

Oxidative Stress Markers

Treatment with Ag-NPs increased brain GSH levels and decreased TBARS, reflecting reduced oxidative stress.

Behavioral Parameters Characterization

Behavioral Parameters Characterization of *Cissampelos pareira* on Immobility time in Force Swim Test, Locomotor Activity in Actophotometer and fall off Time in Rotarod.

Table No. 5 Effect of *Cissampelos pareira* (50, 100, 200 mg/kg) on Immobility Time in
Force Swim Test, Locomotor Activity in Actophotometer and fall off Time in Rotarod
where a vs normal; b vs Inducer; bc1 vs Standard, bc2 vs standard bc3 vs standard and
values are reported as p<0.001 and mean ± S.D.

Group	Immobility Time	Locomotor Activity	Grip Strength
Group-I Normal Control	30.82±0.17	214±1.07	74.83±0.37
Group- II Disease Control	38.96±0.19 [a]	95±0.45 [a]	23.83±0.11[a]
Group- III Standard (Imipramine 10mg/kg)	24.45±0.12 [b]	187±0.93 [b]	67.83±0.33 [b]
Group- IV (*Cissampelos pareira* 50mg/kg)	32.83±0.14 [bc1]	146±0.74 [bc1]	54.83±0.27 [bc1]
Group- V (*Cissampelos pareira* 100mg/kg)	30.83±0.13 [bc2]	162±0.81 [bc2]	58.83±0.29 [bc2]
Group- VI (*Cissampelos pareira* 200mg/kg)	28.83±0.12 [bc3]	172±0.88 [bc3]	60.83±0.32 [bc3]

Superscripts in Table no. 5 indicates a is disease control, b is standard group, bc1 is 50mg/kg, bc2 is 100mg/kg and bc2 is 200 mg/kg. In which per activity 3 values mean were calculated and values are expressed as mean ±SD. p<0.01.

In comparison with vehicle-treated, unstressed mice, chronic stress for three consecutive weeks significantly ($p < 0.001$) reduced the immobility duration of mice as given in **Figure 3.** *Cissampelos pareira* (10 mg/kg), blank nanosuspension (50 mg/kg), (100 mg/kg) had no impact on the length of time that the mice remained immobile.

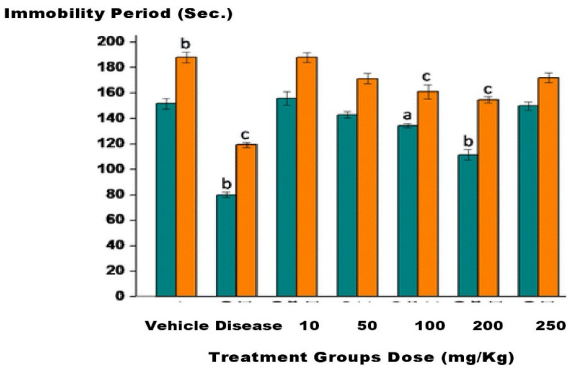


Figure 3: Effect of *Cissampelos pareira* Silver nanoparticles on immobility period of mice using FST Model (n=6). Values are exressed as mean±SEM. Data was analyzed using ANOVA. $p < 0.0001$. $^{a,b}p < 0.01$ and $p < 0.001$ respectively.

Effect on plasma Corticosterone levels. The unstressed mice did not show a significant effect on plasma corticosterone levels by *Cissampelos pareira* nanoparticles (10 mg/kg), AgNPs (50 mg/kg), (100 mg kg^{-1}. Compared to depressed mice treated with a vehicle, animals subjected to persistent unpredictable mild stress had significantly higher plasma corticosterone levels ($p < 0.001$). Nanoparticles dose (10 mg kg^{-1}) shows significantly ($p < 0.01, p < 0.05, p < 0.01$ and $p < 0.001$ respectively) lowered plasma corticosterone content of stressed mice in comparison with their corresponding vehicle-treated controls.

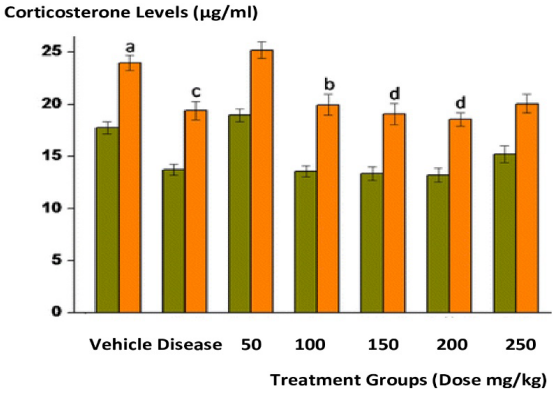


Figure 4: Effect of various drug treatments on the plasma corticosterone levels. $n = 5$ in each group. Values are expressed as the mean ± SEM. Data were analyzed by one-way ANOVA followed by the Tukey–Kramer multiple comparison test.

Biochemical Parameters

Estimation of GSH in Different Grouped Animals

Glutathione (GSH), a major endogenous antioxidant, plays a critical role in protecting brain tissue against oxidative damage. In the present study, depression was induced in mice using the Force Swim Test model, which resulted in a significant decrease in brain GSH levels, indicating heightened oxidative stress associated with depression. Treatment with the standard drug imipramine (10mg/kg) significantly restored GSH levels, confirming its antioxidant and neuroprotective effects. Similarly, *Cissampelos pareira* extract administered at doses of 50, 100, and 200 mg/kg led to a dose-dependent elevation in GSH levels. The 200 mg/kg dose showed the most prominent increase, nearly restoring GSH to normal levels. *Cissampelos pareira* possesses potent antioxidant properties and can effectively mitigate oxidative stress by enhancing GSH levels in the brain, thereby offering neuroprotection in depressed conditions.

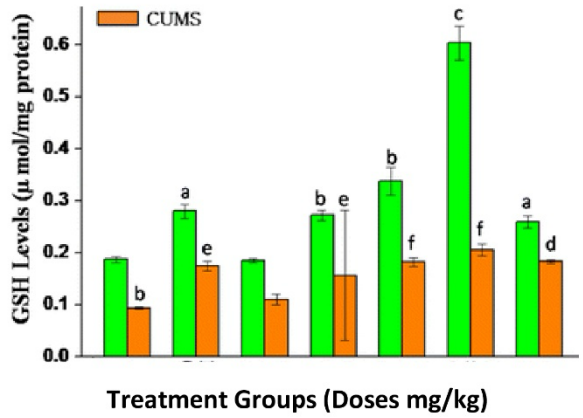


Figure 5: GSH Estimation in different Grouped Animal.

Effect on brain MAO-A levels. On comparing mice treated with the vehicle to unstressed mice, it was found that vehicle treated group shows significantly ($p <$ 0.001) enhanced brain MAO-A activity in the mice. Phytogenic AgNPs Dose (10 mg kg^{-1}), dose (25 mg kg^{-1}), doses (25 mg kg^{-1}) and *Cissampelos pareira* (5 mg kg^{-1}) *per se* administered for 3 consecutive weeks significantly ($p <$ 0.05, $p <$ 0.05, $p <$ 0.001 and $p <$ 0.05, respectively) decreased MAO-A activity of unstressed mice in comparison with the control group treated with the vehicle.

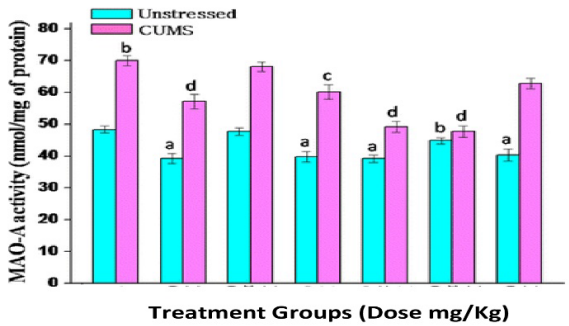


Figure 6: Effect of various drug treatments on brain MAO-A activity. $n =$ 10 in each group. Values are expressed as the mean ± SEM. Data were analyzed by one-way ANOVA followed by the Tukey–Kramer multiple comparison test.

Estimation of TBARs in Different Grouped Animal

Depression is closely associated with oxidative stress, often marked by elevated levels of lipid peroxidation products such as Thio barbituric acid reactive substances (TBARS). Treatment with the standard drug Imipramine(10mg/kg) significantly reduced TBARS levels, indicating its antioxidant and neuroprotective effects. Similarly, administration of *Cissampelos pareira* extract at varying doses (50, 100, and 200 mg/kg) produced a dose-dependent reduction in TBARS levels. The 200 mg/kg dose was particularly effective, showing TBARS values comparable to the imipramine treated group. Cissampelos pareira exhibits notable antioxidant activity and may offer neuroprotection against depression-induced oxidative damage, supporting its potential therapeutic use in depressive disorders.

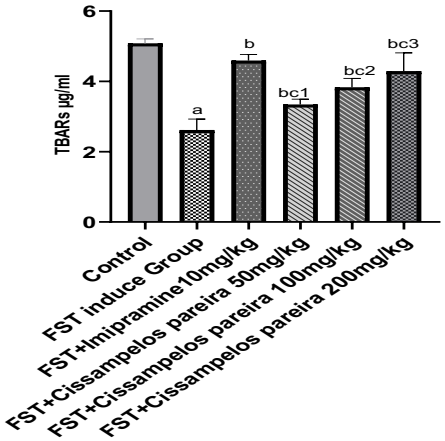
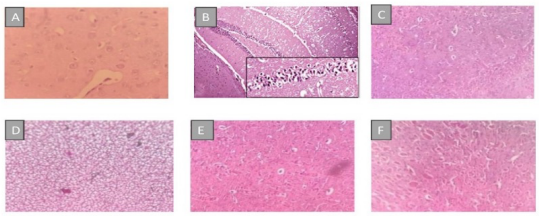


Figure No. 7: TBARs Estimation in Different Grouped Animal



Histological Findings

Histopathological analysis showed improved neuronal integrity and reduced signs of damage in the Ag-NP-treated group. Brain's pathological and biochemical changes can be easily **Figure 8: A** Normal Control. **B** Disease Control (FST). **C** Standard group (Imipramine) **D** Treatment *Cissampelos pareira*(50mg/kg). **E** Treatment *Cissampelos pareira* (100mg/kg*)*. **F** Treatment *Cissampelos pareira*(200mg/kg) revealed using the cellular organizations. Brain comprised of neurons and glial cells that include cerebral cortical cells that diagnose the brain conditions and biochemical fixations like trauma, stroke, infections, demyelination and tumors. Neurological brain associated depressive disorder observed the hippocampus (brain region for memory and emotions) volume reduction. Neurons damage and excess activation of microglia and astrocyte was observed. Glial cell density and neuronal size was also reduced. Beyond structural changes, functional

imaging studies also demonstrate the depression induction and treatment using the test drug molecule.

The histopathological examination of the brain across the different groups will likely reveal distinct changes related to depression and the effects of treatment. In the normal group, brain tissue should exhibit healthy neuronal architecture with no signs of degeneration or inflammation. The Force Swim Test induce group may show neuronal damage, increased apoptosis, and gliosis, indicative of anxiety-induced oxidative stress and neuroinflammation. Treatment with Imipramine (10mg/kg) is expected to provide neuroprotection, preserving neuronal structures and reducing glial activity. In the *Cissampelos pareira* groups, the 50 mg/kg dose may show partial protection with some reduction in neuronal damage and gliosis, while the 100 &200 mg/kg dose should demonstrate stronger neuroprotective effects, similar to Imipramine, with restored neuronal integrity, reduced apoptosis, and decreased neuroinflammation. These findings would suggest that *Cissampelos pareira* exhibits dose-dependent neuroprotective and anxiolytic effects, potentially mediated by its antioxidant properties.

Discussion

The study demonstrates that silver nanoparticles synthesized from *Cissampelos pareira* extracts have strong anti-depressant effects [12]. The behavioral improvements were supported by reduced oxidative stress and preserved brain tissue structure [13]. The observed benefits may be attributed to the high antioxidant content *Cissampelos pareira* which helps counteract reactive oxygen species implicated in depression pathophysiology [14]. Additionally, the use of green synthesis avoids toxic chemicals, making it a safer and environmentally friendly approach [15]. Treatment with the standard antidepressant drug imipramine (10mg/kg) significantly reduced TBARS levels, indicating its antioxidant and neuroprotective effects. Similarly, administration of *Cissampelos pareira* extract at varying doses (50, 100, and 200 mg/kg) produced a dose-dependent reduction in TBARS levels. TBARS readings were similar to those of the imipramine-treated group, indicating that the 200 mg/kg dose was especially effective. GSH levels increased in a dose-dependent manner when 50, 100, and 200 mg/kg of *Cissampelos pareira* extract were given. The greatest rise was seen at 200 mg/kg, which almost brought GSH levels back to normal.

Imipramine treatment (10mg/kg) is anticipated to promote neuroprotection by lowering glial activity and maintaining neuronal structures. While the 200 mg/kg dose should exhibit stronger neuroprotective effects, as compared to Imipramine, with restored neuronal integrity, reduced apoptosis, and decreased neuroinflammation, the 100 mg/kg dose display partial protection in the *Cissampelos pareira* groups with some reduction in neuronal damage and gliosis. These findings open possibilities for developing natural, nanotechnology-based therapies for depression [16]. However, further studies including detailed molecular analyses and human clinical trials are needed to confirm these effects [17]. In addition to its antioxidant effects, Cissampelos pareira modulated neurotransmitter levels, particularly serotonin (5-HT), a neurotransmitter that plays a critical role in regulating mood, depression, and stress responses. Increased serotonin levels were observed after Cissampelos pareira administration, particularly at the higher dose of 200 mg, which further supports its anxiolytic effects. This modulation of serotonin is crucial as serotonin deficiency is often associated with heightened depression. The higher dose of 200 mg/kg produced a more pronounced antidepressant effect compared to the 100 mg/kg dose, demonstrating a dose-dependent relationship. The combined effects of enhanced antioxidant defences, including increased catalase and GSH levels, along with the modulation of neurotransmitters like serotonin, contribute to the overall calming effect on the central nervous system. These findings suggest that Cissampelos pareira exerts its antidepressant actions not only by reducing oxidative stress but also by enhancing neurochemical balance, both of which are essential in depression regulation.

Histopathological analysis of brain tissues further supported these biochemical findings, showing less oxidative damage and improved neuronal health in Cissampelos pareira treated mice as compared to the control group. The preservation of neuronal integrity, as indicated by histopathological observations, aligns with the enhanced catalase activity and overall antioxidant status, underscoring the potential of *Cissampelos pareira* in promoting brain health and resilience against depression related changes. These results provide robust evidence for the antidepressant potential of *Cissampelos pareira* through its antioxidant, anti-inflammatory, and neurotransmitter modulating properties.

Overall, the results of this study suggest that *Cissampelos pareira* could serve as a natural alternative for managing depression, with its rich phytochemical content providing antidepressant benefits. This plant could potentially offer a safer and

more holistic approach to depression management, with fewer side effects compared to
conventional antidepressant drugs.

Conclusion

Cissampelos pareira-derived green-synthesized silver nanoparticles significantly alleviated depression-like behavior and oxidative stress in rodents. This suggests a promising role for these nanoparticles as a natural anti-depressant therapy. 200 mg/kg dose should exhibit stronger neuroprotective effects, as compared to Imipramine, with restored neuronal integrity, reduced apoptosis, and decreased neuroinflammation, the 100 mg/kg dose display partial protection in the *Cissampelos pareira* groups with some reduction in neuronal damage and gliosis. The results demonstrated that *Cissampelos pareira*-derived Ag-NPs significantly reduced depression-like behaviors, lowered oxidative stress levels, and showed protective effects on brain tissues. These findings suggest that green-synthesized Ag-NPs from *Cissampelos pareira* may represent a promising natural therapeutic approach for managing depression.

Acknowledgement

We extend our sincere gratitude to the Rayat Bahra University, Mohali (RBU) for providing the necessary facilities and resources to carry out this research. We are also grateful to the Sophisticated Pharmacology master's research centre lab for conducting the animal experiments in a timely manner, and for the support and guidance of the faculty and staff throughout the project.

Conflict of interest

The authors of the research paper titled " Neuromodulatory Potential of Phytogenic Silver Nanoparticles: Investigating Cell specific delivery and Synaptic plasticity using Depression resistant rodents Model" declare that there are no conflicts of interest that could potentially influence the objectivity or integrity of the research findings presented in this manuscript. We believe in maintaining transparency and scientific integrity throughout the research process. Thus, we affirm that this research paper is free from any conflicts of interest that could compromise the credibility or impartiality of the reported results. If any conflicts of interest arise in the future related to this research or its publication, we commit to disclosing them promptly and transparently to ensure the highest standards of scientific integrity and ethics.

Funding source

This research did not receive any specific grant from funding agencies in the public and commercial sectors. We confirm that we have no financial or personal relationships with any individuals or organizations that could inappropriately influence our work or bias our interpretations. Additionally, there are no competing interests, such as patents or financial support that might be perceived as having influenced the research or the preparation of this manuscript.

Statement of informed consent

We all confirm that all authors voluntarily agreed that we all take part after being fully informed of the research study's purpose, rationale and procedures, usually including approval for publishing the current findings.

Ethics of human and animal experimentation

Experiments with animals was done in accordance with the legal requirements of the relevant local or national authority. Procedures was such that experimental animals do not suffer unnecessarily.

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