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Research Article

Evaluation of the Antipsychotic and Neuroprotective Properties of *Bergenia ciliata* Root Extract in Rat Models

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ABSTRACT

Psychosis, a chronic mental illness, can be treated with plants and traditional herbs, which contain phytochemicals and counter oxidants to support body organs and reduce schizophrenia risk. The study aimed to evaluate the antipsychotic potential of *Bergenia ciliata* by collecting, authenticating, and preparing a methanolic extract from its roots. Two experimental models were used to simulate psychotic symptoms in rats and assess the extract's influence on movement and activity patterns. The objective parameters included behavioral assessments of stereotypic and locomotor activities to quantify the effects of the extract. Biochemical analysis was conducted on brain tissue homogenates, focusing on glutathione (GSH) estimation, acetylcholinesterase (AchE) activity assay, and TNF-α measurement to evaluate inflammation levels. Histopathological analysis of the brain, particularly the frontal cortex and hippocampus was also conducted to examine structural changes in brain tissues. Key observations: This analysis provided insights into the neuroprotective effects of the methanolic root extract on critical brain regions implicated in psychotic disorders. The results showed that the apomorphine control group had high stereotypy levels, while the apomorphine + standard drug group showed a significant reduction in stereotypy scores at 60 and 90 minutes. Both dosages of *B. ciliata* showed significant reductions in stereotypy scores at 60 and 90 minutes, suggesting its potential to mitigate the stereotypy induced by apomorphine. The study also found significant differences in GSH, AchE, and TNF-alpha levels between the normal control (vehicle) and apomorphine 1.5 mg/kg, the std drug (Haloperidol 1-mg/kg), and *B. ciliata* (250 and 500 mg/kg). Recordings in an actophotometer were taken on days 1, 8, 15, and 23. The data revealed no significant differences between groups, except for the positive control, which exhibited a significant decrease in comparison to the healthy animals. A notable increase in locomotor activity was observed following the administration of diazepam in the *B. ciliata* group, particularly when contrasted with the positive control group. Additionally, *B. ciliata* exhibited significant effects on biochemical parameters compared to the positive control. The histology of the brain's cerebellar cortex, hippocampus, and frontal cortex under different conditions showed well-preserved layers and healthy neurons. The diazepam-treated brain showed mild neuronal degeneration with vacuolations and congested blood vessels. Oligodendrocytes and pyramidal cells were less affected, but some neuronal degeneration was evident. Both low and high doses of the test drug maintained normal cortical and hippocampal structure, with healthy neuronal architecture, oligodendrocyte presence, and adequate vascularization. The study suggests that *B. ciliata* may be an effective antipsychotic agent, potentially reducing inflammation and enhancing psychotropic effects.

Introduction

An absence of reality awareness can define a mental health condition called psychosis. Approximately 450 million human beings have been affected throughout the world; one million people endanger their lives and commit suicide annually, and one in every four families has a member who has a mental illness. Schizophrenia, hallucinations, social abandonment, nonsensical activity or expression, trouble paying attention, anxiety, low mood, and thoughts of suicide are some of its symptoms. Patients, particularly

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those from economically prosperous countries like Nigeria, endorse looking for therapy from folk healers since, despite the illness's effect on society, patient care is still inadequate. In their opinion, conventional antipsychotic medications like clozapine, risperidone, and haloperidol (atypical antipsychotics) are less risky, costly, and easier to evaluate than traditional medicine. Extended usage of such medicines causes increased oxidative breakdown and extrapyramidal effects, which can lead to conditions known as heart failure, diabetes, and other serious problems.[1]

The market for herbal medications is always growing. Herbal medications are well-known for having extremely few side effects, making them an excellent treatment option for long-term CNS disorders like psychosis that are essentially untreated. It is also referred to as shaggy leaf bergenia curled bergenia or *Pashanbheda* in Hindi. There are around six species of the family Bergenia, which are found in the subtropical Himalayan Mountains, Central Asia, and the eastern region of Asia. This biennial herb, growing as high as 50 cm in height and spherical, is found across the subtropical Mountain region, ranging from 2000 to 2700 m, throughout Kashmir to Nepal. It is commonly found on hills in and around the Murree region area. Bergenia root is thought to have exactly the beneficial properties of gentiana root, and it is also thought to be demulcent and de-obstruent. It also alleviates discomfort in the chest and ribs caused by severe cold humor and serves effectively as a mild emmenagogue and diuretic. Eliminate kidney stones, blockages in the renal excretory system, and hazardous contaminants that are still present in the intestinal tract. The diffusion is thought to be a lot more potent than the root in treating respiratory conditions, asthma, epilepsy, and spasmodic reactions; the root is efficient in treating long-term venereal diseases.[2] *Bergenia ciliata* (family Saxifragaceae), an extremely advantageous plant, has long been employed as a medication for treating various human illnesses it has been generally referred to as "Zakhmehayat" or "Pakhanabhed."[3] *B. ciliata* is a miraculous plant known for its various health benefits, including treating various ailments such as renal system, gall bladder stones,[4] gastrointestinal issues, pulmonary infections, heart diseases, cataracts, hemorrhoids, kidney, and gall bladder stones. It is also known for its antimicrobial, anticancer, antitussive, antiviral, analgesic, anti-inflammatory, antibacterial, liver disease, and anti-malarial activity.[5] Phytochemical investigations have found gallic acid, bergenin, catechin, gallicin, paashaanolactone, arbutin, β-sitosterol, afzelechin, and other compounds found in the plant. Catechin, a type of phenol linked to (+)-catechin or -the compound epicatechin, has antipsychotic properties. It is believed to be encapsulated in cyclodextrins, improving its flavor and making it suitable for use as an additive. Catechin, a histidine decarboxylase inhibitor, reduces the risk of histamine-related local immunological responses and is effective in treating HIV-related cognitive deterioration. Some flavonoids containing epicatechin can serve as a defense against cytotoxic antioxidants and activate brain-derived neurotrophic networks.[6] In addition, its chemical compounds have been used to treat neurodegenerative conditions like amnesia and Alzheimer's disease.[7] The study explores the potential of *B. ciliata*, a plant with traditional medicinal uses and minimal side effects, as a safer, long-term treatment for psychosis and other neuropsychiatric disorders. With an estimated 450 million people affected globally, traditional medicine is often expensive and side effects are prevalent. This research aims to explore the use of an herbal remedy that could be cost-effective, accessible, and safer, especially in countries with limited healthcare infrastructure. *B. ciliata* is rich in phytochemicals like catechins, bergenin, and flavonoids, known for their neuroprotective and antioxidant properties. The study advocates for the development of phytomedicines to address CNS disorders, which are frequently unresponsive to conventional treatments. If proven effective, *B. ciliata* could become part of a broader category of herbal medications that are more sustainable, affordable, and culturally accepted, especially in regions where traditional medicine is widely practiced. The study contributes to ethnopharmacology and scientific validation, bridging the gap between traditional knowledge and modern science.

Materials And Methods

Plant Material and Authentication

The roots of *B. ciliata* were collected in June at Gangtok, Sikkim and authenticated by Dr. V. Rama Rao along with Dr. S.H Doddamani at Central Ayurveda Research Institute, Bangalore. Authentication number: Authentication/SMPU/CARI/BNG/2024-25/967.

Preparation of Plant Extraction

B. ciliata roots were dried in the shade for several days, then finely powdered and sieved to ensure uniform particle size. About 250 g of powdered roots were extracted using 1000 mL of methanol in three cycles. The solution was filtered using Whatman filter paper no. 1 to remove solid residues. The filtrate was evaporated to dryness, removing all solvents, and a constant weight of the methanolic extract was obtained. The extract was stored for further experimental use to assess its antipsychotic effects. This method ensures optimal extraction of bioactive compounds while maintaining the integrity of the phytochemicals in *B. ciliata* roots.[8-9]

Experimental Design

Stereotype assessment animal model

The study involved Wistar male albino rats, each weighing between 150 and 200 g, housed in polypropylene

cages under controlled conditions. The rats were acclimatized for 7 days before the experiment, and all experiments were conducted in strict accordance with the Experimental Protocols approved by the Institutional Animal Ethics Committee (IAEC) Regs no. KCP-IAEC/14/23-24/09/28/03/24. The rats were housed in groups of six per cage. The study was divided into five groups, with six animals in each group (N = 30), to evaluate the antipsychotic effect of *B. ciliata* using the apomorphine-induced stereotypic behavior model. Acute toxicity data for the plant extract had already been reported previously.[10] Group I (Normal Control): Animals received normal saline as the baseline control. Group II (Disease Control): Rats were administered with apomorphine to induce stereotypic behavior associated with psychosis. Group III (Standard Treatment Group): Rats were treated with std drug haloperidol (1-mg/kg, i.p.). Test Group 1: Animals were given a low dose of *B. ciliata* methanolic extract (250 mg/kg, p.o.), and group V (Test Group 2): Animals received a higher dose of *B. ciliata* methanolic extract (500 mg/kg, p.o.). After 60 minutes, all the groups were administered apomorphine (1.5 mg/kg, s.c.) except normal control. Observations of stereotypic behaviors were made 10, 30, 60, and 90 minutes following apomorphine injection. The study aims to assess the ability of *B. ciliata* extract to mitigate apomorphine-induced stereotypic behaviors in comparison with a standard antipsychotic (haloperidol) using well-established protocols.[11]

Induction of apomorphine

Apart from normal control, all the groups were induced with apomorphine 1.5 mg/kg, S.C. The standard drug group and test drug groups received apomorphine after 1-hour of administration of standard drug haloperidol and test groups *B. ciliata.*

The stereotypic behavior of the rats was assessed using a standardized scoring system based on their activity levels and specific behaviors. The scoring system for behavioral analysis was defined as follows: a score of 0 indicated the animal was asleep or still, while a score of 1 represented active movement. A score of 2 denoted predominantly active behavior with occasional bursts of stereotyped actions such as sniffing and rearing. When constant stereotyped activity, like sniffing, rearing, or head bobbing, was observed alongside ongoing locomotor movement, a score of 3 was assigned. A score of 4 represented continuous stereotyped behavior restricted to a single location. If this stereotyped activity was accompanied by bursts of licking, gnawing, or biting, a score of 5 was given. Scores of 6 and 7 indicated continual licking or biting of the cage grids, respectively. This scoring method allows for the detailed quantification of stereotypic behaviors, enabling precise comparisons across experimental groups.

Sacrifice and Analysis

Following the induction of apomorphine in antipsychotic activity, all animals were sacrificed under a high dose of phenobarbitone sodium for ethical euthanasia, and the brain tissue was isolated. Residual blood was removed by washing the tissue with pre-cooled PBS buffer. The total protein concentration was assessed using the method described by Lowry (1951). The samples were subsequently stored at -20°C for future analysis.

Locomotor Activity Animal Model

The study involved a total of 18 animals, categorized into several groups, including a positive control group and two test drug groups. The positive control group was administered 5 mg/kg of diazepam once per hour prior to the experimental procedure. In contrast, the test drug groups received daily doses of *B. ciliata* extract at 250 and 500 mg/kg, administered over a 23-day period. Diazepam served as the positive control drug. The rats were observed for 10 minutes in an actophotometer, with recordings taken on days 1, 8, 15, and 23, as well as half an hour after the medication was administered. The test drug was given daily throughout the 23 days.[12]

Biochemical Parameters: From Brain Tissue Homogenates

Estimation of glutathione

Glutathione (GSH) serves as a vital antioxidant, playing an essential role in maintaining oxidative homeostasis within the brain. To explore this further, a GSH assay was conducted using a combination of homogenate, trichloroacetic acid (TCA), disodium hydrogen phosphate, and DTNB, with absorbance measured at 412 nm.

The procedure began by mixing 0.3 mL of the homogenate with 0.3 mL of TCA at a concentration of 10% (w/v). The mixture was then centrifuged at 1000 rpm for 10 minutes. Following centrifugation, 0.5 mL of the supernatant was obtained, and 0.3 mL of 0.3 M disodium hydrogen phosphate and 0.25 mL of DTNB were added. The absorbance was measured at 412 nm, and the GSH concentration was calculated and expressed in µg/mg.

Acetyl-choline esterase activity assay (AChE)

A 0.4 mL homogenate was combined with 2.6 mL of a 0.1 M PBS (pH 8) and 100 µL of a DTNB solution in a cuvette. The mixture was thoroughly agitated by bubbling air through the solution, and the absorbance was measured at 412 nm using an LKB spectrophotometer. After the absorbance stabilized, a baseline reading was recorded. Following this, 20 µL of acetylthiocholine was added as the substrate. Changes in absorbance were tracked every 2 minutes over a duration of 10 minutes. The enzyme activity was then calculated based on the rate of change in absorbance per minute. Enzyme activity calculation:

$$R = 5.74 \times 10^{-4} \times A/CO$$

Where, R = rate in moles of substrate hydrolysed/minute/gm tissue. 5.74 × 10^{-4} = constant obtained from the extinction coefficient of thionitro benzoic acid (1.36 × 10^4/molar/centimetre), A = change in absorbance/min, CO = Original concentration of the tissue (mg/mL). The activity was expressed as µM hydrolyzed per min/gram of tissue. [13,14]

Tumor necrosis factor-α (TNF-α)

TNF-alpha is a cytokine produced by various cells, including macrophages, T cells, and endothelial cells. ELISA Procedure (BD OptEIA™ Biosciences Solid Phase Sandwich ELISA).[15,16]

To prepare samples and diluents, briefly add 50 µL of ELISA diluent to each well of a 96-well plate. Carefully pipette 50 µL of each sample and standard into their designated wells. Cover the plate with a sealer and incubate it at room temperature for 2 hours. After incubation, remove the contents of the wells by aspirating or decanting the liquid, then fill each well with 300 µL of pre-mixed wash buffer. Aspirate or decant the Wash Buffer and repeat this washing step four more times for a total of five washes. After the final wash, blot the plate on absorbent paper to ensure complete removal of any remaining buffer for optimal performance. Next, add 100 µL of the detection antibody to each well, cover the plate with a sealer, and incubate it at room temperature for 1-hour. To prepare the enzyme-working reagent, transfer the required amount of enzyme diluent into a clean container and add the specified amount of enzyme concentrate. Mix thoroughly. After washing the wells to remove any unbound detection antibody using the previous wash procedure (five washes), add 100 µL of the enzyme-working reagent to each well. Cover the plate with a sealer and incubate at room temperature for 30 minutes. Then, wash the wells seven times to ensure thorough cleaning. Following the washes, add 100 µL of TMB one-step substrate reagent to each well and incubate the plate in the dark at room temperature for 30 minutes. Finally, after the 30-minute incubation, add 50 µL of stop solution to each well. Measure the absorbance of each well at 450 nm within 30 minutes after adding the stop solution to ensure accurate readings.

Histopathological examination of the brain

After the completion of the experiment, the brains of the rats were carefully removed and placed in a sterile petri dish. The hippocampus and frontal cortex were quickly dissected from the brain to minimize tissue degradation. Immediately following dissection, the isolated brain regions were immersed in 10% formalin to preserve the tissue for histological examination.[17,18]

Statistical Analysis

The data are presented as Mean ± SEM (n = 6). Statistical analyses were conducted using GraphPad. For statistics, ANOVA, followed by Tukey's and Bonferroni tests, was used for multiple comparisons within the groups. A $p < 0.05$ was deemed statistically significant when comparing the untreated normal control group with all other groups.

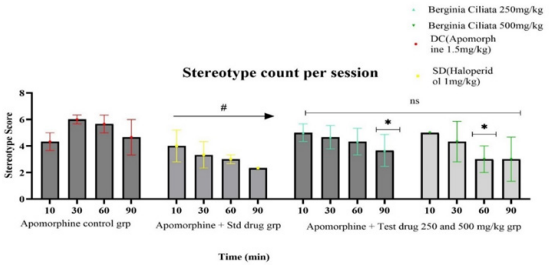
Results And Discussions

Psychosis, a long-term mental disorder characterized by a loss of connection with reality, is a serious public health issue affecting millions globally. It is characterized by perceptual disturbances difficulty distinguishing between reality and illusion, and can persist throughout life. Examples of psychotic disorders include schizophrenia, bipolar disorder, and drug-induced psychosis.[19] Symptoms include hallucinations, delusions, disorganized speech, and cognitive impairments.

Plants contain phytochemicals that play a significant role in preventing and managing diseases by supporting different body functions. Traditional herbs, such as *B. ciliata*, have been proven to have medicinal value and can be used to treat various human conditions. For instance, in schizophrenia, antioxidants have been found to reduce the risk of developing the disorder.[20] Phytochemical investigations of *B. ciliata* have identified compounds such as gallic acid, bergenin, catechin, gallicin, paashaanolactone, arbutin, β-sitosterol, afzelechin, and other active compounds. Other bioactive constituents include tannic acid, mucilage, catechin, glucose, albumin, metarbin, mineral salts, and wax. These bioactive constituents contribute to the plant's wide array of pharmacological effects, making it a promising candidate for treating conditions like psychosis.[21,22] Fig. 1 represents a study on the effects of *B. ciliata* at two different dosages (250 and 500 mg/kg), compared to a standard drug (Haloperidol, 1-mg/kg) on stereotypy counts over time in an apomorphine model.

Key Observations

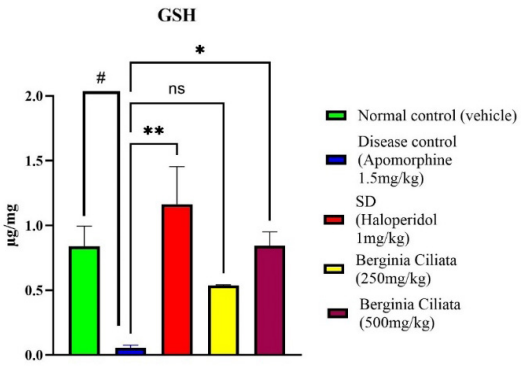
The apomorphine control group shows a steady stereotypy score across all time points (10, 30, 60, 90 minutes),



Values are expressed as Mean ± SEM, (n=6 rats in each group). #$p < 0.05$ indicates a statistically significant difference compared to the apomorphine control group. ns (not significant) $p > 0.05$ indicates no significant difference when compared to the control group. At 60 and 90 minutes, *$p < 0.05$ shows a statistically significant effect for the test drug at 250 mg/kg and 500 mg/kg, respectively.

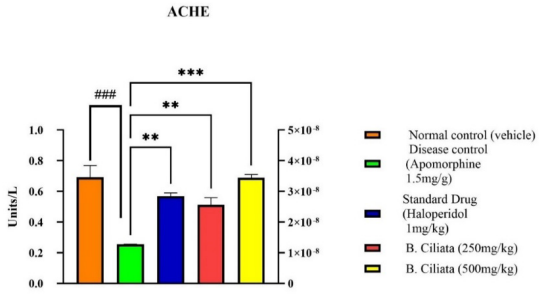
Fig. 1: Graphical representation of apomorphine induced stereotype behavior in rats (Behavioral study)

indicating high stereotypy levels due to the drug. Apomorphine + Standard drug group (Haloperidol 1-mg/kg) demonstrates a notable decrease in stereotypy scores at both the 60 and 90-minute marks (indicated by #p <0.05) when compared to the positive control group. Apomorphine + Test drug groups (*B. ciliata* 250 and 500 mg/kg): Both dosages of *B. ciliata* show significant reductions in stereotypy scores at 60 and 90 minutes, compared to the apomorphine control (indicated by *p <0.05), suggesting its potential in mitigating the stereotypy induced by apomorphine. # p <0.05 denotes a statistically significant difference in stereotypy scores for the standard drug group compared to control.* p <0.05 indicates significant reductions in the stereotypy scores for the test drug groups compared to the Apomorphine control group ns stands for non-significant differences at 30 minutes between groups. The study seems to suggest that *B. ciliata* has a significant anti-stereotypic effect, particularly at later time points, similar to the effects of haloperidol. A study was done to check the level of GSH. Significant differences (Fig. 2) were found between normal control (vehicle) and apomorphine 1.5 mg/kg, apomorphine and haloperidol 1-mg/kg, std drug, apomorphine and *B. ciliata* (500 mg/kg), haloperidol and *B. ciliata* (250 mg/kg), no significant differences were observed between the other group pairs. Study on AchE (Fig. 3): Significant differences were found between normal control (vehicle) and apomorphine, apomorphine and haloperidol, apomorphine and *B. ciliata* (500 mg/kg), apomorphine and *B. ciliata* (250 mg/kg), no significant differences were observed between the other group pairs. Study on TNF-α (Fig. 4): A significant difference was found between apomorphine and *B. ciliata* (500 mg/kg). No significant differences were observed between the other group pairs.



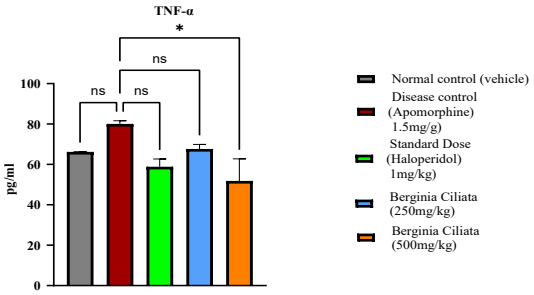
Values are expressed as Mean ± SEM, (n=6 rats in each group). #p < 0.05 indicates a statistically significant difference compared to the apomorphine disease control group vs normal control. ns (not significant) p > 0.05 indicates no significant difference when compared to the disease control group. *p < 0.05 and **p < 0.01 compared to the disease control group.

Fig. 2: Effect of *B. ciliata* in the level of GSH in apomorphine- induced stereotypy in rats



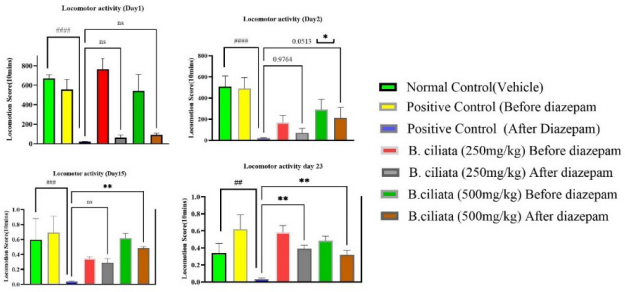
Values are expressed as Mean ± SEM, (n = 6 rats in each group). ###p < 0.001 indicates a statistically significant difference compared to the apomorphine disease control group vs normal control. ***p < 0.001 and **p < 0.01 compared to the disease control group.

Fig. 3: Effect of *B. ciliata* on AchE in apomorphine - induced stereotypy in rats.



Values are expressed as Mean ± SEM, (n=6 rats in each group). *p < 0.05 compared to the disease control group. Rest all other groups were showing non-significant

Fig. 4: Effect of *B. ciliata* on TNF-α in apomorphine - induced stereotypy in rats



Values are expressed as Mean ± SEM, (n = 6 rats in each group). ####p < 0.0001, ###p < 0.001, and ##p < 0.01 compared to normal control, **p < 0.01 and *p < 0.05 compared to the disease control group. Rest all other groups were showing non-significant

Fig. 5: Diazepam associated locomotor activity of over time

The second study was done on diazepam-associated locomotor activity. Recordings in an actophotometer were taken on days 1, 8, 15, and 23.
Key Observations (Fig. 5):

Day 1 locomotor activity

No significant differences (ns) are observed between groups, with all treatments showing similar locomotor scores except positive control, which is a significant decrease compared to normal control.

Day 7 locomotor activity

Significant differences (####$p < 0.0001$) were noted when comparing the normal control group to the positive control group (post-diazepam treatment). The administration of *B. ciliata* at a dose of 500 mg/kg after diazepam led to a notable increase in locomotor activity when compared to the positive control (*$p < 0.05$). On day 15, locomotor activity assessments indicated significant differences (####$p < 0.0001$) between the normal control and both the positive control and other groups. Notably, *B. ciliata* at 500 mg/kg exhibited a significant increase (**$p < 0.01$) in locomotor activity compared to the positive control. By day 23, *B. ciliata* at both 250 mg/kg and 500 mg/kg doses showed the highest locomotor scores, with significant differences (**$p < 0.01$) compared to the positive control and all other groups. The positive control after diazepam treatment shows a major decrease in locomotor activity compared to earlier days. The study suggests that *B. ciliata* influences locomotor activity, with 500 mg/kg showing a consistent increase across days, especially after diazepam administration. Diazepam seems to reduce locomotor activity, and the herbal treatment could counteract this effect. A study for GSH (Fig. 6), AchE (Fig. 7) and TNF-α (Fig. 8) was done in the case of diazepam-associated locomotor activity.

- *GSH*

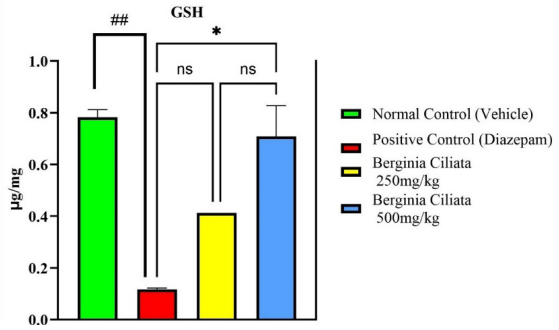
Significant differences were found between normal control (vehicle) and positive control (Diazepam), positive control (Diazepam) and *B. ciliata* (500 mg/kg)
No significant differences were observed between the other group pairs.

- *AchE*

Significant differences were found between positive control (Diazepam) and *B. ciliata* (500 mg/kg), *B. ciliata* (250 mg/kg) and *B. ciliata* (500 mg/kg). No significant differences were observed between the other group pairs.

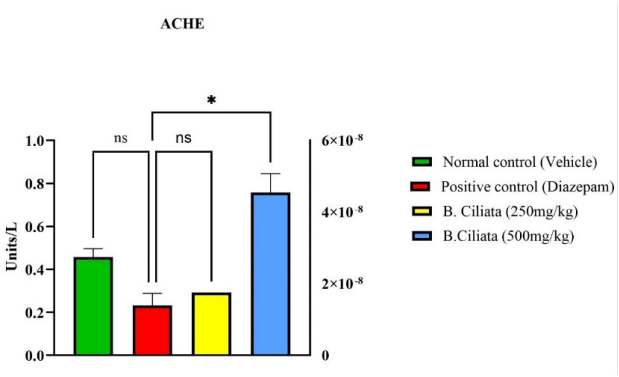
- *TNF-α*

Significant differences were found between normal control (vehicle) and positive control (Diazepam), positive control (Diazepam) and *B. ciliata* (250 mg/kg), positive control (Diazepam) and *B. ciliata* (500 mg/kg), no significant differences were noted among the various group pairs.
The histology of the brain's cerebellar cortex, hippocampus, and frontal cortex under different conditions. Fig. 9 shows the normal structure of the cerebellar cortex is well-preserved, showing distinct layers and cell types. Cortical neurons indicated by an arrow in the image. The neurons,



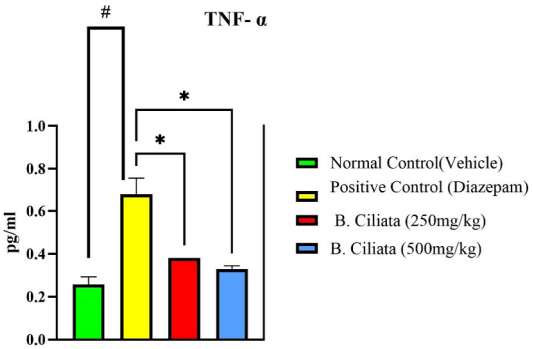
Values are expressed as Mean ± SEM, (n=6 rats in each group). ##p<0.01, compared to normal control, *p<0.01 and nsp>0.05 compared to the disease control group

Fig. 6: Effect of *B. ciliata* on diazepam associated locomotor activity in GSH



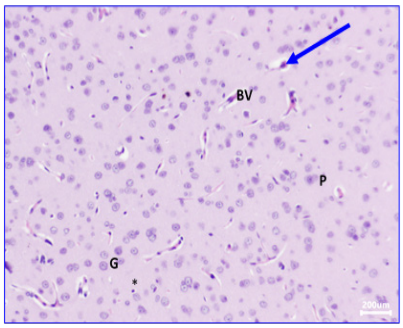
Values are expressed as Mean ± SEM, (n = 6 rats in each group). nsp>0.05 compared to normal control, *$p < 0.05$ compared to the disease control group. Rest all other groups were showing non-significant.

Fig. 7: Effect of *B. ciliata* on diazepam associated locomotor activity in AchE

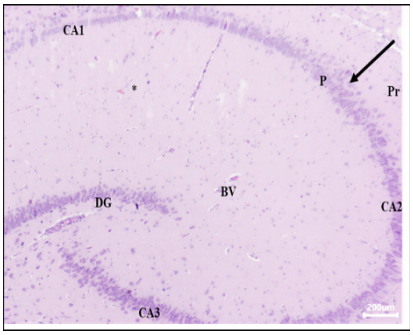


Values are expressed as Mean ± SEM, (n=6 rats in each group). #p<0.05 compared to normal control, *p<0.05 compared to the disease control group.

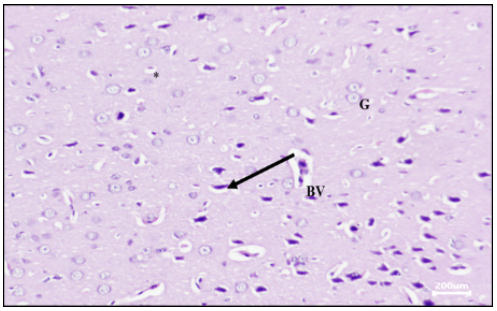
Fig. 8: Effect of *B. ciliata* on diazepam associated locomotor activity in TNF-α



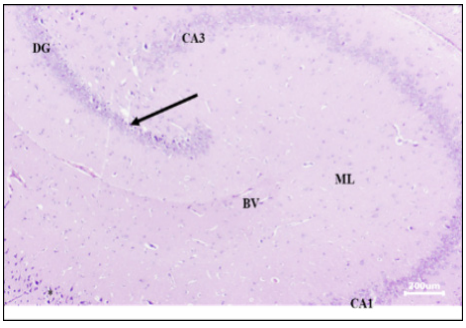
Normal Control: (Untreated)
Fig. 9: Frontal cortex structure
haematoxylin and eosin stain, Scale bar = 200 µm.



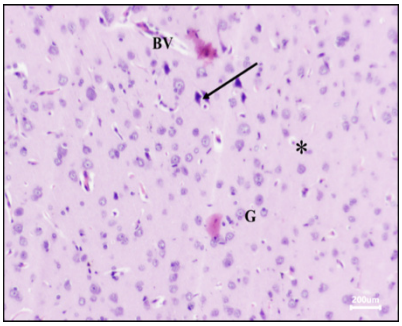
Normal Control (Untreated)
Fig. 10: Hippocampal structure:
haematoxylin and eosin stain, scale bar = 200 µm.



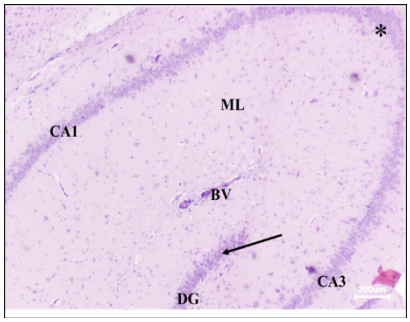
Positive Control (Diazepam Only)
Fig. 11: Frontal cortex structure:
haematoxylin and eosin stain, Scale bar = 200 µm



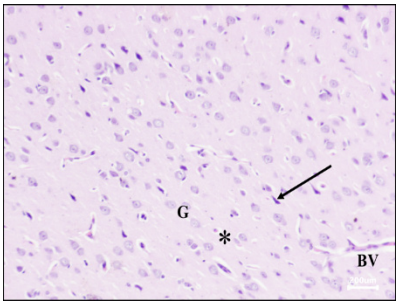
Positive Control (Diazepam Only)
Fig. 12: Hippocampal structure:
haematoxylin and eosin stain, scale bar = 200 µm



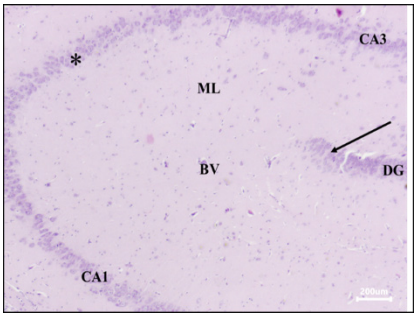
Test Drug (*B. ciliata* 250mg/kg)
Fig. 13: Frontal cortex Structure:
Haematoxylin and Eosin stain, Scale bar = 200µm



Test Drug (*B. ciliata* 250mg/kg)
Fig. 14: Hippocampal structure:
haematoxylin and eosin stain, scale bar = 200 µm



Test Drug (*B. ciliata* 500mg/kg)
Fig. 15: Frontal cortex structure:
haematoxylin and eosin stain, scale bar = 200 µm



Test Drug (*B. ciliata* 500mg/kg)
Fig. 16: Hippocampal structure:
haematoxylin and eosin stain, scale bar = 200 µm

including cortical and pyramidal cells (P), appear healthy with no signs of degeneration or damage. Glial cells (G) are identifiable by their characteristic round shape and dark nuclei - indicated by an asterisk. The granule cells are densely packed, as expected in the cerebellum, and show typical nuclear morphology. The presence of a blood vessel (BV) indicates proper vascularization of the tissue. The hippocampus is divided into regions CA1, CA2, and CA3, which are parts of the cornu ammonis (CA), polymorphic layer (Pr), Fig. 10 The CA1, CA2, and CA3 regions show a typical arrangement of pyramidal cells, which is indicative of a healthy hippocampus. The pyramidal cells are intact with large rounded vesicular nuclei and basophilic cytoplasm, suggesting they are healthy and well-preserved - indicated by the arrow.

Molecular Layer: Contains glial cells, neuronal cells, and blood vessels, indicating proper cellular composition and vascularization -indicated by the asterisk. The presence of numerous ganglion cells in a single layer is a normal feature of the dentate gyrus (DG), indicating it is well-preserved. Proper vascularization is shown by the presence of a blood vessel within the molecular layer Fig. 11. The cortical neurons exhibit mild degeneration with vacuolations, indicating some level of neuronal damage or stress due to diazepam treatment - indicated by the arrow. The presence of a congested blood vessel suggests possible alterations in blood flow or vascular integrity within the cerebellar cortex. Appear normal with characteristic round shape, dark nuclei, and clumped chromatin, suggesting that diazepam might not significantly affect oligodendrocytes indicated by the asterisk. The pyramidal cells show large, rounded nuclei and cytoplasm, which are typical features, indicating that these cells are relatively unaffected. The granular layer remains well-populated with granular cells that have vesicular nuclei and prominent nucleoli, showing that the granular layer maintains its integrity despite diazepam treatment. The histological section of the frontal cerebellar cortex from a diazepam-treated brain shows some signs of mild neuronal degeneration, as indicated by vacuolations in cortical neurons and a congested blood vessel. However, oligodendrocytes, pyramidal cells, and the granular layer appear relatively unaffected. Fig. 12 shows neurons in CA1 and CA3 show degeneration, characterized by vacuolated cytoplasm and pyknosis (dark staining of the nucleus, indicated by an asterisk). Contains pyramidal cells characterized large, rounded nuclei. Cytoplasm with thinning was observed in this layer. Presence of glial cells, neuronal cells, and blood vessels. Contains numerous ganglion cells forming a layer with thinning of the polymorphic layer. Some cells show degeneration with vacuolated cytoplasm and pyknosis (indicated by an arrow). No changes were observed in the granular layer. No interstitial bleeding or infiltration of inflammatory cells was observed. Fig. 13 shows normal architecture with basophilic nuclei located at the periphery (indicated by the arrow). Congested blood vessel (BV): Visible in the image. Oligodendrocytes: Characterized by round cells, dark nuclei with clumped chromatin, and a pericytoplasmic halo- indicated by *. Pyramidal cells (P): Contain large, rounded, basophilic cytoplasm. Contains abundant granular cells and prominent nucleoli (G).

Eosinophilic background: These cells are scattered in the background, formed from neuronal and glial cell processes. The normal histological structure of the frontal cortex is under the influence of a low-dose test drug. Fig. 14 shows the cells in these CA1 and CA3 Regions show pink-staining cytoplasm and blue-staining nuclei (asterisk). Contains pyramidal cells and basophilic cytoplasm.

Molecular Layer (ML): Shows the presence of glial cells, neuronal cells, and blood vessels (BV). Dentate gyrus (DG): Contains numerous ganglion cells and granular cell layers (arrow). No interstitial bleeding or infiltration of inflammatory cells was observed. The image displays (Fig. 15) the normal architecture of cortical neurons, indicating that the overall structure of the frontal cortex is intact.

Basophilic nuclei: These are located at the periphery (indicated by the arrow), which is typical in the molecular layer. These cells have round cell bodies with dark nuclei and clumped chromatin, suggesting healthy oligodendrocyte presence, which is important for myelination in the central nervous system. Identified by their large, rounded vesicular nuclei and basophilic cytoplasm. Pyramidal cells are crucial for cortical function, including cognitive processes and motor control. The granular layer contains cells with vesicular nuclei and prominent nucleoli, indicating active protein synthesis and normal cell function. The presence of blood vessels in the molecular layer shows adequate vascularization. The brain tissue appears healthy, with normal distribution and morphology of neurons, glial cells, and blood vessels. Fig. 16 CA1 and CA3 regions show pink-staining cytoplasm and blue-staining nuclei (asterisk). Contains small pyramidal cells characterized by large, rounded vesicular nuclei and basophilic cytoplasm, indicative of healthy, active neurons. The polymorphic layer includes various cell types, contributing to the complex architecture of the hippocampus. The ML shows the presence of glial cells, neuronal cells, and blood vessels (BV), which is essential for maintaining the structural and functional integrity of the hippocampal tissue. A normal feature of the DG, indicating it is well-preserved. Numerous ganglion cells are present (indicated by the arrow), which are crucial for relaying information in the hippocampus. Granular cell layer contains densely packed neurons that are involved in the processing of information. The neurons and glial cells appear healthy, with proper cellular organization and vascularization.

Conclusion

The findings of this study indicate that *B. ciliata* demonstrates significant potential as an antipsychotic

agent. Key findings include a reduction in GSH levels, an increase in AchE activity, and decreased levels of TNF-α, indicating its role in reducing inflammation, which may contribute to its psychotropic effects. Additionally, the observed effects on apomorphine-induced behavior and diazepam-related locomotor activity further support the viability of *B. ciliata* as a promising alternative for the treatment of psychotic disorders. Its diverse phytochemical profile is likely a crucial factor in this therapeutic potential.

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