

Exploring the Ameliorative Potential of *Barleria cristata* in Cognitive Dysfunction Rat Model: Investigating Behavioral, Biochemical and Cellular Changes

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ABSTRACT

Background: Cognitive impairment is a pervasive and perplexing concern that transcends the boundaries of various neuropsychiatric disorders like schizophrenia and Alzheimer's. As research continues to unravel the intricacies of *Barleria cristata*'s bioactive compounds, it offers promising prospects for the new therapeutic agents in the realm of natural medicine. **Materials and Methods:** In present study the ameliorative effect of methanolic leaf extract of *Barleria cristata* in ketamine induced cognitive dysfunction in rats. Locomotor activity (forced swim test), step down latency and transfer latency were determined for behavioral assessment followed by biochemical estimation of malondialdehyde, reduced glutathione and catalase activity. **Results:** *Barleria cristata* methanolic extract treatment significantly mitigated cognitive impairment in ketamine induced rats with improvement in short and long term memory. Furthermore, it significantly reduced the lipid peroxidation and improved the levels of reduced glutathione and catalase in rats. On histopathological examination *Barleria cristata* methanolic extract revealed dose-dependent substantial neuroprotection, by maintaining normal neurons with less neurodegenerative changes. **Conclusion:** *Barleria cristata* methanolic extract effectively mitigates cognitive impairment, oxidative stress and neurodegeneration in ketamine-induced rats, demonstrating significant neuroprotective properties.

Keywords: *Barleria cristata*, Ketamine, Schizophrenia, Step down latency.

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INTRODUCTION

Cognitive impairment is a pervasive and perplexing concern that transcends the boundaries of various neuropsychiatric disorders like schizophrenia and Alzheimer's, presenting a formidable challenge in the realm of clinical research.¹ Cognitive impairment emerges prominently, characterized by an enduring and profound decline in mental faculties, resulting in a profoundly impactful reduction in an individual's quality of life.²

Schizophrenia, a complex neuropsychiatric disorder, characterized by a diverse array of symptoms. Within this spectrum, cognitive symptoms take center stage, entailing

challenges in learning and memory.³ Pathological features of Alzheimer's disease involve the existence of beta-amyloid plaques and tangles formed by tau proteins, along with Neuroinflammation, oxidative stress and synaptic loss, all of which contribute to the deterioration of cognitive abilities.⁴ Given the brain's heightened vulnerability to oxidative stress, this phenomenon has become a pivotal element in the pathophysiology of psychotic disorders.^{5,6}

The origins of cognitive impairment in these disorders are rooted in intricate neurochemical and neurobiological mechanisms. These encompass a myriad of contributing factors, including the intricate interplay of dopaminergic neurotransmission, the hypoactivity of N-Methyl-D-Aspartic Acid (NMDA) receptors, compromised Gamma-Aminobutyric Acid (GABAergic) activity, neuroinflammatory processes, reduced cholinergic neurotransmission and heightened oxidative stress. These intricacies collectively weave the tapestry of cognitive impairment in these diseases.^{7,8}



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Current medications for cognitive impairment often exhibit limited efficacy across a spectrum of neuropsychiatric disorders. For instance, in the case of Alzheimer's disease, cholinesterase inhibitors and NMDA receptor antagonists offer modest symptomatic relief but fail to halt or reverse the relentless cognitive decline.⁹ Similarly, in schizophrenia, while atypical antipsychotics address positive and negative symptoms, they fall short in effectively ameliorating cognitive deficits, leaving a substantial gap in treatment outcomes.¹⁰ The utilization of existing medications may exacerbate oxidative imbalances, potentially worsening the overall progression of the underlying disorder, thereby limiting their long-term suitability as treatment options.¹¹

In light of these challenges, there is an imperative need to novel and safer innovative therapeutic modalities to address cognitive impairment. Researchers are currently focusing on discovering natural origin potential compounds to alleviate mental disorders.^{12,13} Antioxidants serve as protective agents, safeguarding neurons from the harmful effects of oxidative stress.¹⁴ Various natural compounds, including those derived from plant extracts, have shown promising antioxidant properties.

The current research thrust is anchored in the pursuit of new treatments, for enhancing cognitive functions. *Barleria cristata*, commonly known as the Philippine violet, is a perennial plant native to Southeast Asia.¹⁵ This botanical species has drawn increasing attention in the field of natural medicine due to its potential pharmacological effects, including anti-inflammatory,¹⁶ antioxidant,¹⁷ antidiabetic activity¹⁸ and antimicrobial activity.¹⁹ The plant's phytochemical composition, which includes flavonoids, alkaloids and phenolic compounds, contributes to its diverse therapeutic potential.²⁰ Recently, phenolic from *Barleria cristata* were reported to block carcinogenesis.²¹ This study delves into the effect of *Barleria Cristata* leaves extract in a ketamine-induced psychosis and cognitive dysfunction of rat model by examining the benefits of natural extracts.

MATERIALS AND METHODS

Chemicals

All chemicals employed in the experiment were sourced from Sigma-Aldrich Chemicals Private Limited, Bangalore and met stringent analytical grade standards. The doses are prepared in 0.9% saline water using 5% tween.

Plant Extraction

The *Barleria cristata* leaves were responsibly gathered from the vicinity of Simhachalam Hill in the Visakhapatnam region and authenticated by Prof. Balaram Padal, botanist from Andhra University. The voucher has been submitted in the same. Subsequently, the plant material was subjected to a systematic sequential maceration technique using 70% methanol and the Concentrate (BCME) obtained is used for further evaluation.

Qualitative phytochemical analysis

The BCME was examined for the presence of alkaloids, terpenoids, flavonoids, phenols, saponins and reducing sugars through previously established literature.²²

Determination of *in vitro* antioxidant activity

1,1-Diphenyl,2-Picryl-Hydrazyl (DPPH) activity of BCME was analyzed through Sanchez-Moreno *et al.*, method to examine the *in vitro* antioxidant activity. Different concentrations of BCME were allowed to react with DPPH (0.25% w/v) by incubating in dark for 30 min. Percentage DPPH remained was calculated from the absorbance measured using UV-vis spectrophotometer.²³

Acute toxicity studies

In accordance with the OECD 425 Guideline, albino Wistar mice ($n=6$), irrespective of gender, were randomly chosen. Following a 4 hr fasting period, the mice were orally administered with BCME at an initial concentration of 50 mg/kg and monitored for 3 days. In absence of mortality, the process was repeated with the higher doses.

Experimental animals

Albino Wistar rats of both male and female sex with a weight range of 25-30 g were included in the procedure. They were acclimatized and kept in suitable environment suggested by the Animal Ethical Committee. Animal experimental models were performed with prior approval by IAEC and in CPCSEA registered institutional facility with Regd no: 2003/PO/Re/S/18/CCSEA.

Ketamine induced psychosis

Repeated administration of ketamine has been shown to induce behavioral deficits in rodents, mirroring key aspects of psychosis, including cognitive symptoms.²⁴ The daily injection of ketamine at a dose of 30 mg/kg per day (i.p.) over a continuous period of 10 days, served as the inducing agent. The olanzapine and the BCME extracts were subsequently administered 1 hr after each ketamine dose to evaluate their potential mitigating effects on the behavioral deficits induced by ketamine.^{24,25}

Effect of BCME on ketamine memory model

The experimental rats were methodically distributed into 5 distinct groups, each consisting of six individuals ($n=6$). In Group 1 (control), 0.9% normal saline was administered intraperitoneally. Group 2 (disease control) received the ketamine (30 mg/kg, intraperitoneally) treatment alone. In group 3, (standard) olanzapine (5 mg/kg, oral) dissolved in a solution of 5% tween 80 in normal saline (0.9%) was administered. Meanwhile, Group 4 and Group 5 were the BCME-treated groups, each administered with 100 and 200 mg/kg of BCME oral, respectively dissolved in 0.9% normal saline using 5% tween 80. Following the distinct administrations, various behavioral and biochemical

parameters were meticulously observed to assess the impact of the administered drugs.²⁶

Assessment of behavior in ketamine induced psychosis

To investigate the potential impact of BCME, various behavioral assessments were employed, encompassing novel object recognition test, forced swim test, step-down latency test and transfer latency test.

Novel Object Recognition test

The memory is assessed using a plastic rectangular arena (43 cm × 31 cm × 16 cm). Mice is encountered with two identical white square containers placed equidistantly for maximum 5 min in sample phase. In test phase (on 8th day of drug administration) mirrored the sample phase but replaced one object with a toy to evaluate object recognition. The rat was allowed to explore each object for 60 sec and the total time to 10 min. Cleaning with ethanol (70%) between trials eliminated olfactory cues. Discrimination Index (DI) is calculated as the percentage of time taken to recognize a novel object versus the total time taken to recognize both object types.^{27,28}

Forced Swim Test (FST)

60 min after the acclimatization (1 hr), the rats were individually placed in a cylindrical tank filled with water at a temperature of 25°C and filled to a depth of 30 cm with a span of 6 min. Immobility in this context was defined as the minimal movements necessary for the rats to remain afloat in the water.²⁹

Step-Down Latency Test (SDL)

Initially, the rat was positioned on a platform and the time latency to move was recorded. Following 10 sec of acclimatization in the first step, the rats were returned to cages. In the subsequent step, immediately after the descent, foot shock of 50 Hz was applied for 1 sec. These acquisition steps are performed on the 0th and 4th days. The retention was tested on 2nd, 5th and 9th days, by repeating the same process with 60 sec limit to assess short and long period memory. The duration of descent during was measured.^{26,29}

Transfer Latency Test (TLT)

The Transfer Latency test is conducted with the elevated plus shaped maze, with slight modifications from previous studies.^{30,31} The model consist a pair of open and covered arms each extended from a central platform, elevated to 25 cm from the floor. During the acquisition session on the 0th and 4th days, mice were individually placed on open arm and the latent time to move into a covered arm, with a cut-off time of 5 min.³² Subsequent assessments of memory retention were performed on the 2nd, 5th

and 9th day of the schedule. After each session, an additional 2 min were given for rat to discover surroundings.

Estimation of Biochemical parameters

On the 10th day, following behavioral tests, animals were humanely decapitated and their brains were carefully isolated and washed with buffer (pH 7.4). Brain separated was homogenized and centrifuged in phosphate buffer to collect the supernatant for estimation of biochemical markers and enzymatic activity. This contributed to the understanding of the experimental outcomes.

Thiobarbituric Acid Reactive Substances (TBARS), serving as an indicator of lipid peroxidation, were quantified following Wills ED *et al.*, utilizing spectrophotometric analysis at 532 nm.³³ The assessment of Glutathione (GSH) content in brain tissue was determined as described by Beutler *et al.*³⁴ Catalase activity was determined using the millimolar extinction coefficient of H₂O₂ (0.071 mmol⁻¹ cm⁻¹).³⁵

Histopathological Evaluation

The brains were extracted, washed with saline and promptly immersed in 10% formalin for 72 hr. Following this, the samples were dehydrated in ethanol grades, cleared in xylene and infiltrated. Paraffin embedding was carried out and 4 μm small sections were sliced and then affixed onto glass slides. Subsequently, stained with hematoxylin and eosin and scrutinized under a light microscope.³⁶

Statistical analysis

The results are expressed as mean ± standard error and were statistically validated through One-way Analysis of Variance (ANOVA) followed by Tukey's test using Microsoft Excel at a significance of $p < 0.01$ and $p < 0.001$.

RESULTS

Qualitative phytochemical analysis

On examining the leaf extract BCME (*Barleria cristata* Methanolic Extract) for the identification of secondary metabolites, results revealed the presence of alkaloids, terpenoids, flavonoids and phenols along with other constituents as represented in Table 1. These bioactive compounds are recognized for their capacity to counteract free radicals, which are implicated in the onset of chronic diseases.

Determination of *in vitro* antioxidant activity

Before examining the *in vivo* performance of BCME, *in vitro* DPPH scavenging activity was determined with quercetin as standard. BCME showed good antioxidant potential apparent from continues neutralization of the DPPH free radicals with an increase in concentration as shown in Figure 1 and has an IC₅₀ value of 37.97 μg/mL.

The finding

Acute oral toxicity of *Barleria cristata* (BCME)

BCME exhibited no symptoms of toxicity even at a dose of 2000 mg/kg. Hence 1/10th of it, 200 mg/kg is selected as higher dose and 100 mg/kg as lower dose for further studies.

Novel object recognition

Induction of cognitive impairment on continues ketamine treatment significantly ($p < 0.05$) decreased the total time spent exploring the object even if novel with respect to time (Figure 2a). Treatment with olanzapine, BCME (100 and 200 mg/kg) prevented worsening of the cognitive impairment and were able to improve the attention and memory of the subject compared to disease control significantly ($p > 0.001$) as seen in Figure 2b. BCME (100 mg/kg), though able to improve the differentiation of novel and familiar objects in disease control rats, it showed a discrimination percentage of 39.54% (Figure 2b). Treatment with BCME (200 mg/kg) resulted in discrimination percentage of 50.75% and is equivalent to the standard treatment of 51.68% ($p > 0.05$).

Forced Swim Test

The evaluation is centered on the decrease in immobility, which serves as an indicator of cognitive dysfunction.³⁷ The induced disease made increased immobility compared to the control group ($p < 0.001$). BCME treatment (100 and 200 mg/kg) decreased the immobility compared to disease control ($p < 0.001$) as depicted in Figure 3. However, the effect of BCME 100 mg/kg is significantly lower than the BCME 200 mg/kg and olanzapine ($p < 0.05$). Overall comparison of BCME and standard with the control group indicates improvement of cognitive conditions but not statistically ($p < 0.05$) equivalent to control rats (rats without disease induction).

Step-down latency

Effect of BCME on short term memory

As represented in Figure 4, in the retention test on 2nd day of the study in spite of the short duration of 24 hr a significant ($p < 0.001$) variation in SDL is observed in disease control rats with respect to control group. Evident from the results ketamine model has

induced psychosis with in hours after administration.^{38,39} All the treatment groups showed slight increase in SDL but none of them, standard ($p = 0.0518$), BCME 100 mg/kg ($p = 0.1436$) and BCME 200 mg/kg ($p = 0.0795$) showed significant variation in SDL from the disease control and were significantly ($p < 0.001$) lower than the normal control group. BCME and olanzapine showed insignificant variation at the doses used ($p > 0.98$).

From 5th day retention test, an improvement in the short memory of the disease control rats was observed in group 3, 4 and 5 irrespective of the treatment received in comparison to the non-treated rats in group 2. The SDL values increased significantly ($p < 0.001$) on treatment with olanzapine, BCME (100 mg/kg) and BCME (200 mg/kg) in comparison to diseased. However, a significant ($p < 0.001$) difference still existed with respect to the normal rats. Even though BCME 100 mg/kg showed a significant increase in SDL, this improvement is not comparable to the olanzapine. BCME at a dose of 200 mg/kg showed insignificant ($p = 0.7976$) difference with standard olanzapine. Thus these results indicate a significant improvement in short term memory of rats with cognitive impairment on treatment with BCME.

Effect of BCME on long term memory

Retention test was performed on 9th day of treatment without a prior acquisition test within last 24 hr. Similar to short term memory test, olanzapine, BCME (100 mg/kg) and BCME (200 mg/kg) significantly ($p < 0.001$) increased the SDL values with respect to disease control rats. Continues treatment of rats with ketamine 50 mg/kg alone, resulted in further cognitive impairment and decreased attention span evident from SDL values lower than 10 sec. BCME at 200 mg/kg treatment improved the long-term memory of diseased rats equivalent to the standard treatment group with a $p > 0.05$. Treatment with BCME at 200 mg/kg and olanzapine increased the SDL values to show an insignificant ($p > 0.05$) difference compared to control group. BCME at lower dose of 100 mg/kg was significantly ($p < 0.05$) differing from control group. Thus, BCME at higher dose of 200 mg/kg showed significant improvement in long term memory in spite of ketamine induction and though lower dose improved the memory it was unable to reverse the ketamine effect completely owing to insufficient dose.

Table 1: Phytochemical analysis of BCME.

Sl. No.	Phytochemical constituent	Test employed, reference method adopted	Results
1.	Alkaloids	Dragendroff's test, Mayer's test, Hager's test.	+
2.	Terpenoids	Horizon test.	+
3.	Flavonoids	Alkaline reagent test, Shinod's test.	+
4.	Phenols	Ferric chloride test, Lead tetra acetic acid test.	+
5.	Saponins	Sodium carbonate test.	+
6.	Sugars (Reducing)	Benedict's test, Fehling's test.	+

* '+' indicates presence and '-' indicates absence.

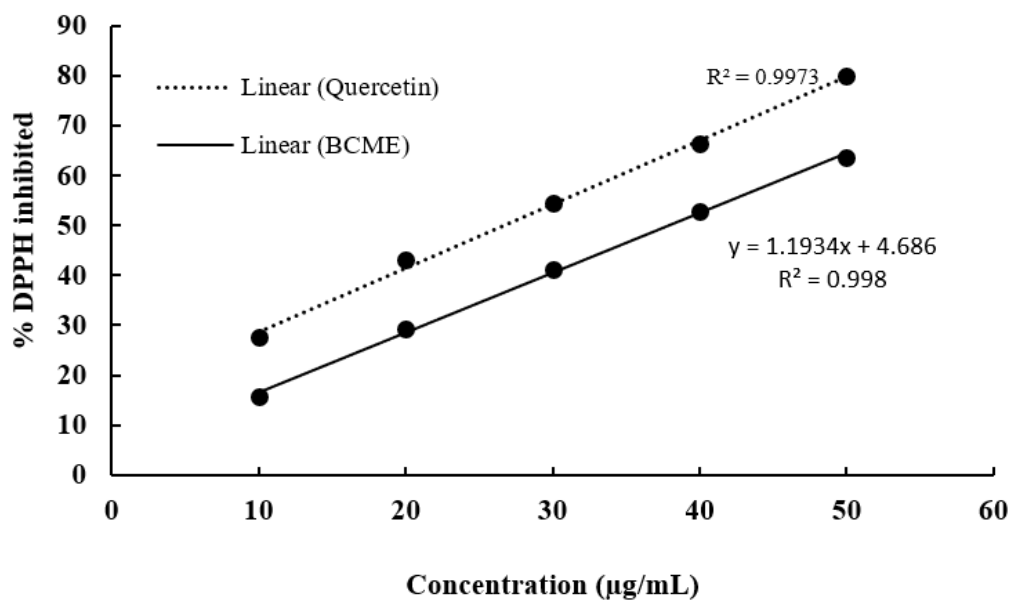


Figure 1: Antioxidant activity of BCME.

Transfer latency test

Effect of BCME on short term memory

On 2nd day retention test, BCME 100 mg/kg and 200 mg/kg has not shown significant difference in treatment than the disease control. However, as seen in Figure 5, BCME and standard showed slight decrease in transfer latency but was not statistically significant compared to disease control. Further acquisition and retention test on 5th day showed significant reduction in latency of BCME 200 mg/kg treated rats and is statistically ($p > 0.05$) comparable to the action of olanzapine. BCME at lower dose also showed protective effect against ketamine action and a specific improvement in rats in comparison to disease control ($p > 0.001$), however is much lesser ($p < 0.05$) than the olanzapine and BCME 200 mg/kg dose. Hence BCME at both the dose was able to prevent the ketamine associated cognitive impairment and significantly improved the short-term memory of rats but only higher dose of 200 mg/kg treated rats equivalent to the standard.

Effect of BCME on long term memory

In retention test on the 9th day of the treatment, rats treated with BCME 200 mg/kg and olanzapine are comparable ($p > 0.05$) to control group. BCME 200 mg/kg showed better results with less latency period than the standard olanzapine however the difference is not significant ($p > 0.05$) enough to declare better therapy by BCME than the olanzapine. Long term memory with BCME 100 mg/kg is significantly improved compared to disease control but not equivalent ($p < 0.05$) to the olanzapine, BCME 200 mg/kg or control group. Yet even at lower doses it prevented further deterioration of condition in diseased rats and also showed improve the memory.

Biochemical Studies

Lipid Peroxidation

Elevated lipid peroxidation in the brain, indicated by increased levels of Malondialdehyde (MDA), is closely linked to cognitive dysfunction in neurodegenerative conditions. Conditions like Alzheimer's disease exhibit heightened lipid peroxidation, underlining its role in cognitive decline. TBARS (malondialdehyde-Thiobarbituric acid adducts) levels of brain homogenates are directly proportional to the lipid peroxidation and oxidative stress in the brain.⁴⁰ BCME (100 mg/kg and 200 mg/kg) significantly reduced the MDA levels ($p < 0.01$) in rats treated for 10 days indicating a reduction in oxidative stress. The MDA levels of BCME 200 mg/kg was comparable to the olanzapine and control (Figure 6a).

Reduced Glutathione Assay

The increase in oxidative stress impact GSH, a pivotal antioxidant. As GSH levels decline due to heightened oxidative stress, the brain's defense against free radicals weakens.⁴¹ Administration of ketamine alone caused oxidative stress evident from the significantly ($p < 0.0001$) decreased GSH levels of rat brain in disease control. Treatment with BCME not only prevented the building up of oxidative stress comparable ($p = 0.978$) to olanzapine but also restored the GSH levels in the brain to near normal as control rats ($p = 0.062$). However, the capacity to improve the GSH levels was observed to be dose dependent. BCME (100 mg/kg) caused a rise ($p < 0.05$) in GSH levels compared to disease control but a significant difference ($p < 0.001$) still existed with respect to the control rats. Consequently, BCME protected the GSH from depletion preventing the oxidative stress (Figure 6b).

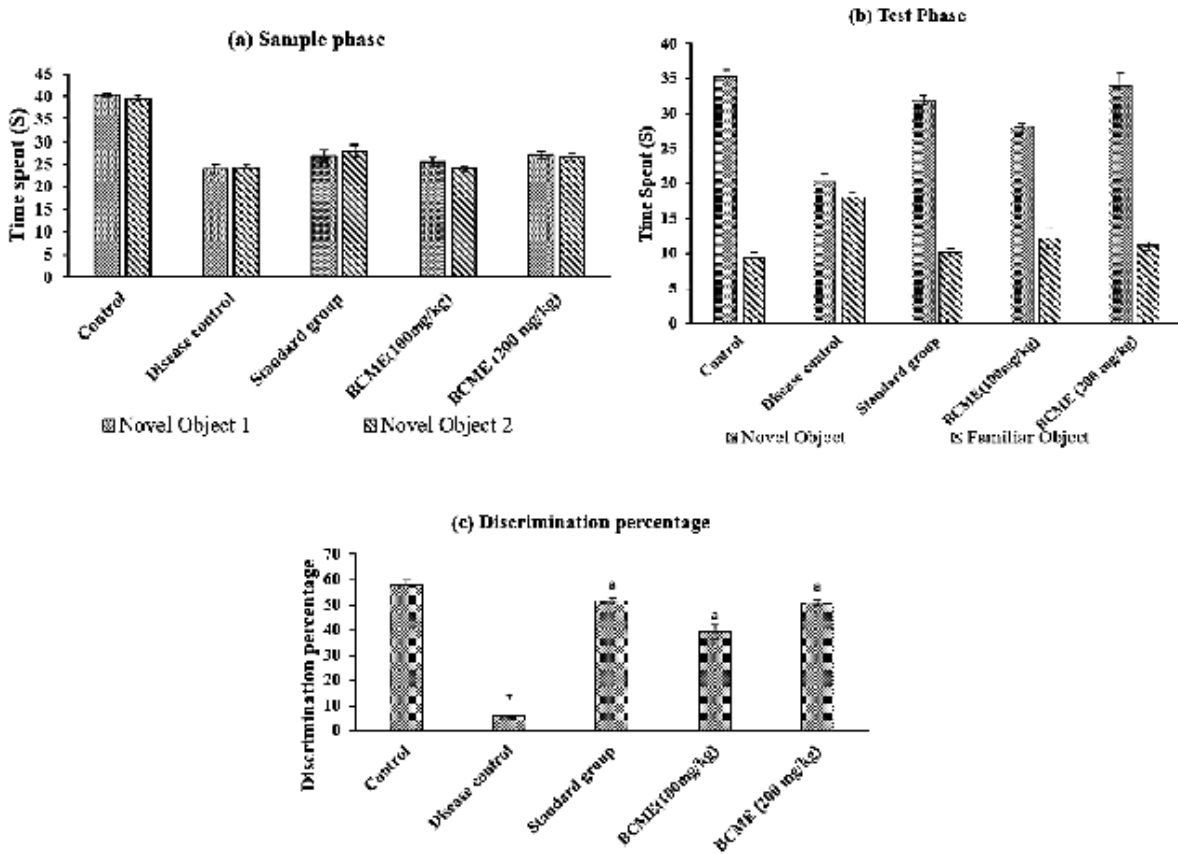


Figure 2: Effect of BCME on Novel object recognition model a) sample phase b) test phase c) discrimination percentage. Results are presented as mean±SE (n=6). ^{***} indicates $p < 0.001$ in comparison to control group and ^a signifies $p < 0.001$ in comparison to Ketamine treated disease control.

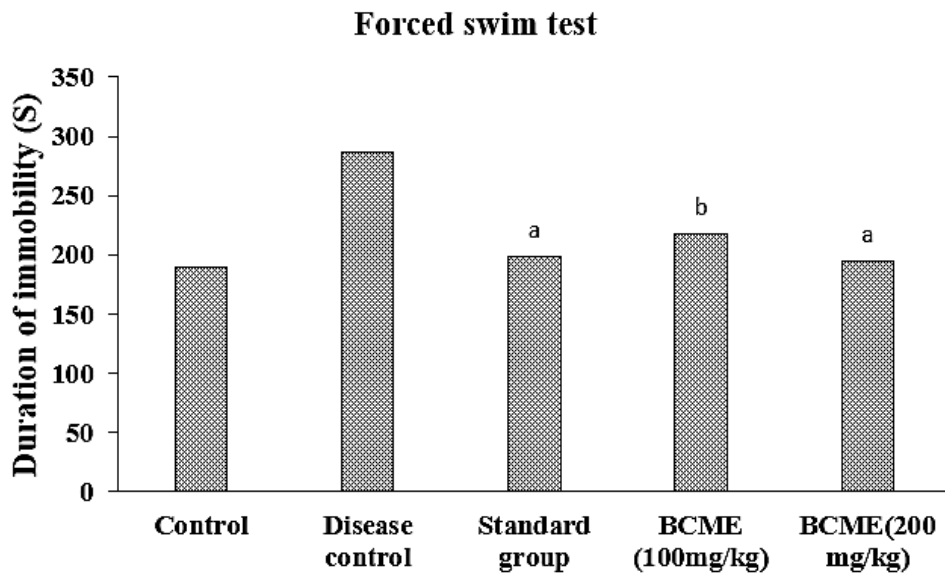


Figure 3: Effect of BCME on forced swim test model. Results are presented as mean±SE (n=6). ^{***} indicates $p < 0.001$ in comparison to control group and ^{a, b} signifies $p < 0.001$ and $p < 0.01$ in comparison to Ketamine treated disease control respectively.

Catalase Assay

Alterations in catalase activity may disrupt the delicate equilibrium between oxidative stress and antioxidant mechanisms, contributing to neuronal damage and cognitive decline.⁴² The test results of catalase activity are similar to the GSH levels of brain homogenates. The depletion of catalase activity in disease control is significant to the level of $p < 0.00001$ and treatment with BCME successfully prevented the depletion of catalase activity, which can be accounted to the antioxidant capability of the BCME (Figure 6c). The rise in catalase enzyme observed with BCME is significant ($p < 0.0001$) compared to disease control at both lower and higher doses and the effect of 200 mg/kg is comparable ($p = 0.99$) to standard olanzapine and control group ($p = 0.99$).

Histopathology

Cognitive impairment manifestation is associated with disruption of neurons specifically in entorhinal cortex and hippocampus.⁴³ In the histopathological assessment, the hippocampus of control rats displayed typical features, portraying intact neurons and undisturbed intracellular brain matrices. In contrast, disease control rats exhibited notable neuronal degeneration and edema with increased gliosis, resulting in observable neuronal loss compared to the control group. Olanzapine treated rats showcased minimal neurodegeneration revealing its neuroprotective action. Interestingly, BCME at both 100 and 200 mg revealed substantial neuroprotection, by maintaining normal neurons with less neurodegenerative changes. BCME at higher dose further exhibited reduced gliosis and both BCME doses

mitigated neuronal loss. This histopathological examination underscores the potential neuroprotective effects of BCME, akin to olanzapine, in alleviating ketamine-induced neuronal damage in the brain as shown in Figure 7.

DISCUSSION

The plant-derived antioxidants, rich in polyphenols, flavonoids and other bioactive compounds, exhibit the capacity to counteract oxidative stress and serve as efficient neuroprotective agents.^{44,45} Therefore *Barleria cristata*, with prior tremendous medicinal properties and promising antioxidant¹⁷ and anti-inflammatory activity¹⁶ was evaluated for the impact on cognitive impairment in ketamine induced rat models. The phytochemicals identified in BCME is in agreement with the earlier literature of other species of *Barleria* genus (*B. acanthoides*, *B. noctiflora*, *B. prionitis*, *B. strigose*), which indicated the presence of primary metabolites like flavonoids, phenolic acids, terpenoids with a prominent antioxidant activity.⁴⁵

BCME antioxidant activity from the *in vitro* antioxidant studies were in alignment with previously reported literature, indicating the efficacy of BCME in mitigating oxidative stress and has a possibility to protect neural cells, which are crucial factors in cognitive improvement. BCME's activity was compared to olanzapine, as the standard drug due to its effectiveness in addressing stereotypic behaviors and cognitive symptoms. The reduced novel object exploration time in sample phase of disease induced groups can be correlated to the fact that cognitive impairment causes easy distraction of the subject decreasing the

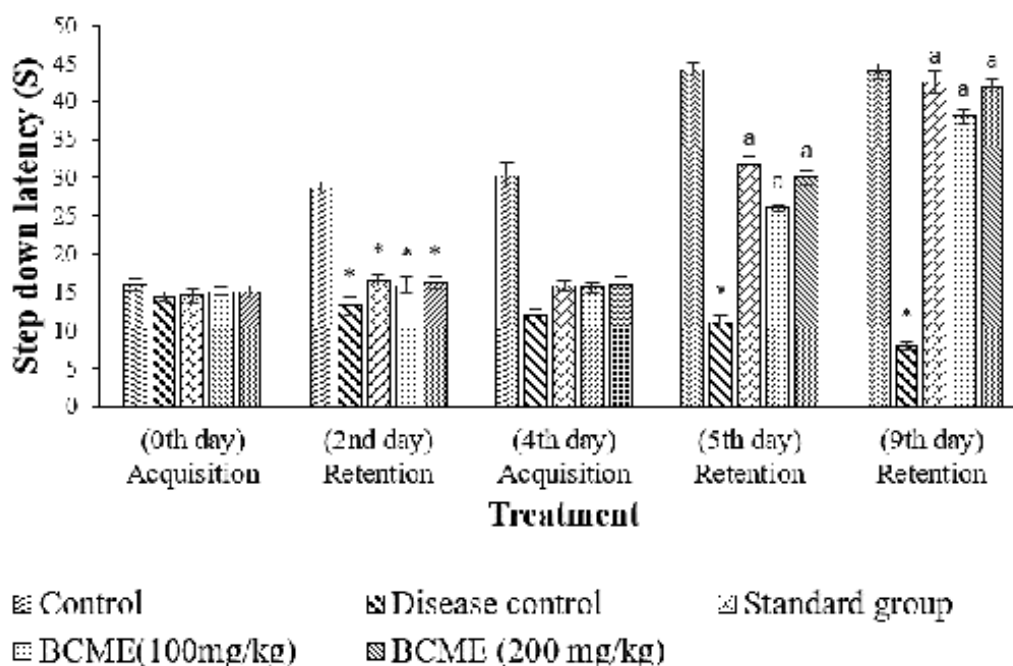


Figure 4: Effect of BCME on step down latency test. Results are presented as mean \pm SE ($n = 6$). ** indicates $p < 0.001$ in comparison to control group and 'a' signifies $p < 0.001$ in comparison to Ketamine treated disease control.

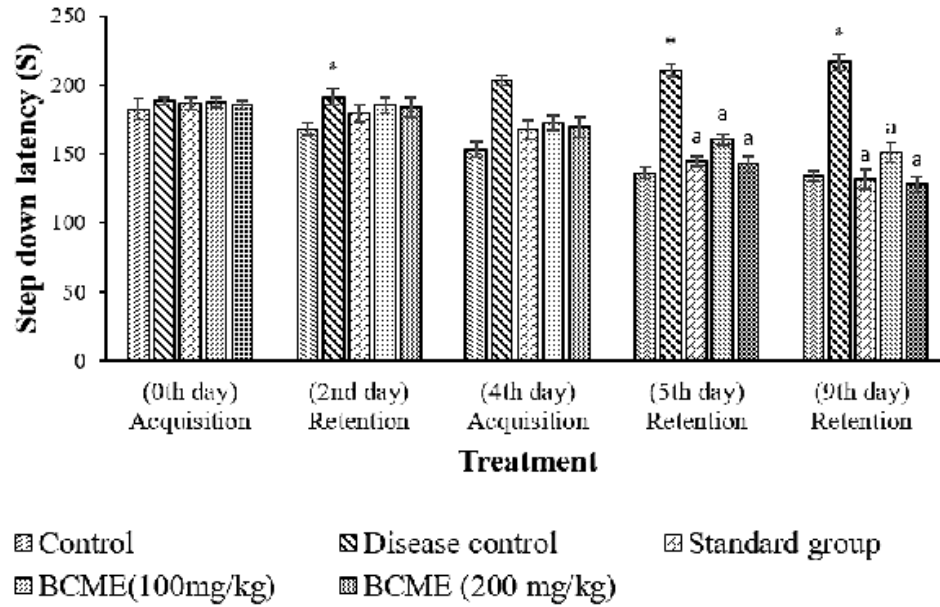


Figure 5: Effect of BCME on transfer latency test. Results are presented as mean±SE (n=6). ‘**’ indicates $p < 0.001$ in comparison to control group and ‘a’ signifies $p < 0.001$ in comparison to Ketamine treated disease control.

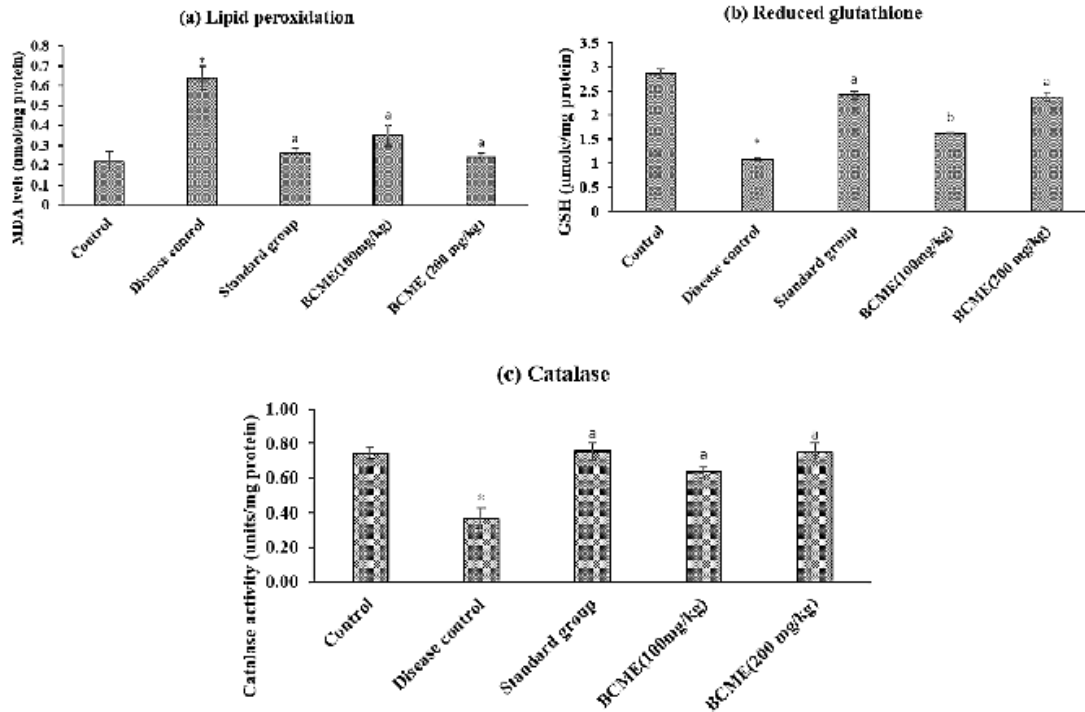


Figure 6: Effect of BCME on lipid peroxidation, reduced glutathione and catalase levels in brain homogenates of rats. Results are presented as mean±SE (n=6). ‘**’ indicates $p < 0.001$ in comparison to control group and ‘a, b’ signifies $p < 0.001$ and $p < 0.01$ in comparison to Ketamine treated disease control respectively.

attention span.⁴⁶ The results demonstrated that BCME, particularly at a higher dose of 200 mg/kg, effectively mitigated cognitive deficits induced by continuous ketamine administration. In the novel object recognition test, BCME improved the ability of rats to distinguish between novel and familiar objects, suggesting an enhancement in recognition memory. Similarly, in the forced

swim test, BCME significantly reduced immobility, indicating an improvement in motivation and overall cognitive function.

The step-down latency and transfer latency tests further corroborated the positive effects of BCME on both short-term and long-term memory. The step-down latency test revealed that BCME improved short-term memory retention, as evidenced by

increased SDL values in the retention tests conducted on the 5th and 9th days of the study. This indicates a potential for BCME to reverse ketamine-induced cognitive deficits over time. Notably, the higher dose of BCME was comparable to the standard treatment with olanzapine, a known antipsychotic with cognitive benefits, underscoring the efficacy of BCME as a potential therapeutic agent. The transfer latency test results reinforced these findings, showing significant reductions in transfer latency in BCME-treated rats, particularly at the higher dose, indicating improved cognitive function and memory retention.

The neuroprotective and cognitive-enhancing effects of BCME may be attributed to its rich phytochemical composition, including flavonoids, alkaloids and phenolic compounds, known for their antioxidant and anti-inflammatory properties.⁴⁷ Flavonoids are known to enhance cognitive function by promoting synaptic plasticity and neuronal survival, while polyphenols are reported efficient for age related cognitive decreased.⁴⁸⁻⁵⁰ These compounds could mitigate oxidative stress and neuroinflammation, which are critical factors in the pathogenesis of cognitive disorders.

The biochemical analyses further support this explanation. Elevated lipid peroxidation, as indicated by increased Malondialdehyde (MDA) levels, is a hallmark of oxidative stress and has been linked to cognitive dysfunction in neurodegenerative diseases such as Alzheimer's.⁵¹ In this study, BCME significantly reduced MDA levels in the brain, suggesting a decrease in lipid peroxidation and oxidative stress. The reduction in MDA levels with BCME treatment was comparable to that achieved with olanzapine, indicating that BCME is effective in mitigating oxidative damage.

Moreover, the reduced Glutathione (GSH) assay revealed that BCME prevented the depletion of this critical antioxidant in the brain. GSH is vital for neutralizing free radicals and protecting neuronal integrity. Ketamine-induced oxidative stress led to a significant reduction in GSH levels, which was ameliorated by BCME treatment in a dose-dependent manner. This restoration of GSH levels highlights BCME's potential to enhance the brain's antioxidant defenses, thereby protecting against oxidative stress-related cognitive impairment.⁴⁷ Catalase is an enzyme that helps decompose hydrogen peroxide, a harmful byproduct of oxidative stress. The significant increase in catalase activity in BCME-treated rats, particularly at the higher dose, suggests that BCME enhances the brain's enzymatic antioxidant defense system. The comparable efficacy of BCME and olanzapine in restoring catalase activity underscores the potency of BCME as an antioxidant.

The hippocampus is crucial for memory and cognitive function and its damage is often associated with cognitive impairments. In ketamine-treated rats, significant neuronal degeneration and gliosis were observed, indicating severe neurotoxicity. However, BCME treatment preserved neuronal integrity, reduced gliosis

and mitigated neuronal loss. These neuroprotective effects were evident at both doses of BCME, with the higher dose showing results comparable to olanzapine. The ability of BCME to maintain normal neuronal structure and reduce neurodegenerative changes suggests that it can effectively protect against ketamine-induced neuronal damage.

Ketamine, employed as a psychosis inducer in this study, acts as an N-Methyl-D-Aspartate (NMDA) receptor antagonist and is known to induce cognitive impairment at lower doses. Recently, novel drugs targeting the glycine site associated with NMDA receptors have shown promise in alleviating cognitive symptoms. The observed prevention of neurodegeneration in ketamine-induced rats by BCME suggests its potential in mitigating the NMDA antagonist effects of ketamine. Studies indicate that flavonoids can attenuate hyper locomotor activity induced by NMDA antagonists.⁵² Therefore, BCME's mechanism for ameliorating cognitive impairment may involve countering ketamine-induced oxidative stress, protecting neurons and preserving synaptic plasticity and memory functions and mitigating NMDA antagonist activity. Additionally, BCME's efficacy in enhancing cognitive function and memory could be linked to its potential role in modulating neurotransmitter systems, particularly the cholinergic and dopaminergic pathways,

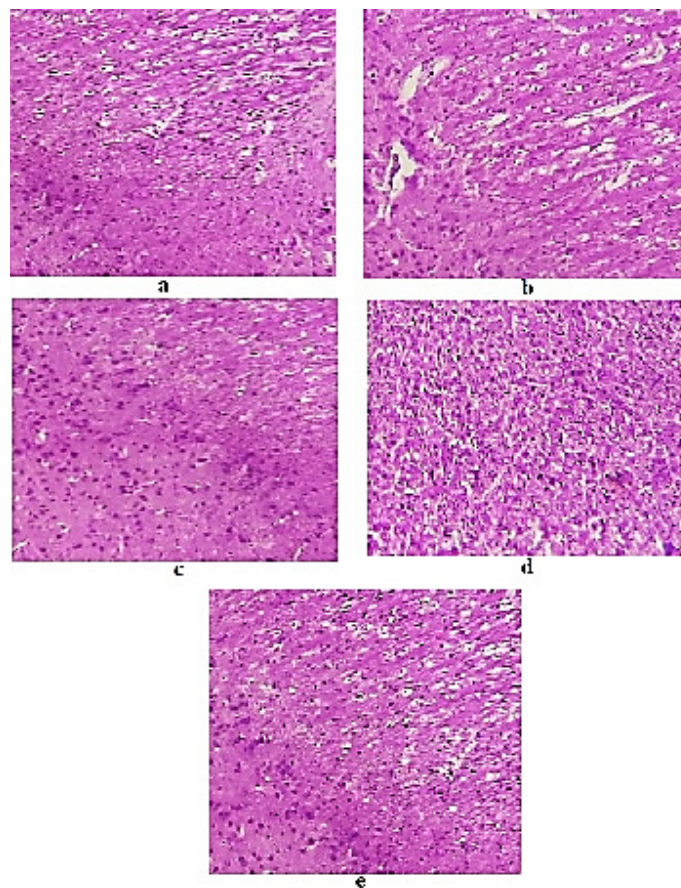


Figure 7: Histopathology images of hippocampus region of a) control group b) disease control c) olanzapine (Standard) d) BCME (100 mg/kg) e) BCME (200 mg/kg) treated rats.

which are crucial for learning and memory processes.⁵³ Further exploration of the plant's therapeutic benefits in this context is warranted, providing a hopeful path for treating cognitive dysfunction in psychotic disorders.

CONCLUSION

This study demonstrates that BCME has significant neuroprotective properties by mitigating cognitive symptoms induced by ketamine in rat, with no observed side effects throughout the study period. The extract's bioactive compounds, including flavonoids, polyphenols and terpenoids, contribute to its antioxidative and anti-inflammatory effects. BCME effectively improved both short and long-term memory, reduced lipid peroxidation and enhanced levels of endogenous antioxidants like GSH and catalase. These promising findings suggest that BCME could serve as a significant lead in developing novel antipsychotic agents. Further clinical investigations are warranted to explore its potential efficacy in patients presenting with predominant psychotic symptoms.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

NMDA: N-Methyl-D-aspartic acid; **GABA:** Gamma-aminobutyric acid; **DPPH:** 1,1-Diphenyl,2-picrylhydrazyl; **BCME:** *Barleria cristata* leaves methanolic extract; **w/v:** Weight/volume; **mg/kg:** Milligram/kilogram; **OECD:** Organization Economic Cooperation and Development; **IAEC:** Institutional Animal Ethical Committee; **CPCSEA:** Committee for the Purpose of Control and Supervision of Experiments on Animals; **FST:** Forced Swimming test; **SDL:** Step-down Latency; **TLT:** Transfer Latency test; **DI:** Discrimination index; **TBARS:** Thiobarbituric acid reactive substances; **MDA:** Malondialdehyde; **GSH:** Glutathione; **ANOVA:** One-way analysis of variance.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

Animal experimental models were performed with prior approval by IAEC and in CPCSEA registered institutional facility with Regd no: 2003/PO/Re/S/18/CCSEA.

SUMMARY

Cognitive impairment is a prevalent issue in neuropsychiatric disorders such as schizophrenia and Alzheimer's disease. Recent research highlights the potential of *Barleria cristata*'s bioactive compounds in addressing cognitive dysfunction. This study investigates the ameliorative effects of Methanolic leaf Extract of *Barleria cristata* (BCME) on ketamine-induced cognitive impairment in rats. Behavioral assessments included locomotor activity, step-down latency and transfer latency tests, while

biochemical evaluations measured Malondialdehyde (MDA), reduced Glutathione (GSH) and catalase activity. BCME significantly improved both short and long-term memory, reduced lipid peroxidation and increased GSH and catalase levels. Histopathological analysis revealed dose-dependent neuroprotection, maintaining normal neuronal structures with minimal neurodegenerative changes. Qualitative phytochemical analysis confirmed the presence of alkaloids, terpenoids, flavonoids and phenols, which are known for their antioxidant properties. *In vitro* antioxidant activity of BCME showed good DPPH scavenging potential with an IC₅₀ value of 37.97 µg/mL. BCME exhibited no toxicity at doses up to 2000 mg/kg, with 200 mg/kg selected as the higher dose for further studies.

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