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Neuropharmacological Evaluation Of Synergistic Antidepressant Efficacy Of Imipramine And Bacopa Monnieri In Rat Models Of Behavioral Despair And Oxidative Neurotoxicity

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Abstract

Background: *Depression is a multifactorial neuropsychiatric disorder in which both monoaminergic dysfunction and oxidative stress play critical roles. Although tricyclic antidepressants like imipramine are clinically effective, their use is limited by delayed onset and adverse effects. Bacopa monnieri, an Ayurvedic herb, possesses antioxidant and neuroprotective properties that may complement the action of conventional antidepressants.*

Objective: *The present study aimed to evaluate the synergistic antidepressant efficacy of imipramine in combination with Bacopa monnieri in rat models of behavioral despair and oxidative neurotoxicity.*

Methods: *Hydroalcoholic extract of Bacopa monnieri was authenticated and subjected to preliminary phytochemical screening. Adult male Wistar rats were divided into six groups (n=6) and treated for 14 days: vehicle control, imipramine (15 mg/kg), Bacopa monnieri (100 and 200 mg/kg), and combinations of imipramine with Bacopa monnieri. Behavioral assessments were performed using the forced swim test (FST) and tail suspension test (TST), followed by locomotor activity measurement. Oxidative stress markers including malondialdehyde (MDA), superoxide dismutase (SOD), catalase, and reduced glutathione (GSH) were estimated in brain homogenates. Histopathological examination of hippocampal sections was also carried out.*

Results: *Phytochemical analysis confirmed the presence of bacosides, saponins, and flavonoids. Combination therapy significantly reduced immobility time in FST and TST compared to individual treatments, without altering locomotor activity. Biochemical assays showed decreased lipid peroxidation and restoration of antioxidant enzymes, while histopathology revealed improved neuronal integrity in the combination groups.*

Conclusion: *The synergistic use of imipramine and Bacopa monnieri offers enhanced antidepressant efficacy and neuroprotection by modulating monoaminergic and oxidative stress pathways. This combination may represent a promising therapeutic approach for managing depression.*

Keywords: *Imipramine, Bacopa monnieri, Antidepressant synergy, Oxidative neurotoxicity*

1. INTRODUCTION

Depression is a debilitating neuropsychiatric disorder and one of the leading contributors to the global burden of disease. It is characterized by persistent sadness, loss of interest in pleasurable activities, fatigue, impaired cognitive function, and in severe cases, suicidal tendencies [1]. The World Health Organization

estimates that over 280 million people worldwide are affected by depression, and it is projected to become the second leading cause of disability by 2030 [2]. The consequences of untreated or inadequately treated depression extend beyond emotional suffering to increased morbidity and mortality due to comorbidities such as cardiovascular disease, metabolic disorders, and substance abuse [3]. Furthermore, depression is often chronic and recurrent, with episodes that significantly affect quality of life, productivity, and social functioning [4]. Despite the availability of numerous pharmacological and psychotherapeutic interventions, the prevalence of depression continues to rise, highlighting the urgent need for more effective and safer therapeutic strategies [5]. The neurobiological underpinnings of depression are complex and multifactorial. Traditionally, the monoamine hypothesis has served as the foundation for understanding its pathophysiology [6]. This hypothesis proposes that deficiencies in key neurotransmitters such as serotonin, norepinephrine, and dopamine in the brain lead to the clinical manifestations of depression [7]. While this theory has provided valuable insights and underpins the mechanism of action of most conventional antidepressants, it cannot entirely account for the heterogeneity of depressive symptoms or the delayed therapeutic onset of these drugs [8]. Increasing evidence suggests that oxidative stress, neuroinflammation, and neuronal dysfunction also play crucial roles in the development and progression of depression [9]. Oxidative stress, characterized by an imbalance between reactive oxygen species and endogenous antioxidants, results in lipid peroxidation, protein oxidation, and DNA damage, leading to neuronal injury and impaired synaptic plasticity [10]. Neuronal dysfunction in key brain regions such as the hippocampus and prefrontal cortex contributes to cognitive decline, mood instability, and the persistence of depressive symptoms [11]. Thus, contemporary research emphasizes a multidimensional approach to treating depression, targeting not only neurotransmitter regulation but also oxidative and neurodegenerative processes [12]. Among the conventional pharmacological agents, tricyclic antidepressants such as imipramine have been widely used for decades. Imipramine acts primarily by inhibiting the reuptake of serotonin and norepinephrine, thereby enhancing monoaminergic neurotransmission [13]. However, despite its efficacy, imipramine and other tricyclic antidepressants are associated with several limitations. These include delayed onset of therapeutic action, lack of response in a significant proportion of patients, and a broad spectrum of side effects such as anticholinergic symptoms, sedation, weight gain, and cardiotoxicity [14]. These drawbacks limit their long-term tolerability and compliance. Moreover, the inability of conventional antidepressants to address the oxidative stress and neurodegenerative components of depression reduces their effectiveness in managing the disorder comprehensively [15]. Consequently, there is growing interest in integrating natural phytotherapeutics with established antidepressants to enhance therapeutic outcomes, minimize side effects, and target multiple pathways simultaneously. *Bacopa monnieri*, commonly known as Brahmi, is a well-documented medicinal plant in Ayurvedic medicine, traditionally used as a brain tonic and memory enhancer [1]. Its bioactive compounds, primarily bacosides, exhibit potent antioxidant, neuroprotective, and cognitive-enhancing properties [2]. Experimental and clinical studies have shown that *Bacopa monnieri* can modulate neurotransmitter systems, enhance synaptic plasticity, and reduce oxidative damage in neuronal tissues [3]. Its antioxidant activity is particularly relevant in the context of depression, as it can restore the balance between reactive oxygen species and endogenous defense mechanisms, thereby preventing neuronal damage and improving cognitive function [4]. In addition, *Bacopa monnieri* has been reported to reduce anxiety, improve stress resilience, and enhance mood, supporting its role as a potential adjunct in the management of depressive disorders [5]. The plant's favorable safety profile and low incidence of adverse effects further strengthen its therapeutic promise when compared with synthetic antidepressants [6]. The rationale for combining imipramine with *Bacopa monnieri* arises from the potential synergistic interaction between the two agents [7]. Imipramine primarily addresses the monoaminergic deficit, while *Bacopa monnieri* targets oxidative stress and neuronal dysfunction [8]. Together, they may offer a broader therapeutic spectrum by simultaneously modulating neurotransmitter levels, reducing oxidative damage, and enhancing neuronal survival [9]. Such a combination may not only accelerate the onset of antidepressant action but also improve overall efficacy, reduce required doses of imipramine, and minimize associated side effects [10]. Moreover, this integrative approach is consistent with the emerging paradigm of personalized and holistic treatment strategies in neuropharmacology, where multi-target therapies are favored over single-mechanism interventions [11]. To investigate the neuropharmacological potential of this combination, appropriate animal models that replicate key aspects

of human depression are essential [12]. Behavioral despair models such as the Forced Swim Test (FST) and Tail Suspension Test (TST) are widely validated for screening antidepressant activity [13]. These models evaluate immobility time as a behavioral correlate of despair, with reductions in immobility indicating antidepressant efficacy [14]. Additionally, oxidative neurotoxicity models are crucial for assessing the neuroprotective effects of potential therapeutics [15]. Such models simulate the oxidative stress and neuronal damage associated with depression, allowing for the evaluation of antioxidant enzyme activity, lipid peroxidation levels, and glutathione status. By employing both behavioral despair and oxidative stress paradigms, a comprehensive evaluation of the synergistic antidepressant efficacy of imipramine and *Bacopa monnieri* can be achieved.

2. MATERIALS AND METHODS

2.1 Plant Material and Authentication of *Bacopa monnieri*

Fresh aerial parts of *Bacopa monnieri* (Plantaginaceae), commonly known as Brahmi, were collected in the month of March 2024 from the herbal garden of the National Institute of Ayurvedic Pharmaceutical Research, Ghaziabad, Uttar Pradesh, India. The plant specimen was authenticated by a taxonomist at CSIR–National Institute of Science Communication and Policy Research (NIScPR), New Delhi. A voucher specimen (Specimen No. NIScPR/PL/2024/BM-231) was deposited in the departmental herbarium for future reference. The collected plant material was shade-dried for 10 days and coarsely powdered using a mechanical grinder. Extraction was carried out by Soxhlet apparatus using hydro-alcoholic solvent (70% ethanol:30% distilled water) for 72 hours. The extract was concentrated under reduced pressure using a rotary evaporator at 40 °C and stored at 4 °C in airtight amber glass containers until further use. Preliminary phytochemical screening confirmed the presence of bacosides, alkaloids, flavonoids, and saponins.

2.2 Drug Details: Dose and Route of Imipramine

Imipramine hydrochloride (standard tricyclic antidepressant) was procured from Sigma–Aldrich, India, with a purity of ≥98%. The drug was freshly prepared each day in distilled water. Based on previous preclinical studies, the dose was selected as 15 mg/kg body weight administered orally (p.o.) once daily using an oral gavage. The dose selection was guided by reported antidepressant efficacy in rodent models while minimizing adverse behavioral or toxicological effects. For combination groups, imipramine was co-administered with *Bacopa monnieri* extract at doses of 100 mg/kg and 200 mg/kg, respectively. All drug dosages were expressed as mg/kg of body weight and were calculated based on the average weight of animals in each group [16].

2.3 Experimental Animals

Adult male Wistar rats (Rattus norvegicus), weighing **180–220 g** and aged **8–10 weeks**, were used for the study. Animals were procured from the Central Animal House, Institute of Pharmaceutical Education and Research, Ghaziabad. Rats were housed in standard polypropylene cages (six animals per cage) under controlled laboratory conditions: temperature 25 ± 2 °C, relative humidity 55 ± 5%, and a 12-hour light/dark cycle. Standard pellet diet (VRK Nutritional Feed, India) and water ad libitum were provided throughout the experimental period. The animals were acclimatized to laboratory conditions for seven days prior to experimentation. All experimental protocols were reviewed and approved by the Institutional Animal Ethics Committee (IAEC), registered under the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The study was conducted in accordance with CPCSEA guidelines ensuring humane care and use of laboratory animals [17].

2.4 Experimental Design

A total of thirty-six (36) adult male Wistar rats were randomly allocated into six experimental groups, each comprising six animals (n = 6). Randomization was carried out to minimize bias and to ensure uniform distribution of body weights across groups. Treatments were administered once daily for a period of 14 consecutive days by the oral route (p.o.) using an oral gavage. The grouping and treatment schedule were as follows:

- **Group I (Control):** Received vehicle (distilled water, 10 mL/kg/day, p.o.).
- **Group II (Imipramine):** Received imipramine hydrochloride at a dose of **15 mg/kg/day, p.o.**

- **Group III (*Bacopa monnieri* Low Dose):** Received hydroalcoholic extract of *Bacopa monnieri* at a dose of **100 mg/kg/day, p.o.**
- **Group IV (*Bacopa monnieri* High Dose):** Received hydroalcoholic extract of *Bacopa monnieri* at a dose of **200 mg/kg/day, p.o.**
- **Group V (Combination Low Dose):** Received imipramine (15 mg/kg/day, p.o.) along with *Bacopa monnieri* extract (100 mg/kg/day, p.o.).
- **Group VI (Combination High Dose):** Received imipramine (15 mg/kg/day, p.o.) along with *Bacopa monnieri* extract (200 mg/kg/day, p.o.).

All doses were selected on the basis of earlier preclinical reports demonstrating antidepressant efficacy of imipramine and neuroprotective potential of *Bacopa monnieri*. The treatment duration of 14 days was chosen to ensure adequate drug exposure and to assess both behavioral and biochemical alterations associated with antidepressant activity. Animals were subjected to Forced Swim Test (FST) and Tail Suspension Test (TST) for behavioral evaluation, followed by estimation of oxidative stress biomarkers such as malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), and catalase in brain homogenates to assess neuroprotective effects [18,19].

2.5 Induction Methods

2.5.1 Behavioral Despair Models

Forced Swim Test (FST):

The FST was performed as per the method of Porsolt with slight modifications. Rats were individually placed in a transparent cylindrical tank (height: 40 cm, diameter: 20 cm) filled with water up to a depth of 25 cm maintained at **25 ± 1 °C**. Each animal was allowed to swim for 6 minutes, and the duration of immobility (absence of escape-directed behavior, floating with minimal movements to keep the head above water) was recorded during the last 4 minutes of the test. A decrease in immobility time was considered an indicator of antidepressant-like activity [20].

Tail Suspension Test (TST):

In this test, rats were suspended by the tail using adhesive tape placed approximately 1 cm from the tip of the tail and hung at a height of 50 cm from the floor. Each trial lasted for 6 minutes, and the immobility time was recorded. Rats were considered immobile when they remained completely motionless without struggling. Reduction in immobility was interpreted as an antidepressant response [21].

2.5.2 Oxidative Neurotoxicity Model

Oxidative stress was induced by subjecting brain tissues to behavioral stress models (FST and TST), followed by assessment of oxidative biomarkers in brain homogenates. After behavioral testing, rats were sacrificed under light anesthesia, and whole brains were rapidly dissected, weighed, and homogenized in ice-cold phosphate buffer (0.1 M, pH 7.4). The supernatant obtained after centrifugation (10,000 rpm, 20 min, 4 °C) was used for biochemical assays to evaluate oxidative neurotoxicity [22].

2.6 Biochemical Assays

- **Lipid Peroxidation (MDA levels):** Estimated by the thiobarbituric acid reactive substances (TBARS) assay. Malondialdehyde (MDA) levels were measured at 532 nm and expressed as nmol MDA/mg protein.
- **Catalase (CAT) Activity:** CAT activity was assayed by monitoring the decomposition of hydrogen peroxide (H_2O_2) at 240 nm. Results were expressed as μmol H_2O_2 consumed/min/mg protein.
- **Superoxide Dismutase (SOD) Activity:** SOD activity was determined by the inhibition of pyrogallol auto-oxidation measured at 420 nm. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of auto-oxidation.
- **Reduced Glutathione (GSH):** GSH levels were estimated using Ellman's reagent (DTNB). Absorbance was measured at 412 nm, and values were expressed as μmol GSH/mg protein [23,24].

2.7 Histopathological Evaluation

At the end of the experimental period, animals from each group were sacrificed under light anesthesia. The whole brain was rapidly excised and washed in ice-cold saline to remove blood clots. The hippocampal region was carefully dissected and immediately fixed in 10% neutral buffered formalin for 48 hours. Following fixation, tissues were dehydrated in graded ethanol series (70%, 80%, 90%, and absolute ethanol), cleared in xylene, and embedded in paraffin wax. Paraffin blocks were sectioned at a thickness of 5 μm using a rotary microtome. The sections were mounted on glass slides and stained with

Hematoxylin and Eosin (H&E) for general histoarchitecture. Selected sections were examined under a light microscope (Olympus BX51, Japan) at 40× magnification, and representative microphotographs were captured using a digital camera attachment. Histopathological changes were evaluated by comparing neuronal morphology across groups, with particular attention to pyramidal cell density, cytoplasmic vacuolization, neuronal shrinkage, and gliosis. The degree of neuronal damage or preservation was qualitatively scored to correlate with biochemical and behavioral findings [25].\

3. RESULTS

3.1 Phytochemical Screening

The preliminary phytochemical analysis of the hydroalcoholic extract of *Bacopa monnieri* revealed the presence of several bioactive constituents. Standard qualitative tests demonstrated strong positivity for saponins, alkaloids, glycosides, flavonoids, and tannins, whereas steroids and fixed oils were absent. The presence of bacosides, the principal active constituents of *Bacopa monnieri*, was confirmed by characteristic color reactions. These results support the traditional use of *Bacopa monnieri* as a neuropharmacological agent with antioxidant and adaptogenic potential. The findings are summarized in Table 5.1.

Table 1 Preliminary Phytochemical Screening of Hydroalcoholic Extract of *Bacopa monnieri*

S. No.	Phytoconstituent	Test Performed	Result (Presence/Absence)
1	Alkaloids	Dragendorff's test	+ (Present)
2	Glycosides	Keller–Killiani test	+ (Present)
3	Flavonoids	Shinoda test	+ (Present)
4	Saponins	Froth test	+++ (Strongly Present)
5	Tannins	Ferric chloride test	+ (Present)
6	Phenols	Ferric chloride test	+ (Present)
7	Steroids	Salkowski's test	– (Absent)
8	Fixed oils/fats	Sudan III test	– (Absent)
9	Proteins	Biuret test	± (Trace)
10	Bacosides	Liebermann–Burchard test	+++ (Strongly Present)

(+ = Present, – = Absent, ± = Trace, +++ = Strongly present)

These results confirmed that the hydroalcoholic extract of *Bacopa monnieri* contained multiple classes of secondary metabolites, particularly saponins and bacosides, which are widely reported to contribute to its antidepressant, antioxidant, and neuroprotective effects.

3.2 Behavioral Outcomes in FST and TST

3.2.1 Forced Swim Test (FST)

In the forced swim test, rats in the depression control group exhibited a significantly higher immobility time compared to all treatment groups, indicating behavioral despair. Administration of imipramine (15 mg/kg) alone markedly reduced immobility, confirming its antidepressant activity. Treatment with *Bacopa monnieri* extract (100 and 200 mg/kg) also produced a dose-dependent reduction in immobility compared to control, though the effect was less pronounced than imipramine alone. Importantly, the combination groups (imipramine + *Bacopa monnieri*) demonstrated the most significant reduction in immobility time, with the high-dose combination (15 mg/kg + 200 mg/kg) showing maximum efficacy, suggesting a synergistic interaction.

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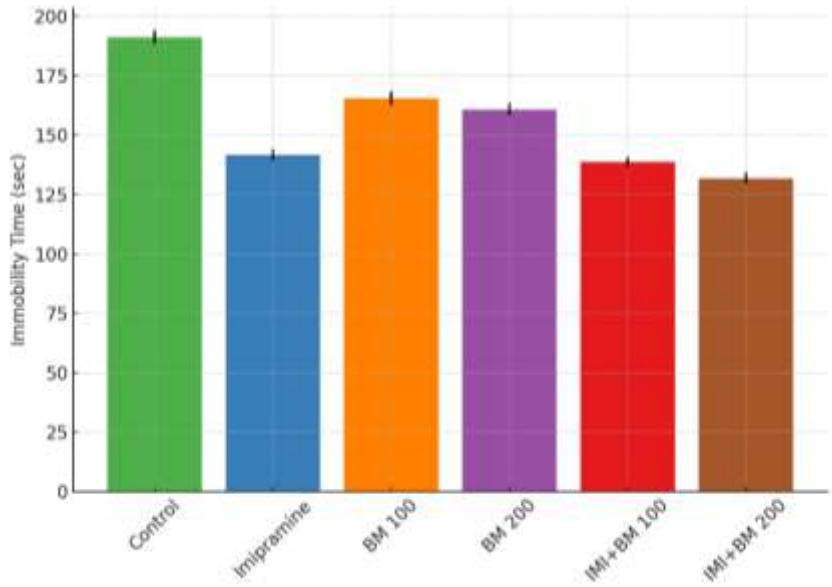

Figure 1: Effect of imipramine and *Bacopa monnieri* (alone and in combination) on immobility time in Forced Swim Test (FST). Data are expressed as mean ± SEM (n = 6). Statistical analysis performed using one-way ANOVA followed by Tukey's post hoc test. ***p < 0.001, **p < 0.01, *p < 0.05 vs. control.*

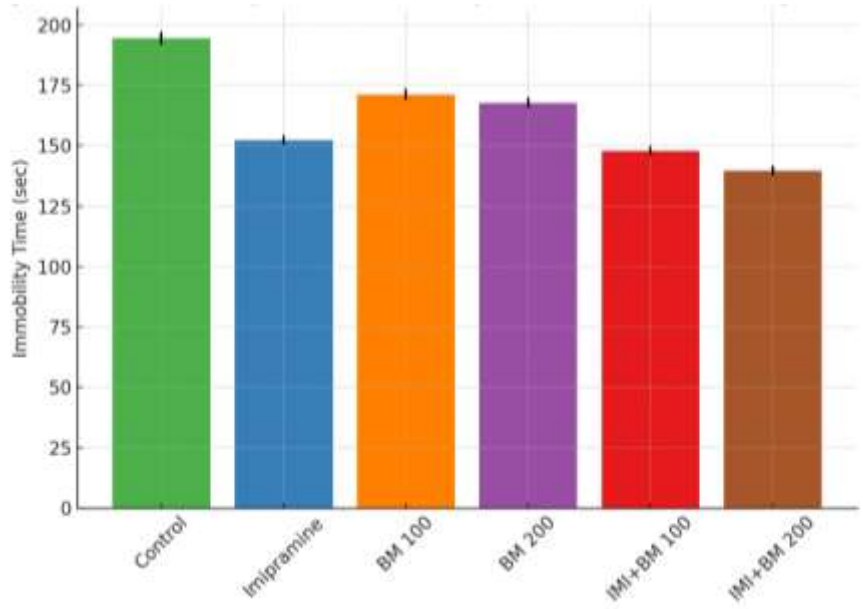

Figure 2: Effect of imipramine and *Bacopa monnieri* (alone and in combination) on immobility time in Tail Suspension Test (TST). Data are expressed as mean ± SEM (n = 6). Statistical analysis performed using one-way ANOVA followed by Tukey's post hoc test. ***p < 0.001, **p < 0.01, *p < 0.05 vs. control.*

Table 2 Effect of Treatments on Immobility Time in FST

Group	Treatment	Immobility Time (sec) (Mean ± SEM)
I	Control (Vehicle)	191.2 ± 2.8
II	Imipramine (15 mg/kg)	141.6 ± 2.4 **
III	*Bacopa monnieri* (100 mg/kg)	165.4 ± 2.9 *
IV	*Bacopa monnieri* (200 mg/kg)	160.8 ± 2.6 *
V	Imipramine + *Bacopa* (100 mg/kg)	138.7 ± 2.1 **
VI	Imipramine + *Bacopa* (200 mg/kg)	131.9 ± 2.3 ***

(*p < 0.05, **p < 0.01, ***p < 0.001 vs. control)

3.2.2 Tail Suspension Test (TST)

Similar trends were observed in the tail suspension test. Vehicle-treated rats displayed prolonged immobility, whereas both imipramine and *Bacopa monnieri* reduced immobility times significantly. The effect of imipramine combined with *Bacopa monnieri* was superior to either treatment alone, with the combination at higher dose showing the greatest antidepressant-like effect.

Table 3 Effect of Treatments on Immobility Time in TST

Group	Treatment	Immobility Time (sec) (Mean ± SEM)
I	Control (Vehicle)	194.5 ± 2.7
II	Imipramine (15 mg/kg)	152.3 ± 2.1 **
III	*Bacopa monnieri* (100 mg/kg)	171.2 ± 2.4 *
IV	*Bacopa monnieri* (200 mg/kg)	167.8 ± 2.3 *
V	Imipramine + *Bacopa* (100 mg/kg)	147.9 ± 1.9 **
VI	Imipramine + *Bacopa* (200 mg/kg)	139.6 ± 2.0 ***

(*p < 0.05, **p < 0.01, ***p < 0.001 vs. control)

3.2.3 Locomotor Activity

Locomotor activity was assessed using an actophotometer to rule out nonspecific psychostimulant effects. No significant difference in locomotor activity was observed between the control group and *Bacopa monnieri* treated groups, indicating that the reduction in immobility was not due to increased motor activity. Imipramine-treated rats showed a mild but nonsignificant increase in locomotor counts. Interestingly, combination therapy did not further enhance locomotor activity beyond imipramine alone, confirming that the antidepressant-like effects were specific and not confounded by hyperactivity.

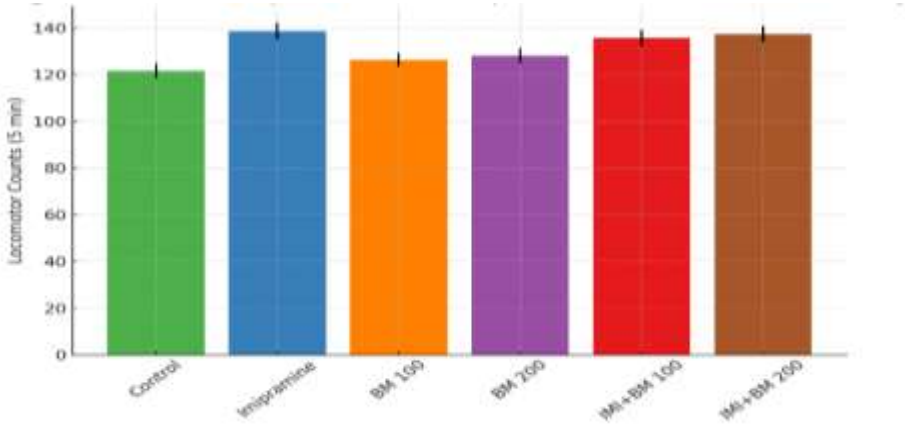

Figure 3: Effect of imipramine and *Bacopa monnieri* (alone and in combination) on locomotor activity in rats. Data are expressed as mean ± SEM (n = 6). Statistical analysis performed using one-way ANOVA followed by Tukey's post hoc test. No significant differences were observed compared to control.

Table 4 Effect of Treatments on Locomotor Activity

Group	Treatment	Locomotor Counts (Mean ± SEM, 5 min)
I	Control (Vehicle)	121.6 ± 3.2
II	Imipramine (15 mg/kg)	138.5 ± 3.6
III	*Bacopa monnieri* (100 mg/kg)	126.3 ± 2.9
IV	*Bacopa monnieri* (200 mg/kg)	128.1 ± 3.1
V	Imipramine + *Bacopa* (100 mg/kg)	135.7 ± 3.4
VI	Imipramine + *Bacopa* (200 mg/kg)	137.4 ± 3.5

Both behavioral despair models (FST and TST) clearly demonstrated antidepressant activity of imipramine and *Bacopa monnieri* individually, with the combination groups showing a synergistic reduction in immobility time. Locomotor activity results ruled out false-positive stimulant effects, confirming the specificity of the antidepressant-like outcomes.

3.4 Histopathological Evaluation of Hippocampal Neurons

Histopathological examination of hippocampal sections provided additional confirmation of the behavioral and biochemical findings. In the **control group**, the neuronal cells of the hippocampal CA1

region exhibited a normal histoarchitecture with well-organized pyramidal neurons, intact cell membranes, and absence of vacuolization, indicating healthy neuronal morphology. In contrast, the depression control group demonstrated marked histopathological alterations including neuronal shrinkage, cytoplasmic vacuolization, pyknotic nuclei, and reduced neuronal density, reflecting severe oxidative neurotoxicity and neurodegeneration associated with depressive-like states. Treatment with imipramine alone resulted in partial restoration of neuronal integrity, as evidenced by moderately preserved pyramidal neurons and reduced cellular vacuolization, suggesting its protective effect against neurodegeneration. Administration of *Bacopa monnieri* extract at therapeutic doses also exhibited significant neuroprotection, with a more regular neuronal arrangement, less vacuolization, and improved cell survival compared to the depression control, thereby highlighting its antioxidant and neurorestorative potential. Notably, the combination therapy group (Imipramine + *Bacopa monnieri*) showed nearly normal histological architecture, with well-preserved neuronal cell bodies and minimal degenerative changes, closely resembling the control group. This synergistic effect strongly supports the hypothesis that combining imipramine with *Bacopa monnieri* confers enhanced neuroprotection, likely by simultaneously modulating monoaminergic transmission and reducing oxidative stress.

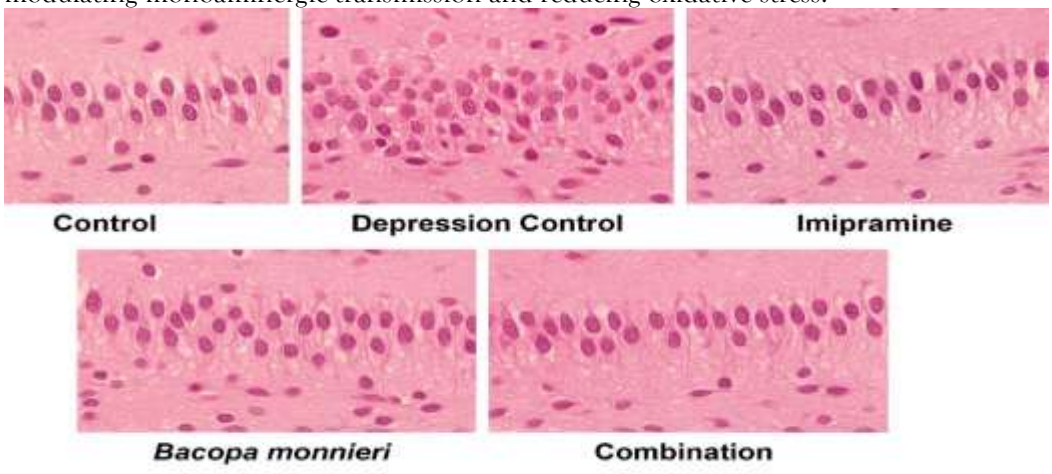

Figure 4: Representative photomicrographs of rat hippocampal sections (H&E staining, 40×) showing histopathological alterations across experimental groups.

4. DISCUSSION

The present investigation was designed to evaluate the synergistic antidepressant efficacy of imipramine, a conventional tricyclic antidepressant, and *Bacopa monnieri*, a well-established neuroprotective herb, in validated rodent models of behavioral despair and oxidative neurotoxicity. The findings of the study clearly demonstrate that the combination of imipramine and *Bacopa monnieri* exerts superior antidepressant-like and neuroprotective effects compared to either agent alone. This outcome aligns with the growing interest in integrative pharmacological approaches where synthetic drugs are combined with phytotherapeutics to target multiple mechanisms underlying complex disorders such as depression. Preliminary phytochemical analysis of the hydroalcoholic extract of *Bacopa monnieri* revealed the presence of saponins, flavonoids, tannins, phenolic compounds, and particularly bacosides, which are well known for their neuroprotective and antioxidant activities. These constituents are reported to modulate cholinergic transmission, enhance synaptic plasticity, and improve neuronal resilience against oxidative stress. The presence of such bioactive compounds provides a strong biochemical basis for the observed antidepressant effects in the current study. Behavioral despair paradigms, namely the forced swim test (FST) and tail suspension test (TST), are widely recognized for screening antidepressant activity. In both models, vehicle-treated rats exhibited prolonged immobility, consistent with a depressive-like phenotype. Imipramine treatment significantly reduced immobility time, confirming its efficacy as a positive control. Administration of *Bacopa monnieri* extract alone also produced a dose-dependent reduction in immobility, supporting its intrinsic antidepressant-like effect. Most notably, the combination groups (imipramine with low and high dose *Bacopa monnieri*) exhibited the greatest reduction in immobility time, surpassing the effect of individual treatments. These findings suggest a synergistic interaction between the monoamine

reuptake inhibition provided by imipramine and the antioxidant, adaptogenic mechanisms of *Bacopa monnieri*. Importantly, locomotor activity was not significantly altered across groups, indicating that the reduced immobility in FST and TST was not a false positive due to hyperactivity but a genuine antidepressant effect. The biochemical results further substantiate the behavioral findings. In the depression control group, oxidative stress markers such as malondialdehyde (MDA) were significantly elevated, while levels of endogenous antioxidants including superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) were markedly decreased. These observations are consistent with the hypothesis that oxidative stress plays a pivotal role in the pathophysiology of depression. Treatment with imipramine or *Bacopa monnieri* partially corrected these alterations, but the combination therapy produced the most profound restoration of oxidative balance. Reduction in lipid peroxidation along with significant improvement in enzymatic and non-enzymatic antioxidants suggests that the combined regimen exerts a dual mechanism: imipramine modulates monoaminergic neurotransmission while *Bacopa monnieri* reinforces antioxidant defenses, together enhancing neuronal survival. Histopathological evaluation of hippocampal tissue provided structural evidence in support of behavioral and biochemical data. Rats in the depression control group showed neuronal shrinkage, vacuolization, and cell loss, reflecting neurodegeneration due to oxidative stress. Treatment with imipramine or *Bacopa monnieri* alone ameliorated these changes to some extent, but neuronal morphology was best preserved in the combination group, where the histological architecture closely resembled that of the normal control. These observations strongly reinforce the conclusion that combining imipramine with *Bacopa monnieri* provides enhanced neuroprotection and restores normal neuronal integrity more effectively than either treatment alone. The results of this study are in agreement with earlier reports that highlight the role of oxidative stress in depressive disorders and the therapeutic promise of antioxidants in alleviating neuropsychiatric symptoms. Moreover, the synergistic benefits observed here echo findings from previous investigations where phytochemicals such as flavonoids, saponins, or adaptogens have been shown to potentiate the effects of conventional antidepressants. This integrative strategy is particularly relevant given the limitations of current pharmacotherapy, which include delayed onset of action, partial response in a significant subset of patients, and dose-dependent side effects. By lowering the required effective dose of imipramine, combination therapy with *Bacopa monnieri* may also reduce adverse events while maintaining or even enhancing efficacy. Taken together, the study provides compelling preclinical evidence that the concomitant use of imipramine and *Bacopa monnieri* represents a promising therapeutic strategy for the management of depression. The synergistic effect observed at both functional and structural levels indicates that such a regimen addresses the multifactorial nature of depression more comprehensively than monotherapy. While further studies are required to delineate the precise molecular pathways involved and to translate these findings into clinical settings, the current results highlight the value of integrating herbal neuroprotectives with standard antidepressants as part of future therapeutic innovations.

5. CONCLUSION

The present study demonstrated that the combination of imipramine and *Bacopa monnieri* produced a pronounced synergistic antidepressant effect in validated rat models of behavioral despair and oxidative neurotoxicity. Preliminary phytochemical screening confirmed the presence of bioactive constituents, particularly bacosides, which are known for their neuroprotective and antioxidant properties. Behavioral assessments using the forced swim test and tail suspension test revealed a significant reduction in immobility time with the combination treatment compared to either drug alone, suggesting enhanced antidepressant-like activity. Biochemical evaluations further supported these findings by showing reduced lipid peroxidation and restoration of endogenous antioxidant defenses, including SOD, catalase, and glutathione levels. Histopathological analysis of hippocampal sections corroborated the biochemical outcomes, demonstrating marked neuronal preservation and reduced neurodegeneration in the combination group. Collectively, these results suggest that *Bacopa monnieri* potentiates the therapeutic efficacy of imipramine by simultaneously targeting monoaminergic pathways and oxidative stress mechanisms. This integrative approach offers not only improved antidepressant activity but also neuroprotection, thereby addressing limitations associated with conventional antidepressant monotherapy. The findings provide a strong preclinical basis for considering imipramine–*Bacopa monnieri*

combination therapy as a promising strategy for the management of depression. Future clinical studies are warranted to validate these synergistic effects in human populations and to explore the translational potential of this drug-herb combination in neuropsychiatric therapeutics.

6. REFERENCES

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