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Research Article

Restorative Effects Of *Bacopa Monnieri* Extract On Olfactory Dysfunction In A Methimazole-Induced Mouse Model

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Abstract

This study investigated the restorative effects of *Bacopa monnieri* L. extract on olfactory dysfunction in a methimazole-induced injury model in mice. Animals were divided into five groups: physiological control, pathological (untreated), two treatment groups receiving *Bacopa monnieri* extract (50 and 100 mg/kg/day), and a positive control group receiving donepezil (1.5 mg/kg/day). After 14 days of treatment, olfactory performance was assessed using a peanut butter odor-finding test, and histological recovery of the olfactory epithelium was evaluated via hematoxylin and eosin (H&E) staining.

Treatment with *Bacopa monnieri* significantly reduced odor-finding latency, increased time spent and entries into the target zone, and improved olfactory epithelial thickness compared with the pathological group. The 100 mg/kg dose produced effects comparable to those of donepezil and closely approximated the physiological control group. These findings highlight *Bacopa monnieri* as a promising natural agent for restoring olfactory function following epithelial injury.

Keywords - *Bacopa monnieri* L., olfactory dysfunction, methimazole, olfactory epithelium, odor-finding test

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1. Background

Bacopa monnieri L., a perennial aquatic herb widely distributed in tropical and subtropical regions—including South and Southeast Asia, China, Australia, and South America—has long been used in traditional medicine for enhancing memory, alleviating anxiety, and supporting neuroprotection. In Vietnam, it grows abundantly in Marsh lands, ponds, and brackish coastal areas, especially in central and southern provinces. Its bioactive components, mainly bacosides A and B, flavonoids, alkaloids, and cucurbitacins, contribute to potent antioxidant, anti-inflammatory, neuroprotective, and cognitive-enhancing effects (Kongkeaw et al., 2014; Shalini et al., 2021). Preclinical evidence suggests that *Bacopa monnieri* promotes neuronal regeneration by upregulating neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF), thereby reducing oxidative stress and supporting synaptic plasticity (Calabrese et al., 2008).

The olfactory epithelium (OE), a peripheral neural tissue located in the upper nasal cavity, plays a central role in odor detection and signal transmission to the brain. It is composed of olfactory receptor neurons (ORNs), supporting cells, and basal cells. Among the basal cells, two main subtypes are identified: horizontal basal cells (HBCs) and globose basal cells (GBCs). ORNs are specialized bipolar neurons capable of continuous regeneration throughout the lifespan. The OE possesses a regenerative capacity following damage caused by chemical agents, microbial infection, or mechanical injury. This regenerative process is modulated by neurotrophic factors, including NGF and BDNF, with NGF being particularly crucial for the differentiation and regeneration of ORNs derived from basal cells (Noda et al., 2019; Hu et al., 2022). However, the regenerative capacity of the OE declines progressively with age and is further impaired under pathological conditions such as chronic inflammation or exposure to neurotoxic agents like methimazole (Suzukawa et al., 2011).

Given its established neurotrophic and antioxidant activities, *Bacopa monnieri* represents a potential natural candidate for promoting olfactory epithelial repair. This study aimed to evaluate the restorative effects of *Bacopa monnieri* extract on olfactory dysfunction induced by methimazole in mice, combining behavioral and histological analyses.

2. Material and methods

2.1. Plant material and extraction

Bacopa monnieri L. was collected from Lao Cai Province, Vietnam. After cleaning, the plant material was dried and cut into small pieces. Extraction was carried out using an ethanol–water (EtOH:H₂O) solvent

system in a 7:3 (v/v) ratio, with a solvent-to-plant ratio of 10:1 (w/w), under reflux at 80 °C for 6 hours. The resulting extract was spray-dried to yield a dry powder. The total bacoside A content was determined by HPLC to be 31.5 % ± 1.2 %.

2.2. Experimental animals

Healthy male *Swiss albino* mice (20–30 g) were obtained from the National Institute of Hygiene and Epidemiology (Hanoi, Vietnam). Mice were housed under controlled conditions (22 ± 2 °C; 12-hour light/dark cycle) with free access to food and water. Animals were acclimatized for 7 days prior to experimentation. All procedures followed the ethical standards for animal research and were approved by the institutional animal care and use committee.

2.3. Experimental design

The study was conducted to evaluate the olfactory function restoration effects of *Bacopa monnieri* extract in a mouse model with methimazole-induced olfactory epithelium damage (dose: 50 mg/kg, intraperitoneal injection). Treatment was administered to the mice for one week prior to the induction of olfactory epithelial injury. After the induction of olfactory epithelial injury, treatment was continued for one week, followed by an evaluation of olfactory function improvement using the peanut butter odor-finding test. Upon completion of the behavioral assessments, the animals were sacrificed for brain perfusion, and hematoxylin and eosin (H&E) staining was performed to examine the extent of damage and the thickness of the olfactory epithelium. Mice were fasted for 24 hours prior to training, with access to water only. On the day of behavioral testing, mice received water, the plant extract, or donepezil by intraperitoneal injection 60 minutes before the test. Experimental animals were divided into five groups, with each group consisting of 10–12 mice, as follows:

- Control group: Mice received no methimazole injection and were administered distilled water (0.1 mL/10 g/day) throughout the experimental period.
- Pathological group: Mice received intraperitoneal injections of methimazole at 50 mg/kg, followed by daily administration of distilled water (0.1 mL/10 g/day).
- *Bacopa monnieri* extract treatment groups: Mice were injected intraperitoneally with methimazole (50 mg/kg), followed by daily treatment with *Bacopa monnieri* extract at doses of 50 mg/kg (BA50) and 100 mg/kg (BA100), respectively.
- Donepezil group: Mice were injected intraperitoneally with methimazole (50 mg/kg) and subsequently treated with donepezil at a dose of 1.5 mg/kg/day (intraperitoneal injection).

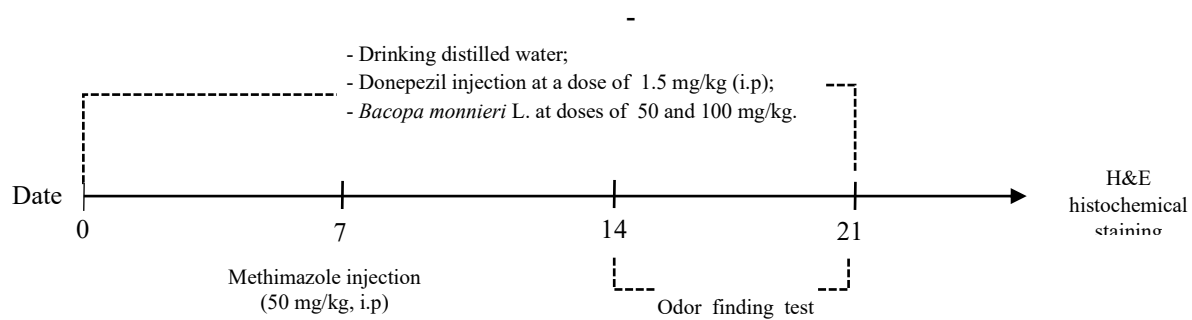


Figure 1. Experimental timeline showing methimazole induction, treatment, and behavioral and histological assessments.

2.4. Odor finding test (OFT)

The experiment was conducted in a wooden box (dimensions: length $\times$ width $\times$ height = 40 $\times$ 20 $\times$ 20 cm) with bedding material at a depth of 5 cm. The experiment consisted of two phases: the training phase and the testing phase. In all phases, each trial utilized a square piece of filter paper (4 $\times$ 4 cm) soaked with peanut butter at a concentration of 1.2 g/mL. The filter paper was buried beneath the bedding, placed centrally within the designated quadrant for each trial to eliminate random factors that might allow the mouse to locate the food by chance. After each trial, the filter paper was replaced, and its placement site was cleaned with

alcohol.

Training Phase: Mice were allowed to explore for 10 minutes to locate the peanut butter and acclimate to the environment, followed by a 5-minute rest in the home cage. Each mouse completed three training sessions before testing.

Testing Phase: Conducted 7 days after methimazole injection. Each mouse was tested for 180 seconds to locate the hidden peanut butter. The latency to find the target, time spent in, and entries into the target zone were recorded using ANY-maze software (Stoelting Co., USA). Trials were terminated if the mouse failed to locate the target within 180 seconds.

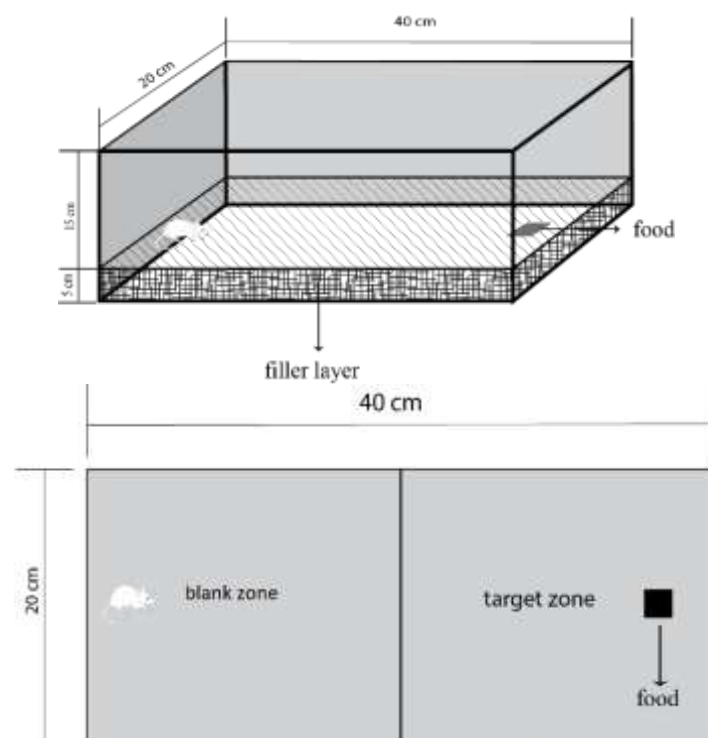


Figure 2. Schematic diagram of the odor-finding test apparatus and behavioral recording setup

2.5. Histological analysis and epithelial thickness measurement

Mice were anesthetized with chloral hydrate (50 mg/kg). Following complete anesthesia, transcardial perfusion was performed with 0.9% saline, after which the heads were decalcified in 0.5 M EDTA (pH 8.0) for one week

at 4 °C. After decalcification, tissues were fixed in 4% paraformaldehyde for at least 2 h, followed by paraffin embedding and sectioning. The tissue sections were cut using an HM 325 microtome (Thermo Scientific) at a thickness of 5 μ m.

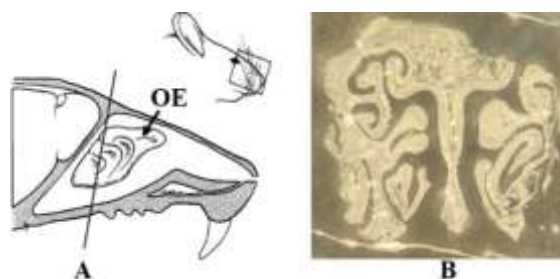


Figure 3. Sectioning sites

(A) Location of tissue sectioning; (B) Paraffin-embedded specimen after sectioning (2X).

Nasal tissue sections were stained with hematoxylin and eosin (H&E) according to the following protocol: deparaffinization in three xylene baths (5 min each); rehydration through graded ethanol baths (100%, 95%, 80%, and 70%), with 15 immersions per bath; rinsing in distilled water (15 immersions); nuclear staining with Harris hematoxylin for 6 min, followed by running tap water for 10 min; microscopic evaluation of nuclear staining; counterstaining with 0.1% eosin B for 1–2 min; rinsing in running water for 1 min. Excess stain was removed by dehydration in 95% and 100% ethanol

(three baths, 5–10 s each), followed by clearing in xylene (three baths, 5–10 s each), and mounting with coverslips.

The olfactory epithelium of mice was examined, and its thickness was quantified using imaging software connected to an Olympus IX73 inverted phase-contrast fluorescence microscope equipped with a 10× objective lens. Measurements were obtained from eight distinct sites on each nasal tissue section, and the mean value was calculated to determine the epithelial thickness.

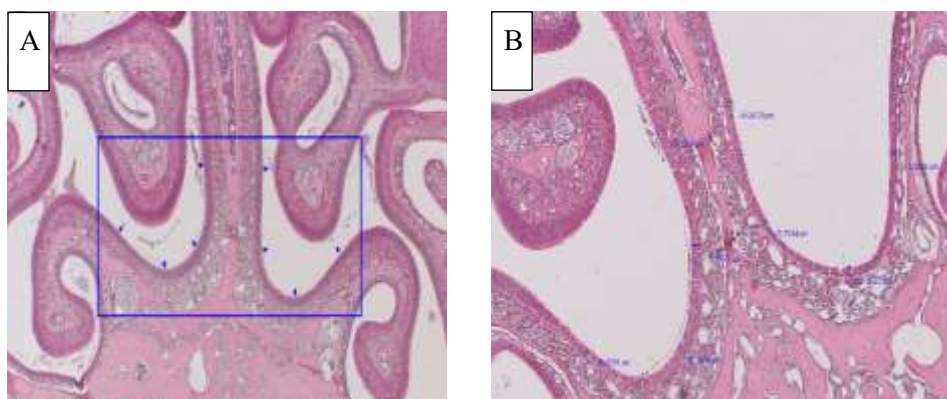


Figure 4. Representative sectioning sites and epithelial thickness measurement areas on nasal tissue samples.

(A) Locations used for epithelial thickness measurement; (B) Measurement performed using imaging software connected to an Olympus IX73 microscope (10X objective).

2.6. Statistical analysis

Data are expressed as mean $\pm$ standard error (SE). Statistical analysis was performed using SPSS 27. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used to compare groups. A p -value < 0.05 was considered statistically significant.

3. Results

3.1. Restoration of olfactory function in the odor-finding test

The olfactory function of experimental animals was evaluated using the odor finding test, which measures the ability of mice to locate the odor of peanut butter. Mice possess highly sensitive olfactory capabilities; after 24 hours of food deprivation, they instinctively search for food when released into the chamber. The OFT assessed key behavioral parameters, including the latency to locate the food source, as well as the duration and frequency of visits to the target zone (the food-containing area), to determine the extent of olfactory recovery. The results are presented in Figures 5 and 6.

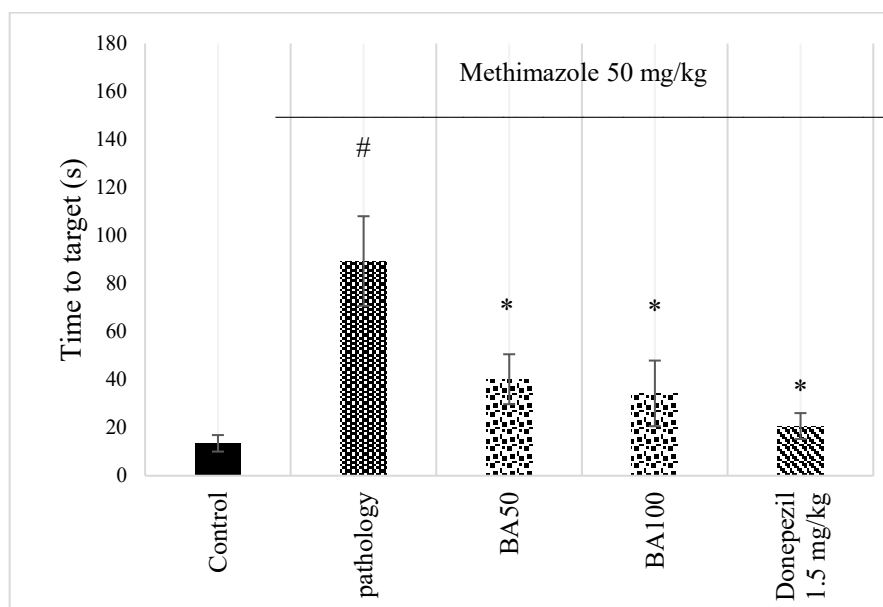


Figure 5. Latency to locate the peanut butter-scented target in the odor-finding test. (#: $P < 0.001$ compared to the control group, *: $P < 0.05$ compared to the pathological group)

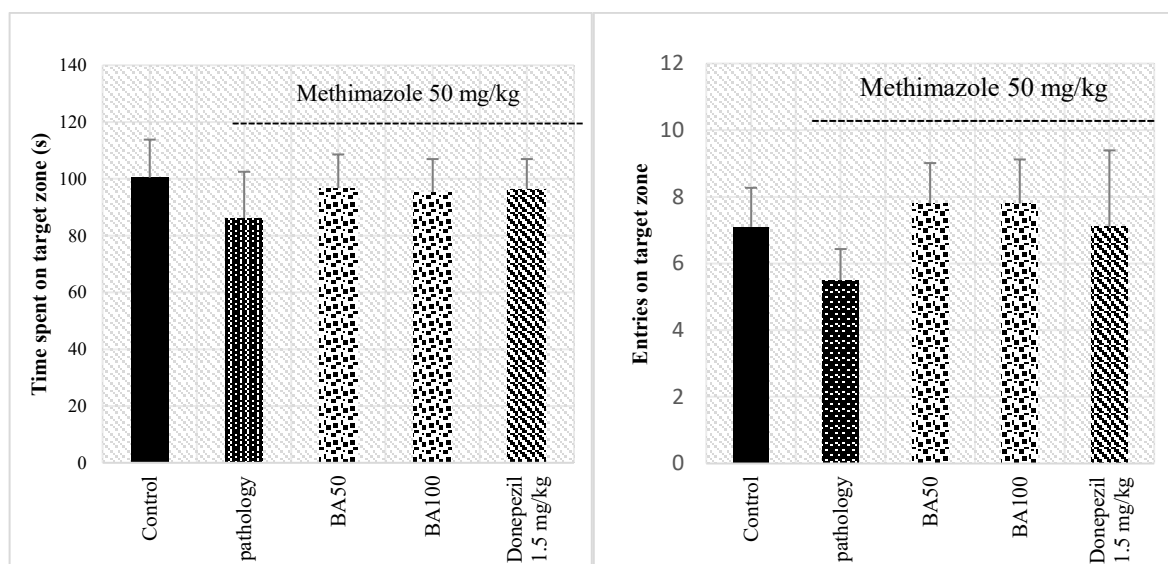


Figure 6. Time spent and number of entries into the target zone in the odor-finding test.

As shown in Figure 5, the latency to reach the target was significantly shorter in the physiological and treatment groups compared with the pathology group. A marked difference was observed between the control and pathology groups ($p < 0.001$), confirming the reliability of the OFT in evaluating olfactory recovery in the methimazole-induced mouse model. Treatment with *Bacopa monnieri* extract (50 and 100 mg/kg) significantly reduced the latency to reach the food compared with the pathology group ($p < 0.05$). Similarly, mice treated with donepezil (1.5 mg/kg) exhibited a shorter latency than the pathology controls ($p < 0.05$), approaching values observed in the control

group. These findings indicate that *Bacopa monnieri* extract (50 and 100 mg/kg) effectively improves olfactory function in the methimazole-induced mouse model.

The improvement in olfactory function was further supported by the behavioral indices of time spent and number of entries into the target zone. As shown in Figure 6, mice in the disease group exhibited shorter durations and fewer visits to the target zone compared with both physiological and treatment groups. Conversely, treated mice displayed increased activity in the food-scented area, indicating restoration of olfactory function and active food-seeking behavior.

3.2. Recovery of olfactory epithelial thickness

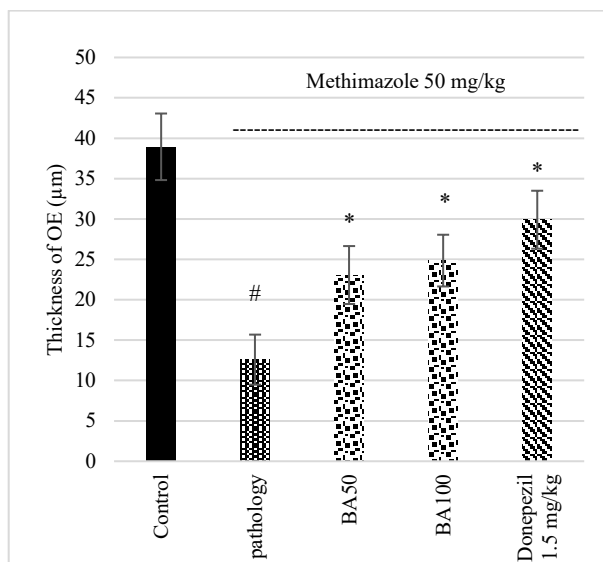


Figure 7. Olfactory epithelial thickness across experimental groups.

(#: $P < 0.001$ compared to the control group, *: $P < 0.05$ compared to the pathological group)

The thickness of the olfactory epithelium revealed that mice in the pathology group exhibited a markedly thinner epithelial layer compared with the control group, with a statistically significant difference ($p < 0.001$). In

contrast, the epithelial thickness in mice treated with *Bacopa monnieri* extract or donepezil was significantly greater than that in the pathology group ($p < 0.05$), indicating structural recovery.

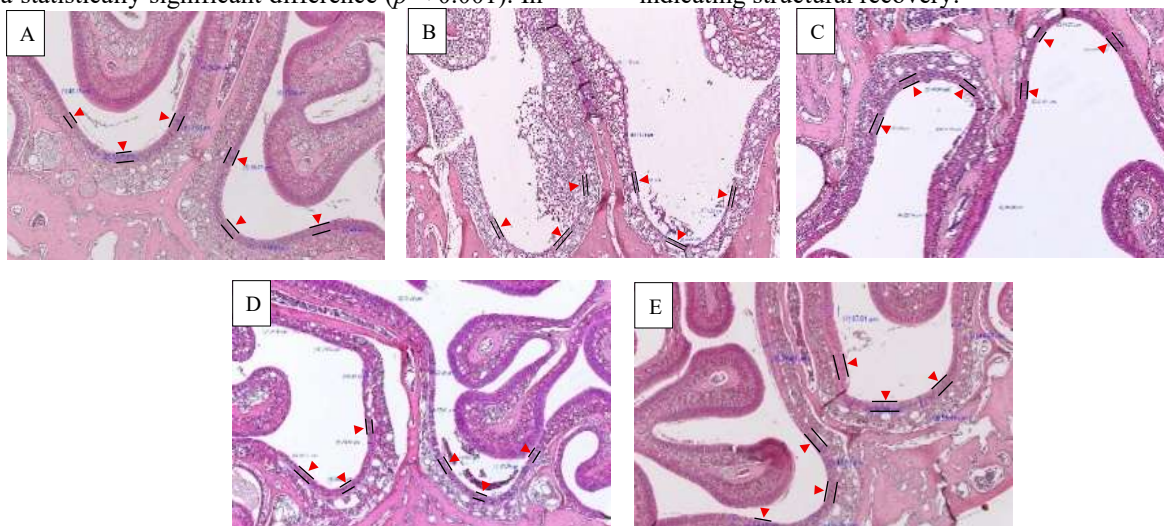


Figure 8. Representative histological images of the olfactory epithelium (H&E staining, 10X)

(A: control, B: Pathology, C: BA50, D: BA100, E: Donepezil)

Histomorphological observation of the olfactory epithelium in mice revealed that, in the control group, the olfactory epithelial layer was thick and uniformly organized. In contrast, mice in the pathology group exhibited a significantly thinner epithelial layer, with loosely arranged cells and occasional tearing within the layer. In the BA50-, BA100-, and donepezil-treated groups, a marked restoration of the epithelial structure was observed. Notably, the epithelial layers in the BA100- and donepezil-treated groups showed a consistent and dense parenchyma, closely resembling that of the control group.

4. Discussion

Methimazole is an antithyroid compound frequently employed to induce olfactory epithelial injury in rodents. Its toxicity results from bioactivated

intermediates generated by flavin-containing monooxygenases in the nasal mucosa, which selectively damage olfactory sensory neurons (Genter et al., 1995; Bergström et al., 2003). This model effectively mimics transient anosmia or hyposmia and has been widely adopted for preclinical evaluation of neuroregenerative therapies. The restoration of olfactory function following methimazole-induced injury primarily depends on the proliferation and differentiation of basal cells—horizontal and globose basal cells—that regenerate olfactory sensory neurons. This process is strongly influenced by neurotrophic factors such as NGF and BDNF and can be compromised by oxidative stress and inflammation (Suzukawa et al., 2011, Brittebo et al., 1995)).

In the present study, both behavioral and histological findings demonstrated that *Bacopa monnieri* extract

promotes recovery of olfactory function. Mice treated with the extract, especially at 100 mg/kg, exhibited reduced latency to locate the target, increased time in the target zone, and improved epithelial structure—comparable to those treated with donepezil, a known neuroprotective agent used clinically for Alzheimer's disease (Lee et al., 2024). Donepezil, an acetylcholinesterase inhibitor commonly prescribed for Alzheimer's disease, enhances cholinergic neurotransmission in the brain. Recent studies have demonstrated that donepezil (1–3 mg/kg, intraperitoneal) not only improves cognitive performance but also enhances olfactory function, particularly in rodent models with olfactory bulb injury. Its therapeutic effects are mediated through stimulation of neurogenesis in the olfactory bulb and attenuation of oxidative stress (Yuan et al., 2021; Yabuki et al., 2017). Our findings revealed that treatment with *Bacopa monnieri* extract, particularly at a dose of 100 mg/kg, significantly improved olfactory recovery compared with the disease group, achieving results nearly equivalent to the physiological control and donepezil-treated groups. Both behavioral olfactory performance and histological outcomes supported this effect: the olfactory epithelium in treated mice displayed markedly increased epithelial thickness, orderly cellular arrangement, and near-normal structural restoration. These results provide supportive evidence for the therapeutic potential of *Bacopa monnieri* in ameliorating methimazole-induced olfactory dysfunction.

These findings are consistent with recent reports on the neuroprotective and neurorestorative effects of *Bacopa monnieri* on both the central and peripheral nervous systems. Multiple preclinical and clinical studies have confirmed its neuroprotective properties. The mechanisms underlying olfactory recovery may be attributed to its ability to stimulate neural stem cell proliferation and differentiation. Specifically, *Bacopa monnieri* activates basal stem cells (HBCs, GBCs) within the olfactory epithelium, promoting their differentiation into new olfactory sensory neurons, thereby re-establishing odor signal transduction pathways. Moreover, bacosides present in *Bacopa monnieri* enhance the expression of neurotrophic factors, creating a favorable microenvironment for neuronal regeneration and repair (Shalini et al., 2021). In addition, *Bacopa monnieri* exhibits potent antioxidant activity, neutralizing free radicals, reducing local epithelial inflammation, and limiting apoptosis, thereby preserving tissue structure and function. It stabilizes neuronal membranes, increases dendritic spine density, and facilitates synaptic connectivity, ultimately improving odor perception efficiency (Calabrese et al., 2008). Collectively, these findings support the hypothesis that *Bacopa monnieri* enhances peripheral neuroregeneration through antioxidant and neurotrophic mechanisms, contributing to structural and functional restoration of the olfactory system.

Limitations

The current study has certain limitations. The methimazole model primarily affects peripheral

epithelial layers and may not fully recapitulate central olfactory processing or chronic neurodegenerative conditions. Moreover, only H&E staining was performed; immunohistochemical markers such as OMP (olfactory marker protein), Ki67 (proliferation), or BDNF would provide stronger mechanistic evidence of regeneration. Future studies should incorporate molecular and immunohistochemical analyses to elucidate the signaling pathways underlying *Bacopa monnieri*-mediated olfactory recovery.

5. Conclusion

The present study demonstrates that *Bacopa monnieri* extract significantly restores olfactory function and epithelial structure in a methimazole-induced mouse model of olfactory dysfunction. Behavioral and histological outcomes indicate that the 100 mg/kg dose achieves efficacy comparable to that of donepezil. These results suggest that *Bacopa monnieri* may serve as a safe, natural therapeutic candidate for the treatment or adjunctive management of olfactory disorders associated with injury, infection, or neurotoxicity.

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Conflict of Interest Statement: The authors declare that they have no conflicts of interest related to this study.

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