Faculty of Medical and Health Sciences, University of Poonch Rawalakot
Journal of Pharma and Biomedics
ISSN: 3007-1984(online), 3007-1976 (Print)
https://www.jpbsci.com/index.php/jpbs

Evaluation of Nootropic and Acetylcholinesterase Inhibitory Activities of *Lentinula Edodes* in Mice

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Received: June 30, 2025; *Revised: September 28, 2025;* *Accepted: October 08, 2025*

A B S T R A C T

Background: Memory is considered as the brain ability to encode, store, and recall knowledge whereas, medicinal compounds which improve memory are called as Nootropics (Smart drugs). Mushrooms have been used from ancient time as traditional medicines, however, few are screened for their nootropic activity. The objective of this study was to evaluate the nootropic activity of *Lentinula edodes* mushroom in mice. Methodology: Elevated plus maze and Morris water maze tests were used for the purpose of the memory assessment. However, open field test was used to rule out the effect of the extract on the motor activity of the mice. Biochemical estimation of acetylcholinesterase was done using Ellman method. Results: In elevated plus maze test, initial transfer latency and retention transfer latency were significantly decreased in the animals treated with the *Lentinula edodes* extract at both the doses of 250mg/kg and 500mg/kg. Whereas, in Morris water maze, time spent in the target quadrant was significantly increased in the animals treated with the extract, however, escape latency was significantly reduced. The level of acetylcholinesterase was also significantly reduced showed the nootropic activity. Conclusion: Based on the above result it is concluded that *Lentinula edodes* possesses significant nootropic activity in mice.

Keywords: Memory, Nootropics, *Lentinula edodes*, Elevated plus maze, Morris water maze, Open field test, Acetylcholinesterase.

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INTRODUCTION

Memory is a neurochemical process involving the encoding, storage, and retrieval of information through the modulation of synaptic signals among neurons. It refers to the brain's ability to encode, store, and recall knowledge. In the early 19th century, neuroanatomists proposed that memories are not simply stored through the formation of new neurons but through the establishment of robust synaptic connections between them (Zlotnik and Vansintjan, 2019). Nootropics, also known as "smart drugs," are compounds that enhance cognitive functions such as memory, learning, and reasoning, especially in individuals with impaired mental performance due to various causes (Malík and Tlustoš, 2022). These substances work through multiple mechanisms, including modulation of cholinergic and dopaminergic pathways, to improve brain function. They have also been studied for their potential therapeutic effects on memory-related disorders such as Huntington's disease, Alzheimer's disease (AD), and Parkinson's disease (Suliman et al., 2016).

Mushrooms, which are macrofungi, have been cultivated and consumed for centuries due to their sensory appeal, rich nutritional profile, functional properties, and relatively easy cultivation requirements (Feeney et al., 2014). Among the various medicinal mushrooms, *Lentinula edodes* (commonly known as shiitake) is considered one of the most important and is the second most widely cultivated mushroom globally. In China, it is known as *Xianggu*, while in Japan, it is called *Shiitake*. It belongs to the Basidiomycota division of fungi (Wasser, 2005). Recent studies have demonstrated the medicinal potential of *L. edodes*, including its activity against hypertension, cancer, diabetes, hypercholesterolemia, influenza, cardiovascular disease, obesity, respiratory ailments, fatigue, sexual dysfunction, aging, allergies, and liver disorders (Bernal-Mercado et al., 2023; Bisen et al., 2010; Ponnusamy et al., 2022). Its longstanding use in traditional Asian medicine and cuisine is attributed to its health benefits and rich flavor (Mizuno, 1995).

Figure 1: *Lentinula edodes.*

Among the various medicinal mushrooms, *Lentinula edodes* (commonly known as shiitake) is considered one of the most important and is the second most widely cultivated mushroom globally. In China, it is known as *Xianggu*, while in Japan, it is called *Shiitake (Xiang et al., 2021)*. The term "shiitake" comes from “shii,” the type of tree on which it grows (the chinquapin tree), and “-take,” meaning mushroom in Japanese. A premium variant of shiitake is also referred to as *Donko*. The species name *edodes* is Latin for “edible” (Bisen et al., 2010). In this study nootropic activity of *Lentinula edodes* mushroom was evaluated in mice using various animal models.

MATERIALS AND METHODS

Drugs and chemicals

Lentinula edodes mushroom, in powdered form was purchased from gluckspilze Austria while physostigmine, scopolamine, and Ellman kit was purchased from Sigma Aldrich, USA.

Animals

In this study male Swiss albino mice (22-25 g) were used. Five groups were made and each group contained 6 mice**.** Animals were kept in a controlled environment using polypropylene cages. The animals were procured from Karachi University**.** The temperature of the area was maintained at approximately 25-30 °C. Animals were given unrestricted availability of water and food. Animals were deprived of food and water for a time of two hours earlier to and following the administration of the drug. Prior to the start of study, mice were acclimatized to the environment of research room for 5 days.

Animal handling

For the handling of animals the recommendations of 8th edition of National Institute of Health (NIH) were followed (Health, 1985).

Dose selection

The doses were selected according to literature survey as under, scopolamine 0.4 mg/kg for inducing memory loss, physostigmine 0.1 mg/kg as standard drug. *L. edodes* extract was given in two doses that were 250 mg/kg, and 500 mg/kg (Choi et al., 2016).

Elevated plus maze

The Elevated Plus Maze (EPM) test was used to evaluate learning and memory in experimental animals by measuring Initial Transfer Latency (ITL) and Retention Transfer Latency (RTL) EPM consist of a plus-like equipment that stands 50 cm above the surface of the ground. It is

composed of two wide-open arms (45 cm × 10 cm), two closed arms (45 cm × 10 cm × 30 cm; length × width × height) and an intermediate platform (10 cm × 10 cm). One parameter of each mouse was checked and recorded on first day of trial which is the initial transfer latency (ITL). The time occupied by a mouse to switch from one of the open arms to any one of the two closed arms with the help of all of its limbs was known as initial transfer latency. Within the duration of 90 seconds if mouse does not move towards any of the closed arms then with the help of hand the mouse was gently led to any one of the two closed arms and the time given to ITL was 90 seconds. Before placing of mice to their cages each mouse was given 120 seconds to move freely within the maze and explore the environment [19]. The same procedure was repeated after 24 hours in order to evaluate Retention Transfer Latency (RTL), on identical criteria and cut-off time. The maze was clean with 70 % ethanol after each trials to remove olfactory signals (Gari and Varshney, 2020).

Table 1: Animal Grouping and administration of drug

Group 1	Vehicle control group	Distilled water 10 mL/kg, *p.o*.
Group 2	Negative control group	scopolamine 0.4 mg/kg, *i.p*.
Group 3	Positive control group	physostigmine 0.1 mg/kg, *i.p*. +scopolamine 0.4 mg/kg, *i.p*.
Group 4	LEE 250 group	LEE 250 mg/kg, *p.o*. +scopolamine 0.4 mg/kg, *i.p*.
Group 5	LEE 500 group	LEE 500 mg/kg, *p.o*. +scopolamine 0.4 mg/kg, *i.p*.

Morris water maze

In rodents an effective model for measuring task learning and remembrance is the Morris water maze test. It consists of a rounded tank which is 25 cm long and 60 cm wide which is full of water and held at a temperature of about 25 degrees Celsius. A nonpoisonous white colored dye was used to create the water cloudy. The tank was separated into four quadrants that were made on the tank by crossing two threads perpendicularly in the center and tightly connecting them to the top of the tank, the quadrants are West, East, South, and North.

A platform (6 x 6 cm) was mounted in the tank's target quadrant (Q4). To make the platform invisible kept the level of water one cm above it. Each mouse was permitted to explore the underwater platform and remain on it for 20 seconds. During the experiment, each mouse was placed in every quadrant (except Q4) without difficulty. The mouse was positioned in such a manner that its face was towards the tank's wall. After the occurrence of each trial, altered the position of each mouse and for the searching of underwater platform each mouse was given the time interval which is 120 seconds. During the learning session, the platform was left untouched and the area remained unchanged. If within the time allocated i.e. 120 seconds the mouse was failed to reach the platform, then the mouse was smoothly moved with the help of hand in the direction of the platform and allow 20 seconds to each mouse to stay on the platform. From 6^{th} to 9^{th} day after receiving the drug every animal's escape latency (EL) was measured. Each mouse was subjected to continuous four days of evaluation. For the search of underwater platform the period of time occupied by the mouse to travel from the initial quadrant to the targeted quadrant is EL. The following pattern was followed for four successive trial days of training.

1st Day: Q1, Q2, Q3 and Q4.
2nd Day: Q2, Q3 Q4 and Q1.
3rd Day: Q3, Q4, Q1 and Q2.
4th Day: Q4, Q1, Q2 and Q3.

For quadrant representation "Q "used

TSTQ (time spent in target quadrant) was measured after the Escape latency period and evaluation. After the removal of platform from the tank, each mouse was given 300 seconds to swim freely. Individual quadrant's average time spent was documented. The average time the mouse was taken to discover the removed platform in the target quadrant (Q4) was also noted. Memory retrieval was regarded the sign of the discovery of the platform. Throughout the preparation and testing days, the observer was remained in the same position (Khatian and Aslam, 2019).

Open field test

The test was carried out in a square arena of 50 x 50 x 40 cm with a marked grid floor of around 16 squares. Each mouse was placed at the center of the arena individually and allowed to explore the arena for the period of 10 minutes. The parameter related to the behavior such as locomotor activity (number of squares crossed) were recorded along with the time spent in each box. The total no of crossings (ambulation) revealed the locomotor activity of each mouse (Seibenhener and Wooten, 2015).

Biochemical estimation of acetylcholinesterase in the brain of mice

For biochemical estimation of acetylcholinesterase, animals

were dissected after the completion of behavioral study. The entire brain was removed carefully from skull and put into a homogenizer comprising of 10 mL normal saline solution and homogenized it by using an ice bath. The centrifugations machine was used to centrifuge the homogenate for 10 minutes at 3000 rpm. Cloudy supernatant liquid was obtained after centrifugation which is used for the assessment of level of acetylcholinesterase in the brain (Sujith et al., 2012). The Ellman method was used to determine acetylcholine level in brain. Spectrophotometric analysis was done and measured the density of yellow colored compound in spectrophotomer at the wavelength of 412nm every minute (Ellman et al., 1961).

Statistical analysis

Data were expressed as ± Standard error of mean (SEM) with confidence intervals (CI) of 95%. The data are interpreted by using one-way ANOVA following Tukey's post hoc test. A probability level of 0.05 or less is accepted as significant.

RESULTS

Initial Transfer Latency (ITL) and Retention Transfer Latency (RTL) in EPM

In Elevated plus maze, significant increase in initial transfer latency was observed in the negative control group as compared to control group. However, animals in positive control group and treated group i.e. *Lentinula edodes* extract at doses of 250 mg/kg and 500 mg/kg showed significant decrease in initial transfer latency when compared with negative control (Scopolamine). It was also noticed that the highest dose of *Lentinula edodes* showed more significant decrease in initial transfer latency (Figure 2a).

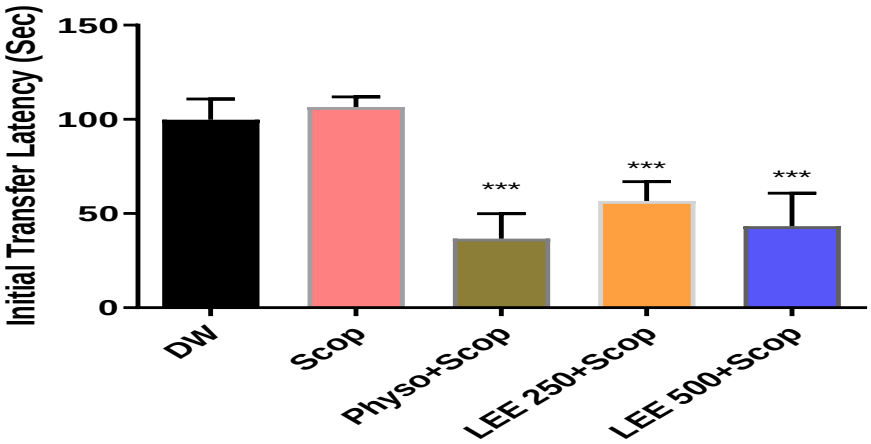

Figure 2a: Effect of *Lentinula edodes* on Initial Transfer Latency (ITL) of mice in EPM.
Animals in each group (n) are = 6; The values in mean ± SEM (One-way ANOVA followed by Tukey's Post hoc test). ap < 0.05, bp < 0.01, cp < 0.001 when contrasted with only scopolamine treated group. *p < 0.05, **p < 0.01, ***p < 0.0001 when compared with DW group. DW: Distilled water, LEE 250: Lentinula edodes extract 250 mg/kg, LEE 500: Lentinula edodes extract 500 mg/kg, Scop.: Scopolamine, Physo.: Physostigmine.

The retention transfer latency was significantly increase in negative control group as compared to control group. However, groups with the co-administration of scopolamine such as *Lentinula edodes* extract at 250 mg/kg and 500 mg/kg doses and with physostigmine significant decrease in retention transfer latency when compared with negative control group (Scopolamine) (Figure 2b).

Morris Water Maze

Effect of *Lentinula edodes* in Morris water maze on Time Spent in Target Quadrant (TSTQ)

In MWM, it was observed that TSTQ decreased significantly in scopolamine treated group of mice. Whereas, animals in the *Lentinula edodes* extract at 250 mg/kg and 500 mg/kg and positive control group along with co-administration of scopolamine showed significant increase in TSTQ when compared with negative control group (Figure 3).

From the sixth to ninth day after the drug administration, the Escape Latency (EL) of the various groups was also evaluated. The escape latency of negative control group was significantly increased when contrasted with control group on the sixth day of drug administration. During the study from sixth to ninth day of drug administration, it was observed that

escape latency of animals in treatement group i.e. *Lentinula edodes* extract at 250 mg/kg and 500 mg/kg doses co-administered with scopolamine significantly decreased when compared with the negative control group (Table 1).

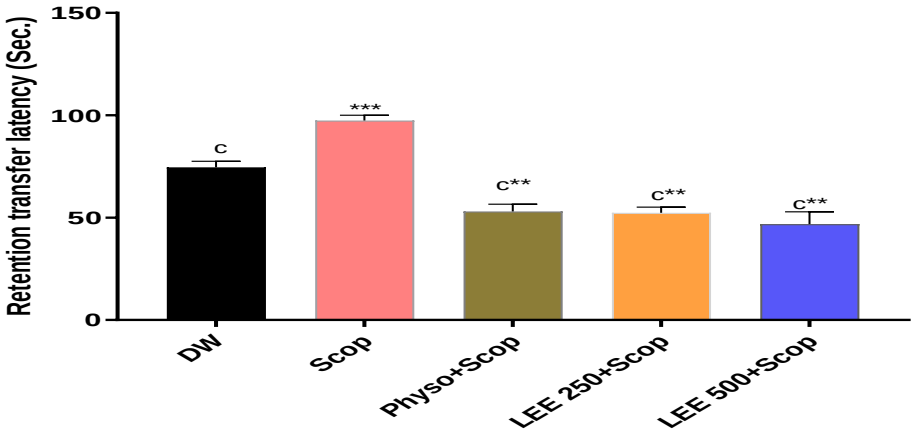

Figure 2b: Effect of *Lentinula edodes* on Retention Transfer Latency (RTL) of mice in EPM.
Animals in each group (n) are = 6; The values in mean ± SEM (One-way ANOVA followed by Tukey's Post hoc test). ap < 0.05, bp < 0.01, cp < 0.001 when contrasted with only scopolamine treated group. *p < 0.05, **p < 0.01, ***p < 0.0001 when compared with DW group. DW: Distilled water, LEE 250: Lentinula edodes extract 250 mg/kg, LEE 500: Lentinula edodes extract 500 mg/kg, Scop.: Scopolamine, Physo.: Physostigmine.

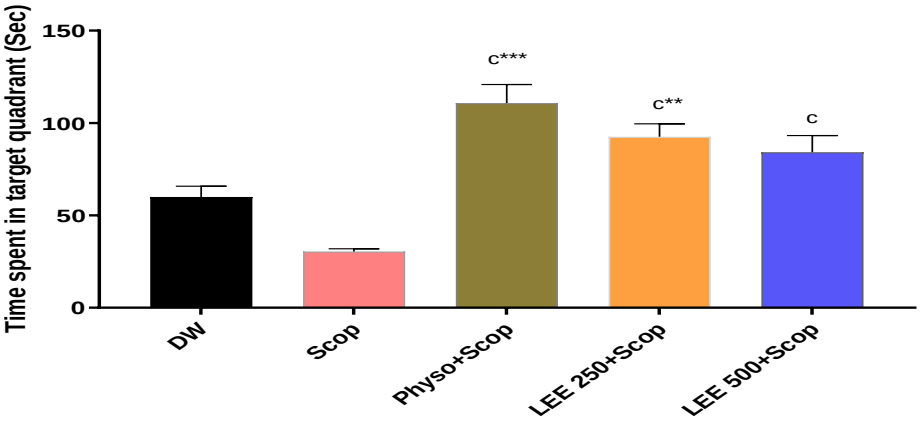

Figure 3: Effect of *Lentinula edodes* on time spent in target quadrant (TSTQ) on the 10th day of the study in MWM.
Animals in each group (n) are = 6; The values in mean ± SEM (One-way ANOVA followed by Tukey's *Post hoc* test). ap < 0.05, bp < 0.01, cp < 0.001 when contrasted with only scopolamine treated group. *p < 0.05, **p < 0.01, ***p < 0.0001 when contrasted with DW group. DW: Distilled water, LEE 250: *Lentinula edodes* extract 250 mg/kg, LEE 500: *Lentinula edodes* extract 500 mg/kg, Scop.: Scopolamine , Physo.: Physostigmine

Table 2: Effect of Lentinula edodes on Escape Latency (EL) in Morris Water Maze.

Group	Dose	EL (Sec) Day 6	EL (Sec) Day 7	EL (Sec) Day 8	EL (Sec) Day 9
Control	10 ml/ Kg	29.4 ± 2.3	14.5 ± 1.3	13.3 ± 1.2	12.5 ± 0.8
Scop.	0.4 mg/ Kg	70.6± 2.5***	71.6± 3.4***	69.6± 4.4***	67.6± 4.8***
Physo + Scop.	0.1 mg/Kg + 0.4 mg/Kg	27.4 ± 1.9	12.8 ± 0.9	11.8 ± 0.9^{c}	11.0 ± 0.8^{c}
LEE 250 + Scop	250 mg/Kg + 0.4 mg/Kg	29.4 ± 2.7	26.4 ± 1.8	12.8 ± 0.8	13.6 ± 0.8^{c}
LEE 500 + Scop.	500 mg/Kg + 0.4 mg/Kg	27 ± 3.5	23.2 ± 2.0	10.8± 0.5^{c**}	10.0± 0.3^{c**}

Animals in each group (n) are = 6; The values in mean ± SEM (One-way ANOVA followed by Tukey's *Post hoc* test). ap < 0.05, bp < 0.01, cp < 0.001 when contrasted with only scopolamine treated group. *p < 0.05, **p < 0.01, ***p < 0.0001 when contrasted with DW group. DW: Distilled water, LEE 250: *Lentinula edodes* extract 250 mg/kg, LEE 500: *Lentinula edodes* extract 500 mg/kg, Scop.: Scopolamine , Physo.: Physostigmine

Locomotor activity using open field test

In open field, no significant change was observed in all the groups (Figure 4).

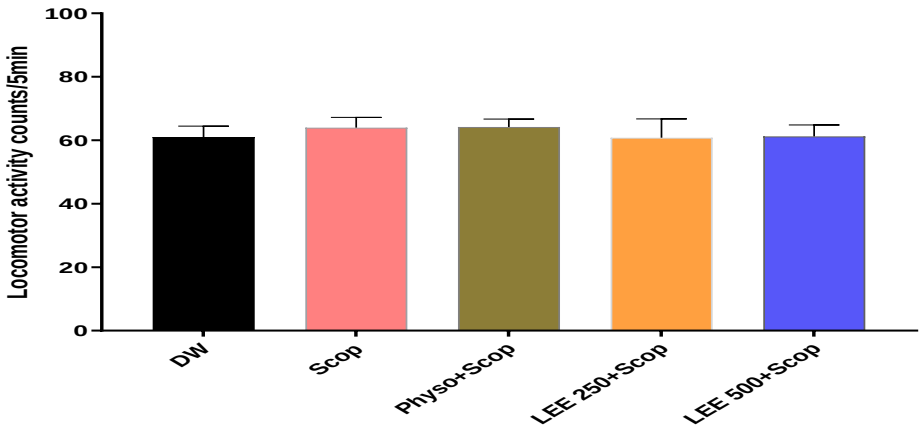

Figure 4: Effect of *Lentinula edodes* on locomotor activity of mice.

Animals in each group (n) are = 6; The values in mean ± SEM (One-way ANOVA followed by Tukey's *Post hoc* test). ap < 0.05, bp < 0.01, cp < 0.001 when contrasted with only scopolamine treated group. *p < 0.05, **p < 0.01, ***p < 0.0001 when contrasted with DW group. DW: Distilled water, LEE 250: *Lentinula edodes* extract 250 mg/kg, LEE 500: *Lentinula edodes* extract 500 mg/kg, Scop.: Scopolamine, Physo.: Physostigmine

Biochemical estimation of acetylcholinesterase in the brain of mice

It was observed that the activity of acetylcholinesterase in brain of mice was significantly increased in negative control group when compared with control group. Whereas the group co-administered with scopolamine in *Lentinula edodes* extract at both doses, and physostigmine showed significant decrease in the activity of acetylcholinesterase in brain of mice when compared with negative control (Figure 5).

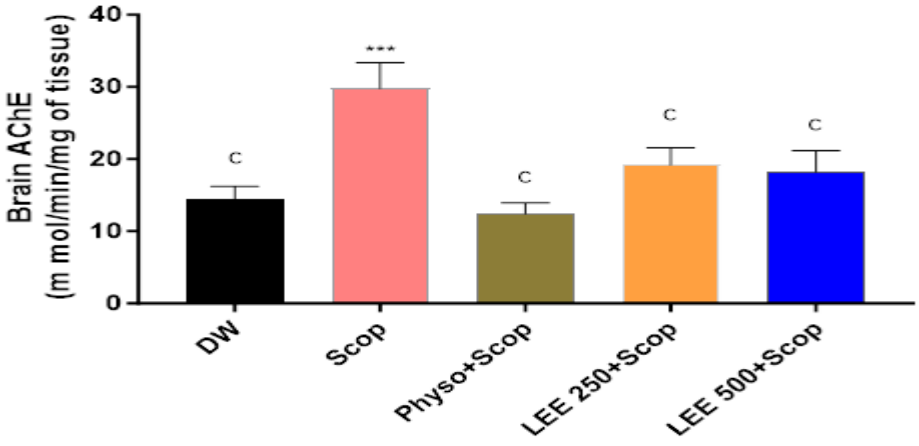

Figure 5: Effect of *Lentinula edodes* on brain acetylcholinesterase in mice.

Animals in each group (n) are = 6; The values in mean ± SEM (One-way ANOVA followed by Tukey's *Post hoc* test). ap <

0.05, bp < 0.01, cp < 0.001 when contrasted with only scopolamine treated group. *p < 0.05, **p < 0.01, ***p < 0.0001 when contrasted with DW group. DW: Distilled water, LEE 250: *Lentinula edodes* extract 250 mg/kg, LEE 500: *Lentinula edodes* extract 500 mg/kg, Scop.: Scopolamine, Physo.: Physostigmine

DISCUSSION

The present study aimed to evaluate the cognitive-enhancing effects of *Lentinula edodes* (L. edodes), commonly known as shiitake mushroom, in a murine model of scopolamine-induced memory impairment. Two doses of L. edodes (250 mg/kg and 500 mg/kg) were administered, with scopolamine serving as a negative control and physostigmine as a positive control. Behavioral assessments included the Elevated Plus Maze (EPM), Morris Water Maze (MWM), and Open Field Test (OFT), while biochemical analysis of acetylcholinesterase (AChE) activity was performed using the Ellman method.

Administration of *Lentinula edodes* at both doses resulted in significant improvements in cognitive functions, as evidenced by reduced initial and retention latencies in the EPM and decreased escape latency in the MWM (Figure 2a, 2b and 3). These findings align with previous studies demonstrating the neuroprotective effects of *Lentinula edodes*. For instance, β-glucan derived from *Lentinula edodes* has been shown to prevent cognitive impairments in high-fat diet-induced obese mice, suggesting its potential in mitigating neuroinflammation and cognitive decline (Pan et al., 2021).

Scopolamine administration induced significant cognitive deficits, as expected, while physostigmine treatment improved cognitive performance, confirming its role as a positive control (Ebert et al., 1998). Notably, co-administration of scopolamine with Lentinula edodes did not exacerbate cognitive impairments, suggesting that *Lentinula edodes* may possess protective properties against scopolamine-induced neurotoxicity. This observation is supported by research indicating that *Lentinula edodes* can modulate the gut-brain axis, thereby influencing cognitive functions (Remya et al., 2019). The 500 mg/kg dose of *Lentinula edodes* exhibited superior efficacy compared to the 250 mg/kg dose, indicating a dose-dependent response. This is consistent with the concept that higher doses of bioactive compounds may exert more pronounced therapeutic effects. However, it is essential to consider the risk of potential toxicity at higher doses, necessitating further studies to determine the optimal therapeutic window.

Biochemical assays revealed a significant reduction in AChE activity in the *Lentinula edodes* -treated groups, particularly at the 500 mg/kg dose (Figure 5). This reduction in AChE activity suggests an increase in acetylcholine levels, which is associated with improved cognitive functions. Similar findings have been reported in studies investigating the AChE-inhibitory effects of various natural compounds, highlighting the relevance of cholinergic modulation in cognitive enhancement (Zaitoun et al.).

The cognitive-enhancing effects of *Lentinula edodes* may be attributed to its bioactive compounds, such as β-glucan, which have been shown to modulate neuroinflammation and enhance synaptic plasticity (Tong et al., 2023). Additionally, the high glutamic acid content in *Lentinula edodes* may contribute to its neuroprotective effects, as glutamic acid plays a crucial role in neurotransmitter stimulation and excitation (Yu et al., 2023). Furthermore, the modulation of the gut-brain axis by *Lentinula edodes* may influence cognitive functions, as alterations in gut microbiota have been linked to neuroinflammation and cognitive decline (Alsegiani and Shah, 2022).

CONCLUSION

In conclusion, the findings of this study suggest that *Lentinula edodes* possesses cognitive-enhancing properties, with the 500 mg/kg dose demonstrating the most pronounced effects. These effects may be mediated through cholinergic modulation and the modulation of the gut-brain axis.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

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