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Brain & Heart

ORIGINAL RESEARCH ARTICLE

L-theanine as a multifunctional neuroprotective and cardiomodulatory agent: Hydroxyl radical scavenging, neurotransmitter modulation, and attenuation of stress-induced cardiac responses

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Abstract

Oxidative stress, resulting from excessive free radical production, plays a pivotal role in the pathogenesis of neurodegenerative and cardiovascular disorders. *L*-theanine, a unique non-protein amino acid present in tea (*Camellia sinensis*), is recognized for its antioxidant, neuroprotective, and anxiolytic properties; however, its direct hydroxyl radical scavenging activity and effects on neurochemical pathways and cardiovascular responses remain inadequately understood. The present study evaluated the *in vitro* hydroxyl radical (•OH) scavenging capacity of *L*-theanine and investigated its effects on dopaminergic and cholinergic neurotransmission, as well as stress-induced cardiac chronotropic responses in Swiss albino mice. Hydroxyl radicals were generated using a ferrous–ascorbate–EDTA system in the presence of *L*-theanine or reference antioxidants (catalase, melatonin, and glutathione), and the hydroxyl radical adducts, 2,3- and 2,5-dihydroxybenzoic acid, were quantified using high-performance liquid chromatography. Mice received intraperitoneal *L*-theanine (5 mg/kg) twice daily for seven days. Striatal dopamine levels and hippocampal acetylcholinesterase activity were subsequently determined, while heart rate was measured non-invasively using a MouseSTAT® Jr. pulse oximeter under acute stress conditions. *L*-theanine significantly inhibited hydroxyl radical formation, with greater scavenging activity than glutathione at 0.075 mM. The treatment also significantly increased striatal dopamine levels and reduced hippocampal acetylcholinesterase activity, indicating enhanced dopaminergic neurotransmission and modulation of cholinergic signaling. In addition, *L*-theanine attenuated the stress-induced increase in heart rate, demonstrating a negative chronotropic effect. These findings demonstrate that *L*-theanine possesses potent antioxidant, neuroprotective, and cardiomodulatory properties, supporting its potential as a multifunctional therapeutic candidate for oxidative stress-associated neurodegenerative and cardiovascular disorders.

Keywords: *L*-theanine; Antioxidant; Dopamine; Acetylcholinesterase; Neuroprotection; Tea; Heartbeat

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Citation: Deb S. *L*-theanine as a multifunctional neuroprotective and cardiomodulatory agent: Hydroxyl radical scavenging, neurotransmitter modulation, and attenuation of stress-induced cardiac responses. *Brain Heart*. doi: 10.36922/BH026280039

Received: July 10, 2026

Revised: August 28, 2026

Accepted: October 8, 2026

Published online: October 22, 2026

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1. Introduction

Tea, produced from the leaves of *Camellia sinensis*, is widely consumed and contains several bioactive compounds with potential physiological and neuropsychological effects.[1] Among these, *L*-theanine, a unique non-proteinogenic amino acid, has attracted considerable interest for its anxiolytic, stress-relieving, and neuromodulatory properties.[2,3] Unlike caffeine, *L*-theanine is associated with a state of relaxed alertness and has been reported to modulate neurotransmitter activity and brain functions.[4]

Neurodegenerative disorders such as Parkinson's and Alzheimer's diseases involve oxidative stress, mitochondrial dysfunction, neuronal loss, and neurotransmitter imbalance.[5] Although previous studies have reported antioxidant and neuroprotective effects of *L*-theanine, its direct hydroxyl radical-scavenging capacity and its effects on neurotransmitter- and enzyme-mediated mechanisms remain insufficiently characterized.[6,7] In particular, the effects of *L*-theanine on striatal dopamine and hippocampal acetylcholinesterase activity warrant further investigation, given their relevance to motor function, cognition, and memory.

Beyond its neuroprotective effects, *L*-theanine has also been implicated in the regulation of physiological responses to stress.[3,8,9] Acute stress activates sympathetic pathways and increases heart rate, while *L*-theanine may attenuate stress-related autonomic responses.[8] However, its effects on stress-induced cardiac chronotropic responses under experimentally induced acute stress remain comparatively unexplored. Examining this response may provide additional insight into the potential cardiomodulatory effects of *L*-theanine.

Therefore, the present study investigated the antioxidant and neuromodulatory effects of *L*-theanine using complementary *in vitro* and *in vivo* approaches. Specifically, its hydroxyl radical-scavenging activity, its effects on striatal dopamine levels and hippocampal acetylcholinesterase activity, and its ability to attenuate stress-induced increases in heart rate were evaluated. Collectively, these investigations aim to provide a more integrated assessment of the antioxidant, neuroprotective, and cardiomodulatory potential of *L*-theanine.

2. Materials and methods

2.1. Examination of the *in vitro* antioxidant activity of *L*-theanine

An *in vitro* hydroxyl radical–generating system was established using phosphate buffer (50 mM, pH 7.2), ethylenediaminetetraacetic acid (EDTA; 240 mM), ascorbate (1 mM), and $FeSO_4$ (200 mM), with minor modifications from the method of Slivkas and Cohen.[10] Hydroxyl radical formation was assessed through the salicylic acid trapping method, wherein radicals react with salicylic acid to yield 2,3-dihydroxybenzoic acid (DHBA) and 2,5-DHBA, serving as reliable markers.[11] The reaction mixture was divided into five aliquots; one served as a blank without salicylic acid, while the others were incubated for 30 min. Following incubation, salicylic acid at 0.75 mM concentration was added to each aliquot, and the samples were further incubated for 5 min. The radical-scavenging potential of *L*-theanine (extrapure, 99%; 44826, Sisco Research Laboratories, India) was evaluated at concentrations of 0.05, 0.075, 0.1, 0.25, and 0.50 mM and compared against equivalent concentrations of known antioxidants, including glutathione, melatonin, and catalase. Reactions were terminated by dilution with chilled 0.1 M perchloric acid (1:9 v/v), and 10 µL of each sample was subjected to high-performance liquid chromatography (HPLC; Waters 2465 Electrochemical Detector, Waters Corporation, United States of America [USA]) for quantification of hydroxyl radical adducts. The mobile phase consisted of heptane sulfonic acid (8.65 mM), EDTA (0.27 mM), acetonitrile (13%), triethylamine (0.43%), and phosphoric acid (0.32%) and was delivered at a flow rate of 0.7 mL/min. Hydroxyl radical scavenging activity was thus determined based on the relative levels of 2,3-DHBA and 2,5-DHBA formed in the presence and absence of *L*-theanine and reference antioxidants.

2.2. Analysis of cardiovascular responses and dopaminergic and cholinergic activities in the brain

A total of 24 Male Swiss albino mice (eight weeks old, 28–32g) were obtained from the Pasteur Institute, Shillong, Meghalaya, India. Animals were housed in polypropylene cages under standard laboratory conditions (25 ± 2°C, 60 ± 5% humidity) with free access to food and water. All experimental procedures were approved by the Institutional Animal Ethics Committee (Approval No. AUS/IAEC/SD-R(a)/2023-1/021) and conducted in accordance with the Committee for Control and Supervision of Experiments on Animals (CPCSEA) guidelines.

Regarding experimental design, Swiss albino mice were divided into two groups of 12 animals each: Control and Treated. They were randomly assigned using a random number generation method. The number of test animals per group was selected based on established practice in comparable preclinical animal studies, balancing statistical reliability with the ethical principle of reducing animal use. The control group received 0.9% saline, while the treated group was administered *L*-theanine intraperitoneally at 5 mg/kg twice daily at fixed intervals for seven consecutive days. On the 8th day, 12 hours after the

final dose, six animals from each group were decapitated for the estimation of dopamine levels. The remaining six animals in each group were subjected to assessment of stress-induced cardiovascular responses. Following completion of the cardiovascular assessment, the animals were sacrificed, and their brains were perfused with 4% paraformaldehyde for subsequent histoenzymological analysis.[12] Hippocampal sections were then prepared using a cryostat (Model 0620E; Thermo Shandon, United Kingdom) and processed for histoenzymological analysis. Biochemical measurements and histological quantification were performed by an investigator who was blinded to the treatment allocation.

2.2.1. Assessment of stress-induced heart rate

To evaluate the effect of *L*-theanine on cardiovascular responses to acute stress, heart rate was measured non-invasively using a MouseSTAT® Jr. Pulse Oximeter (Kent Scientific Corporation, USA).[13] Following seven consecutive days of treatment with either saline or *L*-theanine (5 mg/kg, i.p., twice daily), mice were acclimatized to the recording environment before measurements were performed. Baseline heart rate was first recorded in conscious animals under resting conditions using a paw sensor attached according to the manufacturer's instructions.

Acute stress was subsequently induced by gently restraining each animal in a well-ventilated restrainer for 5 min, following which heart rate was immediately recorded at each minute for a total period of 10 min using the same pulse oximetry system.[14] Measurements were obtained under identical environmental conditions to minimize experimental variability. Heart rate values were expressed as beats per minute (bpm), and the stress-induced chronotropic response was determined by comparing post-stress heart rate with the corresponding resting values. The effect of *L*-theanine treatment on stress-induced cardiac responses was evaluated by comparing heart rate measurements between the control and treated groups.

2.2.2. Assessment of dopamine levels in the striatal region

To evaluate the effect of *L*-theanine on brain biogenic amines and their metabolites, mice were sacrificed through decapitation on the final day of treatment. Following rapid brain extraction, the striatum was dissected within 2 min. Tissues were weighed, sonicated in ice-cold 0.1 M perchloric acid, and centrifuged at 18,000 × *g* for 5 min. The resulting supernatant was injected into the HPLC system at a flow rate of 0.7 mL/min. Calibration curves were generated using known concentrations of standards prepared in perchloric acid, and dopamine levels were quantified in treated samples by comparing with these curves. Electrochemical detection was performed at +740 mV.[15,16]

2.2.3. Acetylcholinesterase histoenzymology in the hippocampus

Acetylcholinesterase activity was assessed by histoenzymology following the method of Banerjee *et al.*[17] Frozen hippocampal sections of 20 µm thickness were prepared using a cryostat, air-dried, rinsed in phosphate buffer solution, and incubated in a green reaction mixture (20 mL) containing 1 mL of 0.1 M sodium citrate, 2 mL of 30 mM copper sulfate, 2 mL of 5 mM potassium ferricyanide, 2 mL distilled water, and acetylthiocholine iodide (11 mg in 13 mL 0.1 M potassium phosphate buffer, pH 6.0), added sequentially with stirring. During the reaction, liberated thiocholine combined with ferricyanide to yield ferrocyanide, which further reacted with copper ions to produce an insoluble brown precipitate. Sections were incubated at 37°C for 45 min, rinsed with distilled water, dipped in histological slide DPX mounting medium, and subsequently examined and photographed under a microscope (Eclipse Ci-L, Nikon, Japan). The mean optical densities of the images were compared using ImageJ software (National Institutes of Health, USA).

2.3. Statistical analysis

Data are presented as mean ± SEM (n = 6 animals/samples per group). A p-value of ≤ 0.05 was considered statistically significant. For experiments involving comparisons among more than two independent groups, such as the hydroxyl radical scavenging assay, one-way ANOVA followed by Tukey's multiple-comparisons test was used. Comparisons between two independent groups, as in the analysis of striatal dopamine levels and hippocampal acetylcholinesterase activity/mean optical density, were performed using an unpaired Student's t-test. Statistical analyses were performed using GraphPad Prism version 10.02.0 for Windows (GraphPad Software, USA).

3. Results

3.1. Comparison of the *in vitro* free radical scavenging activity of *L*-theanine with potent antioxidants

The hydroxyl radical (•OH) scavenging potential of *L*-theanine was systematically examined across a concentration range of 0.05 mM, 0.075 mM, 0.1 mM, 0.25 mM, and 0.50 mM, and its activity was compared with that of well-established antioxidants including glutathione, catalase, and melatonin at equivalent concentrations. Hydroxyl radicals were generated *in vitro* using the

ferrous–ascorbate–EDTA system, and their inhibition in the presence or absence of the test compounds was evaluated. Quantification was carried out through a highly sensitive HPLC method, in which the hydroxyl radicals were trapped by salicylic acid to yield the stable adducts 2,3-DHBA and 2,5-DHBA. The concentrations of these adducts directly reflected the amount of hydroxyl radicals produced in the system and allowed precise estimation of radical scavenging activity.

The results demonstrated a significant decline in the concentrations of both 2,3-DHBA and 2,5-DHBA when the reaction mixtures were incubated with *L*-theanine across the tested concentrations. Specifically, at 0.075 mM and 0.50 mM, *L*-theanine significantly suppressed the formation of 2,5-DHBA compared with the control. In parallel, reductions in 2,3-DHBA were observed at 0.075 mM, 0.1 mM, and 0.50 mM, further underscoring the potent free radical–neutralizing capacity of *L*-theanine (Figure 1). Notably, at the concentration of 0.075mM, *L*-theanine exhibited a superior hydroxyl radical scavenging effect, as evidenced by greater DHBA reduction compared with glutathione, a well-established endogenous antioxidant. These observations demonstrate that *L*-theanine exhibited significant antioxidant activity compared with the untreated control and, under certain conditions, showed activity comparable to or greater than that of glutathione.

3.2. Effect of *L*-theanine on stress-induced heart rate

The effect of *L*-theanine on cardiovascular responses to acute stress was evaluated by measuring heart rate before and following stress exposure using a non-invasive pulse oximetry system. Under resting conditions, heart rate did not differ significantly between the control and *L*-theanine–treated groups (Figure 2A). Exposure to acute stress produced a marked increase in heart rate in both groups; however, the magnitude of this increase was significantly attenuated in mice treated with *L*-theanine. Compared with the control group, where the highest heart rate recorded was 723 bpm and the lowest was 621 bpm, *L*-theanine–treated animals exhibited a significantly lower post-stress heart rate, with a highest of 623 bpm and a lowest of 543 bpm, indicating a reduction in the stress-induced chronotropic response (Figure 2B). These findings suggest that *L*-theanine mitigates stress-induced cardiac activation and exerts a negative chronotropic effect, consistent with its reported stress-relieving and autonomic modulatory properties.

3.3. Effect of *L*-theanine on striatal dopamine level in the mouse brain

Monitoring dopamine levels is a crucial parameter in assessing neuroprotection, particularly in the context of neurodegenerative disorders such as Parkinson's disease.[18] Dopamine is the primary neurotransmitter depleted in the nigrostriatal pathway due to progressive degeneration of dopaminergic neurons. A decline in its concentration directly correlates with the onset and severity of motor impairments, including tremors, rigidity, and bradykinesia.[19] Therefore, quantifying striatal dopamine and its metabolites provides a reliable biochemical index of neuronal integrity and functional preservation.

In the present study, the effect of intraperitoneal *L*-theanine administration on striatal dopamine levels was evaluated. Treatment with *L*-theanine at 5 mg/kg significantly increased dopamine levels in the striatum. The control group had an average dopamine level of 58 pmol/mg of striatal tissue, whereas the treated group showed a significant increase, with an average value of 77 pmol/mg of tissue. Hence, an overall increase of 32.75% was observed following 5 mg/kg of *L*-theanine administration (Figure 3C). Although the change was not large, a significant difference was observed between the control and test groups. In the chromatogram, the dopamine peak appeared at approximately 12.5 min, with the peak maximum occurring at approximately 13 min (Figure 3A,B). The retention time was determined by comparison with the standard curve.

3.4. Neurochemical regulation of acetylcholinesterase by *L*-theanine

Acetylcholinesterase is essential for maintaining cognitive functions.[20,21] It is responsible for hydrolyzing the neurotransmitter acetylcholine in the synaptic cleft, a process vital for key cognitive activities such as learning, memory, and attention.[22] Its activity is precisely controlled to sustain appropriate acetylcholine levels in the brain, as any imbalance may contribute to cognitive decline and dementia during the advanced stages of Parkinson's disease.[23] In the current study, the level of acetylcholinesterase was assessed, and the *L*-theanine–treated group showed a significant decrease in the mean optical density of acetylcholinesterase in the hippocampal region by 31.32% (Figure 4). The control group showed an average mean optical density of 8.3, while the *L*-theanine–treated group showed a mean of 5.7. This suggests that *L*-theanine may help preserve acetylcholine levels in the brain and improve cognitive function.

4. Discussion

L-theanine, a non-protein amino acid naturally present in tea, has been investigated for its anxiolytic, stress-modulating, and neuroprotective properties.[24-26] Oxidative stress and mitochondrial dysfunction are important

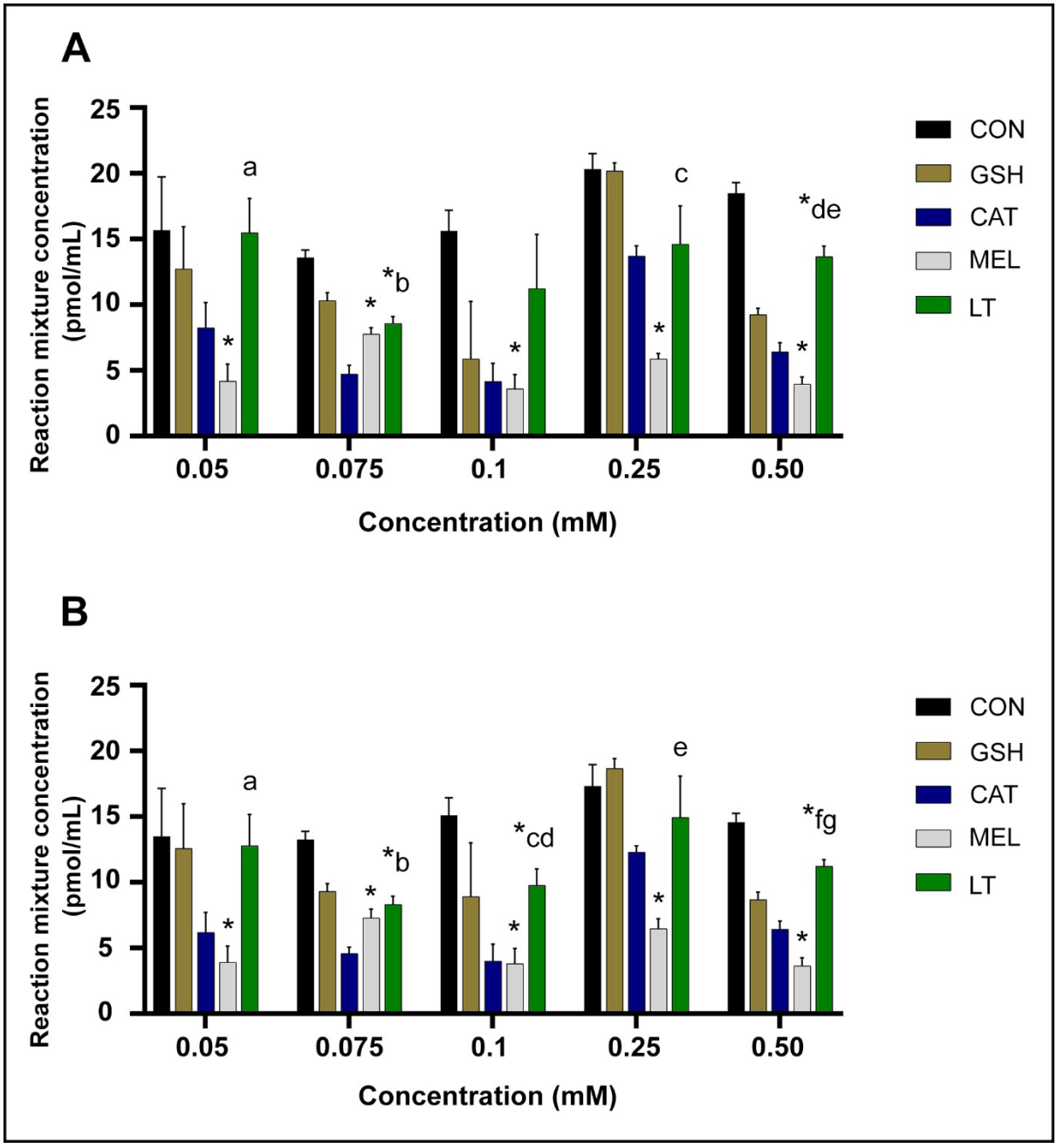


Figure 1. Hydroxyl radical scavenging activity of *L*-theanine. At 0.075 mM, *L*-theanine (LT) demonstrated a stronger reduction in (A) 2,3-dihydroxybenzoic acid (DHBA) and (B) 2,5-DHBA formation compared with glutathione in the ferrous–ascorbate–ethylenediaminetetraacetic acid (EDTA) system. Hydroxyl radical–scavenging activity of *L*-theanine was evaluated using an *in vitro* $FeSO_4$–ascorbate–EDTA hydroxyl radical–generating system, with 2,3-DHBA and 2,5-DHBA formation quantified through high-performance liquid chromatography (HPLC) using the salicylic acid trapping method. Notes: Data are expressed as mean ± SEM. $n = 6$. *$p < 0.05$ indicates a significant difference from the control. For 2,5-DHBA (A), different letters indicate significant differences between GSH and LT at 0.05 mM (a), 0.075 mM (b), 0.25 mM (c), and 0.50 mM (d), and between CAT and LT at 0.50 mM (e). For 2,3-DHBA (B), different letters indicate significant differences between GSH and LT at 0.05 mM (a), 0.075 mM (b), 0.1 mM (c), 0.25 mM (e), and 0.50 mM (f), and between CAT and LT at 0.1 mM (d) and 0.50 mM (g). Statistical analysis was performed using one-way ANOVA.
Abbreviations: CAT: Catalase; CON: Control; GSH: Glutathione; MEL: Melatonin.

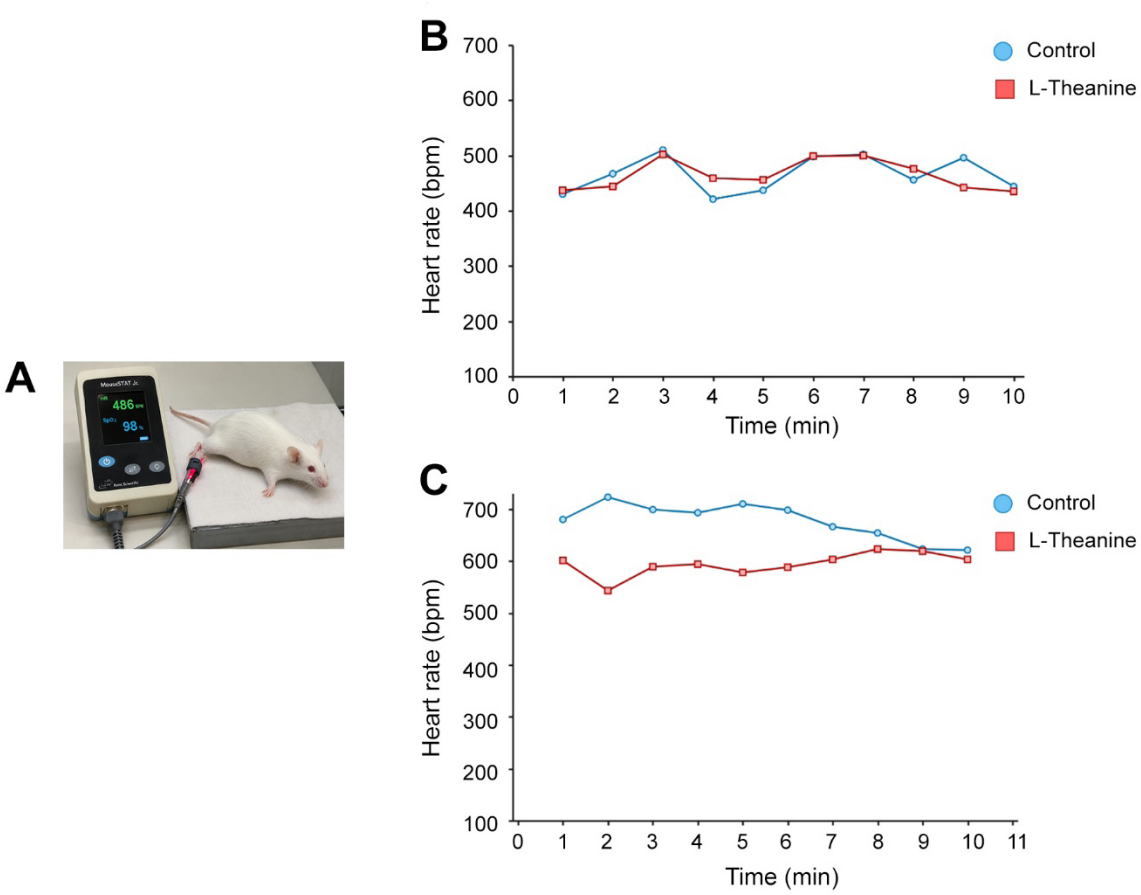


Figure 2. Effect of *L*-theanine on heart rate under resting and acute stress conditions. (A) Heart rate was measured non-invasively using a pulse oximetry system under resting conditions and following acute stress exposure to assess the effect of *L*-theanine on stress-induced cardiovascular responses. (B) Resting heart rate (bpm) measured in conscious, unstressed Swiss albino mice. (C) Heart rate (bpm) recorded during 10 min of restraint-induced stress. Acute stress increased heart rate in both groups, but the increase was significantly lower in *L*-theanine–treated mice.

contributors to neuronal damage in neurodegenerative disorders.[27,28] Although previous studies have primarily associated the antioxidant effects of *L*-theanine with modulation of endogenous antioxidant systems, its direct free-radical-scavenging activity remains less characterized.[29] In the present *in vitro* study, *L*-theanine reduced the formation of both 2,3-DHBA and 2,5-DHBA, indicating hydroxyl radical–scavenging activity. The effects were particularly evident at 0.075 and 0.50 mM for 2,5-DHBA and at 0.075, 0.10, and 0.50 mM for 2,3-DHBA. Notably, at 0.075 mM, *L*-theanine showed greater suppression of 2,5-DHBA formation than glutathione under the conditions tested. These findings support a direct antioxidant effect of *L*-theanine *in vitro*, although the assay conditions do not establish whether a comparable effect occurs *in vivo*.

The present study also demonstrated that *L*-theanine attenuated the increase in heart rate induced by acute restraint stress, while basal heart rate remained unchanged. This finding is consistent with the study of Kimura *et al.*[8], which reported reduced heart-rate responses to an acute stress task following *L*-theanine administration. However, the cardiovascular effects of *L*-theanine have not been uniformly demonstrated. A randomized, double-blind pilot study in healthy men reported no significant differences in heart rate, blood pressure, or heart-rate variability following consumption of an *L*-theanine-containing functional food compared with placebo.[30] Such differences may reflect variations in dose, formulation, duration of exposure, the experimental stressor, and study population. Therefore, the present findings suggest that *L*-theanine can attenuate the cardiac response to acute stress under the experimental conditions used, but they do not establish a generalized cardiovascular or cardioprotective effect.

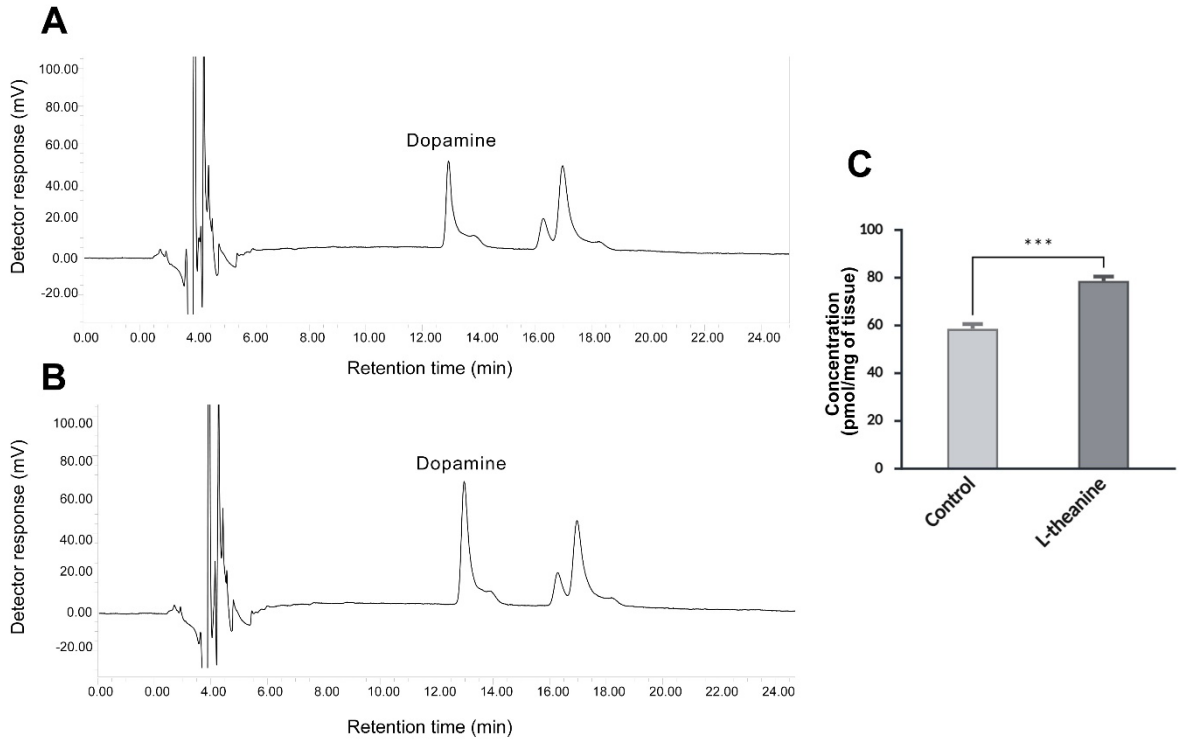


Figure 3. *L*-theanine increases striatal dopamine levels by 32.75% in test animals. (A–B) Representative chromatograms of (A) control and (B) *L*-theanine–administered mice. (C) Graphical representation of dopamine levels (pmol/mg) in the striatum. Notes: Data are expressed as mean ± SEM. ***$p \leq 0.001$ compared with control ($n = 6$). Striatal dopamine levels were quantified using high-performance liquid chromatography (HPLC) following intraperitoneal administration of *L*-theanine, with dopamine concentrations determined by comparing sample retention times and peak areas with those of a standard. Statistical analyses were performed using an unpaired Student's *t*-test.

To further assess its neuromodulatory effects, striatal dopamine levels following *L*-theanine administration were evaluated. Treatment with 5 mg/kg *L*-theanine increased striatal dopamine by 32.75% compared with the control group. Since dopamine depletion in the nigrostriatal pathway is a characteristic feature of Parkinsonian pathology, this finding suggests that *L*-theanine may influence dopaminergic neurotransmission.[31] Previous neurochemical studies have also suggested that *L*-theanine can modulate dopaminergic and other neurotransmitter systems.[30] However, such effects may depend on dose, treatment duration, brain region, and experimental model. Importantly, the present study measured dopamine concentration but did not directly assess dopaminergic neuronal survival, dopamine synthesis, or receptor activity. Thus, the observed increase in dopamine should not be interpreted as direct evidence of preservation of dopaminergic neurons.

In the current study, *L*-theanine treatment also significantly reduced acetylcholinesterase levels in the hippocampus. Because acetylcholinesterase regulates synaptic acetylcholine availability, this finding indicates a potential influence of *L*-theanine on cholinergic neurotransmission. This is in line with a previous study in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced model of Parkinson's disease, which reported that, particularly at 100 mg/kg, *L*-theanine attenuated MPTP-induced motor deficits and several non-motor manifestations, including anxiety, depression, and cognitive-related behavioral alterations, suggesting that its neuroprotective effects may extend beyond dopaminergic motor pathways.[32] A combination of *L*-theanine and spearmint extract has also been shown to increase the level of acetylcholine in the brain.[33] Flavanols in tea were also found to display an acetylcholinesterase inhibition effect.[34]

Overall, the present findings indicate that *L*-theanine possesses hydroxyl radical–scavenging activity *in vitro* and can modulate stress-induced heart-rate responses, striatal dopamine levels, and hippocampal acetylcholinesterase in mice. The findings are broadly consistent with previous reports of stress-modulating and neuromodulatory effects of *L*-theanine, while differences among studies highlight

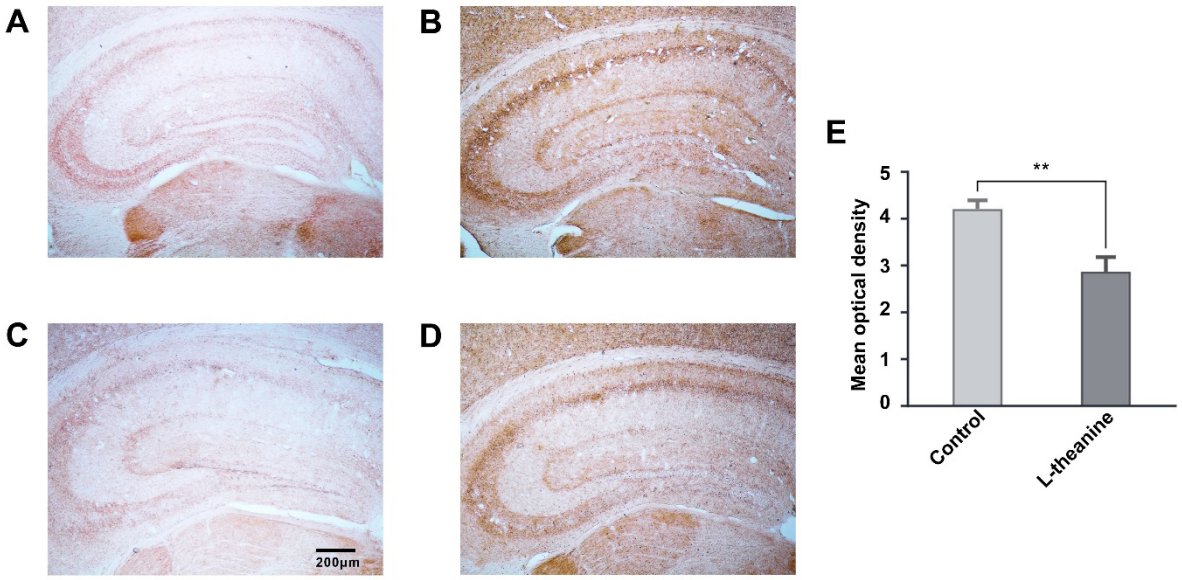


Figure 4. Histoenzymology of acetylcholinesterase reveals that the enzyme is lower in L-theanine–treated test animals than in the control group. (A, C) Hippocampus sections of control animals. (B, D) Hippocampus sections of treated animals. (E) Mean optical density in the hippocampus. Notes: Scale bars: 200 μm; magnification: 4×. Data are expressed as mean ± SEM. $^{**}p < 0.01$ compared with control ($n = 6$). Acetylcholinesterase activity in the hippocampal region was assessed histochemically, and the mean optical density of the stained tissue was quantified to evaluate the effect of *L*-theanine treatment. Statistical analyses were performed using an unpaired Student's *t*-test.

the influence of experimental conditions and biological context. Further studies using complementary measures of neuronal survival, neurotransmitter metabolism, autonomic function, and cognitive performance are necessary to clarify the mechanisms underlying these effects.

Although these findings are promising, the study has certain limitations. The results are derived from experimental models, and their translation to clinical settings requires careful validation through well-designed, long-term human studies. In addition, the present experiments were conducted exclusively in male mice. Future studies should therefore include both sexes to assess potential sex-related differences in the effects of *L*-theanine. Further investigations should explore a broader dose–response relationship and different treatment durations to determine the optimal conditions for its potential neuroprotective effects. Studies examining the interactions between *L*-theanine and other bioactive constituents of tea, as well as its efficacy in clinically relevant models of neurodegenerative and cardiovascular disorders, are also needed.

Comprehensive investigations into its bioavailability, pharmacokinetics, long-term safety, and efficacy are necessary before drawing conclusions regarding its potential therapeutic applications. Such studies may help establish the biological relevance and translational potential of *L*-theanine in conditions associated with oxidative stress and related neurological or cardiovascular dysfunctions.

5. Conclusion

The present study demonstrates that *L*-theanine exhibits multiple biological effects beyond its established anxiolytic properties. Under the experimental conditions used, *L*-theanine showed hydroxyl radical–scavenging activity, increased striatal dopamine levels, reduced hippocampal acetylcholinesterase activity, and attenuated stress-induced increases in heart rate. These findings suggest antioxidant, neuromodulatory, and cardiomodulatory potential in *in vitro* conditions and mouse models examined. However, further studies using appropriate disease models and clinical investigations are required to determine the underlying mechanisms and translational relevance of these findings.

Acknowledgments

The author sincerely appreciate Mr. Deep Choudhury for his invaluable assistance in refining the figures of this manuscript. His meticulous revisions have significantly enhanced the clarity and presentation of the figures.

Funding

The author gratefully acknowledges the financial support provided by the Department of Science and Technology (DST), Government of India, through the INSPIRE Fellowship (DST/INSPIRE Fellowship/2017/IF170756).

Conflict of interest

The author hereby declares that there are no competing interests in publishing this work.

Author contributions

This is a single-authored article.

Ethical approval and consent to participate

The Animal Ethics Committee at Assam University in Silchar, Assam, India, to which Patharkandi College is affiliated, approved the experimental techniques employed in the current work (Sanction No. AUS/IAEC/SD-R(a)/2023-1/021).

Consent for publication

Not applicable.

Availability of data

All data generated during the experimentation are included in the manuscript. Extended findings of this study are available from the corresponding author upon reasonable request.

Further disclosure

During the preparation of this manuscript, ChatGPT was used solely to improve the language, grammar, clarity, and readability of the manuscript. The AI tool was not used to generate scientific content, research findings, interpretations, or references. All scientific content, data, analyses, interpretations, and conclusions were developed and verified by the author, who takes full responsibility for the final content of the manuscript.

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