L-Theanine Attenuates LPS Induced Motor deficit in Experimental Rat Model of Parkinson's Disease: Emphasis on Mitochondrial activity, Neuroinflammation and Neurotransmitters

Shivam Kumar
Indo Soviet Friendship College of Pharmacy
Anupam Awasthi
Indo Soviet Friendship College of Pharmacy
Khadga Raj
Indo Soviet Friendship College of Pharmacy
Shamsher Singh (shamshersinghbajwa@gmail.com)
ISF College of Pharmacy https://orcid.org/0000-0002-2780-6632

Research Article

Keywords: Parkinson's disease, Lipopolysaccharide, L-theanine, Neurotransmitters, NF-κB

Posted Date: September 14th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-867919/v1

Abstract

Parkinson’s disease (PD) is a neurodegenerative disorder characterized by the loss of dopamine neurons in the substantia nigra. The pathogenesis of PD, including oxidative stress, mitochondrial dysfunction, neuroinflammation, and neurotransmitter dysregulation. L-theanine is an amino acid found in green tea and has antioxidant, anti-inflammatory, and neuroprotective effects with a high BBB permeability. Therefore, the current study was designed to investigate the possible neuroprotective effect of L-theanine in lipopolysaccharide (LPS) induced motor deficits and striatal neurotoxicity in a rat model of PD. LPS was infused at a dose of 5 µg/5 µl PBS, stereotaxically into SNpc of rats. Treatment with L-theanine at (50 and 100 mg/kg; po), and Sinemet (36 mg/kg; po) was given from day 7 to 21 in of LPS injected rat. On a weekly basis all behavioral parameters were assessed, and animals were sacrificed on day 22. The striatum tissue of brain was isolated for biochemicals (Nitrite, GSH, catalase, SOD, mitochondrial complexes I and IV), neuroinflammatory markers (IL-1β, TNF-α, and IL-6), and neurotransmitters (serotonin, dopamine, norepinephrine, GABA, and glutamate) estimations. Results revealed that L-theanine dose-dependently and significantly reversed motor deficits, assessed through locomotor and rotarod activity. Moreover, L-theanine attenuated biochemical markers, reduced oxidative stress, and neurotransmitters dysbalance in the brain. L-theanine treatment at 100 mg/kg; po substantially reduced these pathogenic events by increasing mitochondrial activity, restoring neurotransmitter levels, and inhibiting neuroinflammation. Moreover, data suggest that the positive effects of L-theanine on motor coordination may be mediated by the suppression of nuclear factor-κB (NF-κB) induced by LPS.

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the death of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and a substantial decrease in dopamine levels in the striatum (MS Lima et al. 2012). The progressive loss of dopaminergic neurons in the SNpc due to excessive production of free radicals, mitochondrial energy failure, neuroinflammation, and neurotransmitters dysbalance are the main pathogenic characteristics of PD (Kaur et al. 2019). Clinically, PD presents as a motor abnormality such as bradykinesia, stiffness, postural instability, rest tremor, and non-motor symptoms such as olfactory deficiencies, sleep disruption, and psychosis due to autonomic dysfunction (Khan et al. 2019). The nuclear factor-kappa light chain enhancer of B cells (NF-κB) is a transcription factor that controls the production of pro-inflammatory cytokines, Nrf2-Keap1, and the NF-κB inflammatory cascade in neurological disease pathogenesis. The NF-κB has been found to control the production of pro-inflammatory cytokines, Nrf2-Keap1, and the NF-κB inflammatory cascade in the pathophysiology of neurological disorders (Saha et al. 2020).

Lipopolysaccharide (LPS) is an endotoxin that is the major component of gram-negative bacteria and made up of a hydrophobic lipid, a hydrophilic polysaccharide carbohydrate chain and a repetitive hydrophilic O-antigen oligosaccharide side chain. Currently, LPS is the animal model used for studying the relationship between neuroinflammation and PD. LPS is also a potent activator of microglial cells and leads to neuroinflammatory cascades in patients with PD (Ebbensgaard et al. 2018). The LPS infusion

into the SNpc causes microglial activation, leading to the generation of free radical species, like reactive oxygen species (ROS), and propagates the release of pro-inflammatory cytokines (Deng rt al. 2020). LPS treatment has decreased motor activity, reduce dopaminergic neurons in the SNpc, and striatal parts of the brain. Furthermore, LPS generates a hazardous environment for healthy neuronal populations by causing a self-sustaining loop of microglial activation that leads to neuroinflammation and ROS-driven toxicity in the brain (Sharma et al. 2015). Furthermore, preclinical and clinical studies have demonstrated that anti-inflammatory and antioxidant agents may protect dopaminergic neurons against LPS-induced neurotoxicity in a rat model of Parkinsonism (Haslund-Vinding et al. 2017; Esposito et al. 2007).

L-theanine is an active non-protein part chemically known as γ-glutamylethylamide found in green tea. It has antioxidant, anti-inflammatory properties and improves behavioral abnormalities with motor symptoms by regulating catecholamines (dopamine, serotonin) in the brain. Regular L-theanine consumption protects dopaminergic and GABAergic neurons and controls dopamine release in the striatum (Deb et al. 2019). In addition, L-theanine also increases the level of brain-derived neurotrophic factor (BDNF), and nerve growth factor (NGF) indicate, useful for the treatment of PD (Chen et al. 2018). As a result, L-theanine has significant neuroprotective properties, suggesting that it may be a helpful neuroprotective agent (Nathan et al. 2006). Hence, this current research work mainly focused on the neuroprotective effect of L-theanine in LPS induced PD-like symptoms in rats through analysis of behavioral parameters, biochemicals, inflammatory cytokines, neurotransmitters estimation, mitochondrial complexes activity, and NF-κB analysis.

2. Materials And Methods

2.1 Experimental Animals

In this study, forty-one male Wistar rats (180–220 g) were kept under standard laboratory conditions. The standard diet used to feed animals according to Institutional Animal Ethics Committee (IAEC) guidelines. The IAEC approved the experimental protocol with Registration number ISFCP/IAEC/CPCSEA/2019/429.

2.2 Chemicals

LPS purchased from Sigma Aldrich chemicals private limited, Plot #12, Bommasandra-Jigani Link Road, Bengaluru-560100 India. L- theanine was purchased from TCI Chemicals Pvt. Ltd., India. LPS s dissolved in phosphate buffer saline. The test drug used in the present study, L-theanine dissolved in double-distilled water.

2.3 Surgical procedures for the infusion of LPS into the SNpc

The unilateral lesion of the nigrostriatal region was performed to inject LPS into the right substantia nigra. Rats were anesthetized before surgery with ketamine (80 mg/kg; ip) and xylene (5 mg/kg; ip) and placed on a stereotactic frame with nose and ear bars specially adapted for a rat. LPS dissolved in PBS

at the concentration of 1 mg/1 ml. The injection needle was lowered via a drill hole 5.5 mm posterior, 1.5 mm lateral, and 8.3 mm ventral to the bregma. A single injection of 5 µl volume from a stock solution of 1 mg/1 ml of LPS was given for 2 minutes using a Hamilton syringe, and the needle was kept *in situ* for an additional 2 minutes after each operation to prevent reflux along the injection tract.

2.4 Experimental procedure

The rats were divided into 6 groups. Group 1 marked as normal control. Group 2 is considered as sham control. Group 3 received LPS (5µg/5µl). Group 4 & 5 received L-theanine (50 & 100 mg/kg; po) for starting from day 7 of LPS (5µg/5µl) injection to day 21, Group 6 received a Sinemet combination of Levodopa and Carbidopa (36 mg/kg; po) starting from the 7th day of LPS (5µg/5µl) injection. Behavioral parameters recorded on a weekly basis. On day 22, Rats were sacrificed, and the striatum part of the brain was isolated to estimate biochemicals, neuroinflammatory markers, and neurotransmitters.

2.5 Parameters

2.5.1 Assessment of Behavior Parameters

Narrow Beam Walk Test

The narrow beam walk test allows an animal to walk across a narrow beam made up of wooden. This test was used to determine the gait abnormalities and the number of foot slips of experimental rats (Southwell et al. 2009). The apparatus consists of a narrow horizontal beam (1 cm in width, 130 cm in length, and 0.5 mm in thickness), and beam was elevated at 100 cm from the floor. All rats were trained with a narrow beam for 5 days before performing experiments. The latency and their foot slip to cross the beam were recorded in each trial.

Rota-rod Test

The motor coordination and paw grip performance of all animals were evaluated using a Rota-rod apparatus. The Rota-rod apparatus consists of a rod with a diameter of 75 mm and a height of 40 cm divided into four sections (Sharma et al. 2016). Before starting the protocol, all rats were trained for five days to habituate to Rota-rod performance. On an experimental day, rats were placed on the rotating rod at a speed of 25 revolutions per minute (rpm) to assess motor coordination. The average fall-off time was considered as 180 sec. and the average fall-off time was recorded.

Grip Strength Test

Grip strength of forelimbs of all rats was measured using the grip strength apparatus. Rats were trained for five days on grip strength apparatus before experimenting. The grip strength is expressed as a kilogram-force (Kgf) (Singh et al. 2017)

Open-Field Test

The spontaneous locomotor activity of experimental rats was determined using an open field apparatus (Singh et al. 2017). This apparatus is rectangular and made of wood that measures 100 × 100 × 40 cm^3. After animals have become habituated, a single exposure is sufficient to cause movement across the squares from the animal's habituation. The number of grooming and reading sessions was counted in less than five minutes.

2.5.2 Measurement of Biochemical Parameters

Dissection and Homogenization

On day 22, rats were sacrificed by cervical dislocation, and their brains were isolated and used to estimate biochemical, neuroinflammatory, and neurotransmitters. The brains were placed on dry ice to isolate the striatum from the brain during the experimental day. Then, brain tissue samples were homogenized with ice-cold 0.1 M phosphate buffer at pH 7.4 for 10 times (w/v), and the procedure was followed according to the weight of tissue. The striatal tissue of rat was homogenated and centrifuged for 15 min at 10,000 rpm in a refrigerated centrifuge. The oxidative stress, pro-inflammatory markers, and neurotransmitters were measured in this homogenized striatal tissue solution.

Estimation of Lipid Peroxidation (LPO) level

Wills method was used to quantitative measurement of LPO in the striatum (Wills et al. 1971). The procedure consists of 0.5 ml homogenate and 0.5 ml of Tris HCl pipette out in a test tube, incubated at 37°C for 2 hours. The addition of 1 ml of 10% trichloroacetic acid (TCA) was added after 2 hours of incubation and centrifugation at 10,000 rpm for 10 minutes, and the supernatant was collected. 1 ml supernatant was added to 1 ml 0.067 percent thiobarbituric acid, and tubes were placed in boiling water for 10 minutes and then the samples were cooled at room temperature. The amount of LPO was determined using a Shimadzu UV-spectrophotometer at a wavelength of 532 nm.

Estimation of Nitrite level

The homogenate tissue was used to measure nitrite level by using the Griess reagent and earlier reported method by Green et al. 1982 was followed (Green et al. 1982). Equal volumes of Griess reagent and supernatant were incubated in the dark for 10 minutes. The tissue sample was examined at the 540 nm wavelength using a UV-spectrophotometer to determine the nitrite level. The nitrite concentration is determined by comparing it to a sodium nitrite standard curve, and the results are given as a percentage.

Estimation of reduced GSH level

The amount of reduced glutathione (GSH) in the hippocampus was determined using the Ellman method (Ellman et al. 1959). Following the reaction, the yellow colour was formed, and the intensity of the tested sample was determined using a UV-spectrophotometer at 412 nm. The results were calculated, and values were expressed as a percentage.

Estimation of catalase level

The decomposition of H_2O_2 determined catalase activity in striatum homogenates at 240 nm following an earlier published method (Aebi et al. 1984). Homogenate's tissue was added to 1 ml reaction solutions containing 0.1 percent Triton X-100, 15 mM H2O2, and 50 mM potassium phosphate at a pH of 7.4. The units of enzyme activity were calculated using the extinction coefficient of H_2O_2, which is 0.0394 mM-1cm-1. One unit of catalase activity is defined as 1 mmol H_2O_2 consumed/ min/mg of protein.

Estimation of SOD activity

Total SOD activity was determined in brain homogenates using the SOD-inhibitable ferricytochrome c reduction (MacCord et al. 1969). Homogenates of the brain were suspended in 1 ml reaction solutions containing 0.03 percent sodium deoxycholate, 10 mM ferricytochrome c, 50 mM xanthine, 100 mM EDTA, and 50 mM potassium phosphate at a pH of 7.4 with 0.03 percent sodium deoxycholate, 10 mM ferricytochrome c, 50 mM xanthine, 100 mM EDTA. The reaction was initiated using xanthine oxidase, and the absorbance was determined at 550 nm using a UV-1700, Shimadzu. 50% inhibition of cytochrome c reduction is defined as 1 unit of enzyme activity.

Estimation of total protein level

The protein level in the homogenate of striatal tissue was determined using the Lowry technique and the Folin phenol reagent (Lowry et al. 1951).

Mitochondrial Complex-I Activity (NADH Dehydrogenase)

A homogenized brain sample was used to evaluate NADH Dehydrogenase activity. The rate of NADH oxidation was determined using a UV-spectrophotometer at 340 nm (Yu et al. 1980).

Mitochondrial Complex-IV Activity (Cytochrome Oxidase)

The cytochrome oxidase activity was determined in striatum according to the method of Sottocasa, 1967 (Sottocasa et al. 1967).

2.5.3 Estimation of Pro-inflammatory Cytokines

The estimation of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) of striatal was performed using ELISA kits.

2.5.4 Neurotransmitters Estimation

Estimation of Catecholamines (norepinephrine, dopamine, serotonin)

Estimation of striatal catecholamines was performed by high-performance liquid chromatography (HPLC), containing an electrochemical detector (ECD) (Patel et al. 2005).

Estimation of GABA and Glutamate

The previously described technique of derivatizing amino acids in striatal homogenate solution with o-phthalaldehyde/mercaptoethanol (OPA/-ME) was used to quantify amino acids. The concentration of GABA and glutamate in striatal tissue was performed according to the method reported by Lasley and Gilbert (Lasley et al. 2002).

2.5.5 Immunohistochemistry Analysis

Immunohistochemistry was conducted on 5-micron-thick striatum tissue sections that had been post-fixed with 5% paraformaldehyde, as reported by Yildirim et al. The tissue sections were dewaxed twice with xylene for 15 minutes each time, rehydrated with decreasing concentrations of absolute alcohol (100%, 70%, and 50%), and then rinsed with distilled water for 2 minutes. To retrieve antigens, tissue sections were soaked in PBS (pH 7.4) for 5 minutes, and peroxidase activity was stopped by incubation at room temperature for 5 minutes with 3 % hydrogen peroxide. The tissue sections were incubated for 1 hour at room temperature with antibodies against NF-κB and Nrf2 and then rinsed with Tris buffer solution for 10 minutes. Sections were treated for 30 minutes at room temperature with Poly-Horse radish peroxidase (Poly-HRP) and then rinsed twice with PBS for 5 minutes each time and incubated with DAB reagent for 2 minutes. Following DAB reaction, sections were stained with hematoxylin for 3 minutes and then rinsed with double distilled water for 20 minutes. After drying at room temperature, the sections were mounted with DPX and imaged at a magnification of 100X using a fluorescence microscope (Li et al. 2018).

3. Statistical Analysis

All data are presented as mean ± SD and analyzed via GraphPad Prism 5.0 software for Windows. A value of $p < 0.05$ was considered statistically significant.

3. Results

LPS treated rats showed a significant decrease in motor coordination (rotarod activity), narrow beam walking, grip strength, and locomotor (open field) activity at the end of day 21 as compared to the sham and normal control ($p < 0.001$). L-theanine treated rats at the dose of (50 and 100 mg/kg; po) from day 7 to 21 had significantly attenuated the motor coordination, narrow beam walking, grip strength, locomotor activity compared to the LPS treated rats ($p < 0.01$). However, administration of L-theanine (100 mg/kg) had significantly improved compared to motor coordination as compare with the L-theanine (50 mg/kg) ($p < 0.01$). Additionally, the sinemet (levodopa and carbidopa) treated group had shown significant improvement in motor coordination, grip strength, narrow beam walking, locomotor activity compared to LPS, and high dose of L-theanine treated groups ($p < 0.05$). **(**Fig. 2,3,4,5**).**

The level of IL-1β, TNF-α, and IL- 6 were increased in LPS treated rats compared to sham and normal control rats ($p < 0.001$). Consequently, L-theanine treated rats at a dose of 50, 100 mg/kg; po had significantly reduced the neuroinflammatory markers in the striatum compared to LPS treated rats ($p <$

0.05). Moreover, the group treated with a high dose of L- theanine (100 mg/kg) had significantly ameliorated the neuroinflammatory markers in the striatum as compared to L-theanine (50 mg/kg) ($p < 0.01$). Additionally, the standard drug-treated group had shown a significant reduction in the level of neuroinflammatory compared to LPS and high dose of L-theanine treated groups ($p < 0.01$). (Fig. 6)

In addition, as compared to the sham and normal control groups, LPS-treated rats exhibited a substantial reduction in catecholamine (dopamine, serotonin), GABA, and an increase in norepinephrine and glutamate levels in the striatum ($p < 0.001$). However, both low and high doses of L-theanine treated groups had significantly attenuated the reduction in dopamine, serotonin, and GABA levels and decreased norepinephrine and glutamate levels compared to the LPS treated group ($p < 0.01$). Additionally, administration of L-theanine (100 mg/kg) had significantly restored neurotransmitters levels (dopamine, serotonin, GABA) compared to L-theanine (50 mg/kg) ($p < 0.01$). Moreover, standard drug (Sinemet) had shown a significant increase in the level of dopamine, serotonin, GABA and a decrease in the level of norepinephrine and glutamate in the striatum as compared to LPS and a high dose of L-theanine ($p < 0.01$). (Fig. 7, 8)

The level of LPO, nitrite was significantly increased by reducing GSH, catalase, SOD, and mitochondrial complex I and IV activities in LPS treated striatum compared to sham and normal control group ($p < 0.001$). In addition, as compared to the LPS-treated group, treatment with L-theanine at low (50 mg/kg; po) and high dosage (100 mg/kg; po) reduced LPO and nitrite levels, enhanced GSH, catalase, and SOD levels, and restored mitochondrial complex I and IV activities ($p < 0.01$). Moreover, administration of L-theanine (100 mg/kg; po) had decreased the level of LPO, nitrite level and increased the level of GSH, catalase, SOD and restored mitochondrial complex activities as compared to L-theanine (50 mg/kg) ($p < 0.01$). Rats treated with sinemet had shown significantly decrease the level of LPO, nitrite level and increase the level of GSH, Catalase, SOD and improvement in mitochondrial complex I and IV activities as compared to LPS and L-theanine (100 mg/kg) treated rats ($p < 0.05$). (Table 1,2)

Table 1

Effect of L-theanine on LPO, Nitrite, GSH, Catalase, and SOD estimation in LPS treated rats. Data was expressed as Mean ± S.D. [a]p < 0.001 vs Normal control and Sham control, p < 0.01 vs LPS, [c]p<0.01 vs L-theanine (50 mg/kg), [d]p<0.05 vs L-theanine (100 mg/kg). Statistical analysis performed by one-way ANOVA followed by turkey's post hoc test.

Experimental Groups	LPO (nmol/mg pr)	Nitrite (µg/ml)	GSH (µmol/mg pr)	Catalase (U/ mg pr)	SOD (U/ mg pr)
Normal control	1.9 ± 0.4	120.1 ± 7.8	0.142 ± 0.001	4.38 ± 0.36	5.32 ± 0.45
Sham control	1.8 ± 0.3	120.6 ± 7.6	0.140 ± 0.002	4.30 ± 0.39	5.29 ± 0.34
LPS 5µg/5µl	6.2 ± 0.4 [a]	261.7 ± 10.5 [a]	0.025 ± 0.006 [a]	2. 54 ± 0.16[a]	1.45 ± 0.39 [a]
LPS + L-theanine (50 mg/kg)	5.1 ± 0.7 [b]	191 ± 8.9 [b]	0.053 ± 0.005 [b]	2.95 ± 0.18[b]	2.99 ± 0.46 [b]
LPS + L-theanine (100 mg/kg)	4.3 ± 0.9[bc]	158.1 ± 7.7[bc]	0.077 ± 0.008[bc]	3.5 ± 0.25[bc]	3..91 ± 0.38 [bc]
LPS + Sinemet (36 mg/kg)	2.9 ± 0.7[bd]	131.4 ± 8.5[bd]	0.107 ± 0.003[bd]	4.15 ± 0.21[bd]	4.85 ± 0.35 [bd]

Table 2

Effect of L- theanine on mitochondrial complex I and IV in LPS treated rats. Data was expressed as Mean ± S.D. [a]p < 0.001 vs Normal control and Sham control, p < 0.01 vs LPS, [c]p<0.05 vs L-theanine (50 mg/kg), [d]p<0.05 vs L-theanine (100 mg/kg). Statistical analysis performed by one-way ANOVA followed by turkey's post hoc test.

Experimental Grouping	Mitochondrial Complex	
	Complex I (nM/mg protien)	Complex IV (nM/mg protien)
Normal control	12.16 ± 0.12	318.6 ± 12.20
Sham control	11.9 ± 0.13	316.5 ± 7.31
LPS 5µg/5µl	4.01 ± 0.16 [a]	149.6 ± 8.73 [a]
LPS + L- theanine (50 mg/kg)	6.1 ± 0.19[b]	191.0 ± 8.9 [b]
LPS + L- theanine (100 mg/kg)	8.9 ± 0.23[bc]	221.1 ± 6.7[bc]
LPS + Sinemet (36 mg/kg)	11.2 ± 0.21 [bd]	246.4 ± 5.9[bd]

Further, the expression of NF-kB significantly increased after LPS injected in rat striatum compared to sham and normal control group ($p < 0.001$). Rats treated with L- theanine at a dose of 50 mg/kg and 100 mg/kg significantly reduced NF-κB expression in the striatum compared to LPS treated group ($p < 0.01$). Furthermore, rats treated with 100 mg/kg L- theanine significantly attenuated the NF-κB expression compared to 50 mg/kg L-theanine ($p < 0.001$). Moreover, the standard drug-treated group showed decreased considerably NF-kB expression in the striatum than a high dose of L-theanine treated group ($p < 0.01$). (Fig. 9)

4. Discussion

PD is a progressive neurodegenerative movement disorder caused by a number of factors like oxidative stress, neurotransmitters dysbalance, mitochondrial dysfunction, and neuroinflammation. However, current evidence are contrastingly challenging previous opinions and facts towards an active role of oxidative stress, neurotransmitter dysbalance, and neuroinflammation in the progression of motor disturbances, leading to PD. Therefore, to explore these interactions, pure neuroinflammation is driven (LPS induced) animal model of PD was used in the present study (Farooqui et al. 2017). In this study, we have demonstrated that the administration of L-theanine was able to protect dopaminergic neurons against LPS induced neuroinflammatory cytokine release, neurotransmitters imbalance, and mitochondrial dysfunction in the rat brain through analysis of behavioral parameters, biochemical estimation (GSH, LPO, nitrite, catalase, and SOD), neuroinflammatory markers (IL-1β, TNF-α, and IL-6), and neurotransmitters (serotonin, dopamine, noradrenaline, GABA and glutamate) analysis by using HPLC-ECD.

The outcomes of the study revealed the protective effect of L-theanine against LPS induced PD-like symptoms in experimental rats. In order to overcome this, LPS at a dose of 5ug/5ul in PBS was infused stereotaxically into SNpc of rats. This results in an increase in pro-inflammatory cytokine activation, oxidative stress, and neurotransmitter dysregulation. LPS-induced behavioral and motor coordination deficits in rats are similarly likely to be observed in people with Parkinsonism, as indicated by tremor, bradykinesia, and stiffness. (Stigger et al. 2013). Intranigral unilateral infusion of LPS causes the generation of ROS and OH that caused oxidative damage to membrane lipids, leading to the reduction of antioxidant molecules (GSH, catalase, and SOD) in SNpc (Anusha et al. 2017). GSH is an antioxidant enzyme responsible for buffering free radicals through reducing H_2O_2 and organic peroxides (Lobo et al. 2010). Moreover, LPS is highly lipophilic, directly inhibiting the mitochondrial complex I and IV and increasing oxidative stress.

Additionally, it has been shown in pre-clinical and clinical research investigations that catalase and SOD enzymes activity decreased in the substantia nigra and putamen part of the brain. These enzyme alterations may be directly linked to substantia nigra neuron loss and show Parkinson's like symptoms. In PD, these enzyme activities were decreased in the substantia nigra, caudate, and putamen (Jenner et al. 1996).

In the present study, unilaterally infused LPS within SNpc resulted in the activation of neuroinflammatory markers. Their activation released numerous pro-inflammatory substances, which have been implicated in dopaminergic neuronal death (Machado at al. 2011). The direct and indirect dopaminergic pathways of basal ganglia are involved in movements, whereas an imbalance between these pathways results in uncontrolled involuntary movements resulting from progressive dopaminergic neuron degeneration and subsequent changes in striatal neurotransmitter signalling (Crittenden et al. 2011).

In the present research, intranigral injection of LPS induced nigrostriatal area regression and substantially reduced levels of neurotransmitters such as dopamine, serotonin, GABA, and increased the level of norepinephrine, glutamate in the striatum. Although excitotoxicity does not directly cause this damage, it is a major role in the oxidation of catecholamines and their processing by monoamine oxidase enzymes (Wajner et al. 2004).

NF-κB is a transcription factor that has a role in the regulation of the inflammatory response. The NF-κB is primarily found in the cytoplasm of resting cells, complexed with the inhibitory IκB family members. When inflammatory stimuli such as LPS is present, the IB protein is phosphorylated by IB kinase and dissociated from NF-κB, activating the NF-κB signalling pathway. When activated, NF-κB translocate into the nucleus and interacts with the promoter regions of downstream genes, thus controlling their expression in cells. LPS is a potent inducer of NF-κB in neuronal cell death through intracellular ROS production and microglial cells activation (An et al. 2020).

L-theanine treatment improved both motor dysfunction and behavioral deficits. The motor changes and behavioral impairments were measured using narrow beam walk, rotarod test, open field, and grip strength. After the restored motor performance, L-theanine (50 and 100 mg/kg; po) treated rats showed a significant and dose-dependent improvement in neuromuscular coordination and grip strength. We also observed that L-theanine treatment restored the levels of GSH through scavenging superoxide anion and peroxyl radicals. Lipid peroxidation within the membrane and its excessive level is measured as an indicator of oxidative stress, which results in cellular damage through peroxidation in phospholipids membranes (Paradies et al. 1999).

However, L-theanine administration reduced the LPO level in LPS infused rats. This reduction in LPO revealed the antioxidant potential of L-theanine through scavenging ROS, involving superoxide, hydroxyl radical, and peroxyl radicals. RNS (Reactive Nitrogen Species) consists of non-reactive molecule nitric oxide (NO), a chemical messenger that participated in the pathogenesis of PD. NO in combination with O^{2-}, produced harmful and reactive peroxynitrite oxidant ($ONOO^{-}$), responsible for producing lipid peroxidation in the biological membrane (Korkmaz et al. 2006). We also found that administration of L-theanine in LPS treated rats show that neuroinflammatory cytokine markers were brought back to normal. This signified the anti-inflammatory potential of L-theanine towards halting the progression of PD. L-theanine low (50 mg/kg; po) and high dose (100 mg/kg; po) starting from the 7th day of (LPS 5ug/5ul) injection to 21st day, dose-dependently restored the level of dopamine, serotonin, GABA and decreased the level of NE, and glutamate in LPS infused rats. Hence, L-theanine maintained neural circuits and

controlled movement impairments. To further define the mechanism by which L-theanine showed its therapeutic benefits in LPS-induced PD, we examined L-theanine's effects on NF-κB signaling pathways. Thus, L-theanine may exert its inhibitory impact on intracellular ROS generation via inhibiting NF-κB activation triggered by LPS. In the previously published paper, green tea extracts L-theanine reduced the activation of NF-κB in various cell types, including neuronal cells exposed to various oxidative or inflammatory stimuli (Kim et al. 2009). Therefore, it is possible that the ability of L-theanine to prevent ROS generation could be related to its inhibitory effect on LPS-induced stratum neuronal death through inhibition of NFκB. In the present study, we found that theanine prevents the LPS induction of NF-κB by anti-inflammatory impact and could be implicated in the protective effect against LPS-induced Parkinsonism-like symptoms in rats.

Based on our findings, L-theanine has therapeutic potential and needs further exploration to enhance scientific understanding of its role in treating and managing PD. Pertinently, it can be hypothesized that L-theanine reported beneficial effects towards improving motor functions and could be attributed to its neuroprotective abilities, which can be further associated with its anti-inflammatory, antioxidant activity, and capacity to restored the level of striatal neurotransmitters. L-theanine reduces neuroinflammation; reduced neurodegeneration, neurotransmitter alteration, and neuronal destruction. Moreover, our findings proved that treatment with L-theanine might be therapeutically beneficial in treating PD-like symptoms because the study results showed dose-dependently improvement in defects.

5. Conclusion

The positive impact of L-theanine was shown to improved motor functions and locomotor activity, as demonstrated by the open field, rotarod, narrow beam walk, and grip strength efficiency. This is likely due to its antioxidant and anti-inflammatory properties and ability to restored striatal neurotransmitters. The present study findings revealed that L-theanine may be a hallmark for preventing LPS induced degeneration of dopaminergic neurons through decreased oxidative stress and restored GSH, catalase, and SOD activities, reduced rates of neuroinflammatory markers, increased levels of serotonin, dopamine, GABA, and decreased levels of norepinephrine and glutamate. In addition, L-theanine could be useful in treating PD due to its ability to inhibit neuronal cell death via inhibition of NF-κB pathways. Therefore, the outcome of this research showed therapeutic value for L- theanine in neurodegenerative disorders like PD.

Declarations

Data availability statements

The data that support the findings of this study will provide upon reasonable request.

Author’s contribution

Shivam Kumar: Conducted the experiment and writing the manuscript. Anupam Awasthi and Khadga Raj: collected data, read and analysed by taking the help of Dr. Shamsher Singh. Dr. Shamsher Singh:

Designed, Reviewing, and Editing manuscript.

Acknowledgments

The authors are highly thankful to Shri. Parveen Garg, Chairman, ISFCP, for providing an excellent research platform.

Compliance with Ethical Standards

These animals were fed with a standard diet in accordance with Institutional Animal Ethics Committee (IAEC) guidelines. The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (ISFCP/ IAEC/CPCSEA/2019/429).

Conflict of Interest -The authors declare that they have no conflict of interest.

References

1. Aebi Hugo (1984) [13] Catalase in vitro. Methods Enzymol 105:121–126
2. An J, Chen B, Kang X, Zhang R, Guo Y, Zhao J, Yang H (2020) Neuroprotective effects of natural compounds on LPS-induced inflammatory responses in microglia. American Journal of Translational Research 12(6):2353–2378
3. Anusha C, Sumathi T, Joseph LD (2017) Protective role of apigenin on rotenone induced rat model of Parkinson's disease: Suppression of neuroinflammation and oxidative stress mediated apoptosis. Chemico-Biol Interact 269:67–79
4. Chen SQ, Wang ZS, Ma YX, Zhang W, Lu JL, Liang YR, Zheng XQ (2018) Neuroprotective effects and mechanisms of tea bioactive components in neurodegenerative diseases. Molecules 23(3):512–529
5. Crittenden JR, Graybiel AM (2011) Basal Ganglia disorders associated with imbalances in the striatal striosome and matrix compartments. Frontiers in neuroanatomy 5:59
6. Deb S, Dutta A, Phukan BC, Manivasagam T, Thenmozhi AJ, Bhattacharya P, Paul R, Borah A (2019) Neuroprotective attributes of L-theanine, a bioactive amino acid of tea, and its potential role in Parkinson's disease therapeutics. Neurochem Int 129:1–16
7. Deng I, Corrigan F, Zhai G, Zhou XF, Bobrovskaya L (2020) Lipopolysaccharide animal models of Parkinson's disease: Recent progress and relevance to clinical disease. *Brain Behavior Immunity-Health* 4:100060–100067
8. Ebbensgaard A, Mordhorst H, Aarestrup FM, Hansen EB (2018) The role of outer membrane proteins and lipopolysaccharides for the sensitivity of Escherichia coli to antimicrobial peptides. Frontiers in microbiology 9:2153–2166
9. Ellman GL (1959) Tissue sulfhydryl groups. Arch Biochem Biophys 82(1):70–77
10. Esposito E, Di Matteo V, Benigno A, Pierucci M, Crescimanno G, Di Giovanni G (2007) Non-steroidal anti-inflammatory drugs in Parkinson's disease. Exp Neurol 205(2):295–312

11. Farooqui T, Farooqui AA (eds) (2017) Role of the Mediterranean Diet in the Brain and Neurodegenerative Diseases. Academic Press
12. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR (1982) Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. Anal Biochem 126(1):131–138
13. Haslund-Vinding J, McBean G, Jaquet V, Vilhardt F (2017) NADPH oxidases in oxidant production by microglia: activating receptors, pharmacology and association with disease. Br J Pharmacol 174(12):1733–1749
14. Jenner P, Olanow CW (1996) Oxidative stress and the pathogenesis of Parkinson's disease. Neurology 47(6 Suppl 3):161S–161S70S
15. Kaur R, Mehan S, Singh S (2019) Understanding multifactorial architecture of Parkinson's disease: Pathophysiology to management. Neurol Sci 40(1):13–23
16. Khan AU, Akram M, Daniyal M, Zainab R (2019) Awareness and current knowledge of Parkinson's disease: a neurodegenerative disorder. Int J Neurosci 129(1):55–93
17. Kim TI, Lee YK, Park SG, Choi IS, Ban JO, Park HK, Nam SY, Yun YW, Han SB, Oh KW, Hong JT (2009) L-Theanine, an amino acid in green tea, attenuates β-amyloid-induced cognitive dysfunction and neurotoxicity: reduction in oxidative damage and inactivation of ERK/p38 kinase and NF-κB pathways. Free Radic Biol Med 47(11):1601–1610
18. Korkmaz A, Yaren H, Topal T, Oter S (2006) Molecular targets against mustard toxicity: implication of cell surface receptors, peroxynitrite production, and PARP activation. Arch Toxicol 80(10):662–670
19. Lasley SM, Gilbert ME (2002) Rat hippocampal glutamate and GABA release exhibit biphasic effects as a function of chronic lead exposure level. Toxicol Sci 66(1):139–147
20. Li B, Xu P, Wu S, Jiang Z, Huang Z, Li Q, Chen D (2018) Diosgenin attenuates lipopolysaccharide-induced Parkinson's disease by inhibiting the TLR/NF-κB pathway. J Alzheimers Dis 64(3):943–955
21. Lobo V, Patil A, Phatak A, Chandra N (2010) Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev 4(8):118–126
22. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. Journal of biological chemistry 193:265–275
23. MacCord JM (1969) Superoxide dismutase: an enzymatic function for erythrocuprein (hemocuprein). J Biol Chem 244:6049–6055
24. Machado A, Herrera AJ, Venero JL, Santiago M, De Pablos RM, Villaran RF, Espinosa-Oliva AM, Argüelles S, Sarmiento M, Delgado-Cortes MJ, Maurino R (2011) Peripheral inflammation increases the damage in animal models of nigrostriatal dopaminergic neurodegeneration: possible implication in Parkinson's disease incidence. Parkinson's disease
25. Lima MS, Martins MF, Marcia Delattre E, Proenca AB, Mori MA, Carabelli M B and Ferraz C A (2012) Motor and non-motor features of Parkinson's disease–a review of clinical and experimental studies. *CNS Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS Neurological Disorders)* 11(4):439–449

26. Nathan PJ, Lu K, Gray M, Oliver C (2006) The neuropharmacology of L-theanine (N-ethyl-L-glutamine) a possible neuroprotective and cognitive enhancing agent. Journal of Herbal Pharmacotherapy 6(2):21–30
27. Paradies G, Petrosillo G, Pistolese M, Di Venosa N, Serena D, Ruggiero FM (1999) Lipid peroxidation and alterations to oxidative metabolism in mitochondria isolated from rat heart subjected to ischemia and reperfusion. Free Radic Biol Med 27(1–2):42–50
28. Patel AB, de Graaf RA, Mason GF, Rothman DL, Shulman RG, Behar KL (2005) The contribution of GABA to glutamate/glutamine cycling and energy metabolism in the rat cortex in vivo. *Proceedings of the National Academy of Sciences*, *102*(15), pp.5588–5593
29. Valente PF, Segabinazi E, André Luís Ferreira de Meireles, Filipe Mega, Christiano de Figueiredo Spindler, Otávio Américo Augustin, Gabriela dos Santos Salvalaggio (2019) "Severe uncontrolled maternal hyperglycemia induces microsomia and neurodevelopment delay accompanied by apoptosis, cellular survival, and neuroinflammatory deregulation in rat offspring hippocampus." *Cellular and molecular neurobiology* 39, no. 3: 401–414
30. Saha S, Buttari B, Panieri E, Profumo E, Saso L (2020) An overview of Nrf2 signaling pathway and its role in inflammation. Molecules 25(22):5474
31. Sharma N, Nehru B (2015) Characterization of the lipopolysaccharide induced model of Parkinson's disease: Role of oxidative stress and neuroinflammation. Neurochem Int 87:92–105
32. Sharma N, Jamwal S, Kumar P (2016) Beneficial effect of antidepressants against rotenone induced Parkinsonism like symptoms in rats. Pathophysiology 23(2):123–134
33. Singh S, Kumar P (2017) Neuroprotective potential of curcumin in combination with piperine against 6-hydroxy dopamine induced motor deficit and neurochemical alterations in rats. Inflammopharmacology 25(1):69–79
34. Singh S, Jamwal S, Kumar P (2017) Neuroprotective potential of Quercetin in combination with piperine against 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine-induced neurotoxicity. Neural regeneration research 12(7):1137–1144
35. Sottocasa GL, Kuylenstierna BO, Ernster L, Bergstrand A (1967) An electron-transport system associated with the outer membrane of liver mitochondria: a biochemical and morphological study. The Journal of Cell Biology 32(2):415–438
36. Southwell AL, Ko J, Patterson PH (2009) Intrabody gene therapy ameliorates motor, cognitive, and neuropathological symptoms in multiple mouse models of Huntington's disease. J Neurosci 29(43):13589–13602
37. Wajner M, Latini A, Wyse AT, Dutra-Filho CS (2004) The role of oxidative damage in the neuropathology of organic acidurias: insights from animal studies. J Inherit Metab Dis 27(4):427–448
38. Wills ED (1971) Effects of lipid peroxidation on membrane-bound enzymes of the endoplasmic reticulum. Biochem J 123(5):983–991

39. Yu CA, Yu L (1980) Resolution and reconstitution of succinate-cytochrome c reductase. Preparations and properties of high purity succinate dehydrogenase and ubiquinol—cytochrome c reductase. Biochimica et Biophysica Acta (BBA)-Bioenergetics 591(2):409–420

Figures

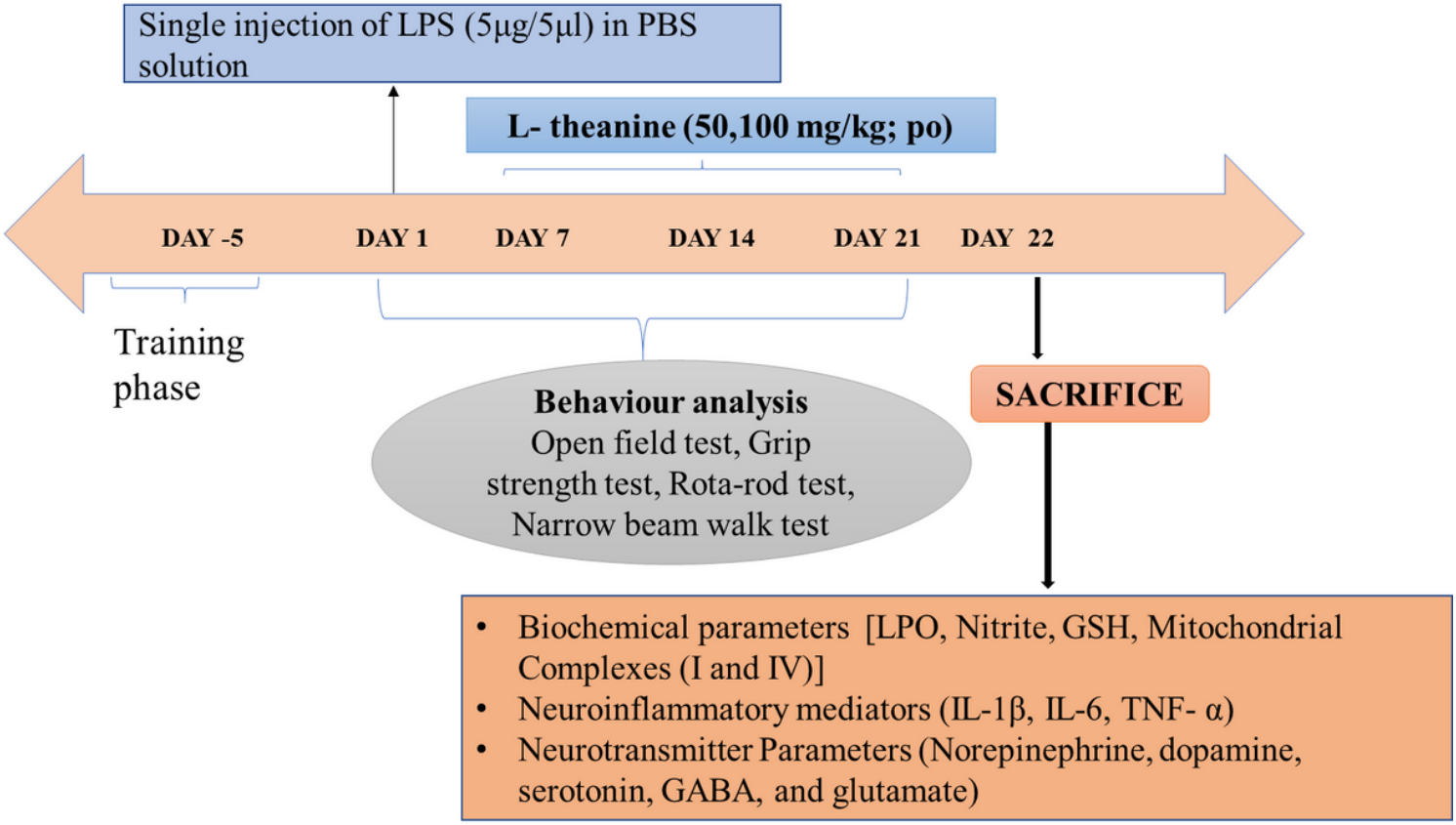


Figure 1

Experimental design

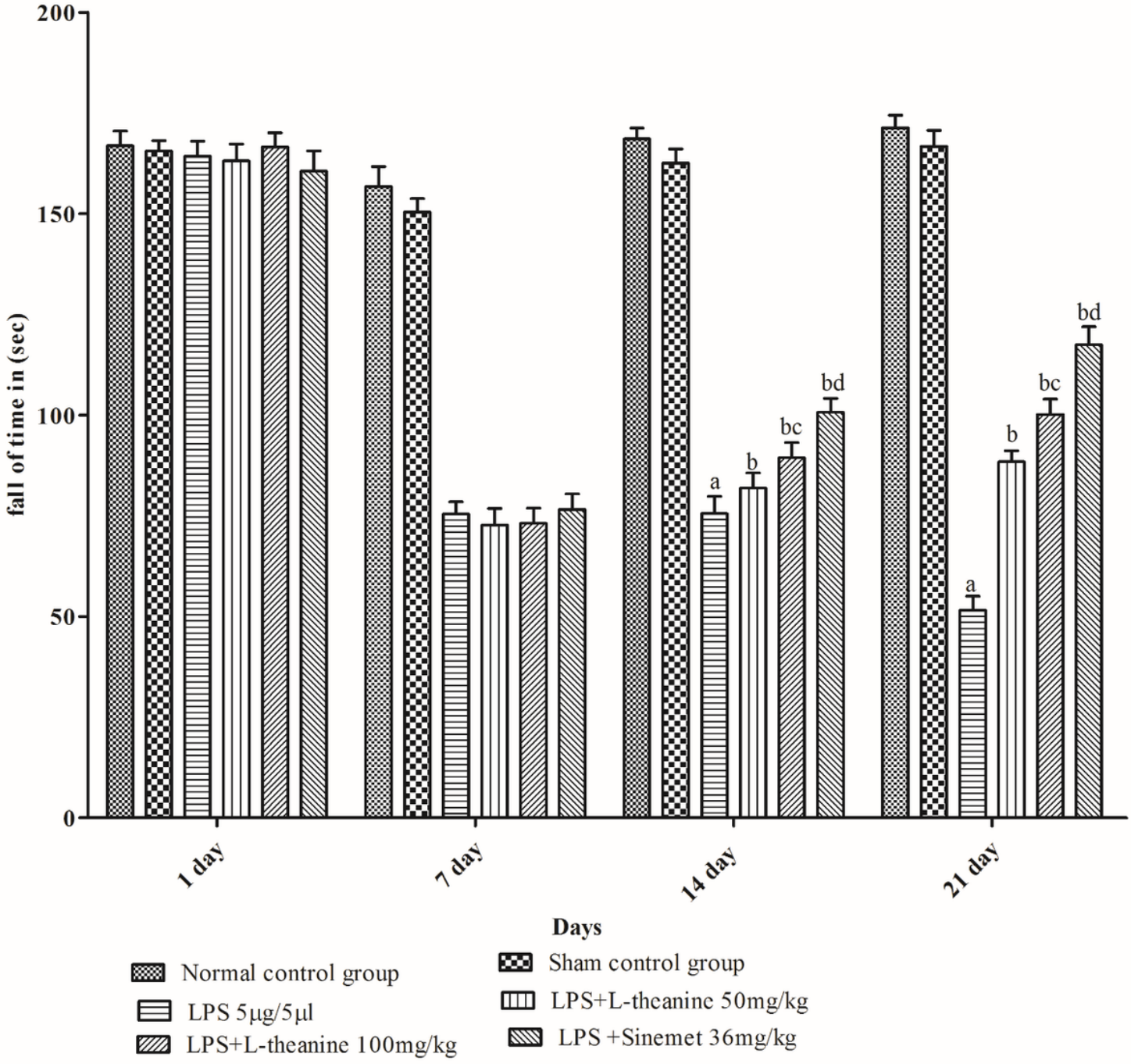


Figure 2

Effect of L-theanine treatment on motor coordination (rotarod activity) in LPS treated rats Effect of L-theanine on motor coordination in LPS treated rats. Data was expressed as Mean ± S.D. $^{a}p<0.01$ vs Normal control and Sham control, $^{b}p<0.05$ vs LPS, cp<0.05 vs L-theanine (50 mg/kg), dp<0.05 vs L-theanine (100 mg/kg). Statistical analysis performed by two-way ANOVA followed by Bonferroni's multiple comparison.

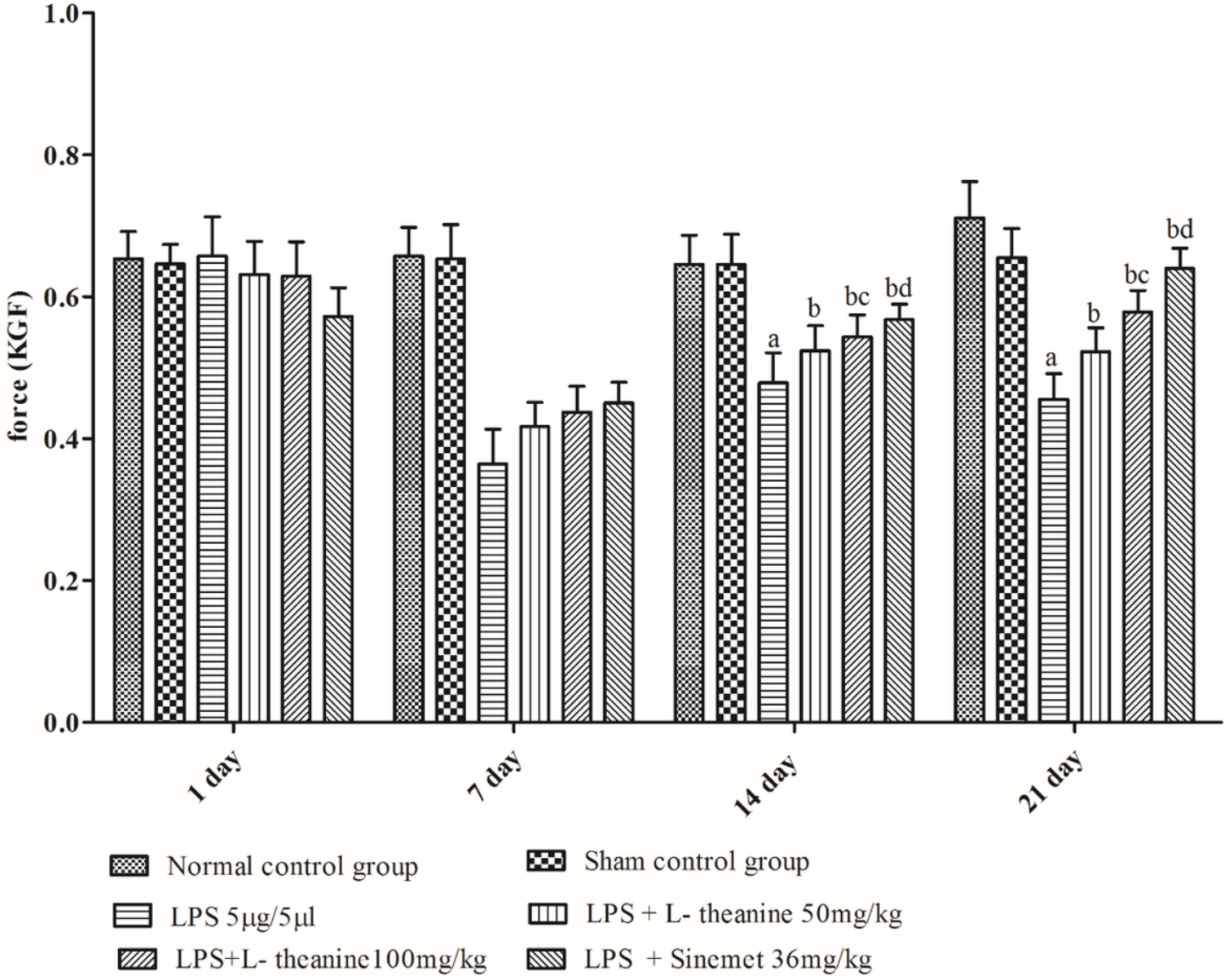
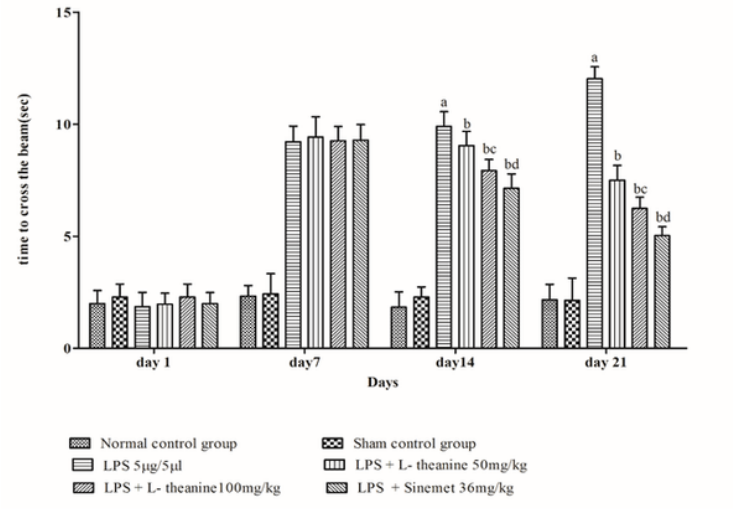
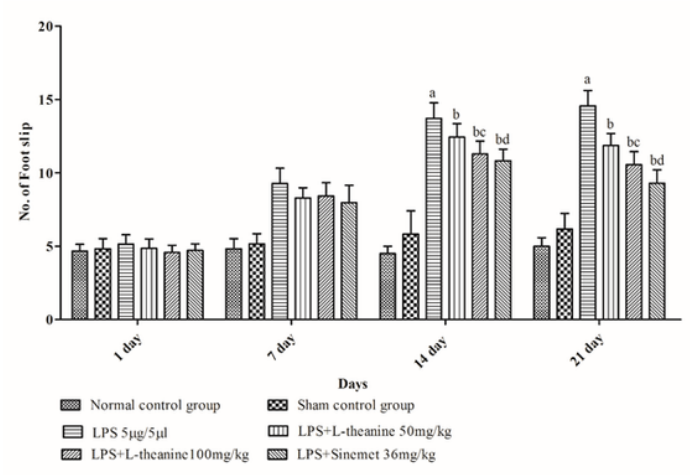


Figure 3

Effect of L-theanine treatment on grip strength activity in LPS treated rats Effect of L-theanine on muscular strength in LPS treated rats. Data was expressed as Mean ± S.D. ᵃp<0.001 vs Normal control and Sham control, ᵇp<0.05 vs LPS, ᶜ p<0.05 vs L-theanine (50 mg/kg), dp<0.05 vs L-theanine (100 mg/kg). Statistical analysis performed by two-way ANOVA followed by Bonferroni's multiple comparison.



A



B

Figure 4

a, b) Effect of L-theanine treatment on narrow beam walking in LPS treated rats a) Evaluation of latency time through narrow beam walk test Effect of L-theanine on motor coordination in LPS treated rats. Data was expressed as Mean ± S.D. ªp<0.001 vs Normal control and Sham control, p<0.01 vs LPS, p<0.05 vs L-theanine (50 mg/kg), dp<0.05 vs L-theanine (100 mg/kg). Statistical analysis performed by two-way ANOVA followed by Bonferroni's multiple comparison. b) Evaluation of number of foot slips through narrow beam walk test Effect of L-theanine on motor coordination in LPS treated rats. Data was expressed as Mean ± S.D. ªp<0.001 vs Normal control and Sham control, p<0.01 vs LPS, p<0.05 vs L-theanine (50 mg/kg), dp<0.05 vs L-theanine (100 mg/kg). Statistical analysis performed by two-way ANOVA followed by Bonferroni's multiple comparison.

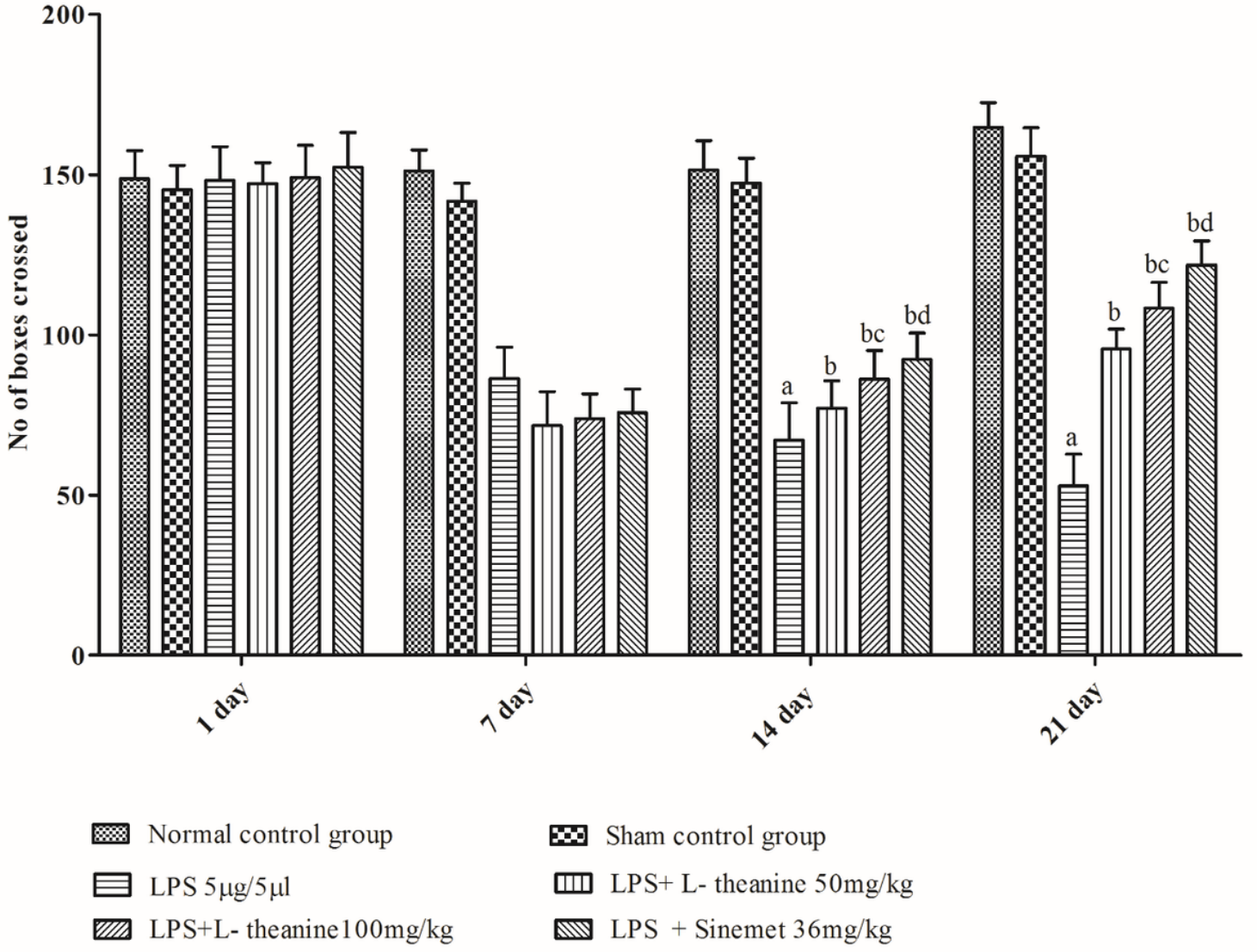


Figure 5

Effect of L-theanine treatment on locomotor (open field) activity in LPS treated rats Effect of L-theanine on motor coordination in LPS treated rats. Data was expressed as Mean ± S.D. ᵃp<0.001 vs Normal control and Sham control, p<0.01 vs LPS, p<0.05 vs L-theanine (50 mg/kg), dp<0.05 vs L-theanine (100 mg/kg). Statistical analysis performed by two-way ANOVA followed by Bonferroni's multiple comparison.

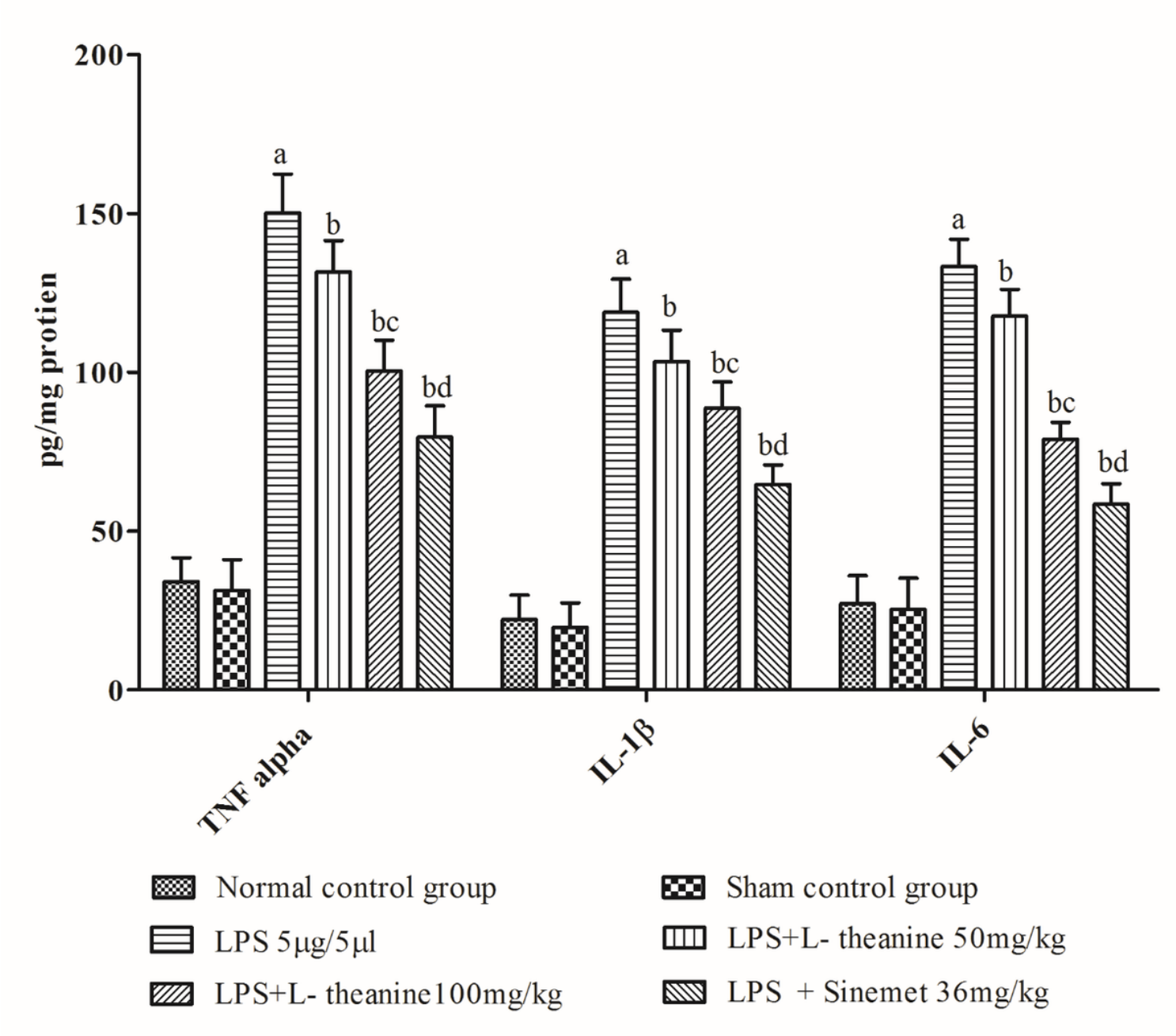


Figure 6

Neuroinflammatory Markers Estimation (TNFα, IL-1β, and IL-6) Through ELISA Kits Effect of L-theanine on TNF-α, IL-1β and IL-6 on LPS treated rats. Data was expressed as Mean ± S.D. ªp<0.001 vs Normal control and Sham control, p<0.05 vs LPS, p<0.01 vs L-theanine (50 mg/kg), dp<0.01 vs L-theanine (100 mg/kg). Statistical analysis performed by one-way ANOVA followed by turkey's post hoc test.

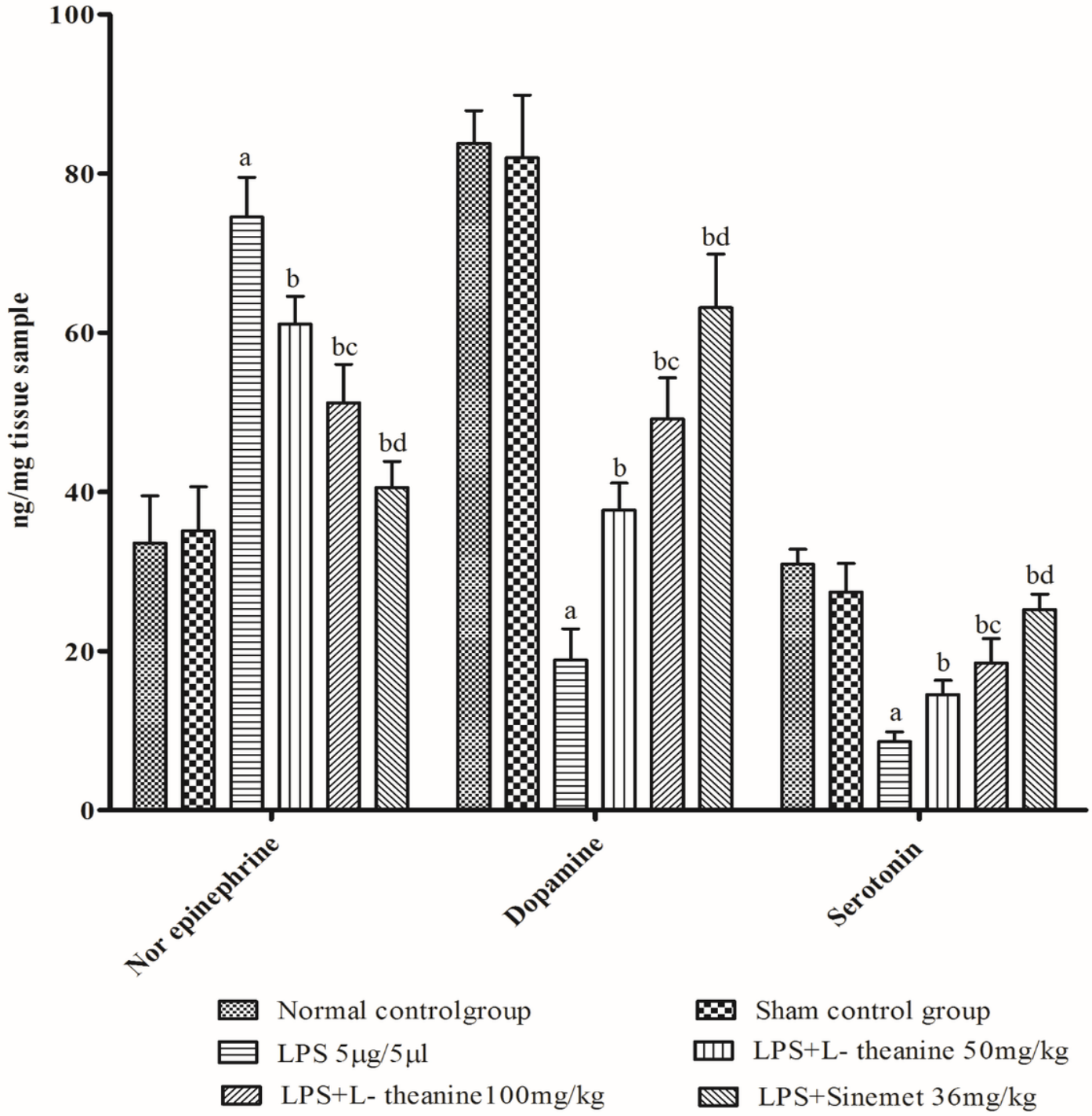


Figure 7

Effect of L-theanine on brain catecholamines (norepinephrine, dopamine, serotonin) in LPS treated rats Effect of L-theanine on norepinephrine, dopamine, serotonin levels in LPS treated rats. Data was expressed as Mean ± S.D. [a]p<0.001 vs Normal control and Sham control, bp<0.01 vs LPS, cp<0.01 vs L-theanine (50 mg/kg), dp<0.01 vs L-theanine (100 mg/kg). Statistical analysis performed by one-way ANOVA followed by turkey's post hoc test.

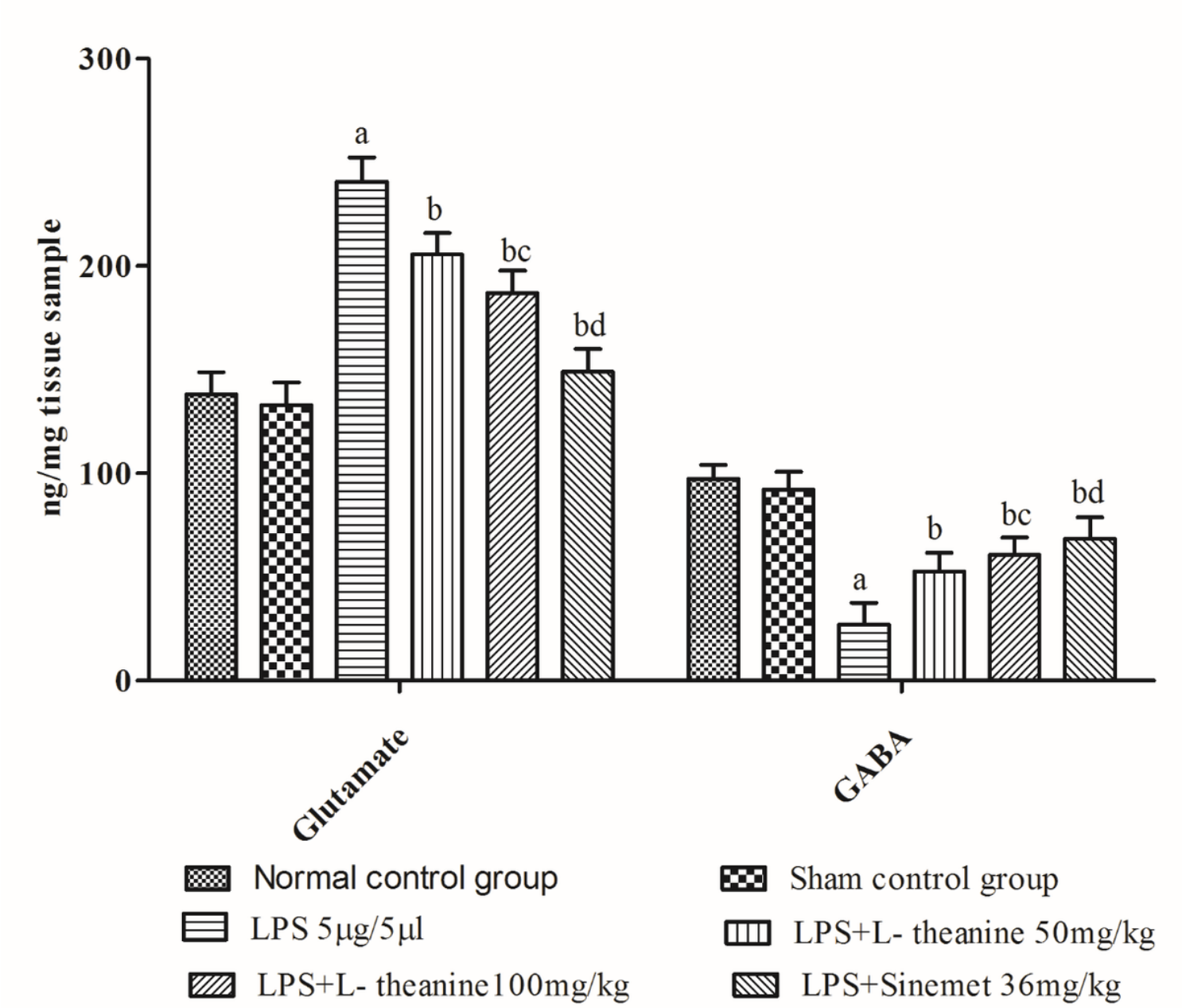
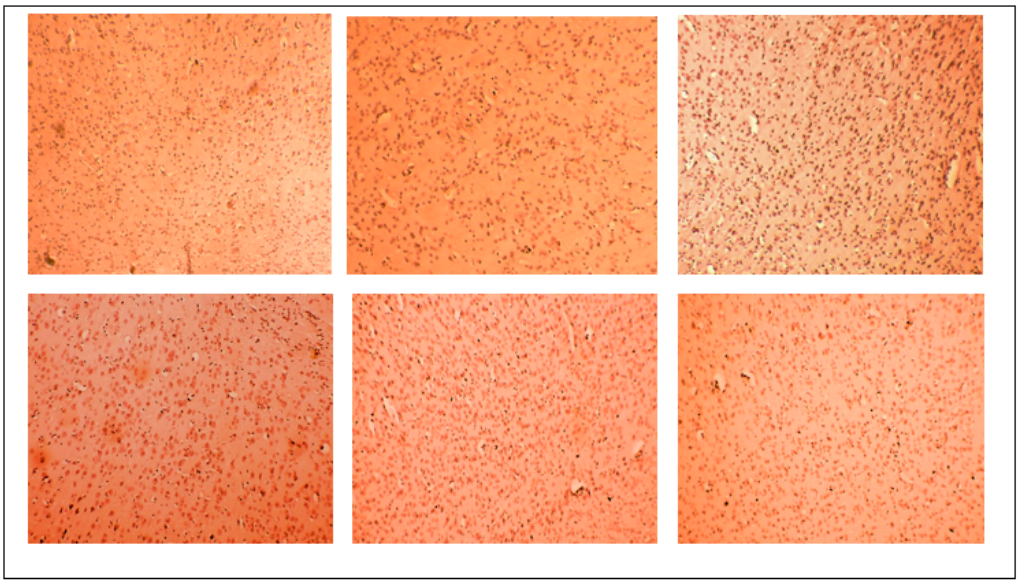


Figure 8

Effect of L-theanine on glutamate and GABA in LPS treated rats Effect of L-theanine on glutamate and GABA on LPS treated rats. Data is expressed as Mean ± S.D. ªp<0.001 vs Normal control and Sham control, p<0.01 vs LPS, p<0.05 vs L-theanine (50 mg/kg), dp<0.05 vs L-theanine (100 mg/kg). Statistical analysis performed by one-way ANOVA followed by turkey's post hoc test.



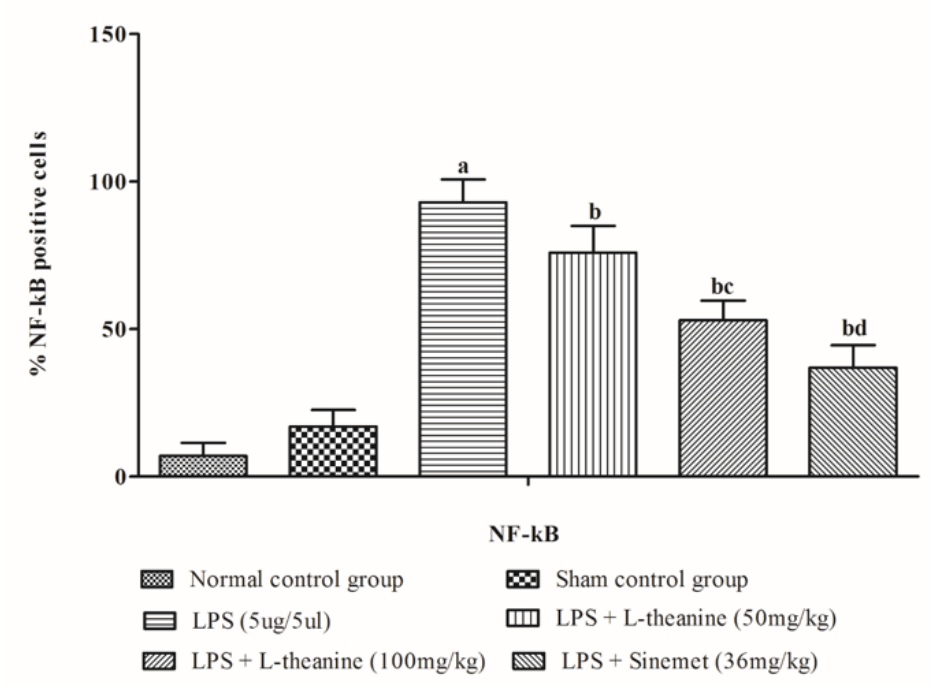


Figure 9

Effect of L-theanine on NF-κB expression in LPS treated rats Effect of L-theanine on NF-κB expression in LPS treated rats. Data was expressed as Mean ± S.D. ªp<0.001 vs Normal control and Sham control, p<0.01 vs LPS, p<0.001 vs L-theanine (50 mg/kg), dp<0.01 vs L-theanine (100 mg/kg). Statistical analysis performed by one-way ANOVA followed by turkey's post hoc test. Arrow indicted the expression of NF-κB 10 X objective with scale bar 20 µm