L-theanine attenuates LPS-induced motor deficit in experimental rat model of Parkinson’s disease: emphasis on mitochondrial activity, neuroinflammation, and neurotransmitters

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Abstract
Rationale  Parkinson’s disease (PD) is a neurodegenerative disorder characterized by loss of dopaminergic neurons. The pathogenesis of PD includes oxidative stress, mitochondrial dysfunction, neuroinflammation, and neurotransmitter dysregulation. L-theanine is found in green tea and has antioxidant, anti-inflammatory, and neuroprotective effects with a high blood brain barrier permeability.

Objective  The objective of this study was to investigate the possible neuroprotective effect of L-theanine in lipopolysaccharide (LPS) induced motor deficits and striatal neurotoxicity in a rat model of PD.

Methods  LPS was infused at a dose of 5 μg/5 μl PBS stereotaxically into SNpc of rats. Treatment with L-theanine (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, and neurotransmitters (serotonin, dopamine, norepinephrine, GABA, and glutamate) estimations.

Results  Results revealed that L-theanine dose-dependently and significantly reversed motor deficits, assessed through loco-motor 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.

Conclusions  These data suggest that the positive effects of L-theanine on motor coordination may be mediated by the suppression of NF-κB induced by LPS. Therefore, L-theanine would have a new therapeutic potential for PD.

Keywords  Parkinson’s disease · Lipopolysaccharide · L-theanine · Neurotransmitters · NF-κB

Introduction
Parkinson’s disease (PD) is a neurodegenerative disorder characterized by the death of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and decreased dopamine level in the striatum (Lima et al. 2012). The progressive loss of dopaminergic neurons in the SNpc occurs due to excessive production of free radicals, mitochondrial energy failure, neuroinflammation, and neurotransmitters dysbalance, which are the main pathogenic pathological features 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). There are more than 10 million people with PD in the globe, and each year, and out of it approximately 60,000 Americans are diagnosed...
L-theanine is a non-protein, non-peptide amino acid that is structurally related to the amino acid γ-aminobutyric acid (GABA), and has been shown to have various health benefits. In particular, it has been studied as a potential neuroprotective agent in various neurological disorders, including Parkinson's disease (PD).

**Materials and methods**

**Experimental animals**

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

LPS (product number: L2143) was purchased from Sigma-Aldrich Chemicals Private Limited, Plot #12, Bommasandra-Jigani Link Road, Bengaluru-560100, India. L-theanine (CAS number: 3081–61-6) was purchased from TCI Chemicals Pvt. Ltd., India. LPS is dissolved in phosphate buffer saline (1 μg/μl). The test drug used in the present study, L-theanine, is dissolved in double-distilled water.

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 (5 μg/5 μl) from a stock solution of 1 mg/ ml of LPS in 1% Monastral blue dissolved in phosphate-buffered saline was given for 2 min using a Hamilton syringe, and the needle was kept in situ for an additional 2 min after each operation to prevent reflux along the injection tract.

Experimental procedure

The rats were divided into 6 groups; each group contains seven animals except the normal control (n = 6). Group 1 is marked as normal control. Group 2 is considered sham control. Group 3 received LPS (5 μg/5 μl). Groups 4 and 5 received L-theanine (50 and 100 mg/kg; po) starting from day 7 of LPS (5 μg/5 μl) 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 (5 μg/5 μl) injection. L-theanine was administered by oral route. Behavioral parameters are 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 (Fig. 1).

Parameters

Assessment of behavior parameters

Narrow beam walk test  The apparatus consists of 2 platforms (8 cm in diameter) joined by a wooden beam (0.5 mm in thickness, 2.0 cm in width, and 120 cm in length) which is made from wood and tested in our pharmacology research lab. This test was used to determine the gait abnormalities and the number of foot slips of experimental rats (Sharma and Nehru 2015). The apparatus consists of a narrow horizontal beam (1 cm in width, 130 cm in length, and 0.5 mm in thickness), and the beam was elevated at 100 cm from the floor. All rats were trained with a narrow beam for 5 days (2 trials per day) before performing experiments. The latency and their foot slip to cross the beam were recorded in each trial.

Rotarod test  Rotarod apparatus was purchased from medicraft INCO (519/E-4C), Haryana, India, consisting of 4 compartments with speeds of 5, 10, 15, 20, and 25, and the unit is revolutions per minute (rpm). The motor coordination and paw grip performance of all animals were evaluated using a rotarod apparatus. The rotarod apparatus consists of a rod with a diameter of 75 mm and a height of 40 cm.
On day 22, rats (Singh et al. 2017). This apparatus is rectangular and made of wood that measures 100 × 100 × 40 cm³. After animals have been habituated for 5 min, a single exposure is sufficient to cause movement across the squares from the animal’s habituation. The floor of the apparatus was divided into 25 rectangular squares by pencil lines. The experimental room was illuminated by a 40-W white bulb located 150 cm above the test apparatus. The animal was kept in the center of apparatus with 0.5 mg/kg apomorphine and placed in the cylinder for 30 min to measure functional motor activity. The number of turns was recorded throughout the test (Huang et al. 2018).

Apomorphine-induced rotation test The apomorphine-induced rotational test has been described as a well-known and widely used method for investigating dopaminergic system impairment and assessing behavioral dysfunction in PD model rats. Rats were put onto the cylinder for a training session at 10 rpm for 10 min to adapt this test. Rats were injected with 0.5 mg/kg apomorphine and placed in the cylinder for 30 min to measure functional motor activity. The number of turns was recorded throughout the test (Huang et al. 2018).

Grip strength test Grip strength is used for measuring neuromuscular functions in rodents by assessing the maximum force displayed by animals. The grip strength of the forelimbs of all rats was measured using the grip strength apparatus. Chatillon force measurement was purchased from Ametek, 8600 Somerset Drive, Largo, FL, 33773, USA, for measurement of grip strength of the forelimb in animals. Rats were trained for 5 days on grip strength apparatus before experimenting. The grip strength is expressed as a kilogram-force (Kgf) (Sharma et al. 2016).

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 cm³. After animals have become habituated for 5 min, a single exposure is sufficient to cause movement across the squares from the animal’s habituation. The floor of the apparatus was divided into 25 rectangular squares by pencil lines. The experimental room was illuminated by a 40-W white bulb located 150 cm above the test apparatus. The animal was kept in the center of apparatus 2 h later, after giving a single exposure to the apparatus, and number of squares cross/5 min, a number of grooming/5 min, and number of rearing/5 min by animal were recorded. Each crossing was considered if the animal put all four paws in another square. After each trial apparatus was cleaned properly and readings were taken, total locomotor activity/5 min was calculated by adding the number of squares crossed, the number of grooming, and the number of rearing.

Measurement of biochemical parameters

Dissection and homogenization On day 22, rats (n = 29) were sacrificed by cervical dislocation, and their brains were isolated and used to estimate biochemical, neuroinflammatory, and neurotransmitters. During the experimental day, the brains were placed on dry ice to isolate the striatum from the brain. Then, brain tissue samples were homogenized with ice-cold 0.1 M phosphate buffer at pH 7.4 for ten times (w/v), and the procedure was followed according to the weight of the tissue. Next, the striatal tissue of the rat was homogenated and centrifuged for 15 min at 10,000 rpm in a refrigerated centrifuge. This homogenized striatal tissue solution measured oxidative stress, proinflammatory markers, and neurotransmitters.

Estimation of lipid peroxidation (LPO) level Wills method was used for quantitative measurement of LPO in the striatum (Wills 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 h. The addition of 1 ml of 10% trichloroacetic acid (TCA) was added after 2 h of incubation and centrifugation at 10,000 rpm for 10 min, and the supernatant was collected. One milliliter supernatant was added to 1 ml 0.067% thiobarbituric acid, and tubes were placed in boiling water for 10 min, 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. One hundred microliters of sample or standard (100, 200, 400, 800, 1000 μg/ml) was added to 400 μl of distilled water. Five hundred microliters of Griess reagent (0.1% N-(1-naphthyl) ethylenediamine dihydrochloride + 1% sulphanilamide and 5% phosphoric acid) was added to the above solution, which was kept at room temperature for 5 min. Equal volumes of Griess reagent and supernatant were incubated in the dark for 10 min. 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 (Green et al. 1982).

Estimation of reduced GSH level The level of reduced glutathione in the striatal homogenate was estimated according to the method described by Ellman (1959). In this, 1 ml of the supernatant was precipitated with 1 ml of 4% sulfo salicylic acid and cold digested at 4 °C for 1 h. Samples were centrifuged at 1200 rpm for 15 min. To 1 ml of the supernatant, 2.7 ml of phosphate buffer (0.1 M, pH 8) and 0.2 ml of 5,5′-dithiobis- (2-nitrobenzoic acid) (DTNB) were added. The yellow color that developed was measured immediately at 412 nm using a spectrophotometer. The glutathione concentration in the supernatant was determined from a standard curve and expressed as μmol per mg protein (Ellman...
The standard curve of absorbance vs protein concentration was plotted and used to determine the unknown protein concentrations.

**Estimation of catalase level** The decomposition of H$_2$O$_2$ determined catalase activity in striatum homogenates at 240 nm following an earlier published method (Aebi 1984). One-milliliter homogenate tissue was added to 1 ml reaction solution containing 0.1% Triton X-100, 15 mM H$_2$O$_2$, and 50 mM potassium phosphate at a pH of 7.4. The units of enzyme activity were calculated using the extinction coefficient of H$_2$O$_2$, which is 0.0394 mM$^{-1}$ cm$^{-1}$. One unit of catalase activity is defined as 1 mmol H$_2$O$_2$ 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 1969). One-milliliter homogenates of the brain were suspended in 1 ml reaction solutions containing 0.03% 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% 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. Fifty percent 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)** Mitochondrial complex I, commonly known as NADH dehydrogenase, is the mitochondrial membrane’s largest protein complex. Complex I can catalyze the dehydrogenation of NADH to produce NAD$^+$. The enzyme activity is calculated by measuring the oxidation rate of NADH at 340 nm using a microplate reader (BioTek, USA). According to the manufacturer’s instructions, each well was added to the mitochondrial complex I activity detection kit (mitochondrial respiratory chain I activity detection kit, Abcam, USA) (Yu and Yu 1980).

**Estimation of pro-inflammatory cytokines** Estimating proinflammatory cytokines (TNF-α, IL-1β, IL-6) of striatal was performed using ELISA kits.

**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). Waters standard system consisting of a high-pressure isocratic pump, a 20 µl manual sample injector valve, C18 reverse phase column, and an electrochemical detector were used in the study. The mobile phase consisted of sodium citrate buffer (pH 4.5)-acetonitrile (87:13, v/v). Sodium citrate buffer consisted of 10 mM citric acid, 25 mM NaH$_2$PO$_4$, 25 mM EDTA, and 2 mM of 1-heptane sulfonic acid. Electrochemical conditions for the experiment were +0.75 V, and sensitivity ranges from 5 to 50 nA. Separation was carried out at a flow rate of 0.8 ml/min. Samples (20 µl) were injected manually. On the day of the experiment, frozen brain samples were thawed and homogenized in a solution containing 0.2 M perchloric acid. After that, samples were centrifuged at 12,000 rpm for 5 min. The supernatant was filtered through 0.22 mm nylon filters before injecting it in the HPLC sample injector. Data were recorded and analyzed with the help of breeze software. The concentration of neurotransmitters and their metabolites was calculated from standard in a concentration range of 10–100 ng/ml (Jamwal et al. 2017).

**Estimation of GABA and glutamate** The previously described technique of derivatizing amino acids in striatal homogenate solution with o-phthalaldehyde/mercaptopetoethanol (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 and Gilbert 2002). Waters standard system consisting of a high-pressure isocratic pump, a 20-µl manual sample injector valve, C18 reverse phase column, and electrochemical detector were used in the study. The mobile phase was composed of 100 mM disodium hydrogen phosphate anhydrous, 25 mM EDTA, and 22% methanol (pH 6.5). Electrochemical conditions for the experiment were +0.65 V, and sensitivity ranges from 5 to 50 nA. Separation was carried out at a flow rate of 1.2 ml/min, and the column temperature was maintained at 40 °C. Samples (20 µl) were injected manually through rheodyne valve injector. On the day of the experiment, frozen brain samples were thawed and homogenized in 0.2 M perchloric acid. After that, samples were centrifuged at 12,000 g for 15 min. The supernatant was dissolved in 0.2 M perchloric acid.
derivatized using OPA/β-ME and then filtered through 0.22 mm nylon filters before injecting it into the HPLC sample injector. Data were recorded and analyzed with the help of breeze software. Concentrations of amino acids were calculated from the standard curve generated by using standard in a concentration range of 10–100 ng/ml. The values are expressed as a percentage of the normal control group.

**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.

**Results**

The open-field test, rotarod test, narrow beam walking, and grip strength were used for motor function detections that represent the ability of motor coordination, balance, and spontaneous movement. The rotarod test assesses balance, motor, and coordination and measures the duration of time that rats maintain their balance on a moving rod. The open-field test is used to measure spontaneous locomotor activity. The narrow beam walk apparatus measured gait abnormalities and foot slips in experimental rats. Therefore, behavior function tests were performed in this study to determine the effects of L-theanine on motor functions. In this study, we observed that 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 motor coordination, narrow beam walking, grip strength, and locomotor activity compared to the LPS-treated rats ($p < 0.01$). However, administration of L-theanine (100 mg/kg) significantly improved compared to motor coordination as compared 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 ($p < 0.05$) (Figs. 2, 3, 4, and 5).

To explore the effect of L-theanine treatment on motor dysfunction of LPS-induced PD rats, apomorphine-induced rotation is commonly used to assess the effect of drug on the dopaminergic system. Our results indicated that L-theanine treatment visibly decreased the number of apomorphine-induced rotations. These findings suggest that L-theanine treatment exerted beneficial effects on motor dysfunction in rats with LPS-induced PD (Fig. 6).

In the progression of PD, inflammation plays a critical role, and raised expression of cytokines in PD is well reported. Therefore, they release proinflammatory cytokines such as TNF-α and IL-1β affect neurons and cause neurodegeneration. The levels 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

**Estimation of dopamine**

Estimation of striatal dopamine was performed using an ELISA kit, and all procedures were carried out according to manufacturer’s instructions. Brain samples were homogenized in PBS according to tissue weight. The homogenates were then centrifuged, and supernatants were collected. Standard working solutions were prepared, and each dopamine concentration was added in duplicate. Samples were added in triplicate in subsequent wells, and 50 μl biotinylated detection Ab working solution was added in all wells; the plate was covered and incubated for 45 min at 37 °C. Following 3 wash steps using 350 μl of wash buffer, 100 μl of HRP conjugate working solution was added in each well; the plate was covered and incubated for 30 min at 37 °C. The solution was aspirated from the wells followed by 5 subsequent wash steps. To each well, 90 μl of substrate reagent was added, and the plate was sealed and incubated for 15 min at 37 °C, followed by the addition of 50 μl of stop solution into each well. The optical density of each well was measured using the UV spectrophotometer (Msibi and Mabandla 2019).

**Immunohistochemistry analysis**

Rat brains ($n = 6$) were immediately fixed in 5% formaldehyde, subsequently embedded in liquid paraffin wax, and then, 5-μm sections were cut, as reported by Yildirim et al. The tissue sections were dewaxed twice with xylene for 15 min each time, rehydrated with decreasing concentrations of absolute alcohol (100%, 70%, and 50%), and then rinsed with distilled water for 2 min. To retrieve antigens, tissue sections were soaked in citrate buffer (pH 7.4) for 5 min, and peroxidase activity was stopped by incubation at room temperature for 5 min with 3% hydrogen peroxide. The tissue sections were incubated for 1 h at room temperature with antibodies against NF-κB and then rinsed with Tris buffer solution for 10 min. Next, sections were treated for 30 min at room temperature with Poly-Horse radish peroxidase (Poly-HRP) and then rinsed twice with citrate buffer for 5 min each time and incubated with DAB reagent for 2 min. Following DAB reaction, sections were stained with hematoxylin for 3 min and then rinsed with double distilled water for 20 min. After drying at room temperature, the sections were mounted with DPX and imaged at a magnification of 10× using a fluorescence microscope (Li et al. 2018).
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 doses of L-theanine-treated groups \( (p < 0.01) \) (Fig. 7).

Degeneration of dopaminergic neurons in the substantia nigra is a hallmark of PD. We investigated the neuroprotective effects of L-theanine on the degeneration of dopaminergic neurons. 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, low and high
doses of L-theanine-treated groups 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) significantly restored neurotransmitter levels (dopamine, serotonin, GABA) compared to L-theanine (50 mg/kg) \((p < 0.01)\). Moreover, the standard drug (sinemet) had shown a significant increase in the level of dopamine, serotonin, and 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 \((p < 0.01)\) (Figs. 8 and 9).

Endogenous antioxidant defense networks consist of enzymes (GSH, SOD, catalase) and chemicals that neutralize oxygen-free radicals that cause oxidative stress when the antioxidant system fails. Antioxidant defense system components such as GSH, SOD, and catalase have been well documented in the PD brain. The level of LPO and nitrite was significantly increased by reducing GSH, catalase, SOD, and mitochondrial complex I and IV activities in LPS-treated striatum compared to the 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 and 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 a significant decrease in the level of LPO and nitrite level and increased 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 \( (p < 0.05) \) (Tables 1 and 2).

Further, the expression of NF-kB significantly increased after LPS injected in the rat striatum compared to sham and
normal control group ($p<0.001$). Rats treated with L-theanine at 50 mg/kg and 100 mg/kg significantly reduced NF-κB expression in the striatum compared to the 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 NF-κB expression considerably in the striatum than a high dose of L-theanine-treated group ($p<0.01$) (Fig. 10).

LPS-treated rats exhibited a substantial reduction level in the striatum ($p<0.001$). However, low and high doses of L-theanine-treated groups significantly attenuated the reduction in dopamine, serotonin levels and decreased norepinephrine and glutamate levels compared to the LPS-treated group ($p<0.01$). Addition, sinemet group had shown a significant increase in the level of dopamine, serotonin, and a decrease in the level of norepinephrine and glutamate in the striatum as compared to LPS and a high dose of L-theanine ($p<0.01$). Data was expressed as mean ± S.D. $^{a}p<0.001$ vs Normal control and Sham control, $^{b}p<0.01$ vs LPS, $^{c}p<0.01$ vs L-theanine (50 mg/kg), $^{d}p<0.01$ vs L-theanine (100 mg/kg). Statistical analysis was performed by one-way ANOVA followed by Tukey’s post hoc test.

**Discussion**

PD is a progressive neurodegenerative movement disorder caused by a number of factors like oxidative stress, neurotransmitter dysbalance, mitochondrial dysfunction, and neuroinflammation. However, current evidence contrasting challenges 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 the possible role of neuroinflammation and dopaminergic loss driven by LPS, the current animal model of PD was used in the present study. In this study, we have demonstrated that the administration of L-theanine and sinemet 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 5 μg/5 μl in PBS was infused stereotaxically into SNpc of rats. This results in an increase in proinflammatory 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 (Pajares et al. 2020). In addition, intranigral unilateral infusion of LPS causes the generation of ROS and OH that cause 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 by reducing H₂O₂ 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 enzyme
The NF-κB is a major transcription factor and modulates inflammatory system through expressing proinflammatory genes including iNOS and COX-2. NF-κB is a transcription factor found in all nucleated cell types mostly in the cytoplasm of resting cells. Additionally, activation of the mitogen-activated protein kinase (MAPK) pathway also plays an essential role in initiating and developing inflammatory processes that are transmitted by sequential phosphorylation events. The three major groups of MAPK cascades are ERK, p38, and c-Jun N-terminal kinase (JNK), and each MAPK is activated by the upstream activation of MAPK kinase (MKK) and MAPK kinase kinase (Son et al. 2020). LPS is a powerful activator of the transcription factor NF-κB, which has been demonstrated to play a key role an important role in regulating the bioactivities induced by LPS through the activation of numerous genes that participate in inflammatory, immune, and acute phase responses (An et al. 2020). Inhibition of MKK and down-regulation of NF-κB by an L-theanine reduced LPS induction of several inflammatory cytokines, including IL-1β, TNF-α, and IL-6, indicating a role for the ERK1/2 pathway in LPS signaling independent of the JNK and p38 pathways.

L-theanine treatment improved both motor dysfunction and behavioral deficits. The rotarod apparatus is used to evaluate the motor coordination in which the animals were trained priorly. The open-field test is used to assess the spontaneous locomotor activity or performance. Gait abnormalities and foot slips count was measured by narrow beam walk apparatus. The grip strength of the fore limbs was measured using digital grip force meter. Therefore, behavior function tests were performed in this study to determine the effects of L-theanine on motor functions. We observed that LPS-treated rats showed a significant decrease in motor coordination (rotarod activity), narrow beam walking (gait abnormalities), grip strength, and locomotor (open field) activity at the end of day 21. L-theanine-treated rats at the dose of (50 and 100 mg/kg; po) from day 7 to 21 had significantly attenuated motor coordination, narrow beam walking, grip strength, and locomotor activity in a dose-dependent manner.

We also observed that L-theanine treatment restored the levels of GSH by scavenging superoxide anions 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). Studies have reported that standard drug (sinemet) showed antioxidant capacity and ability to protect DNA against oxidative-induced damage derived from different mechanisms of action (Colamartino et al. 2015). Similarly, our study found that sinemet improved antioxidant activities and reduced proinflammatory markers in rat brain.

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 participates in the pathogenesis of PD. NO, in combination with $\mathrm{O}_2^-$, produced harmful and reactive proximities 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

### Table 2

| Experimental grouping          | Mitochondrial complex |
|-------------------------------|-----------------------|
|                               | Complex I (nM/mg protein) | Complex IV (nM/mg protein) |
| Normal control                | 12.16 ± 0.12           | 318.6 ± 12.20              |
| Sham control                  | 11.9 ± 0.13            | 316.5 ± 7.31               |
| LPS 5 µg/5 µl                 | 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$^{bd}$         |

$^a p < 0.001$ vs Normal control and Sham control  
$^b p < 0.01$ vs LPS  
$^c p < 0.05$ vs L-theanine (50 mg/kg)  
$^d p < 0.05$ vs L-theanine (100 mg/kg)

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 and Olanow 1996).

In the present study, unilaterally infused LPS within SNpc resulted in the activation of neuroinflammatory markers. Their activation released numerous proinflammatory substances, which have been implicated in dopaminergic neuronal death (Machado et 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 signaling (Crittenden and Graybiel 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 and glutamate in the striatum. Although excitotoxicity does not directly cause this damage, it plays a significant role in catecholamine oxidation and processing by monoamine oxidase enzymes (Wajner et al. 2004).

The NF-κB is major transcription factor and modulates inflammatory system through expressing proinflammatory genes including iNOS and COX-2. NF-κB is a transcription factor found in all nucleated cell types mostly in the cytoplasm of resting cells. Additionally, activation of the mitogen-activated protein kinase (MAPK) pathway also plays an essential role in initiating and developing inflammatory processes that are transmitted by sequential phosphorylation events. The three major groups of MAPK cascades are ERK, p38, and c-Jun N-terminal kinase (JNK), and each MAPK is activated by the upstream activation of MAPK kinase (MKK) and MAPK kinase kinase (Son et al. 2020). LPS is a powerful activator of the transcription factor NF-κB, which has been demonstrated to play a key role an important role in regulating the bioactivities induced by LPS through the activation of numerous genes that participate in inflammatory, immune, and acute phase responses (An et al. 2020). Inhibition of MKK and down-regulation of NF-κB by an L-theanine reduced LPS induction of several inflammatory cytokines, including IL-1β, TNF-α, and IL-6, indicating a role for the ERK1/2 pathway in LPS signaling independent of the JNK and p38 pathways.

L-theanine treatment improved both motor dysfunction and behavioral deficits. The rotarod apparatus is used to evaluate the motor coordination in which the animals were trained priorly. The open-field test is used to assess the spontaneous locomotor activity or performance. Gait abnormalities and foot slips count was measured by narrow beam walk apparatus. The grip strength of the fore limbs was measured using digital grip force meter. Therefore, behavior function tests were performed in this study to determine the effects of L-theanine on motor functions. We observed that LPS-treated rats showed a significant decrease in motor coordination (rotarod activity), narrow beam walking (gait abnormalities), grip strength, and locomotor (open field) activity at the end of day 21. L-theanine-treated rats at the dose of (50 and 100 mg/kg; po) from day 7 to 21 had significantly attenuated motor coordination, narrow beam walking, grip strength, and locomotor activity in a dose-dependent manner.

We also observed that L-theanine treatment restored the levels of GSH by scavenging superoxide anions 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). Studies have reported that standard drug (sinemet) showed antioxidant capacity and ability to protect DNA against oxidative-induced damage derived from different mechanisms of action (Colamartino et al. 2015). Similarly, our study found that sinemet improved antioxidant activities and reduced proinflammatory markers in rat brain.

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 participates in the pathogenesis of PD. NO, in combination with $\mathrm{O}_2^-$, produced harmful and reactive proximities 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 showed that neuroinflammatory cytokine markers were returned 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 5 μg/5 μl) injection to the 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 the 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. The study has found that administration

Fig. 10 Effect of L-theanine on NF-κB expression in LPS-treated rats. Effect of L-theanine on NF-κB expression in LPS-treated rats. The expression of NF-κB significantly increased after LPS injected in rat striatum compared to sham and normal control group (p < 0.001). Rats treated with L-theanine at 50 mg/kg and 100 mg/kg significantly reduced NF-κB expression in the striatum compared to the 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 NF-κB expression considerably in the striatum than a high dose of L-theanine-treated group (p < 0.01). Data was expressed as mean ± S.D. *p < 0.001 vs Normal control and Sham control, †p < 0.01 vs LPS, ‡p < 0.001 vs L-theanine (50 mg/kg), §p < 0.01 vs L-theanine (100 mg/kg). Statistical analysis was performed by one-way ANOVA followed by Tukey’s post hoc test. Arrow indicated the expression of NF-κB 10× objective with scale bar 20 µm
of levodopa (L-dopa) increases dopamine (DA) in the striatum of healthy rats (Huang et al. 2014). Similarly, our study showed that the administration of sinemet increased neurotransmitters level in the striatum compared to L-theanine and LPS-treated rats.

L-theanine, an amino acid derived from Camellia sinensis, penetrates the blood brain barrier through a leucine-prefering transport mechanism. L-theanine has been shown to increase dopamine, serotonin, and GABA levels in the brain, which enables its various neuroprotective properties (Zhao and Zhao 2014). In addition, L-theanine has been shown to protect brains caused by cerebral ischemia–reperfusion, 3-NP-induced striatal toxicity in rats (Thangarajan et al. 2014), rotenone-induced Parkinsonism-like symptoms in rats (Chen et al. 2022), and amyloid-βeta induced cognitive dysfunction and oxidative stress in mice (Kim et al. 2009).

A recent study found that L-theanine possesses antioxidant and anti-inflammatory properties against QA-induced striatal neurotoxicity and neurochemical changes in rats (Jamwal et al. 2017). 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 restore the level of striatal neurotransmitters. L-theanine reduced neurodestruction by attenuating neuroinflammation and prevention of neurotransmitter alteration. Moreover, our findings proved that treatment with L-theanine might be therapeutically beneficial in treating PD-like symptoms because the study results showed dose-dependent improvement in defects.

**Conclusion**

The positive impact of L-theanine was shown to improve motor functions and locomotor activity, as demonstrated by the open field, rotarod, narrow beam walk, and grip strength efficiency, which is likely due to its antioxidant and anti-inflammatory properties and ability to restore 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 reduced levels of norepinephrine and glutamate. In addition, L-theanine could be useful in treating PD due to its ability to inhibit neuronal cell death by inhibiting NF-κB pathways. Therefore, the outcome of this research showed therapeutic value for L-theanine in neurodegenerative disorders like PD.

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**Author contribution** SK: conducted the experiment and wrote the manuscript. AA and KR: collected data, read and analyzed by using the help of SS. SS: designed, reviewed, and edited manuscript.

**Data availability** The data that support the findings of this study will be provided upon reasonable request.

**Declarations**

**Ethics approval** 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 no competing interests.
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