

Vanillin attenuates oxidative stress and neurochemical balance in MPTP-induced Parkinson's disease mice by regulating the TLR-4 inflammatory pathway

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Folia Neuropathol 2024; 62 (1): 76-82

DOI: <https://doi.org/10.5114/fn.2023.131191>

Abstract

This study investigated the protective effect of vanillin against Parkinson's disease (PD). 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; 30 mg/kg) was administered s.c. for 6 consecutive days to induce PD and mice were treated with vanillin (100 and 200 mg/kg, p.o.) for 15 days. Cognitive, motor and non-motor functions were assessed to evaluate the effect of vanillin in PD mice. Levels of dopamine and glutamate and activity of monoamine oxidase B (MAO-B) were estimated in vanillin-treated PD mice. The effect of vanillin on the level of lipid peroxidation and superoxide dismutase in brain tissue of PD mice was estimated. Data of the study revealed that vanillin reversed the altered cognitive, motor and non-motor function in PD mice. Activity of MAO-B and neurochemical level were attenuated with vanillin in PD mice. Inflammatory cytokines, nuclear factor kappa B (NF-κB) and Toll-like receptor 4 (TLR-4) levels were lower in the vanillin-treated group compared to the PD group of mice. Data of the study suggest that vanillin protects against neuronal injury and recovers the altered behaviour in PD mice by regulating neurochemical balance and the TLR-4/NF-κB pathway.

Key words: Parkinson's disease, inflammation, vanillin, neurochemicals, cytokines.

Introduction

Parkinson's disease (PD) is a motor function disorder that occurs due to neuronal loss in the substantia nigra region of brain [20]. Tremor, rigidity and bradykinesia are the classical symptoms that characterize PD, which is also associated with secondary manifestations such as difficulty in swallowing, soft speech, dementia and postural disbalance [6]. The literature reveals that aggregation of protein, i.e. α -synuclein, altered metabolism, apoptosis, inflammation, dysregulation of calcium homeostasis and excitotoxicity are the most common pathogenic pathways involved in development of PD [12]. The inflammatory pathway was recently explored more for the progression of degeneration of dopaminergic

neurons. As Toll-like receptor 4 (TLR-4) phosphorylation activates microglia and astrocytes, these cells support the neurons for normal physiological functioning [10]. Inflammatory cytokine release enhances microglia which promotes misfolding of α -synuclein, contributing to development of PD [13]. Moreover, alteration of astrocyte function promotes generation of ROS, which promotes apoptosis and neurodegeneration [5]. This multidirectional pathway of inflammation is important for neuronal death.

Biochemicals sourced from food and nutrients have shown a promising role in the prevention and management of neurodegenerative disorders including PD. Vanillin is a flavouring agent commonly used as a food ingredient, isolated from vanilla beans [1]. Vanillin was

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Received: 07.12.2022, Accepted: 12.05.2023, Online publication: 13.09.2023

reported to prevent neuronal death by regulating the TLR-4/nuclear factor kappa B (NF- κ B) pathway, which dysregulates cytokine secretion [21]. Moreover, vanillin promotes the regulation of ROS and thereby shows a strong antioxidant property [18]. Thus, the effect of vanillin was evaluated against PD.

Material and methods

Animals

Healthy Swiss albino mice (male; 20-25 g) were housed under controlled conditions of humidity ($65 \pm 5\%$) and temperature ($23 \pm 2^\circ\text{C}$) with a 12 h light/dark cycle. All the mice had free access to water and food. All the experimental protocol was approved by the institutional animal ethical committee (650/02/C/CPC-SEA/25/2021).

Experimental

All the animals were grouped as follows: control; PD which received 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) only; vanillin 100 and 200 mg/kg which received vanillin 100 and 200 mg/kg, p.o. for the period of 15 days after the administration of MPTP. Parkinson's disease was induced in all the groups except the control group by intraperitoneal administration of MPTP (30 mg/kg) for 6 consecutive days.

Estimation of motor function

Motor function and muscle balance were evaluated using rotarod apparatus. All the animals were mounted on the apparatus which rotates at a specific rotation (18 RPM) and the time to fall was recorded as the latency period.

Morris water maze test

Learning and memory were estimated in mice using the Morris water maze (MWM) test as per previously published reports [17]. The MWM apparatus had the following dimensions: circular pool: 120 cm; height: 50 cm; depth: 30 cm. The pool was separated into four different quadrants and the platform was placed in one quadrant. Trials were held on 4 consecutive days. The platform was removed on the 5th day and the time spent in the target quadrant was observed.

Brain tissue homogenate

Striatum and substantia nigra of the brain were isolated from each animal after sacrificing them and brain tissue was homogenized in phosphate buffer (pH 7.4, 0.1 M). Isolated brain was centrifuged at 3000 rpm for 15 min. Neurochemical and biochemical estimation was performed on supernatant solution.

Estimation of dopamine and glutamate

Kits were used to determine the level of dopamine and glutamate in the homogenate of brain tissue of PD mice.

MAO-B assay

An enzyme-linked immunosorbent assay (ELISA) kit was used to determine the level of MAO-B in the homogenate of brain tissue of PD mice and assessment was performed at 450 nm using an ELISA reader.

Estimation of inflammatory cytokines and oxidative stress parameters

Levels of interleukin (IL)-1 β , IL-6, tumour necrosis factor α (TNF- α) and NF- κ B were estimated in the tissue homogenate of PD mice using the ELISA kit as per the direction given by the manufacturer of the kit. Oxidative stress parameters such as lipid peroxidation (LPO) and glutathione (GSH) were determined in PD mice. Malondialdehyde (MDA) level was estimated as per the Ohkawa method at 532 nm. GSH content was determined in the hippocampal tissue by estimating the absorbance at 412 nm.

Western blot assay

Brain tissue was homogenized with tissue homogenizer in RIPA buffer for 3 min and centrifuged for 30 min at 15 000 g at 4°C after incubation for 30 min. Protein assay was used to extract the protein from the supernatant of the sample and SDS-polyacrylamide gel electrophoresis was utilized to separate the protein. The protein sample was filtered with membrane filter, and further incubated with antibodies such as TLR4, MyD88 and β -actin overnight at 4°C . These membranes were incubated with secondary antibody. Blots were observed with the Image Lab software after development of blots with the ChemiDoc MP Imaging System.

Statistical analysis

Data are represented as mean \pm SD ($n = 6$). Analysis of variance (ANOVA) was used for the comparison between different groups followed by the post-hoc Tukey test with SPSS, version 17.0 (SPSS, Inc., Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

Result

Effect of vanillin on motor function

Motor function was assessed by determining number of falls/min in vanillin-treated PD mice using the Rota Road apparatus (Fig. 1). There was a significantly higher number of falls/min (7.9 ± 0.19) in the PD

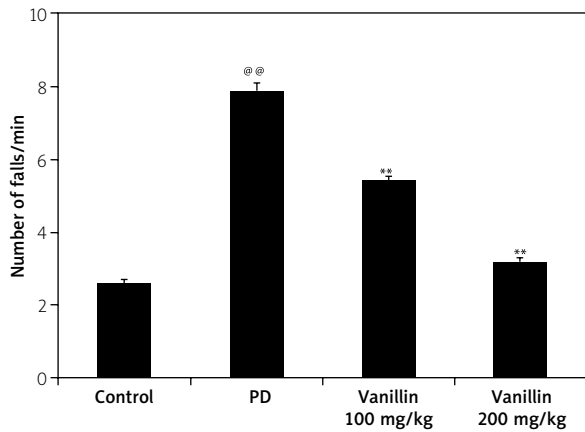


Fig. 1. Vanillin effect on the motor function of MPTP-induced Parkinson’s disease (PD) mice using Rota Road apparatus. Mean \pm SD ($n = 6$); @@ $p < 0.01$ vs. control group, ** $p < 0.01$ vs. PD group.

group of mice than in the control group of mice (2.6 ± 0.1 falls/min). However, treatment with vanillin reduced the number of falls/min to 3.2 ± 0.12 in MPTP-induced PD mice. These results suggest that vanillin improves motor function in PD.

Effect of vanillin on learning and memory

The effect of vanillin on cognitive function was assessed by determining learning and memory function in PD mice using the Morris water maze apparatus as shown in Figure 2. Number of crossings and time spent in the target quadrant were observed to be lower and escape latency higher in the PD group than the control group of mice. However, vanillin treatment reduced escape latency and increased the number of crossings and time spent in the target quadrant in MPTP-induced PD mice.

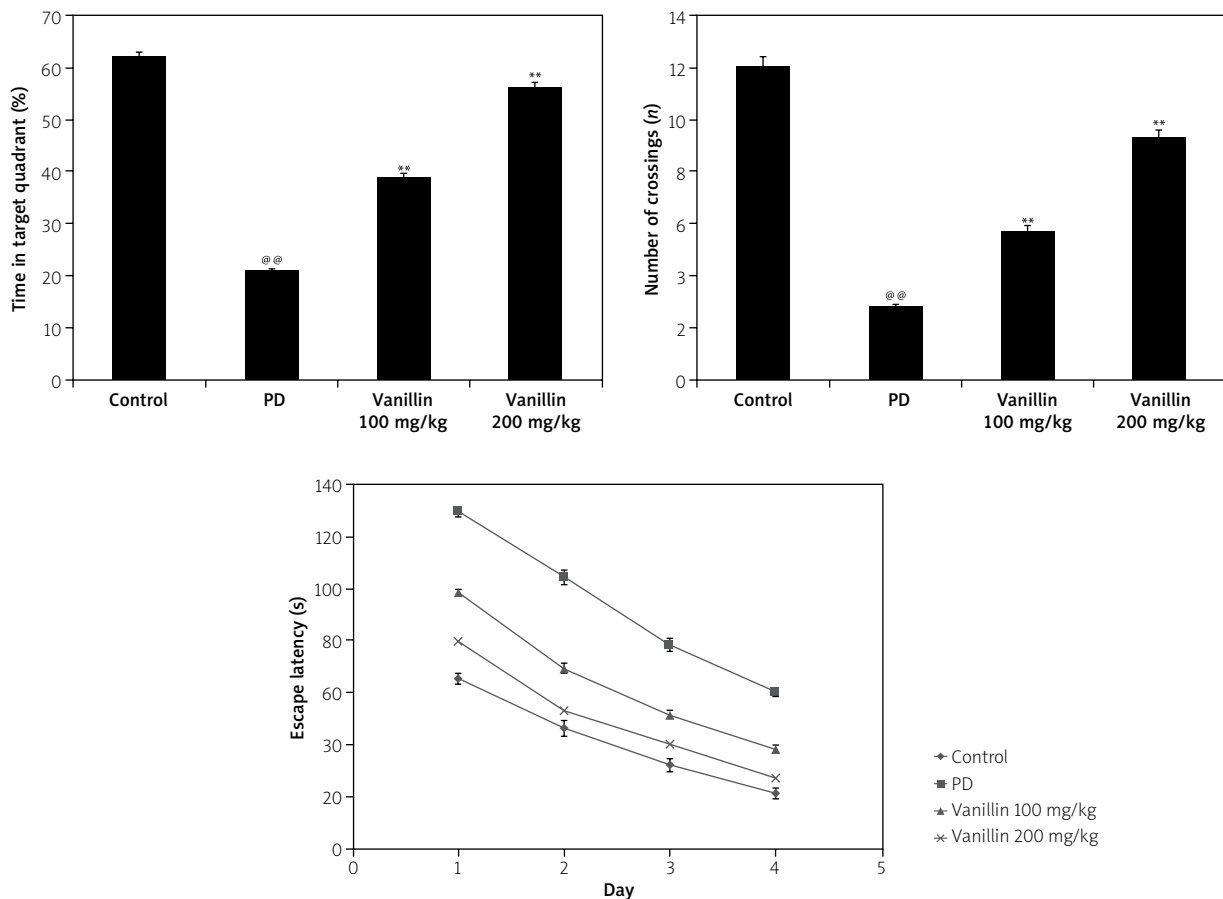


Fig. 2. Vanillin improved cognitive function in MPTP-induced Parkinson’s disease (PD) mice using Morris water maze apparatus. Mean \pm SD ($n = 6$); @@ $p < 0.01$ vs. control group, ** $p < 0.01$ vs. PD group.

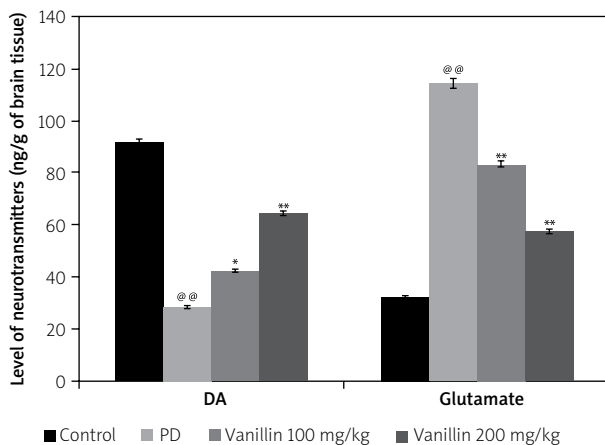


Fig. 3. Vanillin attenuates the level of neurochemicals in the brain tissue of MPTP-induced Parkinson's disease (PD) mice. Mean \pm SD ($n = 6$); @@ $p < 0.01$ vs. control group, ** $p < 0.01$ vs. PD group.

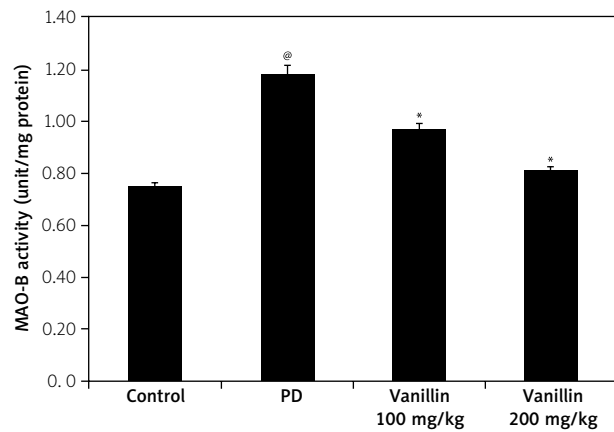


Fig. 4. Vanillin reduced activity of MAO-B in the brain tissue of MPTP-induced Parkinson's disease (PD) mice using ELISA. Mean \pm SD ($n = 6$); @@ $p < 0.01$ vs. control group, ** $p < 0.01$ vs. PD group.

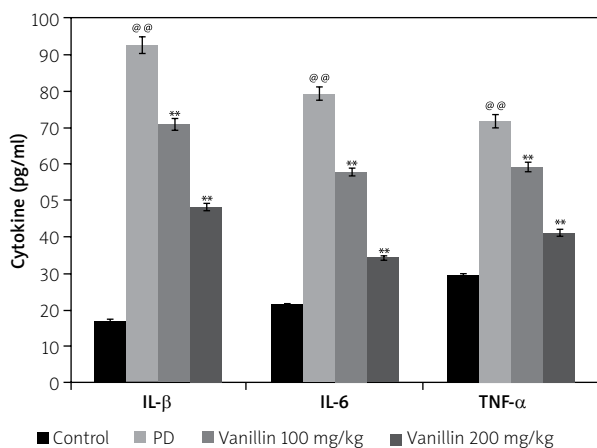
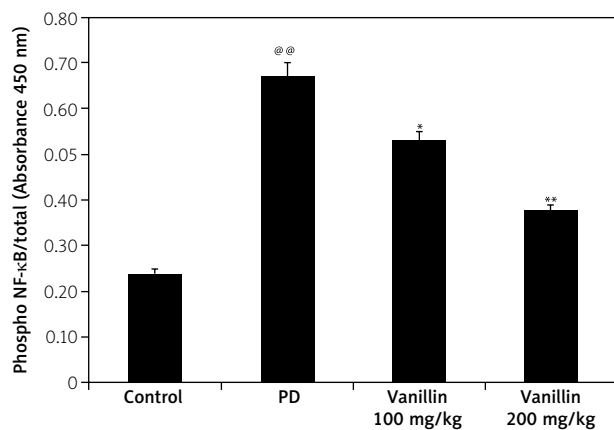


Fig. 5. Vanillin reduced level of inflammatory cytokines in the brain tissue of MPTP-induced Parkinson's disease (PD) mice using ELISA. Mean \pm SD ($n = 6$); @@ $p < 0.01$ vs. control group, ** $p < 0.01$ vs. PD group.



Effect of vanillin on levels of neurochemicals

Neurochemicals such as dopamine and glutamate level were determined in the brain tissue of vanillin-treated PD mice as shown in Figure 3. The level of dopamine was observed to be 91 ± 1.6 ng/g in the brain tissue of control mice, compared to 28 ± 0.58 ng/g in the PD group of mice. Moreover, glutamate level was higher (114 ± 1.95 ng/g) in the brain tissue of the PD group compared to the control group (32 ± 0.54 ng/g). Treatment with vanillin ameliorated the altered level of DA and glutamate up to 64 ± 0.93 and 57 ± 0.96 ng/g in brain tissue of PD mice.

Effect of vanillin on activity of MAO-B

Effect of vanillin on the activity of MAO-B in PD mice was estimated using ELISA as shown in Figure 4. There was significantly higher ($p < 0.05$) activity of MAO-B in brain tissue of PD group than in the control group of mice. Moreover, activity of MAO-B was significantly lower in tissue homogenate of the vanillin-treated group than in the PD group of mice.

Effect of vanillin on level of cytokines

The level of inflammatory cytokines was estimated in vanillin-treated PD mice using ELISA as shown in Figure 5. The level of inflammatory cytokine was higher

in brain tissue of the PD group than the control group of mice. There were significantly lower cytokine (IL-1 β , IL-6, TNF- α and NF- κ B) levels in the vanillin-treated group than the PD group of mice.

Effect of vanillin on markers of oxidative stress

The effect of vanillin on the level of oxidative stress markers such as MDA and reduced glutathione in PD mice was estimated, as shown in Figure 6. There was a significantly higher level of MDA ($176 \pm 4.6 \mu\text{M}/\text{mg}$ of protein) and lower glutathione ($24 \pm 0.8 \mu\text{M}/\text{mg}$ of protein) in the PD group than the control group of mice (MDA: $91 \pm 1.9 \mu\text{M}/\text{mg}$ of protein; reduced glutathione: $68 \pm 1.6 \mu\text{M}/\text{mg}$ of protein). Treatment with vanillin ameliorated the altered level of MDA and reduced glutathione to $104 \pm 2.3 \mu\text{M}/\text{mg}$ of protein and $5.7 \pm 1.5 \mu\text{M}/\text{mg}$ of protein in the brain tissue of PD mice respectively.

Effect of vanillin on the expression of TLR4/MyD88

Expression of TLR-4 and MyD88 proteins was estimated in vanillin-treated PD mice using western blot assay, as shown in Figure 7. In the PD group, expression of TLR-4 and MyD88 proteins was significantly higher than in the control group of mice. Expression of TLR-4 and MyD88 proteins was significantly lower in vanillin-treated PD mice.

Discussion

Parkinson's disease is a neurodegenerative disorder, which dysregulates neuromuscular function and leads to tremor, akinesia and rigidity [6]. Moreover, neurodegeneration led to PD also affected cognitive

function in an individual [8]. The treatment option available for the management of PD has several limitations and specifically no drug is able to cure this chronic disorder. Thus, the present investigation evaluated the effect of vanillin for the management of PD. The literature suggests that MPTP administration reduces the mobility and motor dysfunction in mice, which matches the clinical features of PD and it is an irreversible PD model [14]. In the present study PD was induced in mice by administering MPTP.

Neuromuscular dysfunction in PD altered the muscle coordination, which affected the muscle grip in a PD mouse model, and a report supports it [16]. Parkinson's disease is effectively managed with improvement in muscle grip strength. Treatment with vanillin improves the mean fall time compared to PD mice, which means vanillin promotes the muscle coordination in PD mice. Learning and memory are altered in neurodegenerative diseases including PD [7]. Vanillin improves cognitive function in MPTP-induced PD mice.

There are several etiopathogeneses including the inflammatory pathway involved in the development of PD. Overproduction of cytokine causes neuronal toxicity leading to development of PD. Inflammatory factors such as IL-1 β and TNF- α increase synthesis as they enhance the inflammatory reaction causing an increase in oxidative stress, which promotes neuronal apoptosis [15]. Vanillin was reported for its strong anti-inflammatory property, as it reduces the synthesis of inflammatory cytokines [11]. Data of the present study reveal that treatment with vanillin reduces the level of inflammatory cytokines and oxidative stress in the brain tissue of MPTP-induced PD mice. Microglia cells in the central nervous system (CNS) are activated by the phosphorylation of TLR4, which contributes to the regulation of neurogenesis [2]. TLR4 activates the

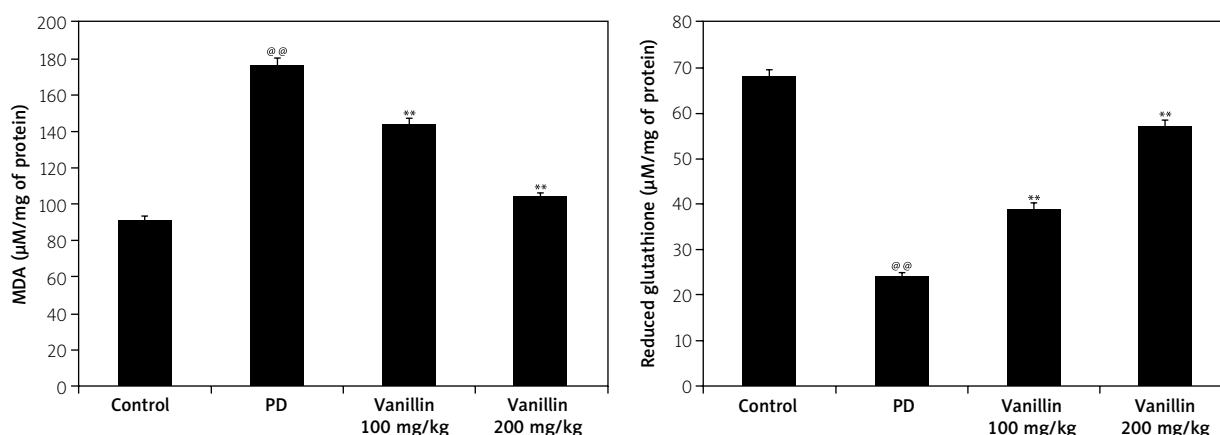


Fig. 6. Vanillin ameliorated the altered level of oxidative stress in the brain tissue of MPTP-induced Parkinson's disease (PD) mice. Mean \pm SD ($n = 6$); @@ $p < 0.01$ vs. control group, ** $p < 0.01$ vs. PD group.

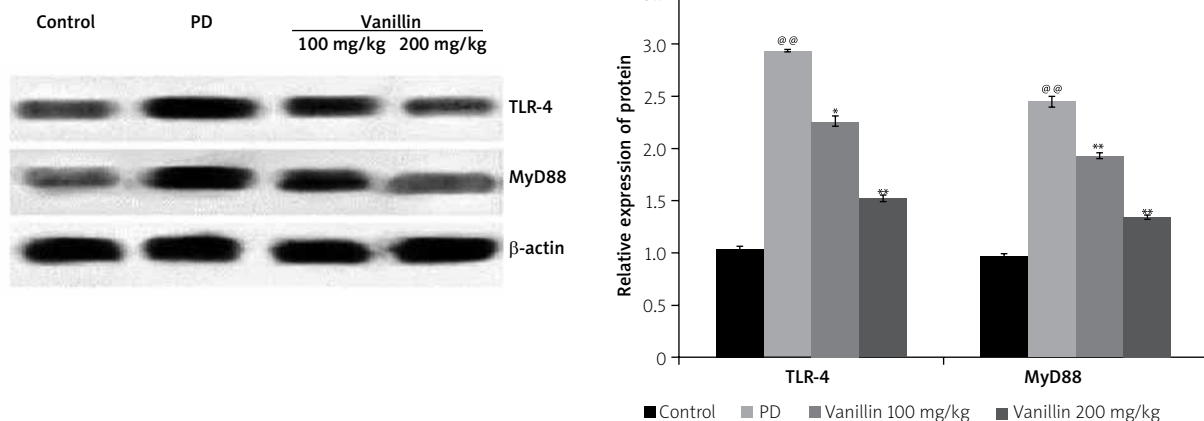


Fig. 7. Vanillin reduced the expression of TLR-4 and MyD88 protein in the brain tissue homogenate of MPTP-induced Parkinson's disease (PD) mice. Mean \pm SD ($n = 6$); ^{@@} $p < 0.01$ vs. control group, ^{**} $p < 0.01$ vs. PD group.

NF- κ B pathway and proinflammatory cytokines, which activate the inflammatory cascade causing neurodegeneration [2]. MPTP was also reported to increase NF- κ B, which is enhanced due to expression of TLR4 and MyD88 that causes an increase in cytokines [19]. All these changes stimulate neuronal injury and amelioration of this pathway protects neurodegeneration and development of PD. Results of the present study suggest that treatment with vanillin ameliorates the altered pathway of TLR4/MyD88 pathway in PD mice.

Neuronal transmission plays a major role in normal functioning of the body, which is regulated by several neurochemicals. Levels of these neurochemicals are altered in neurodegenerative disorders as synthesis of them occurs in neurons [9]. It is well documented that dopaminergic neurons get damaged in PD, which imbalances the level of neurochemicals such as dopamine and acetylcholine [3]. Moreover, recent studies also focus on other neurochemicals which are altered in PD such as GABA and glutamate [4]. Neuronal balance of these neurochemicals protects against the development of PD and data of the investigation reveal that treatment with vanillin ameliorates the altered dopamine, glutamate and enzyme, which regulates the level of these neurotransmitters.

Conclusions

Data of the study reveal that treatment with vanillin ameliorates the altered levels of neurochemicals and oxidative stress in PD mice. Moreover, vanillin mediates behavioural changes and neurodegeneration in PD by regulating the inflammatory TLR pathway.

Acknowledgements

All the authors of present manuscript are thankful to Yunnan Health Training Project of High Level Talents (L-2017013) and an open topic project of Neurology Clinical Medical Center in the First People's Hospital of Yunnan Province (2021LCZXXF-SJ01) for providing necessary fund to conduct the present work.

Disclosure

The authors report no conflict of interest.

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