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Original article

Caffeic acid improves locomotor activity and lessens inflammatory burden in a mouse model of rotenone-induced nigral neurodegeneration: Relevance to Parkinson's disease therapy

Sawsan A. Zaitone[a,b,*], Eman Ahmed[c], Nehal M. Elsherbiny[d,e], Eman T. Mehanna[f], Mohammed K. El-Kherbetawy[g], Mohamed H. ElSayed[h], Duha M. Alshareef[i], Yasser M. Moustafa[b]

[a] Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Tabuk, Tabuk, Saudi Arabia
[b] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Suez Canal University, Ismailia, Egypt
[c] Clinical Pharmacology Department, Faculty of Medicine, Suez Canal University, Ismailia, Egypt
[d] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Tabuk, Tabuk, Saudi Arabia
[e] Department of Biochemistry, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt
[f] Department of Biochemistry, Faculty of Pharmacy, Suez Canal University, Ismailia, Egypt
[g] Department of Pathology, Faculty of Medicine, Suez Canal University, Ismailia, Egypt
[h] Department of Physiology, Faculty of Medicine, Ain Shams University, Cairo, Egypt
[i] Department of Pharmaceutics, Faculty of Pharmacy, University of Tabuk, Tabuk, Saudi Arabia

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ABSTRACT

Background: Caffeic acid phenethyl ester is found in honey bee propolis. It has immunomodulatory, anti-inflammatory and anti-cancer properties. Rotenone is a pesticide commonly used for inducing experimental Parkinson's disease (PD) due to complex I inhibition and microglia activating properties. The current study examined neuroprotective effect of caffeic acid against rotenone-induced neurodegeneration in groups of seven mice.
Methods: Mice received protective doses of caffeic acid (2.5, 5 or 10 mg/kg) daily and nine injections of rotenone (1 mg kg, subcutaneously) - every 48 h. Behavioral evaluation of motor function was done by a battery of tests including open-field test, cylinder test, pole test and rotarod test; all these tests showed motor impairment.
Results: Assay of striatal dopamine highlighted a significant decrease and increases in inflammatory markers. In addition, histopathological assessment of substantia nigra neurons demonstrated low immunostaining for tyrosine hydroxylase (TH) in rotenone treated mice. PCR analysis highlighted upregulation for genes encoding $CD_{11}b$ (a microglia surface antigen), cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS) and nuclear factor-κB (NFκB). Treatment with caffeic acid (5 or 10 mg/kg) amended most of rotenone-induced motor deficits, lessened microglia expression and inflammatory mediators and improved the nigral TH immunostaining.
Conclusion: These results confirmed the anti-inflammatory activity of caffeic acid and highlighted its neuroprotective activity against rotenone-induced neurodegeneration in mice.

Introduction

Some epidemiological studies propose that people expose to pesticides, such as rotenone, may have high chance of developing PD [1]. Chronic inflammation is now assumed to perform a crucial role in the progressive nature of PD through inflammatory-induced neurodegeneration of nigrostriatal dopaminergic neurons [2,3].

Rotenone produces specific inhibition for mitochondrial complex I. Recent evidences suggest rotenone as a model offering

* Corresponding author. Current address: Faculty of Pharmacy, University of Tabuk, Tabuk, Saudi Arabia.
E-mail addresses: szaitone@ut.edu.sa, sawsan_zaytoon@pharm.suez.edu.eg (S.A. Zaitone).

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more benefits over the other experimental models of PD. Rotenone efficiently mimics the behavioral, neurologic as well as pathological features of PD mediated by the selectivity toward dopaminergic neurons [4]. Loss of dopamine leads to motor dysfunctions such as bradykinesia and rigidity [5,6]. Therefore, rotenone model of PD is thought to faithfully recapitulate the neuropathologic and phenotypic features of PD [7].

Microglia, being the brain resident immune cells, are activated by neurotoxins, such as rotenone, leading eventually to worsening of neurotoxicity [8,9] producing a variety of inflammatory mediators including inflammatory cytokines, inducible nitric oxide synthase (iNOS) and nitric oxide [10–12]. Since a current cure for PD does not exist, the keystone of treatment is relieving of symptoms mainly through replacement of dopamine [13].

Caffeic acid is a promising compound for treating neurodegenerative disorders and many other conditions of organ damage as in lung, kidney and liver [14–18]. A promising neuroprotective effect may be expected based on its reported anti-inflammatory activities.

The objectives of the current experiment are to examine the role of caffeic acid in modulating the locomotor dysfunction and microglia activity in rotenone Parkinsonian mice. This aim was achieved through monitoring the motor function by a battery of behavioral tests in addition to exploring the biochemical and histopathologic background for the nigrostriatal system.

Materials and methods

Chemical agents and drugs

Caffeic acid phenethyl ester and rotenone were bought from Sigma-Aldrich (USA). Rotenone was liquified in sunflower oil and used for subcutaneous injection. Caffeic acid preparation was prepared for oral administration by gastric gavage. It was dissolved in sun flower oil to give a stable preparation that can be kept in the refrigerator and used frequently. Three different bottles were labeled as preparation A, B and C (concentration equals 0.025%, 0.05% and 0.1%) and were used to deliver the specified doses to mice in group 3, 4 and 5, respectively.

Animals and experimental conditions

Thirty-five Swiss male albino mice, with body weight 25 ± 5 g, were used to conduct this study. Mice were obtained from Moustafa Rashed Company for experimental animals (Giza, Egypt). Mice were placed in polyethylene plastic cages in groups of 7 mice/cage. Housing was done under hygienic laboratory conditions and normal light-dark cycle with unrestricted access to food and tap water. The protocol of this study was approved by the Research Ethics Committee of Faculty of Pharmacy, Suez Canal University [license number 201706RA1]. The experimental protocol complied with the ARRIVE guidelines and NIH guide for the care and use of laboratory animals.

Study design

Rotenone injection leads to replication of many aspects of the motor and pathological findings of human PD following a previously reported schedule (1415). Random assigning for mice was done into 5 groups -7 mice in each group- as follows. Group (1): Vehicle group: mice received sunflower oil (10 mL/kg, *sc*) every 48 h for a total of 9 injections. Group (2): Rotenone group received rotenone (1 mg/kg, *sc*) every 48 h for a total of 9 injections (volume equals 10 mL/kg). Group (3–5): Mice received rotenone in addition to doses of caffeic acid (2.5, 5 or 10 mg/kg, *po*) every 48 h [19]. Caffeic acid phenethyl ester was given in day 1,3,5,7,9,11,13,15 and 17. Whereas rotenone was injected in days 2,4,6,8,10,12,14,16 and 18. Locomotor function was assessed using a battery of behavioral tests on day 19.

Assessment of locomotor function

Animals were moved while in the home cages to the room of the behavioral platform in the 2 h before beginning of the experiments. The tests were done in the following sequence from 4 to 10 p.m., *i.e.* at the period of normal rodent activity. Behavioral analysis was done by an investigator blinded to the tested groups.

Testing locomotor activity in the open-field test

A plexiglass arena was employed for the assessment of locomotor behavior and non-forced ambulation as shown previously [20]. Mice were introduced individually in the middle of a square arena made of dark plexiglass ($60 \times 60 \times 30$ cm) with a floor painted to form equal squares. Mice were tracked for 5 min and assessed for number of crossed squares, number of stops and rearing (vertical activity) of animals. The number of rears was calculated here as a representation of both exploratory and locomotor behavior [21]. In addition, calculation of the activity index was attained by dividing the total number of traversed squares over the number of stops, reflecting the length of the locomoting intervals [22].

Spontaneous activity in the cylinder test

Mice were put individually in a transparent cylinder. Spontaneous activity was evaluated for a period equals 3 min and number of forelimb and hindlimb steps were counted. Stepping ratio was assessed according to Fleming et al. [23] after doing a little modification. Ratio was calculated by dividing forelimb/hindlimb steps. High ratio will give an evidence for motor dysfunction and difficulty to complete a successful move.

Pole test

Pole test is commonly used to evaluate mice locomotor disorders due to depletion of striatal dopamine [24]. It is a sensitive test for motor dysfunction and usually used to assess the function of the basal ganglia. A wooden pole of 50-cm length was used, and its base was put in the home cage while tilted at about 40-degree angle at the wall side. Mice were sited while their head upwards on the uppermost part of the pole and try to position themselves down and descend back through the length of the pole and reach the cage.

Fixed speed rotarod test

This test measures motor coordination and requires previous training for mice – before starting the experiment – to ensure the ability to accommodate with the rotating rod. The wooden rod is 3-cm in diameter and was fixed at 30-cm above the bench and the rotation speed was constant at 18 rpm. The falling time in seconds is used as a valuable indicator for motor incoordination and the cut-off time was set at 3 min.

Scarification and dissection of the mice brains

Mice were anesthetized and killed through performing a cervical dislocation. Craniotomy was performed to dissect the brains which were immediately washed with cold saline. Midsagittal dissection of the brains into two halves was done, one hemisphere was rapidly frozen by storage at $-80\,^{\circ}$C for subsequent measuring of striatal parameters using ELISA or PCR and for DNA laddering assays. Homogenization was done in phosphate-buffered saline (PBS, pH = 7.4) by a Teflon homogenizer (Glas-Col homogenizer system). The striatal homogenates were transferred into a centrifuge set at 1500 ×g for 15 min. After that, supernatants were kept at −20 °C and used later in ELISA assays. The other hemisphere was fixed overnight in 4% paraformaldehyde, midbrain region identified and then coronally sliced into 2 mm thick slices, identified according to mouse brain atlas, (Bregma −3.88 to −2.78) [25], each processed and embedded in hard paraffin.

DNA laddering assay

Extraction of genomic DNA from the mice striata by Wizard® Genomic DNA Purification kit (Promega, Madison, USA). The concentration and purity of DNA were checked by a Nanodrop® NA-1000 UV/Vis ThermoFisher spectrophotometer (Wilmington, DE, USA). After purification, DNA was subjected to horizontal agarose gel electrophoresis where 10 μL of each sample was mixed with a 2-μL sample of loading dye (ThermoFischer Scientific, Massachusetts, USA) and loaded onto 2% agarose gel stained using ethidium bromide. After that, the DNA ladder was visualized by ultraviolet trans-illumination.

Measurement of striatal dopamine and inflammatory cytokines level

The striatum was dissected, and a homogenate was prepared as described above. The level of dopamine was measured by a double-antibody sandwich ELISA kit for mouse dopamine (Shanghai Crystal Day Biotech Co., Ltd, China). In addition, the striatal homogenate was used to determine tumor necrosis factor-α (TNF-α) using an *in vitro* ELISA kit from RayBiotech Inc. (Norcross, GA, USA) for the quantifiable measurement of TNF-α. A sandwich ELISA kit was used for quantitative detection of interlukin-1β (IL-1β) that was purchased from MyBioSource (CA, USA). Furthermore,

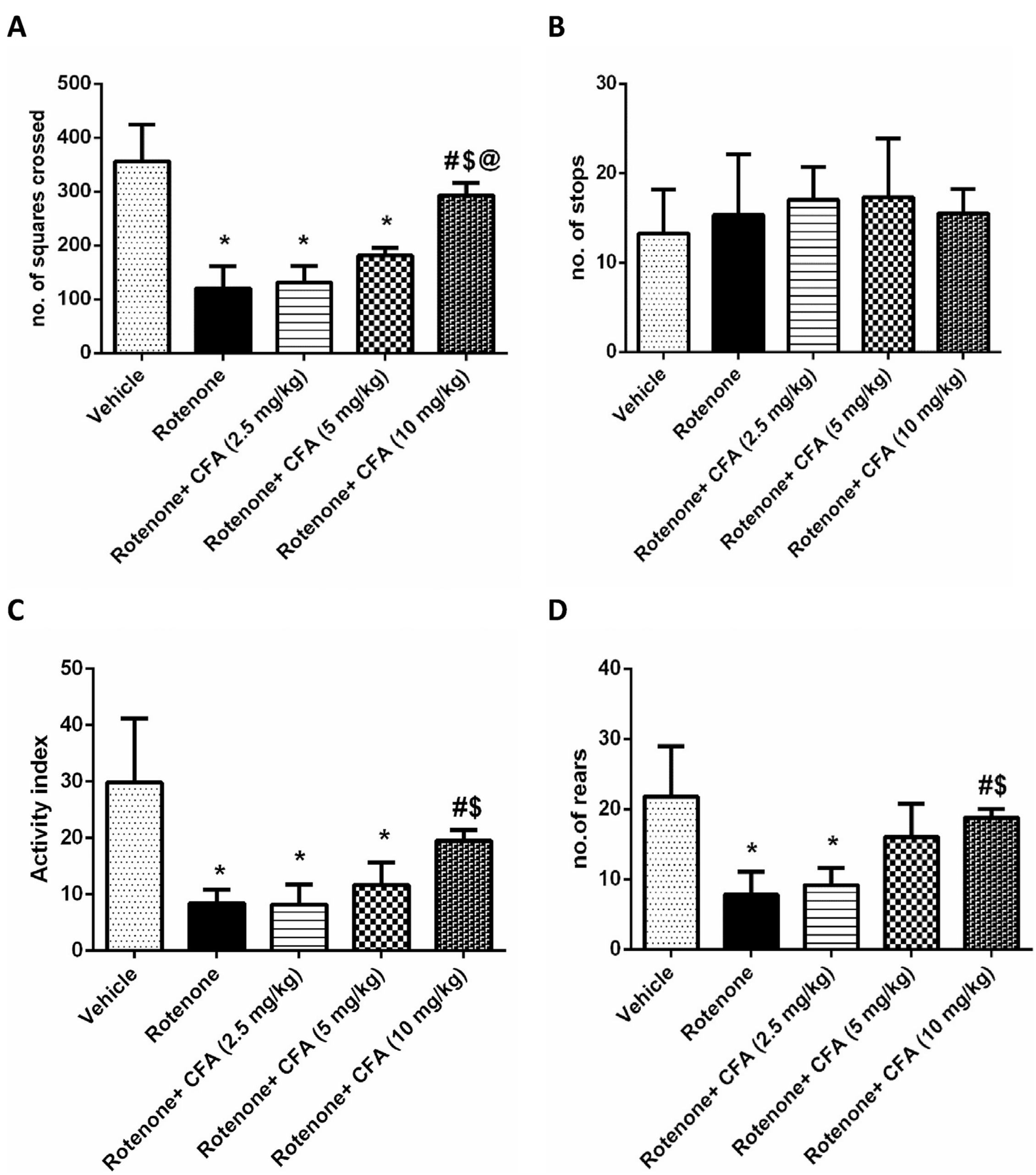

Fig. 1. Effect of caffeic acid on locomotor activity in the open-field test. A) Number of squares, B) number of stops, C) activity index and D) number of rears registered in the open-field test. CFA: caffeic acid. Data are mean ± SD, analysis was done using one-way analysis of variance followed by the Bonferroni's *post-hoc* test. **versus* the vehicle, #*versus* rotenone group, $*versus* rotenone + CFA (2.5 mg/kg) group, @ *versus* rotenone + CFA (5 mg/kg) group, *n* =.6–7.

COX-2 level was measured using a double-antibody sandwich ELISA kit (Glory Bioscience Co. Ltd, USA). Striatal iNOS level was determined using a sandwich-based ELISA kit from Ray-Bio (Norcross, GA, USA, Cat # PEL-iNOS-Y). The assay of NFκB was performed using an ELISA kit from Abcam (Cat# ab176648).

Quantitative analysis of gene expression by real time PCR

Polymerase chain reaction assay was done for determining mRNA expression of $CD_{11}b$, iNOS, COX-2 and NFκB.

Extraction of total RNA

Extraction of total RNA from tissue homogenate was done using SV Total RNA Isolation System from Promega (Madison, WI, USA) according to the instructions of the manufacturer. An ultraviolet spectrophotometer was used for determination of RNA concentrations and purity.

Synthesis of cDNA was done from 1 μg RNA using SuperScript III First-Strand Synthesis System as defined by the manufacturer (#K1621, Fermentas, Waltham, MA, USA). Real-time PCR amplification and analysis were done by the aid of an Applied Biosystem with software version 3.1 (StepOne^{TM}, USA). The reaction contained SYBR Green Master Mix (Applied Biosystems) and gene-specific primer pairs were planned with Gene Runner Software (Hasting Software, Inc., Hasting, NY, USA) from RNA sequences from the gene bank. Calculation of data from real-time assays was done by the v1·7 sequence detection software from PE Biosystems (Foster City, CA, USA). Relative expression of the gene mRNA was determined employing the comparative Ct method. Values were normalized to beta actin and registered as fold change over background levels detected in the control group.

Immunohistochemical analysis

The paraffin imbedded tissues were sectioned (4-μm) and dried for an overnight at 37 °C. Two subsequent sections from each block were then deparaffinized, rehydrated and processed to antigen retrieval using Tris-EDTA at pH = 9 and then primary purified rabbit anti-phospho-tyrosine TH (1:800, R&D Systems Inc., USA) were added to the specimens and allowed to incubate for an overnight at 4 °C. After this process, biotinylated secondary antibodies were added for an hour, and the reaction was detected by Power-Stain™ 1.0 poly HRP-3,3′-diaminobenzidine tetrachloride (DAB) kit from Genemed Biotechnologies (CA, USA). After counterstaining using Mayer's hematoxylin and covered. The TH immunostained sections were examined and the boundaries of the substantia nigra pars compacta in each tissue section were outlined and imaged at × 100 magnification. Photography was performed using an Olympus CX21 light microscope (Tokyo, Japan). Then, images from the 2 sections were analyzed for TH positive neurons (neurons with visible nuclei and clear staining for TH) and averaged. Counting was done using ImageJ software (NIH, USA) in a blind manner.

Statistical analysis of the data

The statistical tests were made applying GraphPad Prism software version 6.00 (GraphPad Softwares Inc., USA). Quantitative data with Gaussian distribution are expressed as means ± standard deviations (SD). The statistical differences between groups were judged by one-way analysis of variance with subsequent application of Bonferroni's *post-hoc* test. However, data showed non-Gaussian distribution are presented as boxplots and quartiles. Statistical analysis was performed by Kruskal-Wallis test followed by Dunn's test. Data are two-tailed and all probable assessments between the groups were considered. The percent of survival in the groups was compared by the aid of the Chi-square test. The differences were considered significant when value of $p<0.05$.

Results

At the end of day 18, survival % in each of the study groups was determined. Vehicle treated group showed 100% survival (7 out of 7 mice), however, the other study groups showed 85.7% survival % (6 out of 7 mice). Analyzing these data using Chi-square test should non-significant differences between all the study groups and the vehicle control group.

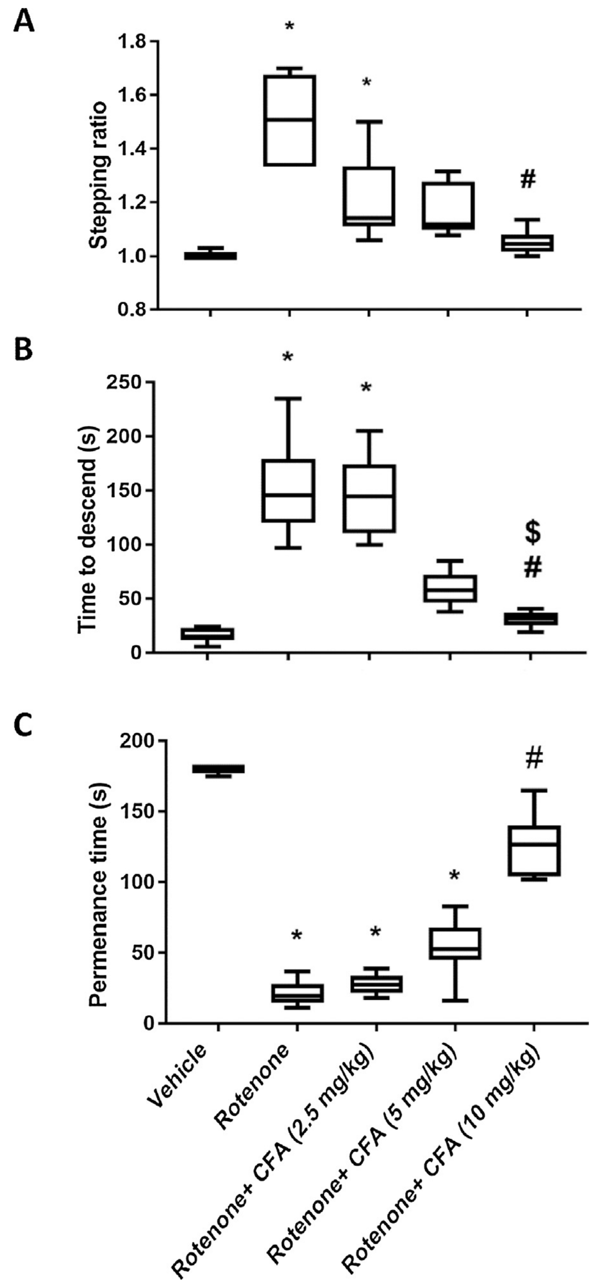

Fig. 2. Effect of caffeic acid on locomotor dysfunction in the cylinder test, pole test and rotarod test. Mice were monitored in the cylinder test to calculate forelimb/hindlimb steps ratio (A). In pole test, the time consumed by the mouse to descend to the home cage is determined (B). The rotarod permeance time was calculated with a cut-off time equals 180 s (C). CFA: caffeic acid. Data are in boxplots representing medians and quartiles for the study groups. Analysis was done applying Kruskal-Wallis test followed by Dunn's test for multiple comparisons at p < 0.05. *versus the vehicle #versus rotenone group and $versus rotenone + CFA (2.5 mg/kg) group, *n* =.6–7.

Motor function assessment

Rotenone injected mice exhibited decreased locomotor activity in the open-field test designated by the low number of squares traversed in the arena compared to the vehicle group (120.5 ± 40.92 *vs.* 356.5 ± 68.26, Fig. 1A). Caffeic acid (2.5 or 5 mg/kg) did not increase the number of squares crossed by mice *versus* rotenone group. However, the large dose (10 mg/kg) produced an increment in the number of squares *versus* rotenone group, and *versus* the lower doses of caffeic acid (2.5 or 5 mg/kg) (Fig. 1A). Regarding number of stops, rotenone group was not significantly different from the vehicle group (15.33 ± 6.77 *vs.* 13.25 ± 4.92) and one-way analysis of variance did not display a significant change among the study groups (Fig. 1B). Further, rotenone group showed lower activity index (8.44 ± 2.39 *vs.* 29.79 ± 11.35) and rearing behavior (7.8 ± 3.27 *vs.* 3.27) *versus* vehicle group ($p<0.05$). Caffeic acid in the high dose (10 mg/kg) increased the activity index and rears *versus* rotenone group and *versus* rotenone + caffeic acid (2.5 mg/kg) group (Fig. 1C and D).

In cylinder test, rotenone treated mice presented a significant rise ($p<0.05$) in the stepping ratio compared to mice received the vehicle, indicating greater usage of the forelimb than the hindlimb (median = 1.5 *vs.* 1 in the vehicle group). Administration of caffeic acid (10 mg/kg) decreased ($p<0.05$) stepping ratio *versus* rotenone group (Fig. 2A). Regarding the pole test, rotenone control mice showed prolonged latency to orient downwards *versus* vehicle (median = 146 s *vs.* 15 s in the vehicle group); reflecting poor coordination in rotenone treated mice. Caffeic acid (10 mg/kg) shortened the latency to orient downwards ($p<0.05$) *versus* rotenone group (Fig. 2B). Rotarod test indicated that rotenone-treated mice displayed a shorter permeance time than the vehicle group. Treatment with caffeic acid (10 mg/kg) prolonged the permanence time *versus* rotenone group (Fig. 2C).

DNA laddering

Fig. 3 shows that caffeic acid (5 mg/kg) markedly decreased the striatum nuclear DNA fragmentation induced by rotenone. Treatment with 10 mg/kg of caffeic acid completely abolished the smear indicative of DNA damage producing an intact band of nuclear DNA.

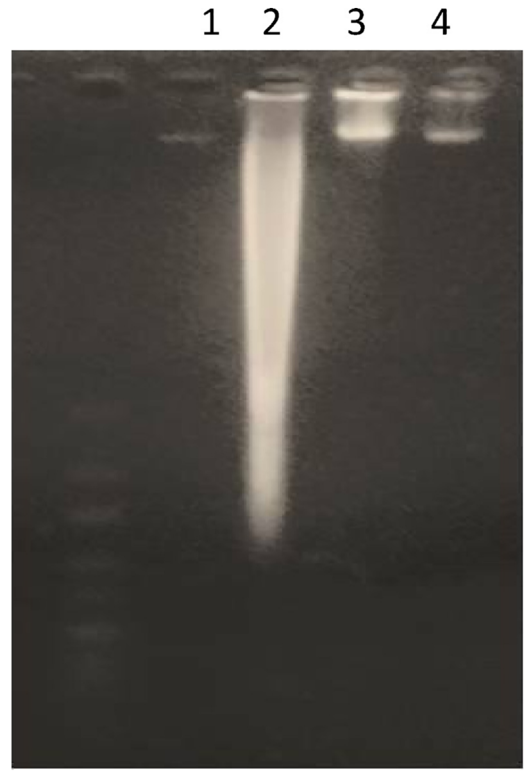

Fig. 3. Agarose gel electrophoresis for DNA from striatal specimens. DNA samples were loaded onto agarose gel 2% (w/v) and 1 h electrophoresis was performed at 90 V and 110 mA using a 100 bp ladder. Internucleosomal DNA fragmentation was detected with mixed smearing. DNA isolated from mice kidneys treated with the vehicle [Lane 1] and rotenone group [Lane 2], rotenone + CFA (5 mg/kg) [Lane 3] and rotenone + CFA (10 mg/kg) [Lane 4].

Striatal dopamine and inflammatory markers

The current model showed low content of striatal dopamine in rotenone group in contrast to the vehicle group (53.33 ± 8.16 *vs.* 198 ± 61.87). A protective dose of caffeic acid (10 mg/kg) improved dopamine level (Fig. 4A). Measuring of inflammatory markers, TNF-α and IL-1β, highlighted greater values (approximately 2-fold and 3-fold increases) in rotenone group in contrast to the vehicle group. Treatment with caffeic acid (10 mg/kg) reduced the elevated levels of these cytokines (Fig. 4B and C). Additionally, measuring striatal COX-2, iNOS and NFκB by ELISA showed greater values in rotenone group compared to the vehicle (3.28-fold, 10.82-fold and 3.2-fold increases, respectively, Fig. 4D-F). The two higher doses of caffeic acid (5 or 10 mg/kg) downregulated the expression of striatal COX-2 and NFκB in comparison to rotenone control group. Further, all the tested doses of caffeic acid (2.5, 5 or 10 mg/kg) were able to downregulate the expression of iNOS.

Immunohistochemistry for tyrosine hydroxylase (TH)

Immunostaining for TH located the dopaminergic neurons and showed their distribution being aggregated and abundant in the vehicle group while dispersed and decreased number in the rotenone group (84.86 ± 21.17 *vs.* 7.72 ± 4.34). However, mice treated with caffeic acid (5 or 10 mg/kg), showed a dose-dependent rise in the number of TH immunostained neurons in contrast to rotenone control group and restored their distribution (Fig. 5).

PCR analysis for $CD_{11}b$, iNOS, COX-2 and NFκB

The expression of $CD_{11}b$, iNOS, COX-2 and NFκB genes increased significantly in rotenone group (7.6-fold, 9.8-fold, 7.73-fold and 8.3-fold, respectively) in comparison to the vehicle group. Further, caffeic acid doses differently affected the expression of these genes; the 5 mg/kg dose downregulated the expression of iNOS, COX-2 and NFκB genes without affecting expression of $CD_{11}b$. However, the highest dose of caffeic acid (10 mg/kg) downregulated the expression of the four genes in contrast to rotenone control group (Fig. 6).

Discussion

Some experiments have demonstrated that caffeic acid has attenuated 6-hydroxy-dopamine (6-OHDA) neurotoxicity tested *in vitro* [26,27] and *in vivo* [14], as well as N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced harm in dopamine producing neurons in mice [16]. In addition, the conclusions of this study highlighted that caffeic acid protected against neurotoxic effect of rotenone mouse model of PD. Neuroprotection is revealed through the noticeable locomotor and coordination improvements after caffeic acid administration. These findings are supported by the rise in striatal dopamine, the key factor in PD pathogenesis, together with the reduction in microglial activation.

Caffeic acid dissolved in sunflower oil to provide a stable preparation and was administered systemically *via* oral gavage in 3 doses along with subcutaneous injections of rotenone. However,

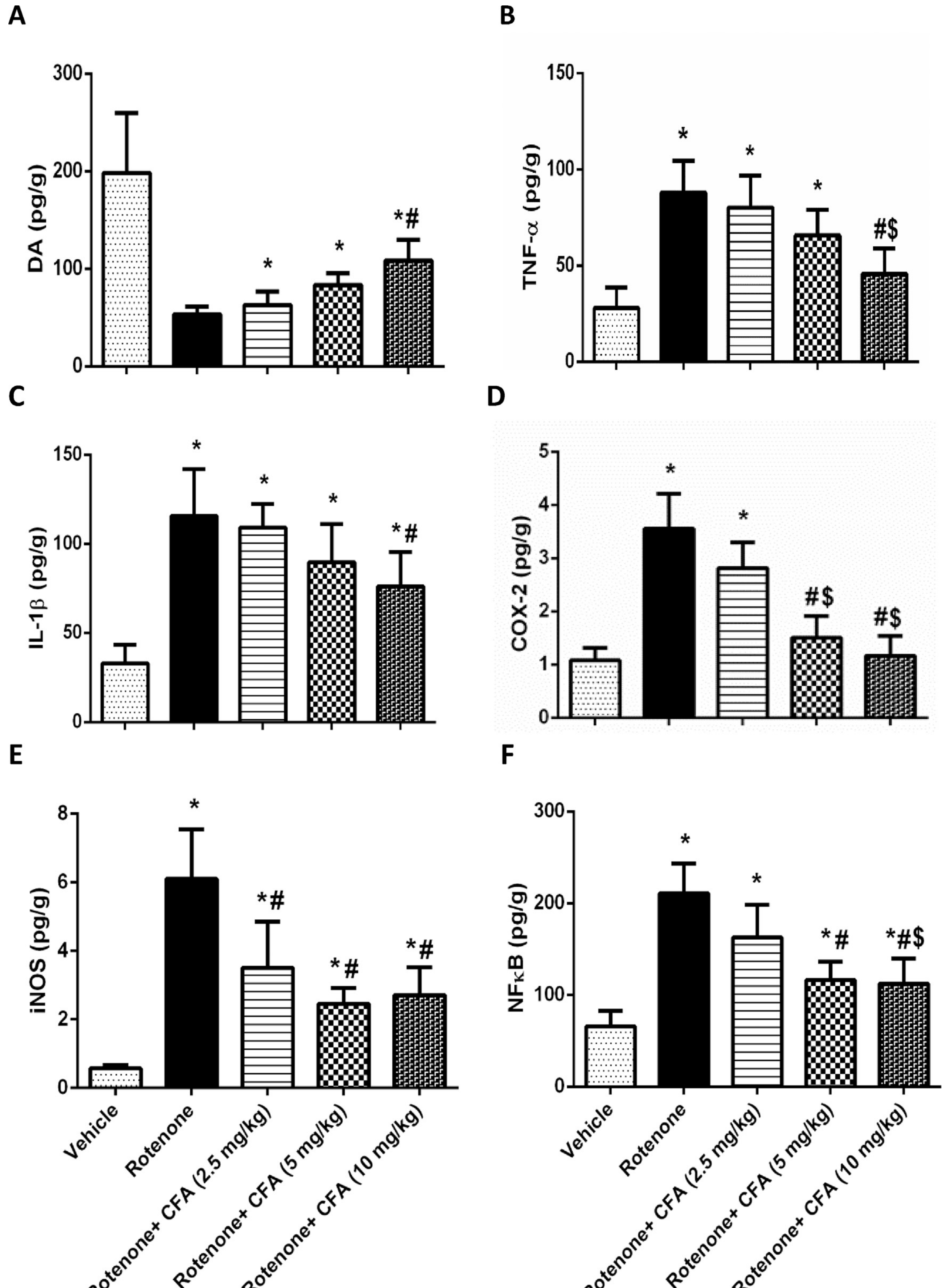

Fig. 4. Effect of caffeic acid on the striatal level of dopamine and inflammatory markers in parkinsonian mice. A) dopamine, B) TNF-α, C) IL-1β, D) COX-2, E) iNOS and F) NFκB measured by ELISA in striatal homogenates. DA: dopamine, TNF-α: tumor necrosis factor-α, IL-1β: interlukin1β, COX-2: cyclooxygenase-2, iNOS: inducible nitric oxide synthase, NFκB: nuclear factor-κB. Data are mean ± SD, analysis was done using one-way analysis of variance followed by the Bonferroni's *post-hoc* test. **versus* the vehicle, #*versus* rotenone group and $*versus* rotenone + CFA (2.5 mg/kg) group, *n* =.6–7.

some studies have used caffeic acid orally but as a supplementation added to food [28] or drinking water [29].

A clear parkinsonian phenotype was produced by rotenone that was highlighted by decreased motor functions in the open-field test; this took place in harmony with previous studies which reported induction of motor deficits by rotenone in animals in open-field test [30–32]. In addition, rotenone treated mice showed difference in hindlimb and forelimb mobility in the cylinder test. Similar motor dysfunctions were

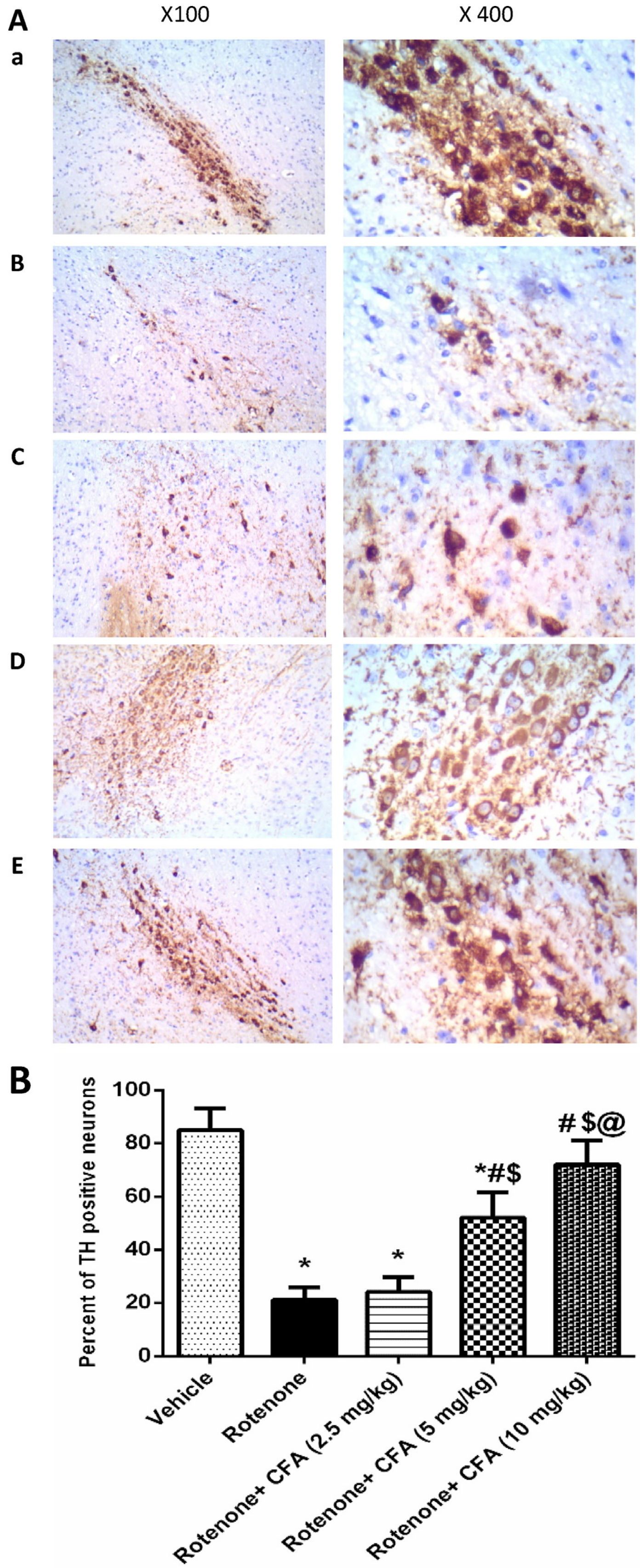

Fig. 5. Effect of caffeic acid on the immunostaining for tyrosine hydroxylase in parkinsonian mice. A) photomicrographs for TH positive neurons in experimental groups and B) Column chart for mean value of percent of positive neurons. Images were analyzed using ImageJ program to count the TH positive neurons. CFA: caffeic acids, TH: tyrosine hydroxylase. Data are mean ± SD, analysis was done using one-way analysis of variance followed by the Bonferroni's *post-hoc* test. **versus* the vehicle, #*versus* rotenone group, $*versus* rotenone + CFA (2.5 mg/kg) group and $*versus* rotenone + CFA (5 mg/kg) group, *n* =.6–7.

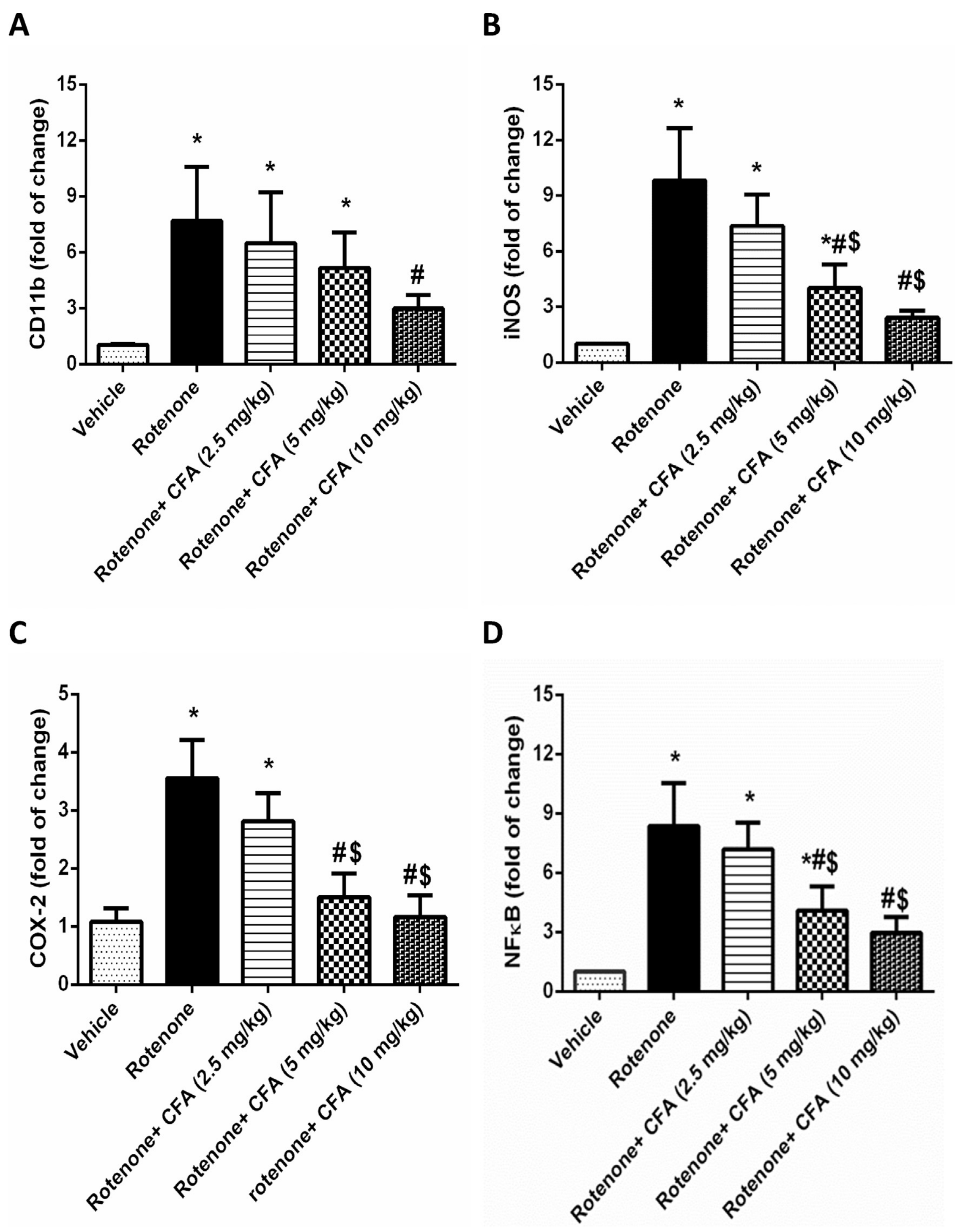

Fig. 6. Effect of caffeic acid on the striatal expression of $CD_{11}b$, iNOS, COX-2 and NFκB in parkinsonian mice. A) $CD_{11}b$, B) iNOS, C) COX-2 and D) NFκB. CFA: caffeic acid, $CD_{11}b$: cluster of differentiation 11b, iNOS: inducible nitric oxide synthase, COX-2: cyclooxygenase-2, NFκB: nuclear factor-κB. Data are mean ± SD, analysis was done using one-way analysis of variance followed by the Bonferroni's *post-hoc* test. * *versus* the vehicle, # *versus* rotenone group and $ *versus* rotenone + CFA (2.5 mg/kg) group, n = 6.

reported by previous PD rotenone rodent models [33]. In accordance with some previous studies [34–36], rotenone produced poor coordination in animals tested in rotarod test as well as pole test.

These behavioral changes observed in the current study were associated with decreased striatal dopamine level, similar to what was previously reported [37–39]. However, one study was carried few years ago and showed that acute and sub-chronic rotenone injection in mice did not produce a decline in dopamine concentration in the striatum [40]. This can be explained as that rotenone toxicity to nigrostriatal dopaminergic neurons varies according to different experimental conditions including dose and duration of exposure. Caffeic acid in the highest dose was able to elevate dopamine levels in striatum.

Several studies support the theory that PD pathogenesis is mainly an inflammatory process. Induction of NFκB has been documented in basal ganglia of MPTP-treated [41] and rotenone-treated rodents [42]. The NFκB activation is followed by stimulation of gene transcription of many proinflammatory mediators in the microglia including iNOS, IL-1β, COX-2 and TNF-α [43,44]. In this study, the expression of NFκB was greater in rotenone-treated mice; which is normally associated with neuronal destruction. A decline in NFκB gene expression was noticed in mice treated with higher doses of caffeic acid. One previous study concluded that caffeic acid phenethyl ester modulated NFκB and suppresses acute inflammation [45]. Consistently, results of another study demonstrated that caffeic acid is a powerful inhibitor for NFκB activation; this may explain its various anti-inflammatory and immunomodulatory activities [46].

In line with this, the data of the current study suggest that neuronal supportive cells as microglial cells have primary role in rotenone-induced neurodegeneration; a marked rise in the expression of CD_{11}b and the proinflammatory mediators iNOS and COX-2 as well as increased protein levels of TNF-α and IL-1β were distinguished in rotenone-treated group and this came on line with one previous study [42]. Caffeic acid in the higher 2 doses brought the values of iNOS and COX-2 back near to normal, whereas the highest dose only was able to markedly lower the values of TNF-α, IL-1β as well as gene expression of CD_{11}b. Thus, caffeic acid which is previously acknowledged for its anti-inflammatory properties [47], is able to halt the inflammatory burden in rotenone-treated mice. A previous study documented that the biosynthesis of leukotriene C4 and D4 in mouse mast tumor cells was repressed entirely by caffeic acid; this may add to its anti-inflammatory effect [48]. Furthermore, anti-inflammatory actions of caffeic acid in the carrageenan air pouch inflammation model in rats and in cultured human oral epithelial cells was linked to COX-2 inhibition [49].

Repeated rotenone injection resulted in histopathological abnormalities with low viability in TH immunostaining. Previous studies highlighted nigral neurodegeneration in rodents injected with rotenone [50,51]. Caffeic acid protected against these histopathological abnormalities and led to increased number of surviving neurons. Similarly, caffeic acid was reported to provide neuroprotection in animal models od neuronal injury such as pentylenetetrazole-induced seizures [52], 5-S-cysteinyl-dopamine induced neurotoxicity [53], 6-OHDA neuronal loss [14] and MPTP neurodegeneration [16]. Another example from neurodegenerative disease highlighted that caffeic acid provides protection for the PC12 cells against toxicity induced by β-amyloid peptide [54].

Taken together, our data gave an evidence that caffeic acid lessened the inflammatory burden in the rotenone model of PD; this involved suppression of microglia cells and downregulation of the inflammatory mediators such as COX-2, iNOS and NFκB. These favorable effects contributed, at least partly, to the neuroprotective activity of caffeic acid and the improvements registered in locomotor activity.

Disclosure of interest

Authors declare no conflicts of interests.

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