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ORIGINAL ARTICLE

Methyl jasmonate ameliorates rotenone-induced motor deficits in rats through its neuroprotective activity and increased expression of tyrosine hydroxylase immunopositive cells

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Abstract

Decreased tyrosine hydroxylase (TH) activity, due to degeneration of dopaminergic neurons contributes to the low dopamine content and the motor deficits that characterized Parkinson's disease (PD). This study examines the effect of methyl jasmonate (MJ), a neuroprotective bioactive compound isolated from jasminum grandiflorum, on motor functions, immunopositive cells of TH, dendritic neurons and dopamine contents in rotenone (Rot)-treated rats. Rats pretreated daily with MJ (100 mg/kg, i.p) for 21 days also received Rot (2.5 mg/kg, i.p.) 30 min after each pretreatment for every 48 h for 21 days. Motor functions were assessed on day 22. The specific brain regions of the rats were processed for determination of dopamine contents, immunopositive cells of TH, neuronal cell morphology and dendritic aborizations. Rot impaired locomotion and rearing behavior, and decreased dopamine content in the striatum, prefrontal cortex and midbrain. It further reduced the expression of TH in the substantia nigra and striatum relative to vehicle-control ($p < 0.05$). Histopathologic studies revealed that Rot-treated rats had degenerated neurons with pyknotic nuclei and loss of nigrostriatal neuronal cells. Rot also altered the nigrostriatal dendritic neuronal networks, decreased the dendritic length and spine density. However, pretreatment with MJ improved motor deficits, increased TH activity and dopamine contents in the specific brain regions of Rot-treated rats. MJ also attenuated the cyto-architectural distortions, loss of neuronal cells and dendritic aborizations of the striatum of Rot-treated rats. These findings suggest that MJ may reverse the motor deficits associated with PD by modifying the key pathological abnormalities involved in the disease progression.

Keywords Methyl jasmonate · Rotenone · Motor deficits · Tyrosine hydroxylase · Dendritic neuronal aborizations

Introduction

The deficiency of dopamine in the brain of patients with Parkinson's disease (PD) has been implicated in the genesis of the debilitating motor symptoms associated with this neurodegenerative disorder (Nishijima et al. 2018). The loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and the consequent degeneration of their projecting nerve fibers in the striatum have been closely linked with the shortage of striatal dopamine concentration in parkinsonia patients (Nishijima et al. 2018). The decrease in tyrosine hydroxylase (TH), due to degeneration of dopaminergic neurons may contribute to the drastic falls in dopamine concentration and the manifestations of the clinical motor symptoms encountered by patients with PD (Haavik and Toska 1998; Tabrez et al. 2012; Johnson et al. 2018). Indeed, the use of neurotoxin animal models has provided a close association between the activity of TH, striatal dopamine and the manifestations of the

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motor symptoms of PD (Sherer et al. 2003a; Johnson et al. 2018). Rotenone is one of the best neurotoxins widely used to induce the biochemical, anatomical, and behavioral features similar to those observed in PD patients (Alam and Schmidt 2002; Dhanalakshmi et al. 2016). Rotenone is a known potent inhibitor of mitochondrial electron transport chain complex-I that leads to the production of free radicals resulting in oxidative stress-mediated neuroinflammation that orchestrate the death of dopaminergic neurons (Sherer et al. 2003a; Gao et al. 2003; Hirsch and Hunot 2009). Previous studies have established that deficit in motor function induced by rotenone was accompanied by decreased immunopositive cells of TH, the rate-limiting enzyme in the biosynthesis of dopamine (Sherer et al. 2003b; Johnson et al. 2018). This finding was further supported by a significant decrease in dopamine concentration in the striatum of rotenone-treated rats (Sherer et al. 2003a, b; Johnson et al. 2018). Decrease in TH and dopamine concentrations particularly in the striatum and midbrain has been consistently implicated in the motor symptoms in parkinsonian patients and in experimental animals exposed to neurotoxins (Nagatsu 1990; Haavik and Toska 1998; Sherer et al. 2003a, b; Azmy et al. 2018). However, compounds with neuroprotective effect have been shown to prevent the loss of dopaminergic neurons and TH-positive cells induced by dopaminergic neurotoxins (Tabrez et al. 2012; Dhanalakshmi et al. 2016; Johnson et al. 2018; Azmy et al. 2018). These findings further reinforced the current notion that existing pharmacological therapies for PD are grossly inadequate, as they cannot alter the pathological features associated with the disease (Bezard et al. 2001; Johnson et al. 2018; Nishijima et al. 2018). These drugs only provide symptomatic relief while the disease progresses unabated and sometimes worsen despite therapeutic interventions (Bezard et al. 2001; Tabrez et al. 2012; Johnson et al. 2018). Thus, an alternative approach focused on the use of phytochemicals that can offer neuroprotection by virtue of their anti-oxidative, anti-inflammatory, and anti-apoptotic activities are being investigated as potential candidates for the treatment of PD (Hirsch and Hunot 2009; Tabrez et al. 2012; Dhanalakshmi et al. 2016; Azmy et al. 2018).

Methyl jasmonate (MJ), known as methyl 3oxo-2-(2-pentenyl) cyclopentaneacetate (Fig. 1) was first isolated from the essential oil of jasmine plant (*Jasminum grandiflorum)* by Demole et al. (1962). MJ is well recognized as a hormone that helps plants to adapt to external stressors through the formation of defensive chemical substances that protect plants against a wide range of biotic and abiotic stressors (Bowles 1990; Cesari et al. 2014). Previous preclinical studies have

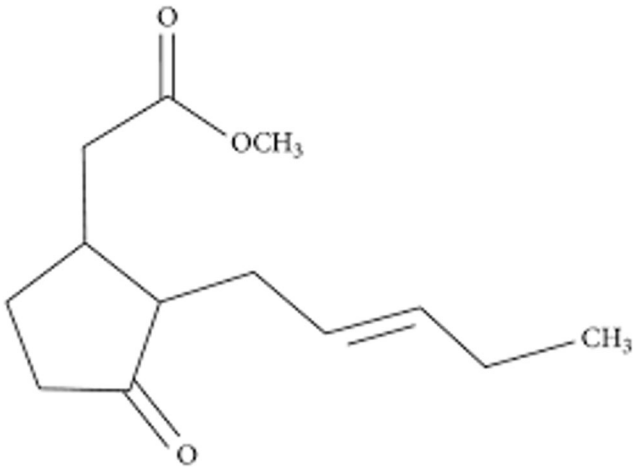

Fig. 1 Chemical structure of MJ (Bowles 1990)

shown that MJ and its derivatives exhibited potent anti-inflammatory property by decreasing the release of pro-inflammatory cytokines and down regulating NF-kB expressions in macrophage cells (Dang et al. 2006; Lee et al. 2011). In addition, we have also reported in our recent studies that MJ attenuated memory dysfunctions through mechanisms related to neuroprotection, decreased brain levels of biomarkers of oxidative stress and neuroinflammation in lipopolysaccharide-treated mice (Eduviere et al. 2016; Umukoro and Eduviere 2017). These findings further suggest the potential benefits of MJ in neurologic diseases like PD, whose pathological abnormality is strongly linked to oxidative stress-mediated degeneration of dopaminergic neurons. This present investigation is a part of our ongoing studies of the effects of MJ on rotenone-induced parkinsonia-like symptoms in rodents.

Materials and methods

Drugs and reagents

Methyl jasmonate (MJ) and rotenone (Rot) were obtained from Sigma Aldrich (Germany). Dopamine ELISA kit was purchased from Abnova (Germany). Tyrosine hydroxylase (TH) primary antibodies were products of Santa Cruz (USA).

Laboratory animals

Male Wistar rats (170–200 g) used in the study were purchased from the Central Animal House, University of Ibadan, Ibadan, Nigeria. They were acclimatized in the Department of Pharmacology and Therapeutics animal holding facility for 2 weeks. They were housed in polycarbonates fabricated animal cages with free access

to standard rodent pellet diet (Vital feeds®, Jos, Nigeria) and water ad libitum. The experimental procedures were carried out in accordance with the National Institutes of Health (NIH Publication No. 8523, revised 1981) Guidelines for the Care and Use of Laboratory Animals and approval was obtained from the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC/18/0055).

Preparation and selection of the dose of MJ

MJ was prepared according to the procedure earlier described (Eduviere et al. 2016). Briefly, MJ was dissolved in 95% ethanol (EtOH) and this solution was further diluted with distilled water. The final concentration of (EtOH) in the solution used for the study did not exceed 1%. Preliminary investigations revealed that 100 mg/kg of MJ was the most effective dose against rotenone-induced parkinsonian-like behaviors in mice hence this dose was used for this present study.

Treatments and experimental design

Rats were randomly distributed into four groups ($n = 7$). Rats in group 1, which served as control, were given vehicle [1% ethanol in sunflower oil (10 mL/kg, i.p)], group 2 also had vehicle (1% ethanol in sunflower oil (10 mL/kg, i.p), group 3 MJ (100 mg/kg in sunflower, i.p) while group 4 received levodopa-carbidopa (LD-CD) (10 mg/kg in sunflower; i.p) for 21 consecutive days. In addition, rats in group 2–4 also received Rot (2.5 mg/kg in sunflower oil, i.p.) 30 min after each treatment for every 48 h for 21 days (Fathalla et al. 2016). Treatments were terminated on day 21 and test for motor functions was done 24 h later (Betarbet et al. 2000) (Fig. 2).

Test for locomotor activity

The effect of MJ on locomotor activity and rearing behavior in rotenone-treated rats was carried out on day 22 using the Ugo Basile activity cage, Comerio, Italy. The rats were placed individually in the activity cage and the horizontal activity (locomotion) and vertical activity (rearing) counts were recorded for a period of 5 min.

Preparation of brain tissues for estimation of dopamine contents

Immediately after the behavioral test, the animals were anaesthetized with ether and the brains were removed for dissection. The prefrontal cortex, striatum and midbrain were dissected on cold iced tray, homogenized in cold sodium phosphate buffer (0.1 M, pH 7.4) and centrifuged at 10,000 rpm for 10 min at 4 °C. The supernatant of each brain region was aliquot and stored at -20 °C until use for determination of dopamine levels.

Estimation of brain dopamine level

The concentrations of dopamine (pg/g tissues) in the striatum, prefrontal cortex and midbrain tissues of rats were determined using ELISA kit (Abnova, Germany) according to the manufacturer's instructions.

Determination of brain tyrosine hydroxylase expression

Rats under deep anaesthesia with ether were perfused through the aorta with sterile normal saline and followed by a cold fixative consisting of sodium phosphate buffered formalin. Sections of the striatum and substantia nigra (20 μm thick) were cut using a

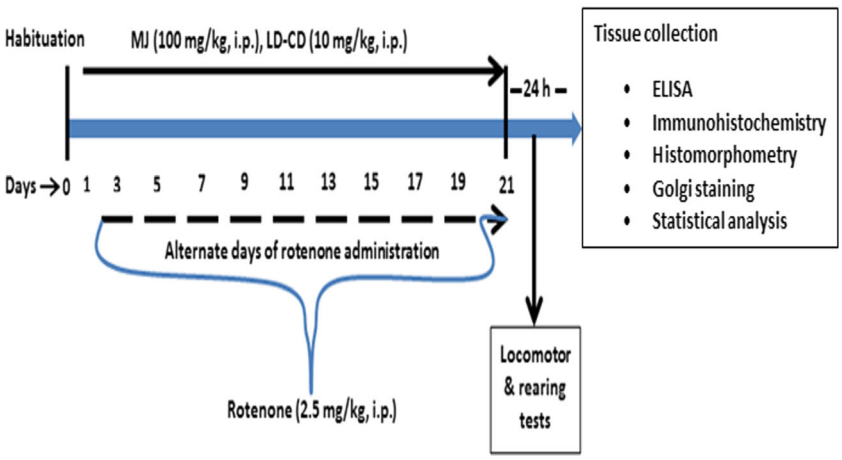

Fig. 2 Schematic presentation of the experimental design of the study

cryostat and collected in 100 mmol/L PBS containing 0.3% Triton X-100 (PBS-T). The tissues were processed on slides and incubated with the TH primary antibodies (diluted 1: 10000) for 3 days at 4 °C. After several washes, the sections were incubated with the appropriate biotinylated antibody to rabbit (1:2000) for 2 h at 23 °C. The sections were then incubated with avidin peroxidase for 1 h at 23 °C. Then, the sections were washed several times with PBS-T between each incubation, and labeling was revealed by 3,3′-diaminobenzidine (DAB) with hematoxylin counter staining. The photomicrographs of stained slides were acquired using Leica ICC50 E Digital Camera (Germany) connected to a computer interface (Magnafire) and an Olympus BX-51 Binocular research microscope. TH immunopositive cell expressions were defined and analyzed with the aid of Image J software (NIH, Bethesda, MD, USA).

Histopathological evaluation of the substantia nigra and striatum in rotenone-treated rats

Hematoxylin and eosin staining technique was used to evaluate the neuroprotective effect of MJ on viability of neuronal cells in the substantia nigra and striatum of Rot-treated rats. The fixed brain tissues were processed to obtain paraffin wax embedded tissue blocks, which was sectioned in the sagittal plane using microtome (Leica, Germany). The paraffin embedded blocks were trimmed, sectioned, and processed through the stages of fixation, dehydration, clearing, infiltration and staining using haematoxylin and eosin. Slides were viewed using Leica DM 500 digital light microscope (Germany) and images were captured with a digital camera (Leica ICC50 E, Germany) connected to a computer interface (MagnaFire). Numbers of viable neuronal cells were counted in the substantia nigra and striatum using Image J software (NIH, Bethesda, MD, USA).

Evaluation of dendritic arborisation of the substantia nigra and striatum of rotenone-treated rats

Golgi staining technique was used to further evaluate the neuroprotective effect of MJ on dendritic neuronal networks of the substantia nigra and striatum of rats treated with Rot. Staining with Golgi silver impregnation was done according to the procedure described by Angulo et al. (1996). The perfused brains were immersed in potassium dichromate solution for 24 h daily for 5 d. The brain tissues were then treated with silver nitrate for 3 days (3 changes every 24 h). The whole brain tissues were then infiltrated in molten wax for 30 min, followed by embedding into paraffin wax and stored at 4 °C overnight. The paraffin embedded blocks were trimmed and sectioned at 60 μm and serially transferred through graded alcohol beginning with 80%, 90%, and two changes of 100% for 2 min and rinsed in xylene for 10 min. The stained brain tissues were mounted on glass slides with DPX mountant and thereafter viewed with Leica DM 500 digital light microscope (Germany) and images were captured with a digital camera (Leica ICC50 E, Germany) connected to a computer interface (MagnaFire). The dendritic length and spine density were determined in sections obtained from the substantia nigra and striatum using Image J software (NIH, Bethesda, MD, USA).

Statistical analysis

Data were analyzed using Graph Pad Prism® software version 5 (Graph Pad Software, San Diego, CA, USA) and expressed as mean ± standard error of the mean (SEM). Statistical analysis was done using one-way analysis of variance (ANOVA) followed by Newman-Keuls post hoc test. *P*-values less than 0.05 were considered statistically significant.

Results

Methyl jasmonate attenuates rotenone-induced deficits in locomotor activity and rearing behavior

The effects of MJ on Rot-induced deficits in locomotor activity and rearing behavior in rats are presented in Fig. 3. One-way ANOVA revealed that there were significant differences between treatment groups: locomotor activity ($[F_{3,\ 31}] = 17.44$, $p < 0.0001$) and rearing behavior ($[F_{3,\ 31}] = 9.64$, $p = 0.0001$). Post hoc analysis by Newman-Keuls test showed that Rot (2.5 mg/kg, i.p) administered every 48 h for 21 days reduced locomotion (Fig. 3a) and rearing frequency (Fig. 3b) when compared with vehicle-controls. As shown in Fig. 3a-b, MJ (100 mg/kg, i.p) or LD-CD (10 mg/kg, i.p) significantly ($p < 0.05$) attenuated Rot-induced deficits in locomotor activity and rearing behavior in rats.

Methyl jasmonate reverses rotenone-induced depletion of dopamine content in rat's brain

Figure 4 shows the effect of MJ on the concentrations of dopamine in the striatum, prefrontal cortex and midbrain of Rot-treated rats. As presented in Fig. 4a-c, Rot

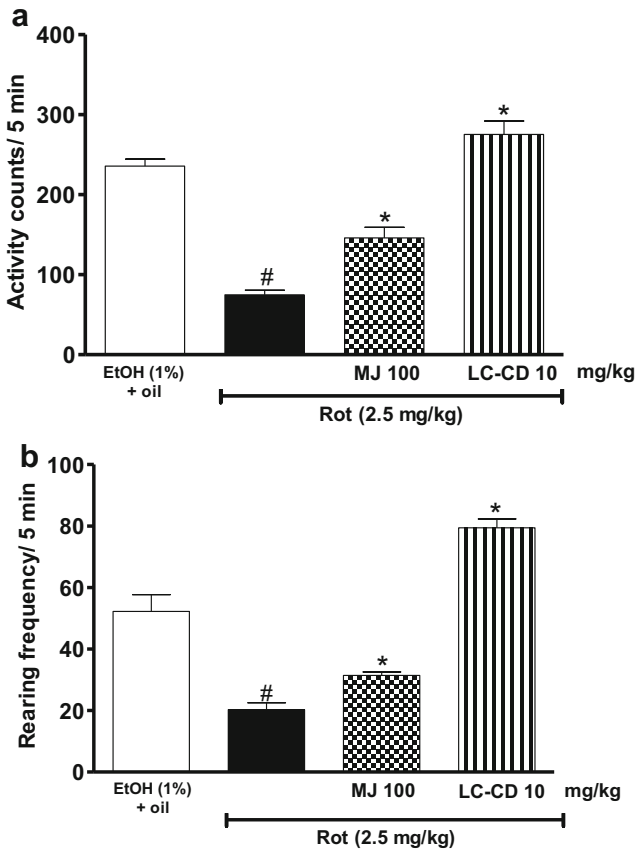

Fig. 3 Methyl jasmonate (MJ) or levodopa-carbidopa (LD-CD) increases locomotor activity (**a**) and rearing behavior (**b**) in rotenone (Rot)-treated rats. Data are expressed as mean ± SEM of 6 animals per group. $^{\#}p < 0.05$ vs vehicle ethanol (EtOH, 1%), $^{*}p < 0.05$ vs rotenone (One-Way ANOVA followed by Newman-Keuls post hoc test)

(2.5 mg/kg, i.p) given alternately (48 hourly) for 21 days produced a significant ($p < 0.05$) decrease in the concentrations of dopamine in the striatum, prefrontal cortex and midbrain of rats when compared with vehicle-controls. However, MJ (100 mg/kg, i.p.) exhibited significant ($p < 0.05$) inhibitory activity against Rot-induced depletion of dopamine contents in the striatum (66.7%), prefrontal cortex (73.9%) and midbrain (57.3%). LD-CD combination also inhibited Rot-induced depletion of dopamine level in striatum (56%), prefrontal cortex (52.1%) and midbrain (75.8%) of rats.

Methyl jasmonate reduces rotenone-induced loss of tyrosine hydroxylase immunopositive cells

The effect of MJ on tyrosine hydroxylase (TH) immuno-expression induced by Rot in rat's brain is shown in Figs. 5 and 6. There was a significant reduction in expression of TH immunopositive neurons in the substantia nigra (21%) and striatum (10%) of rats treated with Rot (Fig. 7a-b). However, pretreatment with MJ (100 mg/kg, i.p.) or LD-CD (10 mg/kg, i.p) produced a significant ($p < 0.05$) increase in the expression of TH immunopositive cells in the substantia nigra and striatum of Rot-treated rats (Fig. 7a-b).

Methyl jasmonate protects striatal neurons of rats treated with rotenone

Histopathologic studies of the brain sections stained with hematoxylin and eosin showed normal cyto-architecture of the substantia nigra and striatum with normal nuclei, neuropil and capillaries in vehicle-control rats. However, rats treated with Rot had numerous degenerated neurons with pyknotic nuclei and cytoplasmic pigmentation in the substantia nigra and striatum (Figs. 8 and 9). As shown in Fig. 10a-b, Rot produced a significant ($p < 0.05$) decrease in the population of viable neuronal cells in the substantia nigra and striatum of rats. On the other hand, pretreatment with MJ (100 mg/kg, i.p) restores the normal cyto-architecture of the neurons of these brain regions of rats treated with Rot (Figs. 8 and 9). As shown in Fig. 10a-b, MJ (100 mg/kg, i.p) pretreatment abrogates Rot-induced neuronal cell loss in the striatum in a significant manner ($p < 0.05$) (Fig. 10a) but was not significant ($p > 0.05$) in the substantia nigra (Fig. 10b). Pretreatment with LD-CD (10 mg/kg, i.p) did not significantly ($p > 0.05$) prevent the neuronal cell loss in the substantia nigra of Rot-treated rats.

Methyl jasmonate protects against rotenone-induced loss of dendritic networks

The photomicrographs of the effects of MJ on dendritic neuronal networks of the substantia nigra and striatum of rats treated with Rot are presented in Figs. 11 and 12. Staining with Golgi silver impregnation revealed that Rot caused degeneration of the dendritic networks of the substantia nigra (Fig. 11) and striatum (Fig. 12) of rats as shown by loss of fine branching of the dendrites. Quantitative morphometric analysis of the pathological changes induced by Rot further revealed loss of dendritic spines and reduced dendritic length in these brain regions of rats (Fig. 13a-d). However, MJ (100 mg/kg, i.p) restores the cyto-architecture of the dendritic neurons and improved their aborizations in these brain regions of Rot-treated rats (Figs. 11 and 12). The histomorphometric analysis revealed that pretreatment with MJ (100 mg/kg, i.p) significantly ($p < 0.05$) increased the dendritic spine density and length in the substantia nigra (Fig. 13a-b) and striatum

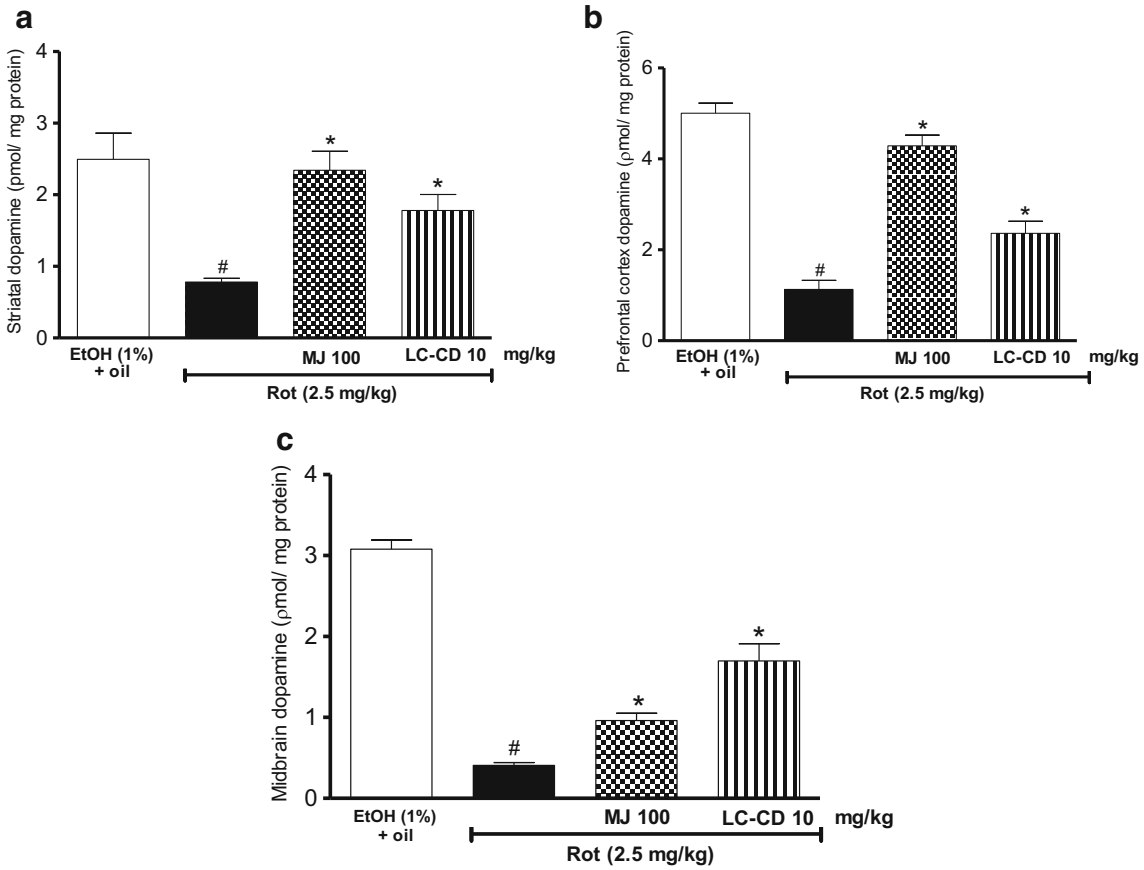

Fig. 4 Methyl jasmonate (MJ) or levodopa-carbidopa (LD-CD) attenuates rotenone (Rot)-induced dopamine depletion in striatum (**a**), prefrontal cortex (**b**) and mid-brain (**c**) of rats. Data are expressed as mean ± SEM of 6 animals per group. $^{\#}p < 0.05$ vs vehicle ethanol (EtOH, 1%), $^{*}p < 0.05$ vs rotenone (One-Way ANOVA followed by Newman-Keuls post hoc test)

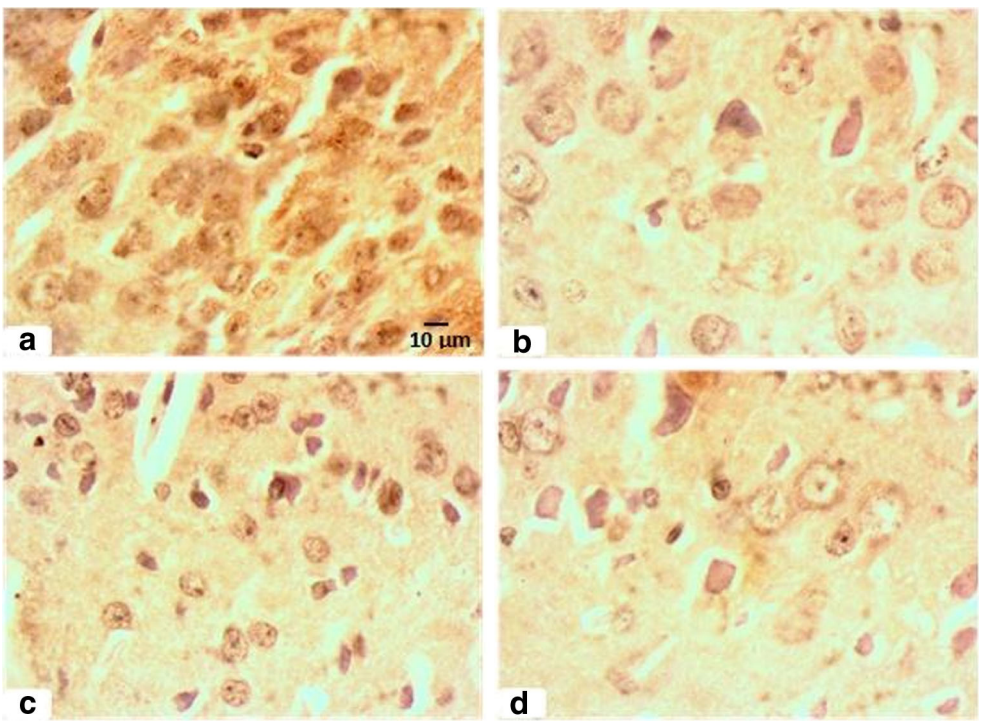

Fig. 5 Representative light photomicrographs of immunohistochemistry staining of tyrosine hydroxylase enzyme in neurons of the substantia nigra of rats treated with vehicle (1% ethanol) (**a**), 2.5 mg/kg of rotenone (**b**), 100 mg/kg of MJ + 2.5 mg/kg of rotenone (**c**) and 10 mg/kg of levodopa-carbidopa + 2.5 mg/kg of rotenone (**d**)

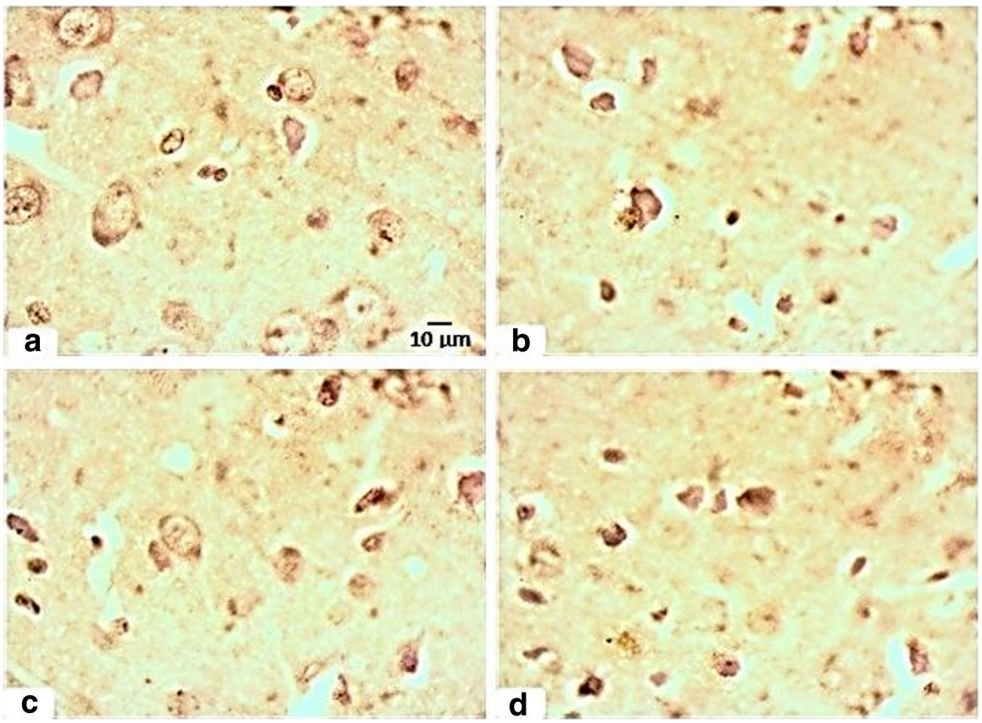

Fig. 6 Representative light photomicrographs of immunohistochemistry staining of tyrosine hydroxylase enzyme in neurons of the striatum of rats treated with vehicle (1% ethanol) (**a**), 2.5 mg/kg of rotenone (**b**), 100 mg/kg of MJ + 2.5 mg/kg of rotenone (**c**) and 10 mg/kg of levodopa-carbidopa + 2.5 mg/kg of rotenone (**d**)

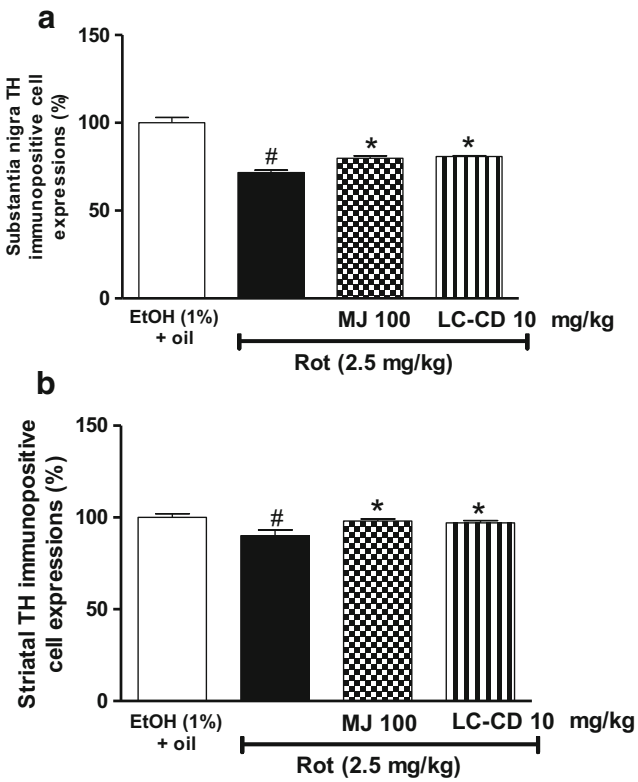

Fig. 7 Methyl jasmonate (MJ) or levodopa-carbidopa (LD-CD) up-regulates tyrosine hydroxylase (TH) enzyme expressions in the substantia nigra (**a**) and striatum (**b**) of rotenone (Rot)-treated rats. Each column represents the mean ± SEM of 6 animals per group. $^{\#}p < 0.05$ vs vehicle, ethanol (EtOH, 1%), $^{*}p < 0.05$ vs rotenone (One-Way ANOVA followed by Newman-Keuls post hoc test)

(Fig. 13c-d) when compared with Rot groups. Levodopa-carbidopa (10 mg/kg, i.p) significantly ($p < 0.05$) increased the dendritic spine density in the substantia nigra but not in the striatum. The dendritic length was however, increased in both the substantia nigra and striatum ($p < 0.05$) relative to Rot alone (Fig. 13a-d).

Discussion

The results of this present study showed that MJ reverses motor deficits as shown by enhanced locomotion and rearing behavior in rotenone-treated rats. Rotenone is a well-known neurotoxin widely used to replicate the pathological changes and behavioral symptoms akin to those seen in patients with PD (Alam and Schmidt 2002; Fathalla et al. 2016). The toxic effect of Rot to dopaminergic innervations is believed to be due to inhibition of mitochondrial electron transport chain complex-I that orchestrate the death of dopaminergic neurons via multiple mechanisms (Hirsch and Hunot 2009; Sherer et al. 2003a, b; Gao et al. 2007). Specifically, previous studies have shown that Rot-induced inhibition of mitochondrial electron transport chain complex-I causes increased free radical generation, which in turn leads to microglia activation (Sawada et al. 2006). Activated microglia are known to cause translocation

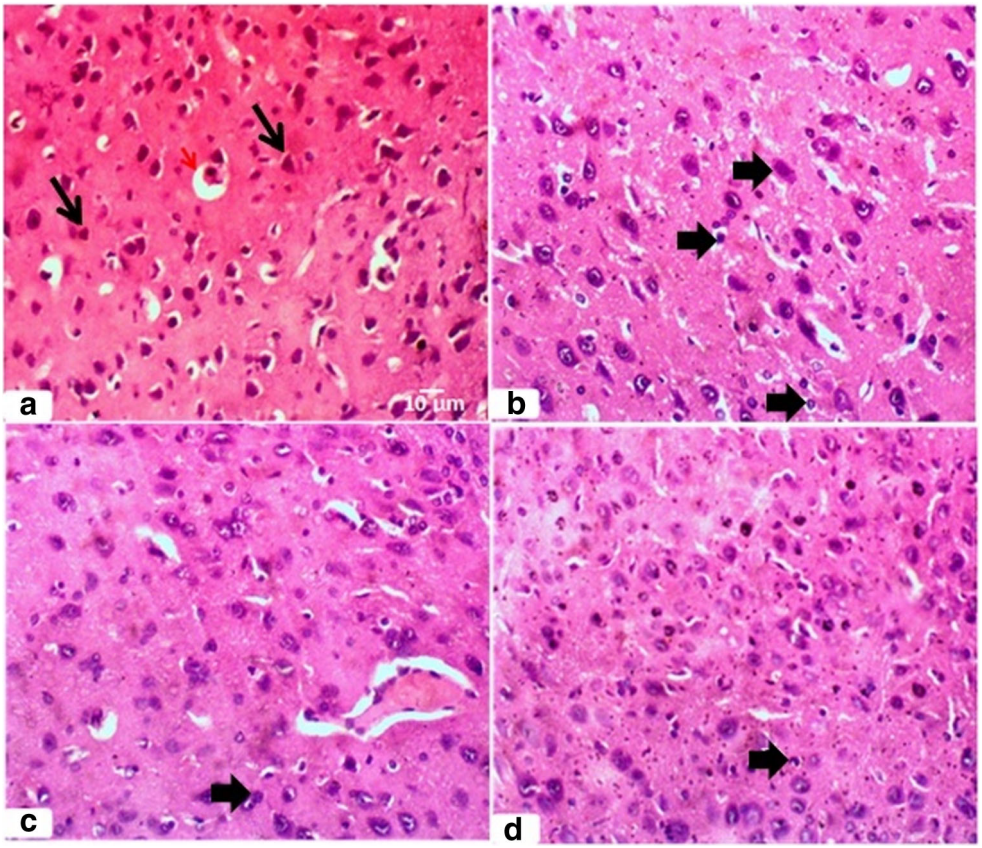

Fig. 8 Representative light photomicrographs of hematoxylin and eosin stained sections of the substantia nigra of rats treated with vehicle (1% ethanol) (**a**), 2.5 mg/kg of rotenone (**b**), 100 mg/kg of methyl jasmonate + 2.5 mg/kg of rotenone (**c**) or 10 mg/kg of levodopa-carbidopa + 2.5 mg/kg of rotenone (**d**). Rats treated with vehicle (**a**) had no visible lesions, neurons (black arrow), neuropil and capillaries (red arrow). Rotenone-treated rats (**b**) showed numerous pyknotic nuclei and cytoplasmic pigmentation (arrow head). Rats pretreated with methyl jasmonate (**c**) or levodopa-carbidopa (**d**) before rotenone injection had few pyknotic nuclei and cytoplasmic pigmentation (black arrow). Mag. × 400 (**e**)

of the redox-sensitive nuclear factor kappa-B to the nuclear compartment of cells that orchestrate the death of dopaminergic neurons and loss of TH enzyme (Sherer et al. 2003a, b; Sawada et al. 2006). The loss of TH cells and dopaminergic neurons that serves as a local source of dopamine has been reported to be responsible for the motor deficits caused by Rot (Alam and Schmidt 2002; Dhanalakshmi et al. 2016). However, drugs that attenuate microglia activation, free radical generation and depletion of striatal dopamine have been found to improve motor deficits in animals and patients with PD (Höglinger et al. 2003; Dhanalakshmi et al. 2016; Fathalla et al. 2016; Nishijima et al. 2018). The findings from our study further confirmed the results of previous investigations, which have shown that Rot produced hypolocomotion and loss of rearing behavior in rodents (Höglinger et al. 2003; Fathalla et al. 2016). Meanwhile, previous studies have shown that MJ inhibits microglia activation and oxidative stress (McKenzie and Klegeris 2018) as well as modulates mitochondrial dynamics (Goldin et al. 2008). However, further investigations are necessary to establish the role of mitochondrial dynamics in the ability of MJ to attenuate motor deficits induced by Rot in rats.

The decrease in TH due to degeneration of dopaminergic neurons have been reported to play a prominent role in the reduced brain concentrations of dopamine and the manifestations of the clinical motor symptoms in patients with PD (Haavik and Toska 1998; Tabrez et al. 2012; Johnson et al. 2018). The changes in TH expression have been used to show the rate of dopamine turnover or as an indirect measurement of dopaminergic activity, hence could serve as a predictor of the severity or progression of the disease (Haavik and Toska 1998; Tabrez et al. 2012; Johnson et al. 2018). Consequently, TH enzyme is being viewed as an additional target for identifying new therapeutics for the disease (Nagatsu 1990; Haavik and Toska 1998;

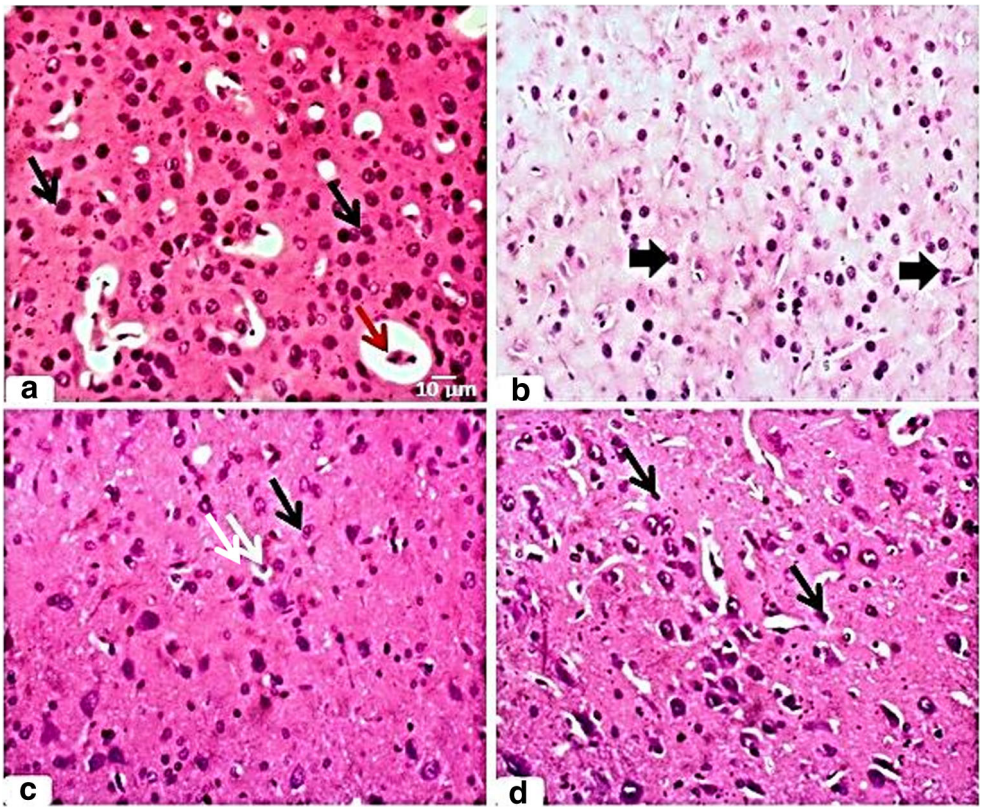

Fig. 9 Representative light photomicrographs of hematoxylin and eosin stained sections of the striatum of rats treated with vehicle (1% ethanol) (**a**), 2.5 mg/kg of rotenone (**b**), 100 mg/kg of methyl jasmonate + 2.5 mg/kg of rotenone (**c**) or 10 mg/kg of levodopa-carbidopa + 2.5 mg/kg of rotenone (**d**). Vehicle-treated rats (**a**) shows normal neurons (black arrow), neuropil and capillaries (red arrow) and no observable lesions. Rotenone-treated rats (**b**) displayed cyto-architectural neuronal distortions with ischaemic necrosis of pyramidal neurons (arrow head). Rats pretreated with methyl jasmonate (**c**) or levodopa-carbidopa (**d**) before rotenone injection exhibited reduced cyto-architectural neuronal distortions with gliosis (white arrow). Mag × 400

Tabrez et al. 2012; Fathalla et al. 2016). Our finding is in agreement with previous reports showing that Rot depletes immunopositive cells of TH in the striatum (Sherer et al. 2003a, b; Azmy et al. 2018). The results of our study also confirmed the reports of earlier investigations, which showed that Rot that depleted dopamine contents in the striatum, midbrain and prefrontal cortex in animal models of PD (Höglinger et al. 2003; Sharma and Nehru 2013), which was salvaged by MJ pretreatment. Although L DOPA is the gold standard for the treatment of PD, its long-term use is known to cause serious side effects such as on and off phenomenon, dyskinesias (abnormal involuntary movements) and motor fluctuations (Oertel and Quinn 1997; Shin et al. 2009; Tabrez et al. 2012; Nishijima et al. 2018; Johnson et al. 2018). Moreover, the brain availability of L-DOPA is limited by the presence of L-DOPA decarboxylase enzyme, hence; its action is usually enhanced by concurrent administration of carbidopa, an inhibitor of L-DOPA decarboxylase (Sherer et al. 2003a; Tabrez et al. 2012). In accordance with our investigations, earlier studies had also shown that LD-CD increased dopamine levels and TH in the substantia nigra and striatum of Rot-treated rats (Shin et al. 2009; Tabrez et al. 2012; Fathalla et al. 2016). Thus, the findings that MJ increased TH immunopositive cells and dopamine contents in the brain of Rot-treated rats further suggest a potential benefit in PD.

The loss of dopaminergic neurons has been highlighted as the major reason for the reduced nigrostriatal dopamine contents and TH enzyme in animals or patients with PD (Haavik and Toska 1998; Tabrez et al. 2012). Our findings from the histological studies showed that Rot caused cyto-architectural distortions and loss of neuronal cells of the substantia nigra and striatum of rats. These observations are in agreement with previous investigations that have implicated degeneration of dopaminergic neurons in Rot-induced parkinsonia-like features in rodents (Alam and Schmidt 2002;Sherer et al. 2003a; Höglinger et al. 2003; Villalba et al. 2009; Sharma and Nehru 2013). Meanwhile, pretreatment with MJ reduces the cyto-architectural distortions and loss of

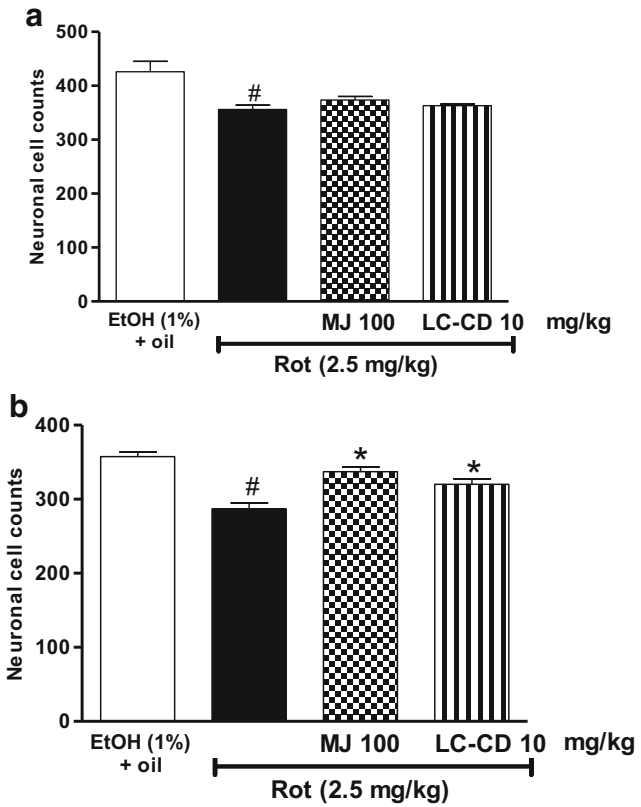

Fig. 10 Methyl jasmonate (MJ) attenuates loss of striatal (**a**) but not substantia nigra (**b**) neuronal cells induced by rotenone (Rot) in rats. Each column represents the mean ± SEM of 6 animals per group. $^{\#}p < 0.05$ vs vehicle, ethanol (EtOH, 1%), $^{*}p < 0.05$ vs rotenone (One-Way ANOVA followed by Newman-Keuls post hoc test)

neuronal cells of the striatum of rats treated with Rot, which suggest neuroprotective activity. We had earlier reported that MJ exhibited neuroprotective property as revealed by its ability to reduce the cyto-architectural distortions and also increased the population of viable neuronal cells in lipopolysaccharide-treated mice (Eduviere et al. 2016; Umukoro and Eduviere 2017). Taken together, the findings that MJ reduced the cyto-architectural distortions and loss of neuronal cells in the brains of rats treated with Rot further suggest an action related to neuroprotection.

Golgi impregnated morphological studies showed that Rot produced severe distortion of the dendritic networks of the substantia nigra and striatum in rats. Rot-treated animals have been reported to have severe loss of dendritic spines and reduced dendritic length in the striatum of rat, a consistent neuropathologic phenomenon also observed in postmortem PD brain (Stephens et al. 2005; Zaja-Milatovic et al. 2005). Other neurotoxins such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 6-hydroxydopamine that selectively destroy dopaminergic neurons have also been reported to caused loss of striatal neurons and distortion of dendritic networks in rodents (Villalba et al. 2009; Tendilla-Beltrán et al. 2019). Dendritic spines are tiny protuberances of dendrites that are involved in the regulation of excitatory and inhibitory synapses in the mammalian brain (Deutch et al. 2007) and thus play a major role in synaptic

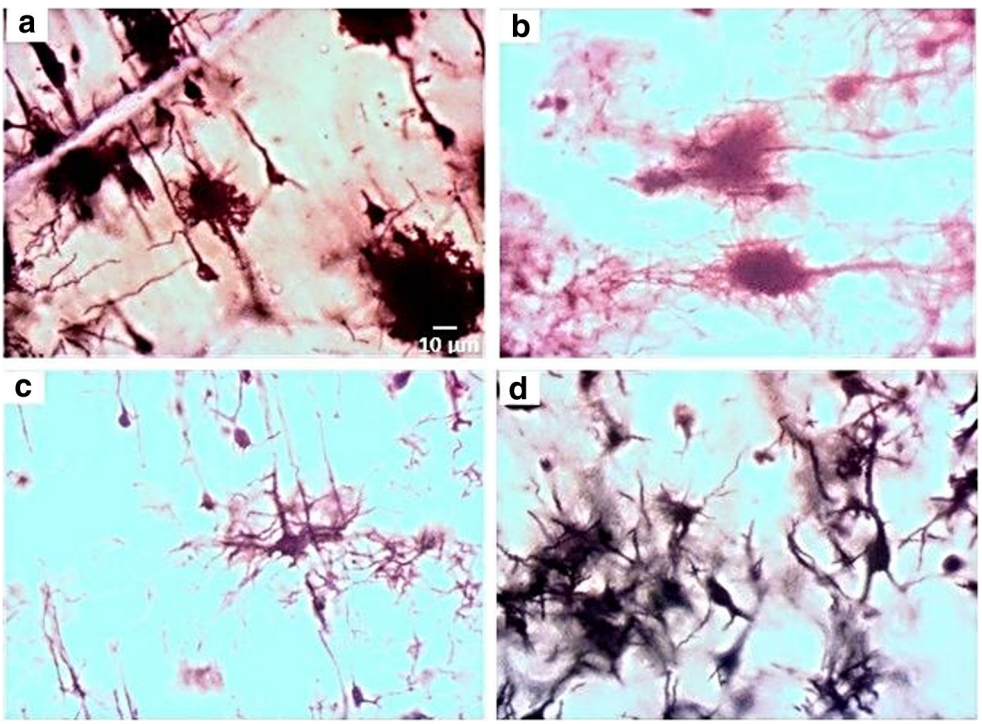

Fig. 11 Representative light photomicrographs of Golgi Stain of the substantia nigra of rats treated with rats treated with vehicle (1% ethanol) (**a**), 2.5 mg/kg of rotenone (**b**), 100 mg/kg of methyl jasmonate + 2.5 mg/kg of rotenone (**c**) or 10 mg/kg of levodopa-carbidopa + 2.5 mg/kg of rotenone (**d**)

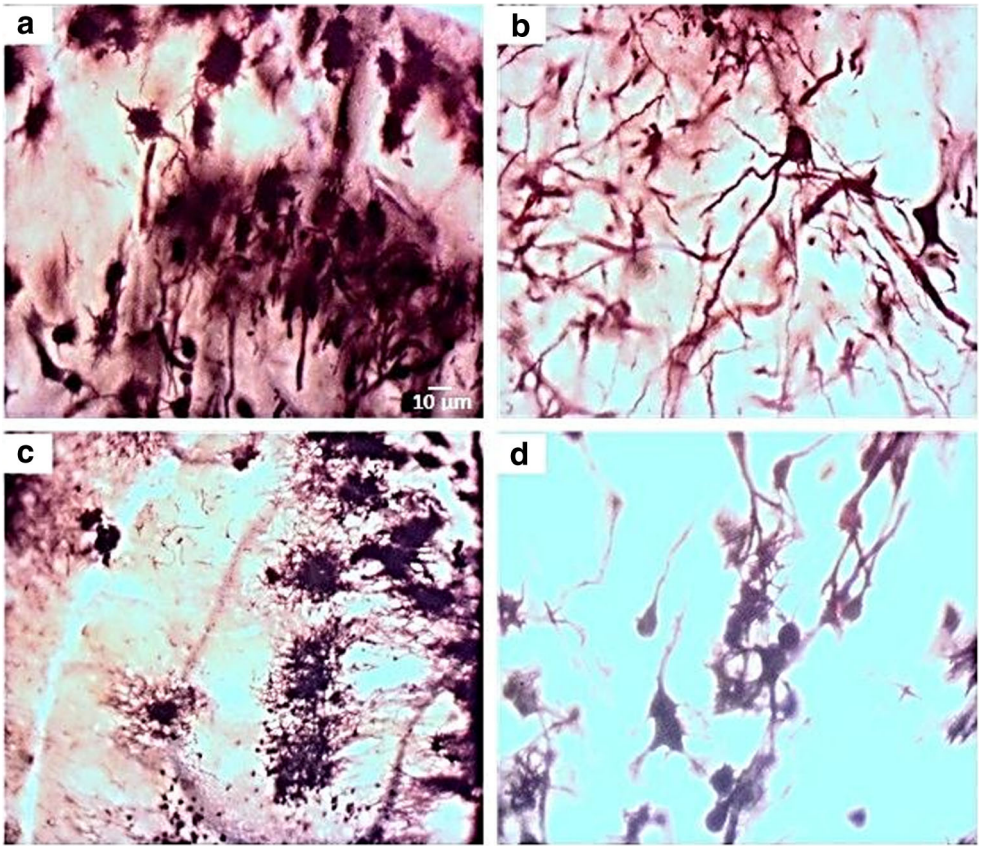

Fig. 12 Representative light photomicrographs of Golgi Stain of the striatum of rats treated with vehicle (1% ethanol) (**a**), 2.5 mg/kg of rotenone (**b**), 100 mg/kg of methyl jasmonate + 2.5 mg/kg of rotenone (**c**) or 10 mg/kg of levodopa-carbidopa + 2.5 mg/kg of rotenone (**d**)

communications. The decreased level of dopamine has been reported to cause the neurons of the striatum to fire uncontrollably resulting in degeneration of dendritic spines or loss of dendritic arborisation that characterized PD (Smith et al. 2009; Janakiraman et al. 2017; Nishijima et al. 2018). However, our findings showed that MJ produced a significant reversal of the morphological distortions of the striatal dendritic spine and also increased striatal dendritic spine density in rats treated with Rot. Regrettably, LD-CD did not prevent Rot-induced loss of striatal dendritic spines. Accordingly, similar studies have also shown that L DOPA failed to increase spine density in mice exposed to MPTP (Shin et al. 2009). Besides, the loss of dendritic spine has also been implicated in the pathological complications observed in advanced cases of PD (Stephens et al. 2005; Zaja-Milatovic et al. 2005; Bhullar and Rupasinghe 2013). Thus, the improvement in Rot-induced motor impairments with MJ pretreatment may be related to attenuation of degeneration of the dendritic neuronal networks, which constitutes the neuroprotection strategy.

In advanced stages of PD, the efficacy of L DOPA has been reported to be compromised due to loss of striatal dendritic spines (Villalba et al. 2009; Tabrez et al. 2012; Sharma and Nehru 2013). Surprisingly, it has been observed that no amount of dopamine could attenuate the loss of striatal dendritic spines (Ingham et al. 1989; Stephens et al. 2005; Bhullar and Rupasinghe 2013). Consequently, it has been suggested that natural products such as phytochemicals and neurotrophic factors that can confer neuroprotection should be investigated as potential therapeutic agents for PD (Gao et al. 2007; Wang et al. 2010; Xu et al. 2010; Tabrez et al. 2012; Agrawal et al. 2012; Airavaara et al. 2012; Ojha et al. 2015). Indeed, a new paradigm shifts involving the use of phytomedicines that could target multiple pathways involved in the pathophysiology of PD and confer neuroprotection have being the subject of extensive investigations (Hirsch and Hunot 2009; Tabrez et al. 2012; Agrawal et al. 2012; Dhanalakshmi et al. 2016; Azmy et al. 2018). This is based on the belief that neurodegeneration cascade can be abrogated with the use of neuroprotectants thereby salvaging dopaminergic neurons in parkinsonia brain (Wang et al. 2010; Tabrez et al. 2012; Agrawal et al. 2012; Ojha et al. 2015). Indeed, several phytochemicals have been found to attenuate parkinsonia-like symptoms by

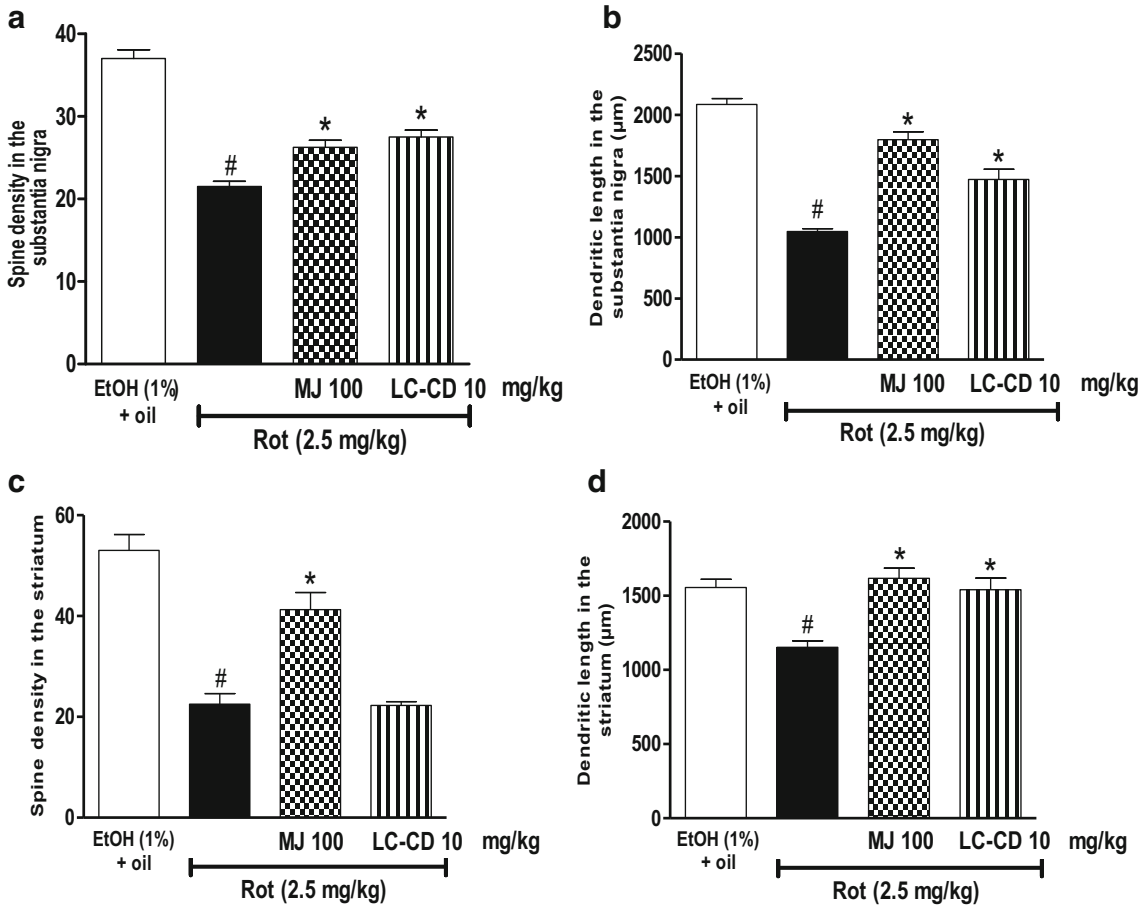

Fig. 13 Methyl jasmonate (MJ) or levodopa-carbidopa (LD-CD) increases dendritic spine density and dendritic length in the substantia nigra (Fig. 13a-b) and striatum (Fig. 13c-d) of rat treated with rotenone (Rot). Each column represents the mean ± SEM of 6 animals per group. $^{\#}p < 0.05$ vs vehicle, ethanol (EtOH, 1%), $^{*}p < 0.05$ vs rotenone (One-Way ANOVA followed by Newman-Keuls post hoc test)

targeting various neurodenerative processes in PD (Gao et al. 2007; Xu et al. 2010; Wang et al. 2010; Agrawal et al. 2012; Ojha et al. 2015). Catalpol, a bioactive compound found in a number of Chinese medicinal herbs, for example, was reported to mitigate the neurotoxic effect of MPTP through elevation of TH activity and dopamine levels (Xu et al. 2010) and brain derived neurotrophic factor (Airavaara et al. 2012). Moreover, epidemiological studies have shown that regular intake of fruits, vegetables and fish in large quantity was inversely associated with PD risk (Gao et al. 2007; Alcalay et al. 2012). It has been reported that the Mediterranean dietary patterns are emerging as neuroprotective measures for PD (Okubo et al. 2012). Thus, the intake of phytochemicals with neuroprotective activity such as MJ with a proven high safety profile (Cesari et al. 2014; Eduviere et al. 2016; Umukoro and Eduviere 2017) might be useful in PD. However, the major limitation in this study was the use of large magnification for the visualization of the neuronal cells and this might have contributed to the reduced surface area and poor localization of different sub-fields of the striatum and substantia nigra.

Conclusion

The results of this study suggest that methyl jasmonate reverses motor deficits through increase in dopamine content, tyrosine hydroxylase immnopositive cell expressions and dendritic aborization in the striatum of rotenone-treated rats.

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Compliance with ethical standards

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

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