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Methyl jasmonate abrogates rotenone-induced parkinsonian-like symptoms through inhibition of oxidative stress, release of pro-inflammatory cytokines, and down-regulation of immnopositive cells of NF-κB and α-synuclein expressions in mice

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ABSTRACT

Oxidative stress and neuroinflammation play key roles in the initiation and progression of Parkinson's disease (PD), a neurodegenerative disorder, associated with the loss of nigrostriatal dopaminergic pathway. Thus, compounds that can mitigate oxidative stress and neuroinflammation are being investigated as promising agents for the treatment of PD. This study was designed to evaluate the effects of methyl jasmonate (MJ), a potent antioxidant and anti-inflammatory compound on parkinsonian-like symptoms and the underlying biochemical changes induced by rotenone (Rot) in mice. To this end, the effects of graded doses of MJ (25, 50 and100 mg/kg, i.p.) on motor dysfunctions, cognitive and depressive-like disorders induced by Rot (2.5 mg/kg, i.p.) were evaluated. The specific brain regions (striatum, prefrontal cortex and hippocampus) of the animals were processed for various biochemical studies. Rot-treated mice showed reduced motor activity, postural instability, cognitive and depressive-like disorders. Rot also increased brain levels of malondialdehyde (MDA), nitrite, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and acetyl-cholinesterase (AChE) activity. Moreover, Rot reduced the concentration of glutathione (GSH) and increased immnopositive cells of NF-κB and α-synuclein expressions in these brain regions. However, pretreatment with MJ, attenuated the parkinsonian-like symptoms and reduced the brain levels of MDA/nitrite, TNF-α and IL-6 induced by Rot. MJ also reduced AChE activity and down-regulate the expressions of NF-κB and α-synuclein in the brain of Rot-treated mice. These findings suggest that MJ has anti-parkinsonian-like activity, which may be related to the inhibition of oxidative stress, release of pro-inflammatory cytokines, and down regulation of NF-κB and α-synuclein expressions.

1. Introduction

Movement disorders such as tremor at rest, rigidity, akinesia (difficulty in initiating movement), bradykinesia (slowness of voluntary movements), gait, and postural abnormalities are common disabling motor symptoms of Parkinson's disease (PD) (Dauer and Przedborski, 2003; Hisahara and Shimohama, 2011; Cooper et al., 2018). These symptoms are mainly due to the loss of dopaminergic neurons in the substantianigra pars compacta (SNpc) and the consequent degeneration of their projecting nerve fibers in the striatum (ST) leading to reduced striatal dopamine content (Dauer and Przedborski, 2003; Schapira and Jenner, 2011). However, non-motor abnormalities such as anxiety, apathy, cognitive and depressive symptoms are also prominent features of PD that often contribute to the reduced quality of life or productivity of individuals suffering from the illness (Cooper et al., 2018). Thus, the disease presents a huge source of burden to the patients and their relatives as well as the society at large (Schenkman et al., 2001).

Although the exact pathogenesis of PD is yet to be clearly elucidated, increased oxidative stress and neuroinflammation have been reported to be associated with the initiation and progression of the illness (Jenner, 2003). Several studies have shown that the substantia nigra of PD patients had elevated levels of oxidized lipids and decreased GSH (Zeevalk et al., 2008; Ganesan et al., 2015). The presence of reactive oxygen species (ROS)-generating enzymes such as tyrosine

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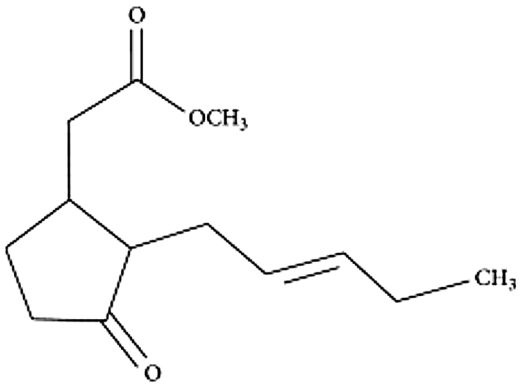
hydroxylase and monoamine oxidase, also make the dopaminergic neurons to be more prone to oxidative stress (Schapira and Jenner, 2011). In almost two decades ago, wealth of new information have emerged implicating oxidative stress-derived neuroinflammation and cytokine-dependent toxicity as the major contributors to the degeneration of nigrostriatal pathway and the hastened progression of PD (Qian et al., 2010; Ganesan et al., 2015). The existence of ongoing inflammatory processes in PD is supported by evidence of activated microglia, accumulation of cytokines, up regulation of nuclear factor kappa B (NF-κB) pathway in the brains of individuals (McGeer et al., 1988; Hirsch and Hunot, 2009) and experimental animals with the disease (Blesa et al., 2010). Thus, it has been noted that the course of the disease spirals out of control as a result of activation of microglia, over production of cytokines and other inflammatory mediators, as well as ROS (Whitton, 2007; Qian et al., 2010). Studies have also shown that mice expressing increased levels of human α-synuclein exhibited increased microglial activity and higher levels of proinflammatory cytokines that preceded loss of dopaminergic neurons (Theodore et al., 2008; Cooper et al., 2018). Moreover, neurotoxins such as 6-hydroxydopamine (6-OHDA), Rot, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and paraquat have also been shown to replicate most of the phenotypic and pathological features of PD through the induction of oxidative stress and neuroinflammation (Alam and Schmidt, 2002; Dhanalakshmi et al., 2016). Rot for example, has been considered as one of the best neurotoxins for induction of biochemical changes and behavioral symptoms similar to those observed in patients with PD (Alam and Schmidt, 2002; Dhanalakshmi et al., 2016). Rot is a well-known potent inhibitor of mitochondrial electron transport chain complex-1 (Sherer et al. 2003a,b) that leads to the production of free radicals (Chen et al., 2008). It also activate the microglial cells and trigger neuroinflammatory responses that orchestrate dopaminergic cell death by releasing cytotoxic inflammatory cytokines, such as interleukin-1 β (IL-1 β), IL-6 and tumor necrosis factor-α (TNF-α). TNF-α has been implicated in the loss of dopaminergic neurons by activating intracellular signaling pathways (NF-κB), Jun N-terminal kinases (JNK), cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) expressions, which further amplify the inflammatory cascades (Hirsch and Hunot, 2009).

Although the pharmacological treatment of the motor symptoms of PD involves the use of dopaminergic and central anti-cholinergic drugs, the quality of life of patients with the illness still remains very low (Hisahara and Shimohama, 2011; Cooper et al., 2018). The clinical usefulness of these drugs in PD is also associated with a lot of limitations such as high incidence of disabling side-effects, poor adherence to treatment and treatment failures in certain patients (Obeso et al., 2000). Moreover, these drugs can not alter the underlying cause of the illness but only provide symptomatic relief. Furthermore, other non-motor symptoms like depression and loss of cognition associated with the disease are insensitive to anti-parkinsonian drugs (Raffaele et al., 2002; Cooper et al., 2018). Depression and cognitive dysfunction are known to worsen the disease as they affect the mental and emotional well-being of the individuals (Raffaele et al., 2002). Thus, there is a need to search for newer drugs that can target the multiple pathological derangements and attenuate the motor and non-motor symptoms associated with PD.

Methyl jasmonate (MJ) also known as methyl 3oxo-2-(2-pentenyl) cyclopentaneacetate (Scheme 1) was first isolated from jasmine, a perennial climbing plant (*Jasminum grandiflorum)* that is highly reputed for its sweet and scented flowers. Jasmine is widely used for depression, nervousness, tension, alertness, and memory deficit in aromatherapy (Kuroda et al., 2005). MJ is well recognized as a hormone that help 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). MJ has won international recognition over the years, as a potential anticancer agent for the treatment of various tumors (Cesari et al., 2014). Studies had



Scheme 1. Chemical structure of MJ **(**Cesari et al., 2014**).**

shown that MJ selectively kills cancer cells, leaving normal cells intact; raising the hope of it becoming an effective and safe chemotherapeutic drug against neoplastic diseases (Roteim et al., 2005; Cesari et al., 2014). Moreover, *in vivo* studies have also confirmed that MJ did not produce local or systemic adverse effects, irrespective of the route of exposure in both humans and laboratory animals (Belsito et al., 2012; Cesari et al., 2014). Meanwhile, previous preclinical investigations have revealed that MJ has sedative effect and potentiated GABA-mediated inhibitory neurotransmission suggesting its benefits in neuropsychiatric diseases (Hossain et al., 2004). However, over the years, there is increasing interest in the development of MJ as a therapeutic drug for the treatment of diseases with inflammation as a major underlying factor. This interest arose from the observation that MJ shares a similar chemical structure with anti-inflammatory prostaglandins hence; its effects on inflammation were screened in culture macrophage cells (Dang et al., 2012). The results of these studies confirmed that MJ and its derivatives demonstrated potent anti-inflammatory property by decreasing the release of pro-inflammatory cytokines and down regulating NF-kB expressions (Lee et al., 2011; Dang et al., 2012). In addition, we have also reported in our recent studies that MJ attenuated memory dysfunction through mechanisms related to neuroprotection, decreased brain levels of biomarkers of oxidative stress and neuroinflammation in lipopolysaccharide (LPS)-treated mice (Eduviere et al., 2016; Umukoro and Eduviere, 2017). These findings further suggest its potential benefits in neurodegenerative diseases like PD, whose pathological abnormality is strongly linked with oxidative stress/neuroinflammation. Thus, this present study was designed toevaluate the effect of MJ on Rot-induced parkinsonian-like behaviors and the underlying biochemical changes in rodents (Fig. 1).

2. Materials and methods

2.1. Drugs and reagents

Methyl jasmonate (MJ), rotenone, N-(1-naphthyl) ethylenediaminedihydrochloride, sulphanilamide, thiobarbituric acid (TBA), 5,5′-Dithio-bis-(2-nitrobenzoic acid), and acetylthiocholine, were obtained from Sigma Aldrich (Germany).Bromocriptine (Brom) was obtained from Novartis Pharmaceuticals, Lagos, Nigeria. Tumor necrosis factor–alpha (TNF-α), interleukin-6 (IL-6) ELISA kits were obtained from Biolegend (USA). Nuclear factor kappa B (NF-κB) and alpha synuclein primary antibodies were products of Santa Cruz (USA).

2.2. Laboratory animals

Sixty male Swiss mice (22–25 g) used in the study were procured from the Central Animal House, University of Ibadan, Ibadan, Nigeria. They were acclimated in the Department of Pharmacology & Therapeutics animal holding facility for two weeks. They were housed

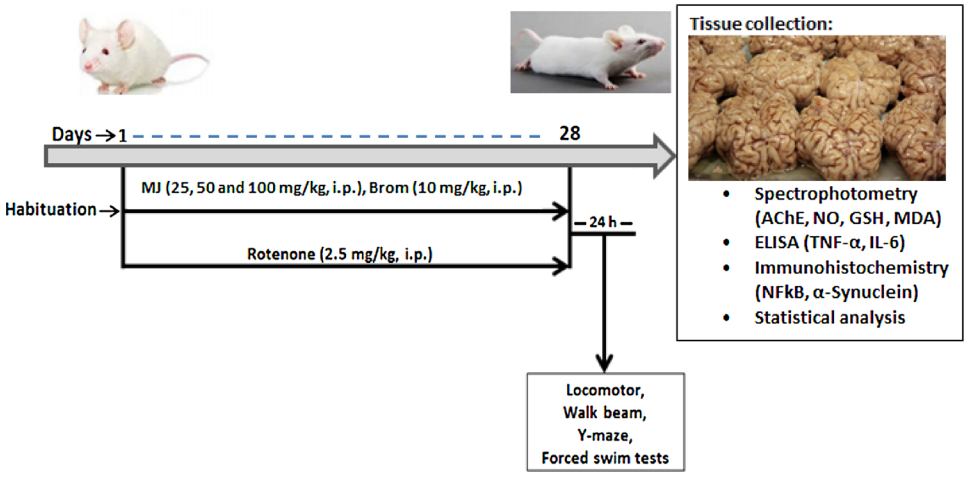


Fig. 1. Design of the experimental procedures.

in polycarbonates fabricated animal cages with free access to standard pellet diet (Vital feeds®, Jos, Nigeria) and water *ad libitum*. The experimental procedures were approved by the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC/18/0055).

2.3. Preparation 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 ethanol in the solution used for the study did not exceed 1%. The doses of 25, 50 and 100 mg/kg of MJ were selected based on the results obtained from previous investigations (Umukoro and Eduviere, 2017).

2.4. Induction of Parkinsonian-like behaviors in mice

Parkinsonian-like behaviors were induced in mice *via* intraperitoneal injection of Rot (2.5 mg/kg in sunflower oil) according to the procedure earlier described (Morais et al. (2012). Male mice were divided into 6 groups (n = 10). Mice in group 1, which served as control received vehicle (1% etOH, 10 mL/kg in sunflower oil), group 2 also had vehicle (1% ethanol; 10 mL/kg in sunflower oil), group 3–5 received MJ (25, 50 and 100 mg/kg in sunflower oil) while group 6 were given Brom (10 mg/kg in sunflower oil) intraperitoneally for 28 consecutive days. In addition, mice in groups 2–6 also received i.p. injection of rotenone in multiple doses (during 28 consecutive days, 28 × 2.5 mg/kg in sunflower oil; each injection was given 30 min after treatment with vehicle, MJ or Brom. Thereafter, the tests for neurobehavioral phenotypes (locomotor activity, motor coordination, cognition and depression) were carried out in this order, 24 h later (Fig. 1).

2.4.1. Test for spontaneous locomotor activity

The test for spontaneous locomotor activity (SMA) was done on day 28 after Rot administration using activity cage (Ugo Basile, Italy). Mice were placed individually in the activity cage and the number of times the animals breaks the horizontal beam (locomotor activity) or vertical beam break (rearing activity) was recorded for a period of 5 min.

2.5. Test for motor coordination

The sensorimotor function of the animals was assessed using the walk beam test (WBT) as previously described (Hilber et al., 1999; Omorogbe et al., 2018). The WBT is used to evaluate fine motor coordination and balance or postural instability in rodents. The walk beam consists of a piece of wood of length 100 cm, width 2 cm, and elevated to a height of 40 cm.The WBT consists of two phases; the training and test phase. The animals were trained to walk on the beam for 2 min before test session. The test phase was carried out on day 28 for a period of 2 min and the number of times the back foot slips off the bar was counted. The total distance travelled across the beam was also recorded (Omorogbe et al., 2018).

2.6. Evaluation of memory function

The Y-maze test was used to evaluate the eff ;ect of MJ on Rot-induced memory deficit according to the method described by Casadesus et al. (2006). This test was also carried out immediately after the walk beam test on day 28. Each mouse was placed at the end of arm A and allowed to explore all the three arms freely for 5 min. The number of arm visits and sequence (alternation) of arm visits were recorded by a blind observer. The percentage alternation, which is used as an index for spatial memory was then calculated (Casadesus et al., 2006). After each test, the apparatus was also cleaned with 70% ethanol to remove residual odor. Alternation behaviors were defined as consecutive entries into all the three arms (*i.e.* ABC, CAB or BCA but not BAB) (Casadesus et al., 2006).

2.7. Test for depressive-like behavior

The forced swim test (FST) was used to evaluate the effect of MJ on Rot-induced depressive-like behavior in mice (Porsolt et al., 1977). This test was performed on day 29 between the hours of 9 am-12 pm. Each mouse was placed in a vertical glass cylinder (26 cm high, 12 cm in diameter) that was filled with water at 25 °C to a depth of 16 cm for 6 min. The period of immobility (s) was recorded for 4 min after the initial 2 min delay by a blind observer. The animals were considered immobile whenever they remained motionless and hung passively.

2.8. Preparation of brain tissues for biochemical studies

Immediately after behavioral tests on day 29, mice were anaesthetized using ether. The anaesthetized animals were perfused with cold sodium phosphate buffer (0.1 M, pH 7.4), and the brains were carefully removed and placed on ice. Dissection of the brain region of interest was done on cold iced tray. The striatum, prefrontal cortex and hippocampal regions of the brains were individually homogenized in iced cold phosphate buffer, 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 for determination of acetyl-cholinesterase activity, nitrite, reduced GSH, MDA, and pro-inflammatory cytokines.

2.8.1. Estimation of brain acetyl-cholinesterase activity

Acetyl-cholinesterase enzyme activity was determined in the brain regions using the method described by Ellman et al. (1961). Briefly, 0.1 mL of the brain tissue supernatant was diluted with 1.4 mL phosphate buffer saline (PBS), and incubated with 0.05 mL DTNB (0.01 M in 0.1 M PBS) for 10 min at room temperature. The mixture was added into a cuvette and the initial absorbance level measured at 412 nm in a UV/VIS spectrophotometer (INESA). Thereafter, 0.05 mL of acetylthiocholine iodide (0.028 M in 0.1 M PBS) was added as a substrate and the reaction was allowed to proceed for 2 min before measuring absorbance at the same wavelength. The rate of acetyl-cholinesterase activity was expressed as μmol/min/g tissue was calculated using the formula below:

$R = 5.74 \times 10^{-4}$ x A/Co

Where, R = Rate in moles of substrate hydrolyzed/minute/g tissue

A = Change in absorbance/min, Co = Original concentration of the tissue

2.8.2. Determination of brain nitrite level

The level of nitrite is commonly used as an indicator of the concentrations of nitric oxide in biological tissues. The Griess method (Green et al., 1981) was used to estimate the nitrite level in the brain regions. The Griess reagent freshly prepared by mixing equal ratio of reagent A. Supernatant from tissue homogenates was incubated with the Griess reagent (1% sulfanilic acid in 5% phosphoric acid and 0.1% of N-1-naphthyl ethylenediamine dihydrochloride) and the absorbance was measured at 540 nm. Brain tissue nitrite concentration (μM per gram tissue) was extrapolated from sodium nitrite (0–100 μM) standard curve.

2.8.3. Determination of reduced glutathione contents

The level of reduced GSH in the striatum, prefrontal cortex and hippocampus of mice was determined as earlier described (Moron et al., 1979). Brain tissue supernatant was diluted 10 times in phosphate buffer (0.1 M, pH 7.4) and deproteinized with 20% trichloroacetic acid. The mixture was centrifuged at 4000 rpm for 10 min at 4 °C. The supernatant was removed into new tubes and mixed with 5^1, 5^1-Dithiosnitrobenzoic acid (0.0006 M). The absorbance was read within 10 min in a UV/Vis spectrophotometer (INESA 752 N) at 412 nm. The concentration of GSH (μM GSH/g tissue) in the samples was extrapolated from the standard curve generated using standard glutathione.

2.8.4. Estimation of brain malondialdehyde levels

Brian level of MDA, an index of lipid peroxidation was measured using the thiobarbituric reacting substance (TBARS) assay as described by Ohkawa et al. (1979). Supernatants (0.1 mL) of the striatum, prefrontal cortex or hippocampus of mice were diluted twenty times using 0.15 M Tris-KCl buffer, and mixed with 0.5 mL TCA (30%), and 0.5 mL thiobarbituric acid (0.75%). The reaction mixture was heated at 80 °C in a water bath for 45 min. The heated tubes were placed on iced cold water for 10 min to stop the reaction, thereafter centrifuged at 4000 rpm for 5 min. The supernatant was carefully removed and the absorbance read at 532 nm in a UV/VIS Spectrophotometer (INESA, 752 N). The concentrations of MDA in the brain tissue were calculated using an index of absorption for MDA (molar extinction coefficient 1.56×10^5/M/cm) and expressed as ηmol MDA/g tissue.

2.8.5. Enzyme-linked immunosorbent assay for tumor necrosis factor (TNF-α) and Interleukin-6 (IL-6)

The concentrations of TNF-α and IL-6 were determined using supernatants obtained from the striatum, prefrontal cortex and hippocampus according to the manufacturer's instructions. TNF-α and IL-6 were measured by specific mouse TNF-α (BioLegend ELISA MAX™ Deluxe kit, USA) and IL-6 (BioLegend ELISA MAX™ Deluxe kit, USA) with sensitivity limit of 4 pg/mL. All the measurements were done at room temperature in accordance to Biolegend instructions using microplate reader with 450 nm filter. The concentration of TNF-α and IL-6 from the tissues were extrapolated from the standard curve of TNF-α and IL-6 standards included in the assay kits. The level of TNF-α and IL-6 in the different brain regions were expressed as pg/g tissues.

2.9. Immunohistochemical determination of nuclear factor-kappa B and alpha-synuclein protein expressions

Brains of mice were removed following transcardial perfusion with sterile normal saline and sodium phosphate buffered formalin. After perfusion, the brains were post-fixed for 48 h with paraformaldehyde in 100 mmol/L phosphate buffer and then transferred sucrose solution (15%) in 0.1 M PB containing 0.1% sodium azide at 4 °C. Section of the striatum, prefrontal cortex and hippocampus, 20 μm thick, were done using a cryostat and collected in 100 mmol/L PBS containing 0.3% Triton X-100 (PBS-T). After several washes, the slices were stored until use in a free-floating state at 4 °C for immunohistochemical analysis. The process for the immunohistochemical staining begins with deparaffinization and hydration of the tissue using xylene and decreasing concentration of alcohols (100, 90, and 80%) in 5 min intervals. This was followed by washing the slides with normal saline twice, incubation with peroxidase block for 5–10 min at room temperature (25 °C). The slides were again rinsed with normal saline, placed in citrate buffer tank and heated for 3–5 min over a water bath for antigen retrieval. The slides were again washed thrice with phosphate buffer saline (PBS) containing 0.02% Tween 20, before blocking with milk solution for 5–10 min at room temperature. The tissues on slides were incubated with the NF-κB or α-synuclein primary antibody (1:300) for 20–30 min at room temperature (25 °C). Thereafter, the slides were incubated with one-step horseradish peroxidase (HRP) polymer for about 20–30 min, and rinsed with PBS repeatedly (4–6 times). Drops of ready to use 3, 3′-diaminobenzidine (DAB) reagents were dropped on each slide and allowed to incubate for 6–10 min at room temperature and then followed by washing with PBS 7–9 times. The slides were counter stained with hematoxylin for 30–60 s, rinsed with normal saline and dried. The photomicrograph of stained slides was acquired using Leica ICC50 E Digital Camera (Germany) connected to a computer interface (Magnafire) and an Olympus BX-51 Binocular research microscope. Immunopositive cell expression was defined and analyzed with the aid of Image J software (NIH, Bethesda, MD, USA).

2.10. Statistical analysis

Data were presented as mean ± standard error of mean (SEM) and analyzed using GraphPad Prism® software version 5 (GraphPad Software, San Diego, California, USA). Statistical analysis were done using analysis of variance (ANOVA) and significant main effects were further analyzed by *post hoc* test using either Newman-Keuls (one away ANOVA) or Bonferroni's multiple comparison test (two-way ANOVA) as appropriate. P-values less than 0.05 were considered to be statistically significant.

3. Results

3.1. Methyl jasmonate attenuates rotenone-induced locomotor impairment in mice

As presented in Fig. 2A and B, One-way ANOVA showed that there were significant differences between treatment groups: horizontal counts (locomotor activity) ($[F_{6, 34}] = 8.072$, $p < 0.0001$) and vertical counts (rearing activity) ($[F_{6, 34}] = 100.7$, $p < 0.0001$). Moreover, analysis by Newman-Keuls post hoc test revealed that mice treated with Rot (2.5 mg/kg, i.p.) daily for 28 days exhibited impaired

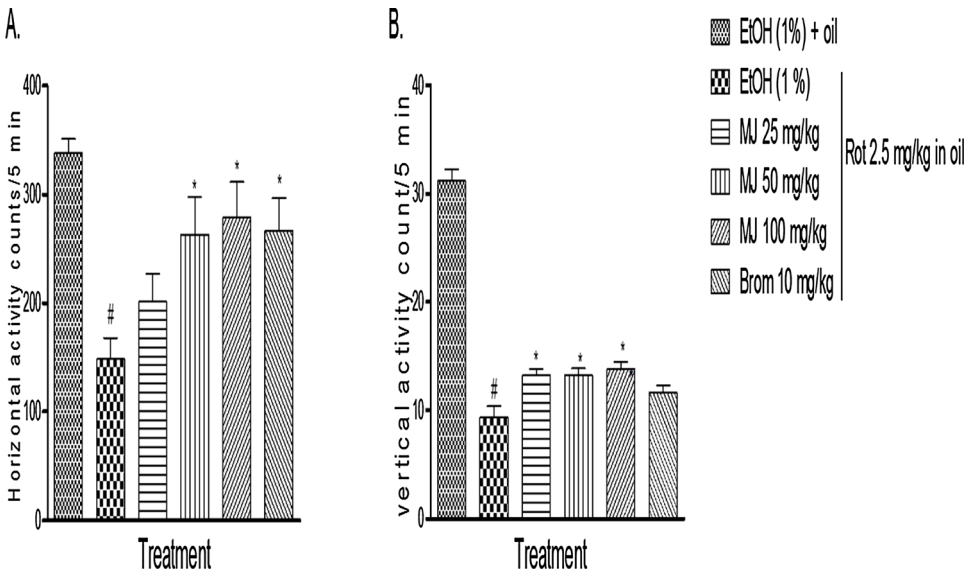


Fig. 2. Methyl jasmonate (MJ) and bromocriptine (Brom) attenuates rotenone-induced impaired locomotor activity (A) and rearing behavior (B) in mice. Data are expressed as mean ± SEM of 6 animals per group, $^{\#}p <$ 0.05 *vs* 1% ethanol (EtOH), $^{*}p <$ 0.05 *vs* rotenone (Rot) group (One-way ANOVA followed by Newman-Keuls post hoc test for multiple comparison).

locomotor activity and rearing behavior as shown by a significant ($p < 0.05$) decrease in horizontal and vertical activity counts when compared with controls. However, pretreatment with MJ (25, 50 and 100 mg/kg, i.p) or Brom (10 mg/kg, i.p.) significantly ($p < 0.05$) ameliorated Rot-induced impairment in locomotor activity (Fig. 2A) and rearing behaviors in mice (Fig. 2B).

3.2. Methyl jasmonate attenuates motor incoordination in the walk beam test

One-way ANOVA showed that there was a significant main effect in the number of foot slip ($[F_{6, 37}] = 30.43$, $p <$ 0.0001) and the total distance travelled ($[F_{6, 40}] = 5.928$, $p =$ 0.0003) on the beam walk test (Fig. 3A and B). *Post hoc* analysis using Newman-Keuls test revealed that Rot (2.5 mg/kg, i.p.) given for 28 consecutive days impaired motor coordination as evidenced by a significant ($p < 0.05$) increase in number of foot slips and reduced distance travelled in the WBT in mice when compared with vehicle (Fig. 3A and B). As shown in Fig. 3A and B, MJ (25, 50 and 100 mg/kg, i.p.) significantly ($p < 0.05$) attenuated motor incoordination induced by Rot in mice. Brom (10 mg/kg, i.p.) also reversed ($p < 0.05$) the Rot-induced motor incoordination in mice (Fig. 3A and B).

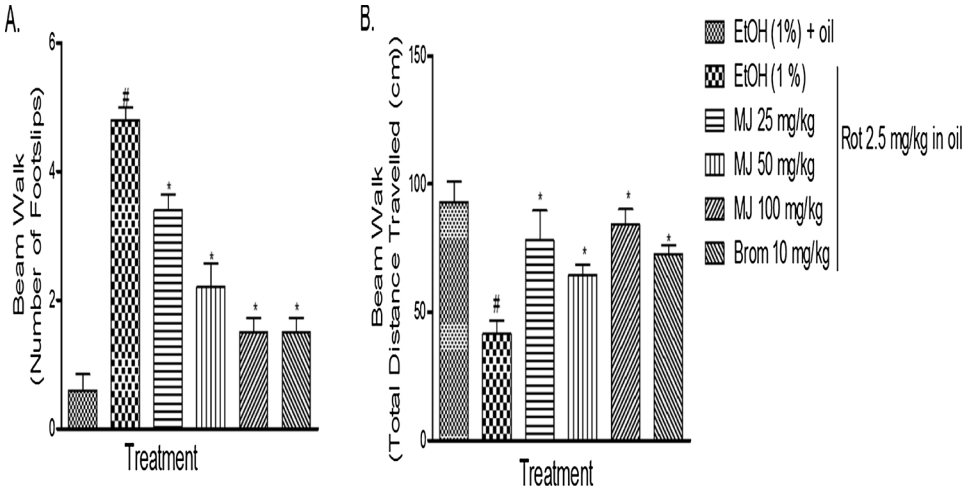


Fig. 3. Methyl jasmonate (MJ) or bromocriptine (Brom) improves rotenone-induced impairment in motor coordination: Foot slip on the walk beam (A) and Total distance travelled (B) on the walk beam in mice. Each bar represents the mean ± SEM of 6 animals per group, $^{\#}p <$ 0.05 *vs* 1% ethanol (EtOH), $^{*}p <$ 0.05 *vs* rotenone (Rot) group (One-way ANOVA followed by Newman-Keuls post hoc test for multiple comparison).

3.3. Methyl jasmonate ameliorates memory deficit and depression-like behavior induced by rotenone in mice

The effects of MJ on Rot-induced memory deficits and depression-like symptom are presented in Fig. 4A and B. One-way ANOVA revealed that were significant differences between treatment groups: percentage alternation ($[F_{6, 34}] = 9.251$, $p <$ 0.0001) and immobility time ($[F_{6, 32}] = 32.24$, $p <$ 0.0001). As presented in Fig. 4A, daily doses of Rot (2.5 mg/kg, i.p) for 28 days impaired memory performance as shown by a significant ($p < 0.05$) decrease in alternation (%) when compared with vehicle group in the Y-maze test in mice. Daily injection of Rot (2.5 mg/kg, i.p.) for 28 days also produced depression-like effect as it significantly ($p < 0.05$) increased immobility time (s) in the forced swim test in mice relative to vehicle. However, pretreatment with MJ (25, 50 and 100 mg/kg, i.p.) or Brom (10 mg/kg, i.p.) significantly ($p < 0.05$) reversed memory deficit and depression-like behavior induced by Rot in mice (Fig. 4A and B).

3.4. Methyl jasmonate inhibits acetyl-cholinesterase activity in the brains of rotenone-treated mice

The effects of MJ on Rot -induced changes in the activity of AChE in the striatum, prefrontal cortex and hippocampus of mice are shown in Fig. 5. One-way ANOVA showed that there were significant differences between groups in brain regions [$F_{2, 63} = 69.37$, $p <$ 0.0001]. As

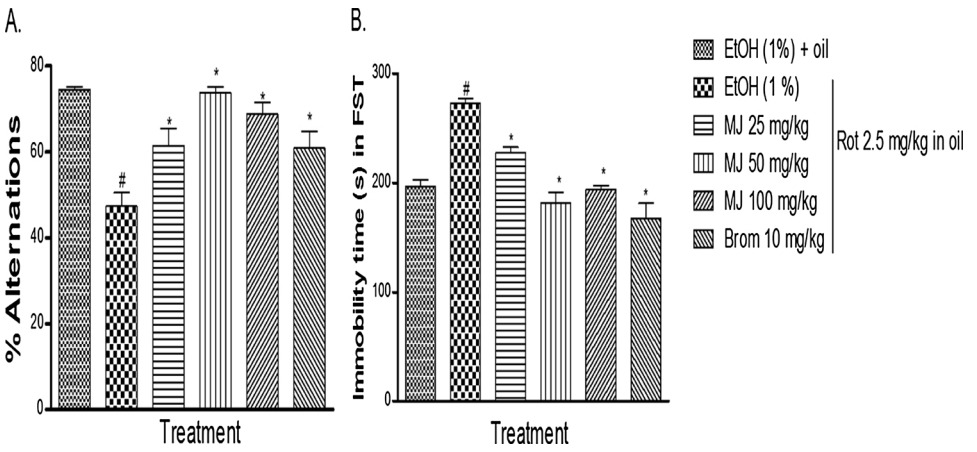


Fig. 4. Methyl jasmonate or bromocriptine (Brom) attenuates memory deficit (A) and depression-like behavior (B) induced by rotenone in mice. Each column represents the mean ± SEM of 6 animals per group, $^{\#}p <$ 0.05 compared with 1% ethanol (EtOH), $^{*}p <$ 0.05 compared with rotenone (Rot) group (One-way ANOVA followed by Newman-Keuls post hoc test for multiple comparison).

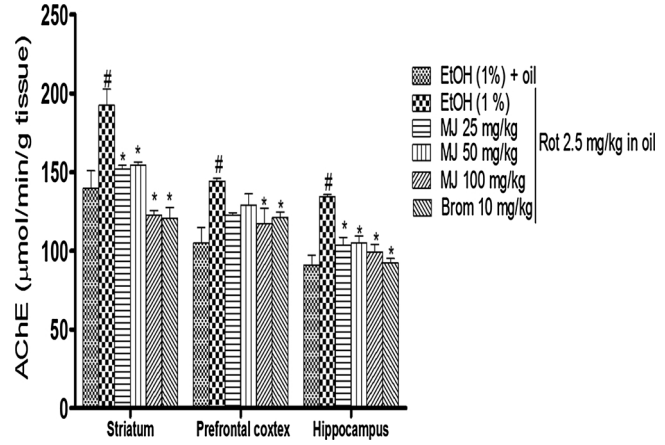


Fig. 5. Methyl jasmonate (MJ) or bromocriptine (Brom) inhibits rotenone-induced acetyl-cholinesterase activity (AChE) in mice. Each column represents the mean ± SEM of 6 animals per group, $^{\#}p <$ 0.05 compared with 1% ethanol (EtOH), $^{*}p <$ 0.05 compared with rotenone (Rot) group (One-way ANOVA followed by Newman-Keuls post hoc test for multiple comparison).

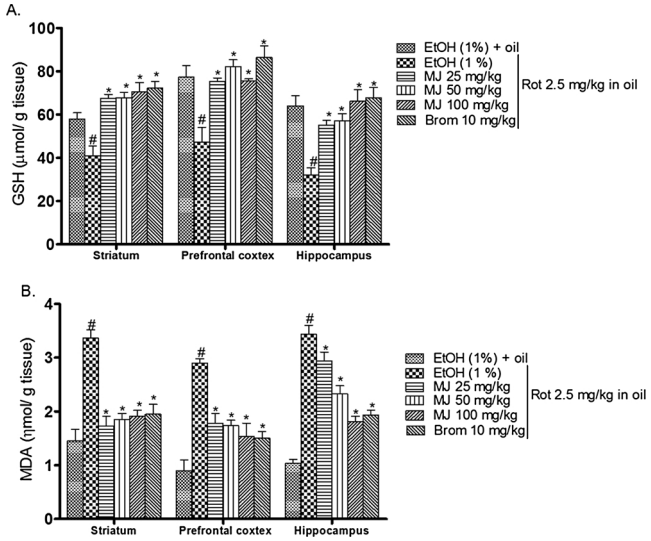


Fig. 6. Methyl jasmonate (MJ) or bromocriptine (Brom) reverses altered brain levels of glutathione (A) and malondialdehyde (B) induced by rotenone in mice. Each column represents the mean ± SEM of 6 animals per group, $^{\#}p <$ 0.05 compared with 1% ethanol (EtOH), $^{*}p <$ 0.05 compared with rotenone (Rot) group (One-way ANOVA followed by Newman-Keuls post hoc test for multiple comparison).

shown in Fig. 5, Rot (2.5 mg/kg, i.p.) injection caused a significant ($p < 0.05$) increase in AChE activity in the striatum, prefrontal cortex and hippocampus of mice when compared with vehicle. However, pretreatment with MJ (25, 50 and 100 mg/kg) produced a significant ($p < 0.05$) decrease in acetyl-cholinesterase activity in the striatum and hippocampus relative to rotenone groups (Fig. 5). As shown in Fig. 5, Brom (10 mg/kg, i.p.) significantly reduced acetyl-cholinesterase activity in the striatum, prefrontal cortex and hippocampus of Rot-treated mice.

3.5. Methyl jasmonate elevated glutathione and reduced malondialdehyde brain levels in rotenone-treated mice

As shown in Fig. 6A and B, injection of Rot (2.5 mg/kg, i.p) for 28 consecutive days produced a significant decrease ($p < 0.05$) in GSH level in the striatum (2 fold), prefrontal cortex (2 fold) and hippocampus (2 fold) of mice relative to vehicle controls. In addition, Rot caused a significant increase ($p < 0.05$) in lipid peroxidation end product (MDA) in the specific brain regions [striatum (2 fold), prefrontal cortex (3 fold) and hippocampus (3 fold)] when compared with vehicle-controls in mice (6B). As presented in Fig. 6A, animals pretreated with MJ (25, 50 and 100 mg/kg, i.p.) or Brom (10 mg/kg, i.p.) prevented the depletion of GSH contents in the striatum, prefrontal cortex and hippocampus ($p < 0.05$). The increases in MDA levels in the striatum, prefrontal cortex and hippocampus induced by Rot was significantly ($p < 0.05$) attenuated by MJ or Brom (Fig. 6B).

3.6. Methyl jasmonate decreases brain nitrite levels in rotenone-treated mice

Rot (2.5 mg/kg, i.p.) given daily for 28 days significantly ($p < 0.05$) elevated the levels of nitrite in the striatum (37.2%), prefrontal cortex (32.6%) and hippocampus (40.6%) when compared with vehicle (Fig. 7). As presented in Fig. 7, MJ (25, 50 and 100 mg/kg, i.p.) or Brom (10 mg/kg, i.p.) reduced the concentrations of nitrite in the striatum and hippocampus ($p < 0.05$) but not in the prefrontal cortex when compared with Rot groups.

3.7. Methyl jasmonate decreases brain concentrations of tumor necrosis factor-α and interleukin-6 in mice treated with rotenone

There was a significant ($p < 0.05$) increase in the concentrations of TNF-α in the specific brain regions [striatum (2 fold), prefrontal cortex (3 fold) and hippocampus (2 fold)] of mice treated with Rot (2.5 mg/kg, i.p.) for 28 days when compared with vehicle-controls (Fig. 8A). As presented in Fig. 7B, significant increases ($p < 0.05$) in the levels of IL-6 were also observed in the striatum (3 fold), prefrontal cortex (2 fold) and hippocampus (4 fold) of mice treated with Rot (2.5 mg/kg, i.p.) in comparison with vehicle-controls. However, MJ (25, 50 and 100 mg/kg, i.p.) or Brom (10 mg/kg, i.p.) demonstrated significant ($p < 0.05$)

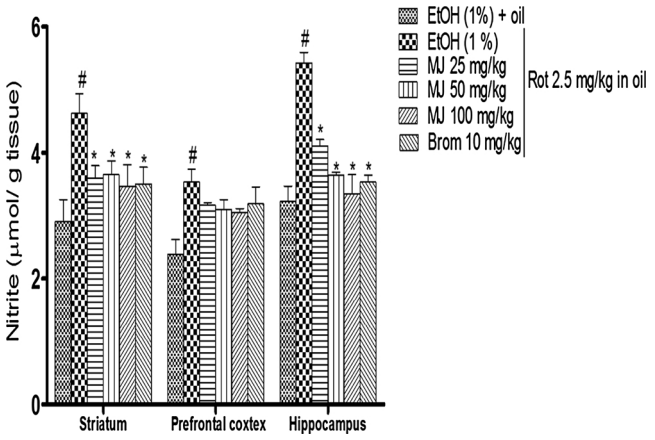


Fig. 7. Methyl jasmonate (MJ) or bromocriptine (Brom) decreases nitrite concentration in the brains of mice treated with rotenone. Each bar represents the mean ± SEM of 6 animals per group, $^{\#}p < 0.05$ compared with 1% ethanol (EtOH), $^{*}p < 0.05$ compared with rotenone (Rot) group (One-way ANOVA followed by Newman-Keuls post hoc test for multiple comparison).

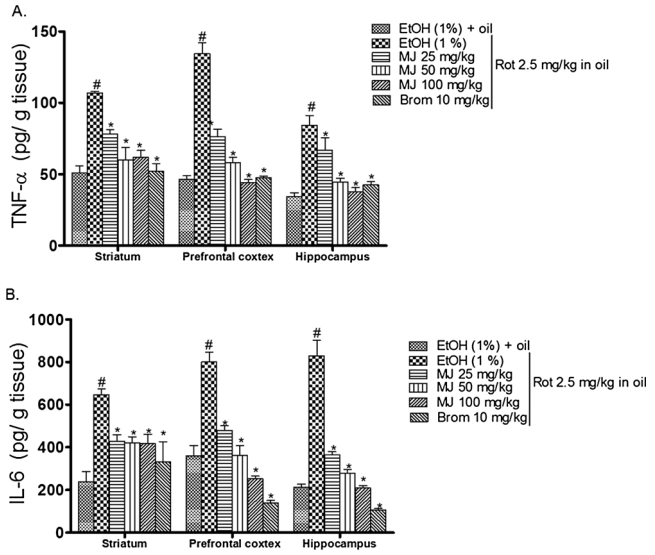


Fig. 8. Methyl jasmonate (MJ) or bromocriptine (Brom) reduces the brain level of tumor necrosis factor-α (TNF-α) (7A) and interleukin-6 (IL-6) (7B) induced by rotenone in mice. Each column represents the mean ± SEM of 6 animals per group, $^{\#}p < 0.05$ compared with 1% ethanol (EtOH), $^{*}p < 0.05$ compared with rotenone (Rot) group (One-way ANOVA followed by Newman-Keuls post hoc test for multiple comparison).

inhibitory activity against Rot-induced elevation of TNF-α and IL-6 levels in the striatum, prefrontal cortex and hippocampus respectively (Fig. 8A and B).

3.8. Methyl jasmonate down regulates rotenone-induced NF-κB expression in mice brains

Immunohistochemical findings from the three brain regions (striatum, prefrontal cortex and hippocampus) revealed immune-reactivity against the p65 subunit of NF-κB in rotenone-treated mice (Fig. 9). As shown in Fig. 10, the intensity of the expression of NF-κB was significantly elevated in the brain regions of the striatum (3 fold), prefrontal cortex (2 fold) and hippocampus (3 fold) of mice treated with Rot (2.5 mg/kg, i.p.) when compared with vehicle controls. However, pretreatment with MJ (100 mg/kg) significantly ($p < 0.05$) reduced the expression of NF-κB in striatum, prefrontal cortex and hippocampus of mice relative to Rot groups. Similar effects were also observed in the Brom-treated group, which showed a significant ($p < 0.05$) reduction in the intensity of NF-κB expression (Fig. 10).

3.9. Methyl jasmonate down regulates rotenone-induced increased α-synuclein immunoreactivity in the prefrontal cortex and hippocampus of mice

As presented in Fig. 11, injection of Rot (2.5 mg/kg) daily for 28 days produced a marked expression of α-synuclein immunoreactivity in the striatum, prefrontal cortex and hippocampus of mice. Intraperitoneal administration of MJ (100 mg/kg) or Brom (10 mg/kg) significantly ($p < 0.05$) reduced Rot-induced increased expression of α-synuclein in the prefrontal cortex when compared to Rot groups (Fig. 12). Brom (10 mg/kg), but not MJ (100 mg/kg) significantly decreased α-synuclein in the striatum. On the other hand, MJ (100 mg/kg) significantly ($p < 0.05$) down-regulated α-synuclein immunoreactive cells expression in the hippocampus when compared with Rot-treated group (Fig. 12).

4. Discussion

The loss of multiple neuronal pathways underpins the wide range of debilitating motor and non-motor symptoms associated with PD, a progressive neurodegenerative disorder connected with the aging process (Li, 2005). The disabling motor symptoms in patients with PD is well depicted to be related to the degeneration of nigrostriatal dopaminergic neurons that results in deficiency of dopamine, the neurotransmitter, implicated in the regulation of posture, voluntary movements, and other motor functions (Alam and Schmidt, 2002; Dhanalakshmi et al., 2016). The decreased levels of dopamine cause the neurons of the striatum to fire uncontrollably; thereby preventing the patients with PD from having a normal coordinated motor functions (Alam and Schmidt, 2002; Dhanalakshmi et al., 2016). Rot, a potent inhibitor of mitochondrial electron transport chain complex-1, is known to replicate the motor and non-motor symptoms associated with PD patients *via* up-regulation of inflammatory and oxidative signaling pathways (Alam and Schmidt, 2002; Dhanalakshmi et al., 2016).

Different studies have shown that Rot-treated mice and rats exhibited impaired locomotor activity and postural instability (Pont-Sunyer et al., 2015; Cooper et al., 2018). Impaired locomotor function, evidenced by reduced or slowness of voluntary movements (bradykinesia) and difficulty in initiating movement (akinesia) due to muscle rigidity is a prominent feature of PD (Dauer and Przedborski, 2003; Schapira and Jenner, 2011). Postural instability characterized by loss of balance and fine motor coordination is also a major incapacitating motor symptom experienced by patients with the disease (Pont-Sunyer et al., 2015; Cooper et al., 2018). Hence, the effect of MJ on Rot-induced motor deficits was assessed using activity cage and in line with earlier reports (Alam and Schmidt, 2002), Rot impaired locomotor activity, which was attenuated by MJ. The effect of MJ on Rot-induced motor impairment was further assessed using the walk beam test. The walk beam test is used to test for sensorimotor performance, fine motor coordination and postural stability in rodents (Hoglinger et al., 2003; Swarnkar et al., 2010). Our findings are in accordance with literature, which showed that Rot caused postural instability and motor incoordination as evidenced by increased number of foot slips and reduced distance travelled in the walk beam test (Hoglinger et al., 2003; Swarnkar et al., 2010). However, pretreatment with MJ, enhanced the performance of Rot-treated mice in the walk beam test suggesting improved postural stability and motor coordination. Previous studies have shown that Brom, an ergot alkaloid used for the treatment of PD, reversed Rot-induced motor deficits in rodents, an action ascribed to D_2-dopamine receptor stimulation in sub-cortical regions of the brain (Hisahara and Shimohama, 2011; Alam and Schmidt, 2002). Thus, the finding that MJ attenuates Rot-induced motor deficits suggests that it might be beneficial in conditions associated with movement disorders

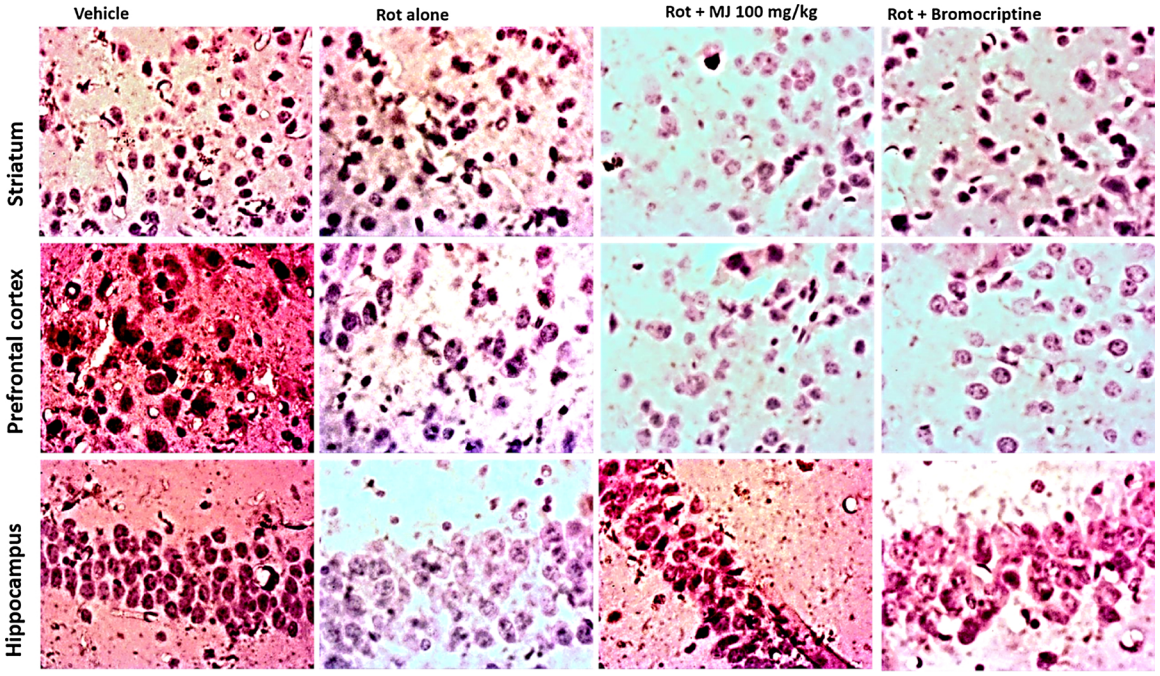


Fig. 9. Representative light photomicrographs of immunostained NF-κB in the specific brain regions of rotenone-treated mice. Brain sections were immunostained with antibody against p65 subunit of NF-κB and counterstained with hematoxylin. Vehicle (1% ethanol), rotenone (Rot, 2.5 mg/kg), methyl jasmonate (MJ, 100 mg/kg), bromocriptine (10 mg/kg).

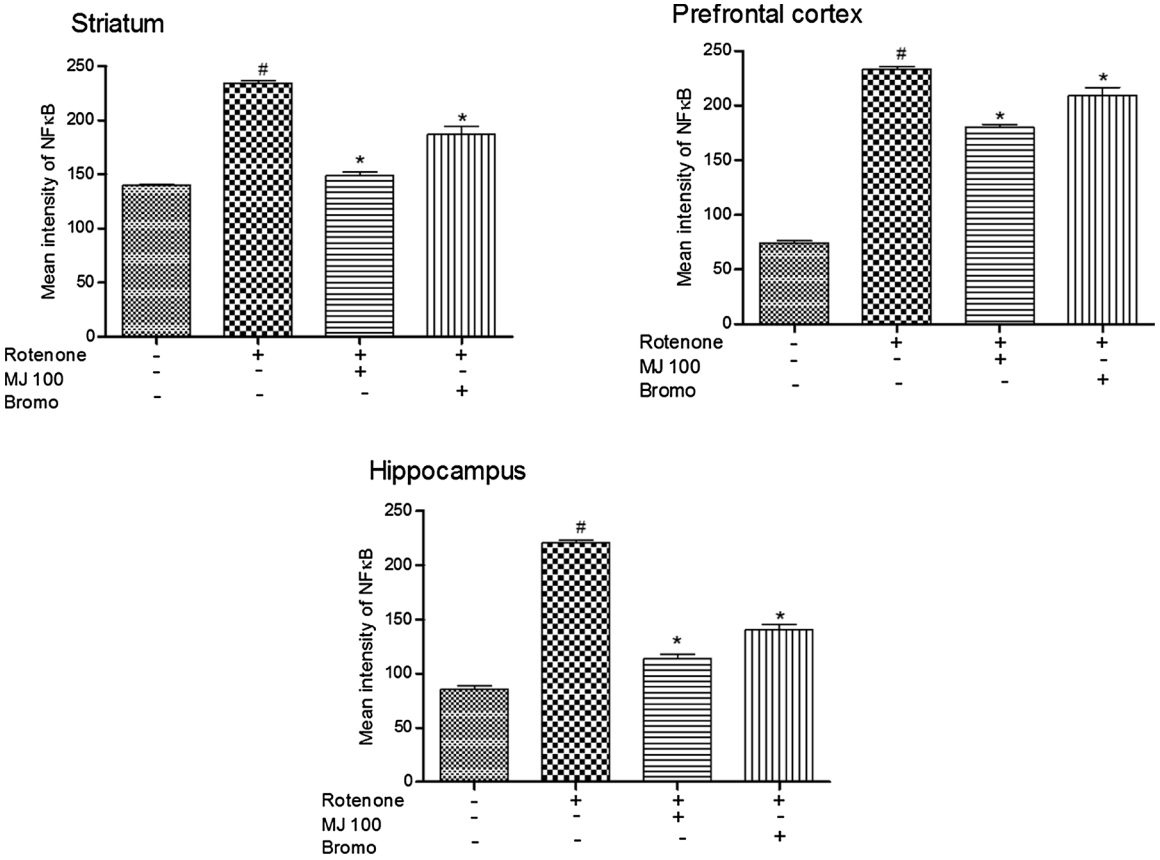


Fig. 10. Methyl jasmonate (MJ) or bromocriptine (Brom) down regulates NF-kB expressions in the brain regions of mice treated with rotenone. Each column represents the mean ± SEM of 6 animals per group, $^{\#}p < 0.05$ compared with 1% ethanol (EtOH), $^{*}p < 0.05$ compared with rotenone (Rot) group (One-way ANOVA followed by Newman-Keuls post hoc test for multiple comparison).

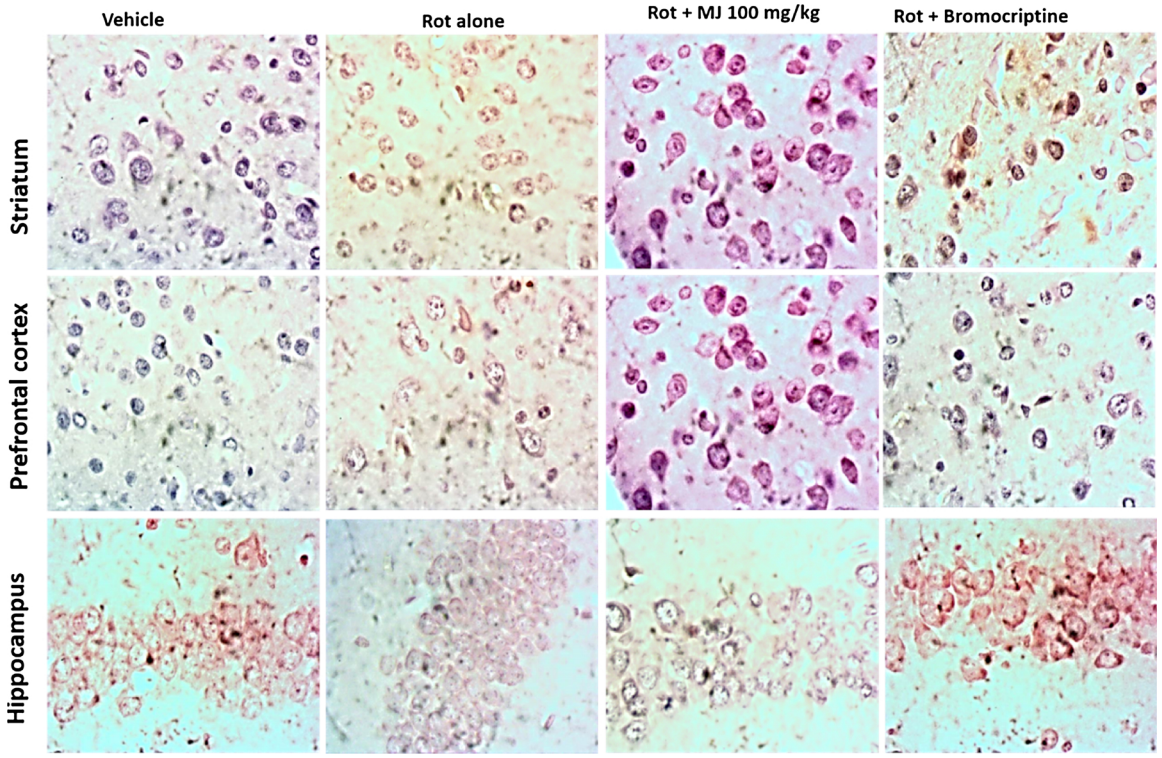


Fig. 11. Representative light photomicrographs of immunostained α-synuclein in the specific brain regions of rotenone-treated mice. Brain sections were immunostained with antibody α-synuclein and counterstained with hematoxylin. Vehicle (1% ethanol), rotenone (Rot), methyl jasmonate (MJ, 100 mg/kg), bromocriptine (10 mg/kg).

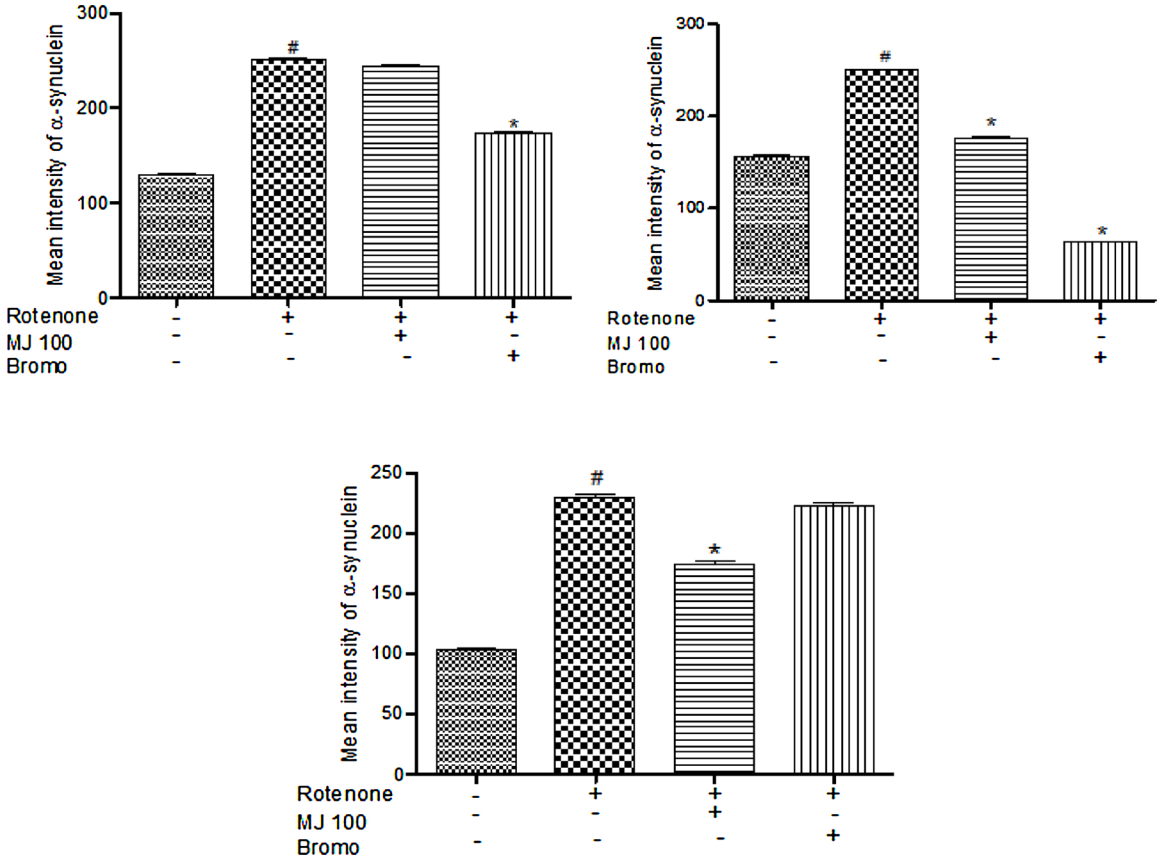


Fig. 12. Methyl jasmonate (MJ) or bromocriptine (Brom) down regulates α-synuclein expressions in the brain regions of mice treated with rotenone. Bars represent the mean ± SEM of 6 animals per group, $^{\#}p <$ 0.05 compared with 1% ethanol (EtOH), $^{*}p <$ 0.05 compared with rotenone (Rot) group (One-way ANOVA followed by Newman-Keuls post hoc test for multiple comparison).

like PD.

Studies have shown that depression is a common non-motor symptom in patients with PD that often precedes the motor anomalies (Cooper et al., 2018; Litteljohn et al., 2009). The pathology of depression in PD is rather complex but may be due to changes in monoaminergic pathways (Morais et al., 2012; Azmy et al., 2018). This has been ascribed to reduced dopaminergic, serotonergic and noradrenergic inputs to the striatum and the consequent decline in neuronal efficiency involved in reward and motivation (Hoglinger et al., 2003; Litteljohn et al., 2009; Morais et al., 2012). Moreover, studies have also shown that Rot, MPTP and 6-OHDA produced depressive-like behavior accompanied by reduced dopamine contents and hippocampal turnover of serotonin and its metabolite (5-hydroxyindoleacetic acid); which are major molecular substrates implicated in the pathology of depression (Santiago et al., 2010; Morais et al., 2012). The results of our study support previous reports (Hoglinger et al., 2003; Morais et al., 2012), which have shown that Rot caused depressive-like behavior as evidenced by increase in duration of immobility in mice. The finding that MJ shortened immobility time in the FST, therefore, suggests its potential usefulness in the management of depression associated with PD.

Loss of cholinergic neurons and increased acetyl-cholinesterase enzyme (AChE) has been implicated in cognitive deficits in patients with PD (Sedelis et al., 2001; Chtourou et al., 2015). Moreover, preclinical studies have also shown that Rot depletes brain acetylcholine due to increased expression of cortical and sub-cortical acetyl-cholinesterase activity and degeneration of cholinergic neurons (Hoglinger et al., 2003). Besides, inhibitors of AChE such as donepezil and rivastigmine are drugs of choice for the treatment of cognitive deficits in patients with the disease (Schenkman et al., 2001; Santiago et al., 2010; Cooper et al., 2018). In accordance with existing data (Hoglinger et al., 2003), the results of our study showed that Rot impaired memory performance and also increased AChE activity in specific region regions of mice. However, pretreatment with MJ abrogated the memory deficit and increased AChE activity induced by Rot in the specific brain regions. In another study in our laboratory, we have also shown that MJ reverses memory impairment induced by LPS in mice *via* inhibition of AChE (Eduviere et al., 2016; Umukoro and Eduviere, 2017). Overall, these findings suggest that the improvement in memory function in Rot-treated mice by MJ may be related partly to enhancement of central cholinergic transmission through inhibition of AChE activity.

There are increasing evidences supporting the involvement of oxidative stress and neuroinflammation in the onset and progression of PD (Mogi et al., 1996; Hunot et al., 1996; Azmy et al., 2018). Positive correlation between the brain levels of IL-6 and postural instability has been established in patients with PD (Scalzo et al., 2010). TNF-α and IL-6 have been implicated in neuronal cell death through mechanisms associated with induction of oxidative stress and stimulation of apoptotic factors (Azmy et al., 2018), which further create a vicious cycle of neuroinflammation that typified the progressive loss of nigrostriatal dopaminergic neurons (Azmy et al., 2018; Pan et al., 2016; Cooper et al., 2018). Previous investigations have identified increased levels of oxidative/nitrergic stress and pro-inflammatory cytokines in the brain of animals exposed to Rot, MPTP and 6−OHDA (Szabo et al., 2007; Azmy et al., 2018). Different studies have shown that Rot-induced inhibition of mitochondrial electron transport chain complex-I causes increase in free radical generation and microglia activation in cortical and sub-cortical brain regions, both of which are known to trigger metabolic degradation of neuronal lipids and DNA (Sian et al., 1994; Sherer et al., 2003a,b; Dawson and Dawson, 2003). Production of reactive oxygen and nitrogen species is well-known to be exacerbated by increased microglia activation, neurodegeneration, mitochondrial dysfunctions, and impaired antioxidant response mechanisms (Lev et al., 2009). Thus, Rot-induced depletion of endogenous antioxidant molecules (catalase, superoxide dismutase and glutathione) may contribute to its toxicity on dopaminergic neurons (Sherer et al. 2003a,b; Pan et al., 2016). Indeed, deficiency of striatal GSH has been reported in literature and is believed to contribute to α-synucleinopathy in PD (Sian et al., 1994; Pearce et al., 1997). Recently, altered cellular expression of the antioxidant enzyme gene, nuclear factor erythroid 2-related factor 2 (Nrf2) has been implicated in the pathogenesis of PD (Lastres-Becker et al., 2016). Rot-induced PD has also been linked to dysfunctional Nrf2-mediated antioxidant response *via* mechanisms related to suppression of Nrf2 nuclear translocation and signaling (Abdelsalam and Safar, 2015; Pan et al., 2016). Studies have shown that the up-regulation of Nfr2 by antioxidant molecules might prevent death of dopaminergic neurons and thereby retard the progression of the disease (Lim et al., 2008; Lastres-Becker et al., 2016). Our results showed that both MJ and Brom reduced pro-inflammatory cytokines (IL-6 and TNF-α), MDA and nitrite levels in specific brain regions of Rot-treated mice. Depletion of endogenous antioxidant molecule (GSH) induced by Rot was also attenuated by MJ. Previous studies have also confirmed the antioxidant and anti-inflammatory properties of MJ (Dang et al., 2006; Lee et al., 2011; Eduviere et al., 2016; Umukoro and Eduviere, 2017) and Brom (Ogawa et al., 1994; Kondo et al., 1994) in various experimental models. Taken together, these findings suggest that the positive effects of MJ on Rot-induced motor and non-motor deficits in Rot-treated mice may be mediated through inhibition of oxidative/nitrergic stress and release of pro-inflammatory cytokines in these brain regions of mice. Meanwhile, it has been reported that Brom did not only improve motor deficits by activating dopamine D_2 receptor, but also *via* neuroprotective mechanisms resulting from antioxidation, mitochondrial stabilization or induction of the anti-apoptotic Bcl-2 expression (Kitamura et al., 2003; Ogawa et al., 1994; Yoshikawa et al., 1994; Takashima et al., 1999). Pretreatment with Brom was shown to reduce levodopa-induced neurodegeration and offers complete protection against 6-OHDA-induced depletion of striatal dopamine and its metabolites (Ogawa et al., 1994; Takashima et al., 1999). Moreover, Brom was also reported to elevate Nfr2 expression, hence, providing neuroprotective or antioxidant defense mechanisms against oxidative stress-induced loss of dopaminergic neurons (Lim et al. (2008). However, further studies are necessary before any valid conclusion can be made on the role of Nfr2 signaling pathway in the protective effect of MJ against Rot-induced parkinsonian-like disorder in mice.

Progressive degeneration of dopaminergic neurons has been linked to increased release of pro-inflammatory cytokines *via* activation of NF-κB-dependent p53 signaling pathway in PD (Yan et al., 2014). Previous studies have shown that Rot-mediated microglia activation causes nuclear translocation of the oxidative stress-sensitive NF-κB thereby forming adducts with dopaminergic synthesizing enzymes and misfolding of proteins on the surface of dopaminergic neurons (Sherer et al., 2003a, Sawada et al. 2006), an action that has been linked to selective dopaminergic degeneration and α-synuclein aggregation (Sherer et al., 2003b). Moreover, Rot-induced PD has been shown to cause rapid phosphorylation of NF-κB inhibitor accompanied by increased NF-κB-dependent p53 nuclear translocation to the nucleus of dopamine neurons, which is known to produce neuronal cell death that characterized PD (Wang et al., 2002). It is worthy of note that the substantia nigral of dopaminergic neurons is particularly more vulnerable to neuroinflammatory-mediated neurotoxicity due to the presence of large number of mitochondrial, microglial cells, catalytic metals like irons and low levels of reduced GSH in the striatum (Sian et al., 1994; Qian et al., 2010). Indeed, clinical studies have also shown that nigrostriatal dopaminergic system had increased expression of NF-κB (Mogi et al., 2007), which further implicates the role of this inflammatory protein in the progression of PD (Wang et al., 2002; Ghosh et al., 2007; Yang et al., 2010). In our study, the increase in NF-κB expression in the striatum, prefrontal cortex and hippocampus of Rot-treated mice was in line with earlier report showing that Rot caused dopaminergic neuronal cell death *via* up-regulation of NF-κB-dependent p53 expression in brain-region dependent manner (Wang et al., 2002). Thus, inhibition of expression of NF-κB has been reported to prevent the loss of dopaminergic neurons in animals with PD (Ghosh et al., 2007;

Yang et al., 2010). The results of our study showed that MJ inhibits NF-κB-dependent p53 expression in specific brain regions of Rot-treated mice. It is instructive to note that previous studies have also shown that MJ and its derivatives demonstrated NF-κB inhibitory activity in culture cells (Lee et al., 2011; Dang et al., 2012). Besides, MJ has been reported to selectively kill cancer cells through several related mechanisms including inhibition of NF-κB expression and mitochondrial apoptotic and non-apoptotic pathways (Kim et al., 2004; Roteim et al., 2005). However, further studies are needed to establish whether inhibition of NF-κB-dependent p53 expression contributes to the ability of MJ or Brom to alleviate the parkinsonian-like motor and non-motor symptoms induced by Rot in mice.

Over the past decades, oxidative and inflammatory damage to cellular proteins including α-synuclein and cytosolic deposit of the mutant or wild-type α-synuclein have been highlighted as a major pathological hallmark and indicator of the severity of PD (Theodore et al., 2008). Alpha-synuclein is a soluble protein that is prominently expressed in presynaptic terminals of neurons and accumulation of α-synuclein as intracellular filamentous dimmers has been associated with degeneration of dopaminergic neurons (Cooper et al., 2018). Increased expression of wild-type α-synuclein is known to cause neurological deficits and loss of dopamine neurons (Cooper et al., 2018). Moreover, increased accumulation of wide-type α-synuclein in dopaminergic neurons reduces mitochondria complex I activity and induces production of ROS leading to neuronal cell death (Cooper et al., 2018). Meanwhile, increased formation of ROS and mitochondrial dysfunction caused by Rot has been reported to correlate with α-synucleinopathies and impaired dopamine biosynthesis (Norris et al., 2005). This finding is in agreement with the results of our study, which showed that Rot produced a significant increase in the expression of immunopositive cells of α-synuclein in the striatum, prefrontal cortex and hippocampus of mice. However, pretreatment with MJ reduced Rot-induced increased expression of α-synuclein in the specific brain regions of mice, though with mild effect on striatal α-synuclein immunopositive cells. However, Brom was more effective than MJ in reducing α-synuclein immunopositive cells in striatum and prefrontal cortex but not in the hippocampus of Rot-treated mice. The reason (s) for the differential effects of MJ or Brom on the specific brain regions on α-synuclein immunopositive cells are not apparent in this study but requires further investigations. In addition, the implication of MJ-induced down-regulation of the expression of α-synuclein immunopositive cells in the hippocampus of Rot-treated mice is also open to further studies and may also be linked to the non-motor beneficial effects observed herein.

Although the choice of doses of MJ used in this study was based on results obtained from previous studies, the reason for the lack of dose-dependency in some of the activities exhibited by MJ requires further investigations (Eduviere et al., 2016). Meanwhile, its high safety profile both *in vivo* and *in vitro* studies (Belsito et al., 2012; Cesari et al., 2014) contrasts sharply with Brom. Despite the fact that Brom relieves parkinsonian-like symptom without causing loss of dopaminergic neurons and dyskinesia or wearing-off effect that occur with long-term L-dopa therapy, its clinical efficacy has been compromised by several severe side effects (Hisahara and Shimohama, 2011). Notable among these adverse effects of Brom, include abnormal involuntary movements, hypersexuality, stereotyped behaviors, hallucination and mental confusion (Hisahara and Shimohama, 2011). Furthermore, it has also been reported that Brom produces valvulopathy, which is a fibrotic heart disease mainly induced by ergoline derivatives in clinical settings (Serratrice et al., 2002; Hisahara and Shimohama, 2011). Besides, it is also known to be less effective in patients with advanced cases of PD (Ogawa et al., 1994), hence the need to search for newer agents still persist. It is worthy of note that MJ forms a major component of many functional fruits and vegetables, and has also been recognized as safe food additive by food regulating bodies (Environmental Protection Agency, 2013; Cesari et al., 2014). Furthermore, animal and human toxicological studies have also confirmed that MJ is very safe irrespective of the route of administration (Belsito et al., 2012). Besides, earlier studies have also shown that MJ has an oral LD_{50} value greater than 5000 mg/kg and 90 days oral treatment of methyl dihydrojasmonate, an active metabolite of MJ, showed no observed adverse effects (Belsito et al., 2012). Taken together, these findings might encourage the development of MJ as a promising agent for the treatment of patients with PD.

5. Conclusion

The results of this study suggest that MJ attenuates motor and non-motor features induced by Rot in mice through mechanisms associated with inhibition of acetyl-cholinesterase activity, oxidative stress, release of pro-inflammatory cytokines, and down regulation of NF-κB and α-synuclein expressions.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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