Demethoxycurcumin, a Natural Derivative of Curcumin Abrogates Rotenone-induced Dopamine Depletion and Motor Deficits by Its Antioxidative and Anti-inflammatory Properties in Parkinsonian Rats

Muthu Ramkumar[1], Srinivasagam Rajasankar[2], Veerapan Venkatesh Gobi[1], Udaiyappan Janakiraman[3], Thamilarasan Manivasagam[3], Arokiasamy Justin Thenmozhi[3], Musthafa Mohamed Essa[4,5,6], Ranganathan Chidambaram[7], Saravana Babu Chidambaram[8], Giles J. Guillemin[9]

[1]Department of Anatomy, Bharath University, Selaiyur, Chennai, [2]Department of Anatomy, Velammal Medical College and Hospital, Madurai, [3]Department of Biochemistry and Biotechnology, Annamalai University, Annamalainagar, Tamil Nadu, India, [4]Department of Food Science and Nutrition, CAMS, Sultan Qaboos University, Muscat, [5]Ageing and Dementia Research Group, Sultan Qaboos University, Muscat, Oman, [6]Food and Brain Research Foundation, Chennai, Tamil Nadu, India, [7]Department of Radiology, Sri Lakshminarayana Institute of Medical Sciences, Puducherry, [8]Department of Pharmacology, JSS College of Pharmacy, JSS University, SS Nagar, Mysore, Karnataka, India, [9]Neuroinflammation Group, Department of Biomedical Research, Faculty of Medicine and Health Sciences, Macquarie University, Sydney, NSW, Australia

ABSTRACT

Background: Parkinson's disease (PD) is a progressive neurodegenerative disorder (NDD) associated with the loss of dopaminergic neurons in the substantia nigra and subsequently has an effect on motor function and coordination. The pathology of PD is multifactorial, in which neuroinflammation and oxidative damage are the two of the main protagonists. **Objectives:** The present study aims to assess the potential antioxidant and anti-inflammatory effects of demethoxycurcumin (DMC), a natural derivative of curcumin, against rotenone-induced PD in rats. **Materials and Methods:** Rats were randomized and divided into six groups: control, rotenone (0.5 mg/kg/day, intraperitoneal in sunflower oil) treated for 7 days, rotenone and DMC (5, 10, and 20 mg/kg b.w) cotreated, and DMC (20 mg/kg b.w) alone treated groups. **Results:** Based on the dopamine concentration and biochemical estimations, the effective dose of DMC was selected and the chronic study was performed. At the end of the experimental period, behavioral studies and protein expression patterns of inflammatory markers were analyzed. Rotenone treatment led to motor dysfunctions, neurochemical deficits, and oxidative stress and enhanced expressions of inflammatory markers, whereas oral administration of DMC attenuated all the above. **Conclusion:** Even though further research is needed to prove its efficacy in clinical trial, the results of our study showed that DMC may offer a promising and new therapeutic lead for the treatment of NDDs including PD.

Key words: Demethoxycurcumin, dopamine, neurodegenerative disease, neuroinflammation, oxidative stress

SUMMARY

- Curcumin and their derivatives have been shown to be potent neuroprotective effect
- Demethoxycurcumin (DMC) amolerated the rotenone induced behavioural alterations
- DMC abrogated the rotenone induced dopamine deficits
- DMC attenuated the rotenone induced oxidative stress
- DMC diminished the rotenone mediated inflammation.
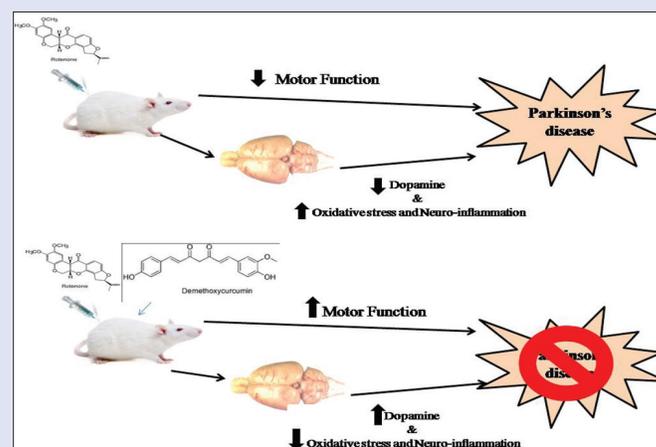
Abbreviations used: COX-2: Cyclooxygenase-2; DA: Dopamine; DMC: Demethoxycurcumin; DMRT: Duncan's multiple range test; GSH: Reduced glutathione; GPx: Glutathione peroxidase; IL-1 β: Interleukin-1 β; IL-6: Interleukin-6; iNOS: Inducible nitric oxide synthase; PD: Parkinson's disease; SN: Substantia nigra; SOD: Superoxide dismutase; TBARS: Thiobarbituric acid reactive substances; TNF-α: Tumor necrosis factor-α.

Correspondence:

Dr. Srinivasagam Rajasankar,
Department of Anatomy,
Velammal Medical College and Hospital,
Madurai - 625 009, Tamil Nadu, India.
E-mail: anatomysrs@yahoo.com
DOI: 10.4103/pm.pm_113_17

Access this article online

Website: www.phcog.com

Quick Response Code:

INTRODUCTION

The disabling symptoms of Parkinson's disease (PD) are related to the movement abnormalities such as tetrad of tremor at rest, rigidity, akinesia (difficulty in initiating movement), bradykinesia (slowness of voluntary movements), gait, and posture abnormalities. These symptoms are mainly due to the loss of dopaminergic neurons in substantia nigra pars compacta (SNpc) and the consequent degeneration of their projecting nerve

fibers in the striatum (ST), which leads to shortage in striatal dopamine (DA) content. Although the exact cause of PD remains unclear, enhanced oxidative stress and mitochondrial dysfunction are reported to be associated with the pathological processes underlying PD.[1] Other prominent neuropathological features of PD include gliosis[2] and the presence of intraneuronal proteinaceous inclusions called Lewy bodies (LBs)[3] that further induce the loss of neurons in the substantia nigra (SN).

Using the animal models, the reliability and internal validity mimicking human PD can be achieved. The striatal DA deficiency associated with motor symptoms of PD is evaluated through the use of an animal model as this is not possible in a cellular model. Two types of animal models (neurotoxin and transgenic) resembling the familial and sporadic forms of PD have been used.[4,5] Toxin models represent the classic experimental PD models, which are able to replicate most of the phenotypic and/or pathological features of PD. The neurotoxin agents have been used to induce PD in a broad range of organisms from small single cell yeast to large, nonhuman primates including 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 6-hydroxydopamine (6-OHDA), rotenone, paraquat, and maneb.

Rotenone is considered as one of the best neurotoxins for induction of *in vitro* and *in vivo* models of PD.[3,6] Previous studies indicated that the administration of rotenone to animals induces biochemical, anatomical, and behavioral symptoms similar to those observed in PD patients.[7,8] It is a potent inhibitor of mitochondrial electron transport chain complex-I[9] that leads to production of free radicals resulting in oxidative damage.[10] It also has the ability to activate both the microglial and astroglial cells and trigger the neuroinflammatory responses.[11] Activated microglia might cause dopaminergic cell death by releasing cytotoxic inflammatory cytokines, such as interleukin-1 β, 6 (IL-1 β, 6) and tumor necrosis factor-α (TNF-α). TNF-α damages dopaminergic neurons by activating intracellular death signaling pathways (necrosis factor kappa B [NF-κB], JNK, and p38 pathways) and inducing cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) expressions, which further amplify inflammatory cascades.[12]

Even today, L-DOPA therapy remains the most effective treatment for PD. Substitution treatment of PD by L-DOPA initially reduces motor symptoms. However, long-term administration typically leads to motor complications, including involuntary choreoathetoid movements and motor fluctuations, collectively known as L-DOPA-induced dyskinesia (LIDS), due to excessive response of DA.[13] Therefore, the long-term L-DOPA treatment worsens the neurodegeneration and produces LIDS.[14] The current pharmacological therapies for the PD are inadequate. These are only able to provide symptomatic relief and after long use produce stern side effects and worsen the condition. An alternative therapeutic approach would be the use of natural plant extracts or their associated phytochemicals that are reported to have access to the brain and offer neuroprotection by virtue of their radical scavenging, iron chelating, anti-inflammatory, and anti-apoptotic activities.[15,16]

Curcuma longa (turmeric) has a long history of use in Ayurvedic medicine. The pharmacological properties of turmeric may be due to the presence of curcumin, the active polyphenol which offers anti-parkinsonism effect attributed to its antioxidant,[17] mitochondrial protective,[18] signal modulating,[19] anti-inflammatory,[20,21] and antiapoptotic properties.[22] Commercially available preparations of curcumin contain at least three curcumin compounds including curcumin, demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC). The ratio of curcumin: DMC: BDMC in commercially available preparations is about 66:23:11. DMC and BDMC are also used in the food industry, for domestic cooking and folk medicines.[23]

Curcumin analogs such as DMC and BDMC have also been reported to possess considerable antioxidant, anti-inflammatory, and antiproliferative activities.[24-26] DMC is reported to have better anticancer and anti-inflammatory activity compared with curcumin.[27,28] Few researchers have indicated that the methoxy groups on the phenyl rings in curcumin have important health effects.[17] The curcuminoids, curcumin, DMC, and BDMC strongly inhibited βA-fibril formation in Alzheimer's disease.[29] Based on the above literature, the present study focused on the neuroprotective role of DMC against rotenone-induced motor deficits, neurochemical alteration, and oxidative stress and the expression of inflammatory markers in rats.

MATERIALS AND METHODS

Animals and drug treatment

Male Wistar rats (225–250 g) were procured from the Biogen Laboratory, Bangalore, India. They were kept under the ambient conditions and fed with standard pellet and water *ad libitum*. All the experimental protocols met with the National Guidelines on the Proper Care and Use of Animals in Laboratory Research (Indian National Science Academy, New Delhi, 2000) and were approved by the Animal Ethics Committee (IAEC/KMPC/230/2015–2016).

Experiment I

About 36 rats were randomized and divided into the following groups (*n* = 6): control rats (0.5 ml of sunflower oil intraperitoneal [i.p.] for 3 weeks), rotenone (2.5 mg/kg/day, i.p in sunflower oil for 7 days) alone treated, rotenone (as group II) and low dose of DMC (5 mg/kg/b.w. was administered orally after 1 h after rotenone treatment) treated, rotenone and middle dose of DMC (10 mg/kg b.w.) treated, rotenone and high dose of DMC (20 mg/kg b.w.) treated, and DMC (20 mg/kg/day) alone treated. After the end of the experimental period, a behavior test (narrow beam walking) was carried out and the ST and SN were procured for the estimation of DA and oxidative stress-related indices, respectively.

Experiment II

Based on the DA level, we considered 10 mg/kg of DMC treatment as the effective dose and used it for further experiments. Twenty-four animals were randomized and divided into four groups (*n* = 6): control group (0.5 ml of sunflower oil i.p. for 45 days), rotenone-treated group (2.5 mg/kg/day i.p. in sunflower oil for 45 days) (as group II), DMC (10 mg/kg b.w. p.o. for 45 days) + rotenone (2.5 mg/kg/day i.p. in sunflower oil for 45 days) treated group, and DMC (10 mg/kg) alone treated. After the end of the experimental period, behavior tests (Rotarod and Hang test) were carried out. Then, the animals were sacrificed by cervical dislocation and the SN was dissected, rinsed in ice-cold saline, and stored at −80°C for protein expression studies of inflammatory markers.

Narrow beam walking

Rats were pretrained to cross an elevated and narrow beam to reach an enclosed escape platform in the beam-walking test. After the experimental period, the animals were allowed to transverse the narrow, flat wooden beam (L100 cm × W1 cm), which was placed at a height of 100 cm from the floor. The time taken to cross the starting area to the finishing area was measured.[30]

Rotarod

The rotarod test, in which animals walk on a rotating drum, is widely used to assess motor status in laboratory rodents. Performance is measured

by the duration an animal stays on the drum in relation to the drum speed. Rats were allowed to adjust their posture in order to maintain their balance on a rotating rod at speeds of 5, 10, 15 and 20 rpm. The average retention time on the rod was calculated.[31]

Hang test

Rats were placed on a horizontal grid and were supported until they grabbed the grid with their fore and hind paws. Then, the grid was inverted so that the rats were allowed to hang upside down. The falling time was measured.[32]

High-performance liquid chromatographic analysis of dopamine

Striatal DA levels were determined by the high-performance liquid chromatography (HPLC) with an electrochemical detector by the method of Muralikrishnan and Mohanakumar.[33] Briefly, the procured ST region was immediately sonicated in 0.1 M perchloric acid (ice-cold condition) containing 0.01% ethylenediaminetetraacetic acid (EDTA). Samples were centrifuged (10,000 g for 10 min) and the supernatant was collected and injected (10 μl) into the HPLC system using a rheodyne 7725i injector valve with a 20 μl injection loop. The mobile phase consisting of 7% acetonitrile, 3% methanol, and 90% 20 mM citric acid, 10 mM monobasic phosphate sodium, 3.25 mM octane sulfonic acid, 0.1 mM EDTA, 3 mM heptane sulfonic acid, 2 mM KCL, 6 mL/L o-phosphoric acid, and 2 mL/L diethylamine (pH 3) was allowed to flow at a rate of 0.3 mL/min with a Gold 118 system (Beckman, Fullerton, CA, USA). Results were expressed in ng/mg weight of brain tissue.

Estimation of the thiobarbituric acid reactive substances

Briefly, the tissue was homogenized in chilled 0.1 M phosphate buffer. The assay mixture contained 0.67% thiobarbituric acid, 10% chilled trichloroacetic acid, and homogenate (10%) in a total volume of 3 ml. The reaction mixture was centrifuged at 4000 rpm for 15 min, and the supernatant was boiled for 10 min. After cooling, the samples were read at 535 nm. The rate of lipid peroxidation was expressed as l mol of thiobarbituric acid reactive substances (TBARSs) formed/mg protein.[34]

Estimation of the levels of reduced glutathione

Reduced glutathione (GSH) levels were determined by the method of Jollow *et al.*[35] Postmitochondrial supernatant (PMS) (1.0 ml) was precipitated with 1.0 ml of sulfosalicylic acid (4.0%). The samples were kept at 4°C for 1 h and then subjected to centrifugation at 1200 g for 15 min at 4°C. The assay mixture consists of 0.5 ml of aliquot, 2.3 ml of sodium phosphate buffer (0.1 M, pH 7.4), and 0.2 ml of DTNB. The yellow color thus developed was read immediately at 412 nm on a spectrophotometer.

Determination of glutathione peroxidase activity

To 0.1 ml of PMS, 1.44 ml of sodium phosphate buffer (0.1 M, pH 7.4), 0.1 ml of EDTA (1 mM), 0.1 ml of sodium azide (1 mM), 0.05 ml of glutathione reductase (1 IU/ml), 0.1 ml GSH (1 mM), 0.1 ml NADPH (0.02 mM), and 0.01 ml of hydrogen peroxide (H_2O_2) (0.25 mM) were added. Glutathione peroxidase (GPx) activity was calculated as nmol NADPH oxidized min/mg protein at 340 nm.[36]

Determination of catalase activity

To 0.05 ml of PMS, 1.95 ml of phosphate buffer (0.1 M, pH 7.4) and 1 ml of H_2O_2 (0.09 M) were added. Catalase activity was calculated in terms of nmol H_2O_2 consumed min/mg protein at 240 nm.[37]

Determination of superoxide dismutase activity

Superoxide dismutase (SOD) activity was assayed using an indirect inhibition assay,[38] in which xanthine and xanthine oxidase serve as a superoxide generator and nitro blue tetrazolium (NBT) is used as a superoxide indicator. To 20 μl of PMS, 960 μl of 50 mM sodium carbonate buffer (pH 10.2) containing 0.1 mM xanthine, 0.025 mM NBT, and 0.1 mM EDTA and 20 μl of xanthine oxidase were added. Changes in absorbance were observed spectrophotometrically at 560 nm. The activity was expressed as units/min/mg protein.

Western blotting

Nigral tissues were homogenized in an ice-cold RIPA buffer and the homogenate was centrifuged (12,000 rpm/min for 15 min at 4°C) to remove debris. Protein level was measured by the method of Lowry *et al.*[39] 50 μg of total cellular protein samples was loaded on 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis. Then, the gel was transferred on to a nitrocellulose membrane (PALL Corporation, Biotrace). The membranes were incubated with the blocking buffer consisting of 5% nonfat dry milk powder for 2 h to reduce nonspecific binding sites and then the blots were probed with various antibodies: IL-1 β, IL-6, NF-κB, TNF-α, iNOS, COX-2, brain-derived neurotrophic factor (BDNF), and β-actin (1:2000) with gentle shaking overnight at 4°C. After this, membranes were incubated with their corresponding secondary antibodies (anti-rabbit IgG conjugated to horseradish peroxidase) for 2 h at room temperature. The membrane was washed thrice with TBST for 30 min. Immunoreactive protein was visualized by the chemiluminescence protocol (GenScript ECL kit, USA), scanned bands were quantitated by TotalLab Quant 1.11 software, and the control was set to 100.[32]

Statistical analysis

Statistical analysis was executed by one-way analysis of variance followed by Duncan's multiple range test using Statistical Package for the Social Science (SPSS) software package version 15.0 (GraphPad Software, Inc., USA). All data are expressed as mean ± standard error of mean for six rats from each group. Results were considered statistically significant at *P* < 0.05.

RESULTS AND DISCUSSION

Behavioral indices and dopamine levels

Rats treated with rotenone displayed a significantly increased beam-crossing duration and declined rotarod performance and neuromuscucular strength than the control group [Figures 1-3]. Previous studies indicated the impaired motor functions such as diminished walking balance, vestibular integrity, and muscular coordination in narrow beam walking test,[40] lack of coordination during walking, bradykinesia, and rigidity in the rotarod test[41] and lowered neuromuscular strength in the hang test[32] in various PD models. Administration of rotenone at low doses causes DA neurodegeneration in the nigro-striatal pathway.[9] Striatal DA depletion is the pathological hallmark of PD. The ST harbors a population of dopaminergic neurons that is thought to act as a local source of DA and this neurotransmitter is responsible for balance, control of movements, and walking.[42] Figure 4 reveals a significant decline in the levels of striatal DA in the rotenone alone treated rats as compared with control rats, whereas administration of DMC dose dependently enhanced the levels of dopamine in rotenone-induced PD rats. However, it was observed that both the 10 and 20 mg/kg b.w showed similar induction but more significant than 5 mg/kg b.w. Hence, we have chosen the 10 mg/kg b.w of DMC as the optimum dose for further studies. The decreased levels of DA cause the neurons of the ST to fire uncontrollably, preventing the PD patient from directing motor function. In the present study, rotenone administration caused a
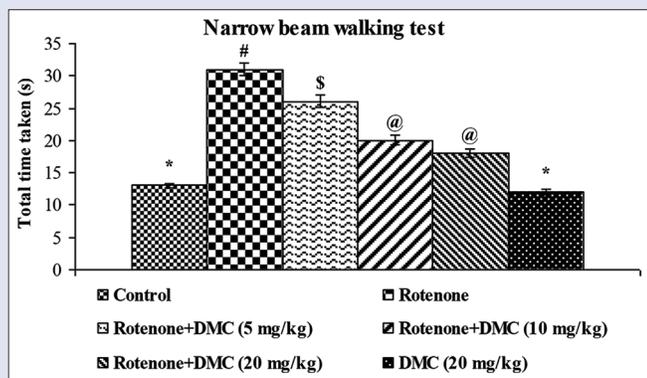
Figure 1: Measurement of balance and muscular coordination by narrow beam walking test. Rotenone-treated rats took more time to cross the narrow beam, whereas administration of demethoxycurcumin significantly attenuated motor deficts induced by rotenone. Results given are mean ± standard error of mean (n = 6), values not sharing common superscript are significant with each other P < 0.05, one-way analysis of variance followed by Duncan's multiple range test
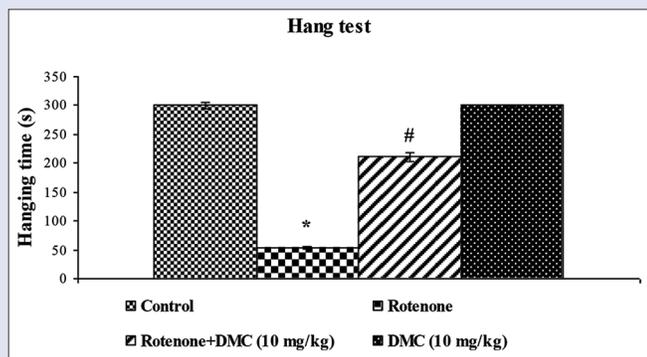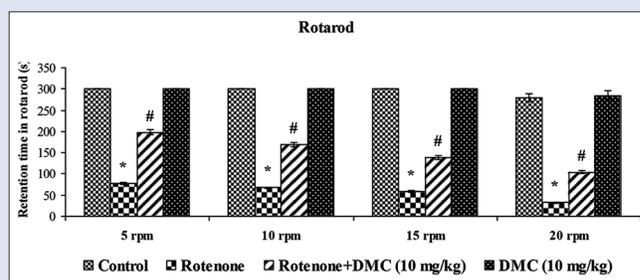

Figure 3: Determination of neuromuscular strength by hang test. Hanging time of rotenone induced rats were drastically decreased, meanwhile treatment with demethoxycurcumin significantly increased hanging time of rotenone induced rats. Results given are mean ± standard error of mean (n = 6), values not sharing common superscript are significant with each other P < 0.05, one-way analysis of variance followed by Duncan's multiple range test

Figure 2: Rotarod performance of control and experimental rats: Rotenone-induced rats illustrate significantly reduced rotarod performance compared to the control rats (different rpm such as 5, 10, 15, and 20). Treatment of demethoxycurcumin to rotenone-induced rats significantly improved the retention time in rotarod as compared to the demethoxycurcumin-untreated rotenone rats. Values are given as mean ± standard error of mean for six rats in each group. *P < 0.05 compared to the control, #P < 0.05 compared to the rotenone control
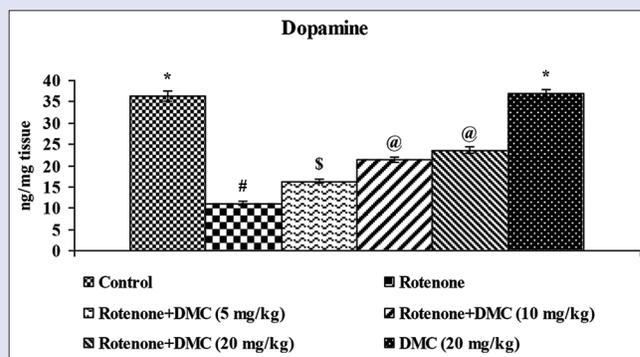
Figure 4: Rotenone-induced depletion of dopamine can be conserved by demethoxycurcumin treatment. Administration of rotenone depleted the levels of dopamine, which is attenuated dose-dependently by treatment of demethoxycurcumin. Results given are mean ± standard error of mean (n = 6), values not sharing common superscript are significant with each other P < 0.05, one-way analysis of variance followed by Duncan's multiple range test

significant reduction in levels of striatal DA, whereas cotreatment of DMC ameliorated the rotenone-induced dopamine depletion. Drugs that are able to ameliorate DA depletion are considered to be neuroprotective. In this context, the cotreatment of DMC improved the behavioral status in the animals intoxicated with rotenone, which may be due to its neuroprotective effect on enhancing the depleted dopamine levels.

Levels and activities of lipid peroxidative products and antioxidants

Oxidative stress is the main trigger of neurodegeneration in PD. The levels of TBARS and GSH and the activities of SOD, catalase, and GPx were assayed in the SN of control and experimental animals to examine the antioxidative role of DMC. The levels of TBARS were significantly elevated, whereas the levels of GSH and activities of SOD, catalase, and GPx were significantly diminished in the rats treated with rotenone. Treatment of DMC significantly and dose dependently attenuated rotenone-induced oxidative stress by diminishing the levels of TBARS and elevating the levels

and activities of antioxidants. On its own, DMC (20 mg/kg) treatment did not show any significant change in the levels and activities of oxidative and antioxidative indices as compared to control rats [Figure 5]. Compared to other tissues, the brain is more vulnerable to oxidative stress because of (i) high metabolic activity and utility of more oxygen, (ii) auto-oxidation of DA, its metabolites, and precursors to form semiquinones and quinones able of adducting sulfhydryl groups of protein, including GSH, and (iii) presence of enhanced iron levels in the SN leading to the reduction of H_2O_2 to form the highly reactive hydroxyl radical (OH) (Fenton chemistry).[1,43,44] Postmortem analysis of the brains from PD patients confirmed the enhanced level of oxidative stress in the SN,[45,46] diminished level of GSH,[47,48] and enhanced lipid peroxidation.[49,50] The extent of lipid peroxidation processes was measured by quantifying the level of TBARS, collective products of lipid peroxidation. Bashkatova *et al.*[51] showed increased NO and TBARS level in the ST and cortex, following the chronic rotenone treatment, which verifies our study. Increased production of reactive oxygen species (ROS) during neurodegenerative disorders is an indication of the oxidative stress and leads to a rapid consumption of endogenous scavenging antioxidants. Rotenone-administered PD rats enhanced the level of TBARS, a marker of lipid peroxidation processes, and reduced the levels and activities of enzymatic antioxidants which are
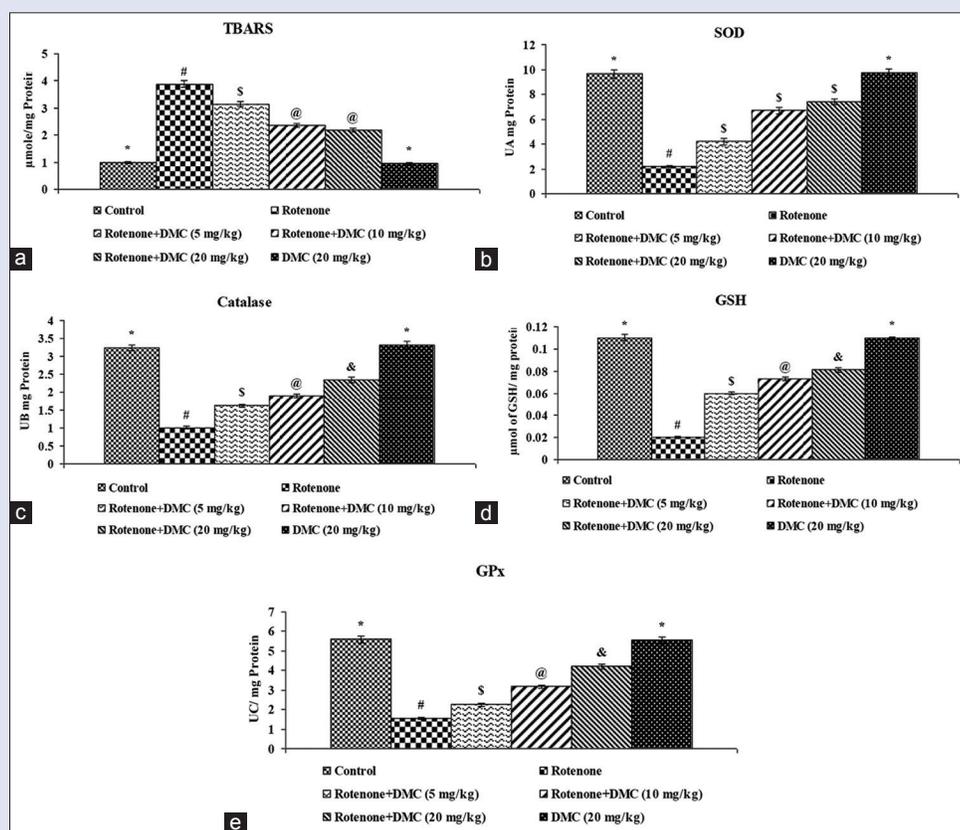
Figure 5: Rotenone induced oxidative stress can be attenuated by demethoxycurcumin. The levels of thiobarbituric acid reactive substances (a), and activities of superoxide dismutase (b) and catalase (c) were increased along with decreased levels of reduced levels of reduced glutathione (d) and activities of GPx (e) in the substania nigra of rotenone treated rats--oxidative stress

consistent with the previous studies.[50,52,53] Previous studies demonstrated decreased GSH level, SOD activity, and oxidative DNA[54] and enhanced levels of MDA[55] in rotenone models of PD.

However, cotreatment of DMC-reduced, rotenone-induced TBARS levels ameliorates lipid peroxidation. The methoxy group present on the phenyl ring is one of the functional groups responsible for the antioxidant property of curcumin. As DMC has one methoxy group, it is also considered as a potent antioxidant.[56] A fault in individual or more endogenous antioxidant molecules, particularly GSH, is an important factor in etiology of PD.[57] Dairam *et al.*[56] demonstrated that the administration of DMC increased the levels of GSH in lead-treated animals, which is consistent with our work. In the present study, diminished activity of SOD in the rotenone-treated rats indicated activation of SOD by ROS[58] and leads to the scenario of increased superoxide radical production. Enhanced superoxide radicals spontaneously dismutated to form H_2O_2. Elevated levels of H_2O_2 could lead to depletion of their metabolizing enzymatic antioxidants such as catalase and GPx. DMC has antioxidant effects and improved the activities of SOD, catalase, and GPx against the 6-OHDA-induced rat model of PD,[59] middle cerebral artery occlusion-induced experimental stroke,[60] and other *in vitro* studies.[61,62]

Protein expressions of interleukin-1 β, interleukin-6, necrosis factor kappa B, tumor necrosis factor-α, inducible nitric oxide synthase, cyclooxygenase-2, and brain-derived neurotrophic factor

Western blot analysis was performed in the SN regions of experimental rats. Rotenone treatment markedly increased the expressions in IL-1 β,

IL-6, NF-κB, TNF-α, iNOS, and COX-2 and reduced the expression of BDNF. Oral administration of DMC significantly attenuated the rotenone-induced inflammation. No significant changes were observed between control and DMC alone treated groups [Figure 6]. Inflammation is one among the several assumed factors that play an important role in PD pathogenesis. Inflammatory mediators modulated the immune and neuronal cells, thereby contributing to the cascade of events leading to neuronal degeneration. These mechanisms comprise microglial activation, astrogliosis, and lymphocytic infiltration. Activation of microglia is related with dopaminergic neuronal loss and α-synuclein that have been reported to play an important role in the initiation and progression of inflammation in PD.[63] Microglia in PD have been observed to grow densely in the ST and SN with increased expression of pro-inflammatory mediators, including TNF-α, IL-1 β, IL-2, IL-4, IL-6, COX-2, and iNOS.[64] In addition, levels of inflammatory cytokines such as TNF-α, IL-6, and IL-10 are elevated in different brain regions of patients with PD.[65]

The pro-inflammatory cytokines TNF-α and IL-1 can also trigger direct toxicity in neurons[66,67] and can potentiate an ongoing inflammatory response by enhancing microglial NO production.[68,69] Upregulation of inflammatory mediators involved in apoptotic cell death through TNF-α-induced signaling pathway, including caspase-1, caspase-3, and TNF receptor R1 (TNF-R1 or p55), were identified in the SN from parkinsonian patients, indicating the occurrence of a proapoptotic environment in PD.[70] Curcuminoids attenuated H_2O_2-enhanced production of pro-inflammatory molecules such as IL-6, TNF-α, monocyte chemoattractant protein-1, and NF-κB.[61]
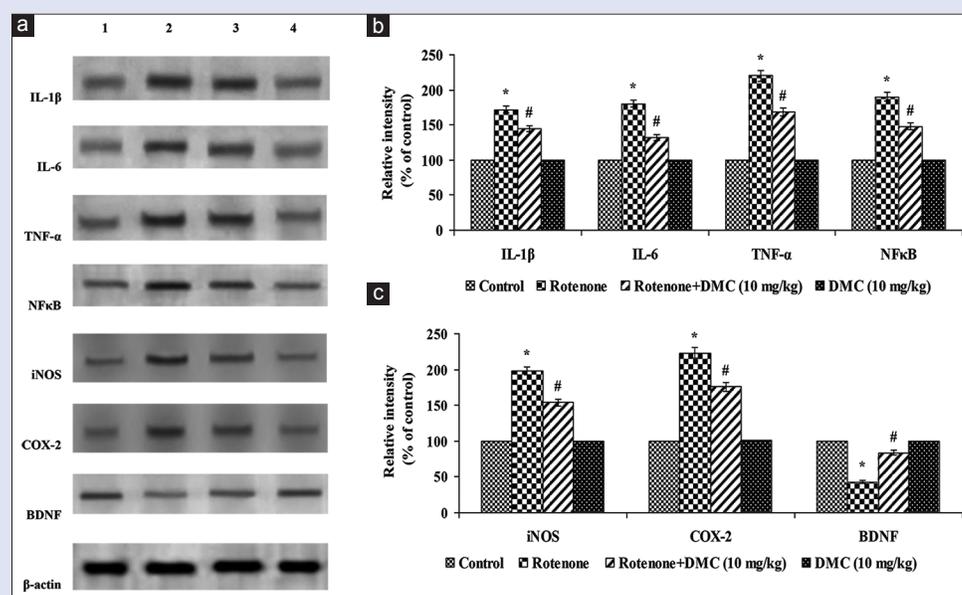
Figure 6: Rotenone persuaded inflammation can be amoliered by demethoxycurcumin administration. Expressions of interleukin-1β, interleukin-6, tumor necrosis factor-α, Necrosis Factor kappa B, inducible nitric oxide synthase, cyclooxygenase-2, and brain-derived neurotrophic factor in the substantia nigra were analyzed by Western blot (a), lane 1: Control, saline control, lane 2: Rotenone, Rotenone-treated only, lane 3: Rotenone - demethoxycurcumin, Rotenone with demethoxycurcumin, lane 4: Demethoxycurcumin, demethoxycurcumin alone administrated. The band density was quantified by scanning densitometry (b and c). Results given are mean ± standard error of mean, (n = 6), values not sharing common superscript are significant with each other $P < 0.05$, one-way analysis of variance followed by Duncan's multiple range test

Recently, Zhang *et al*.[71] reported that DMC exhibited a stronger inhibitory activity on nitric oxide and TNF-α production compared to curcumin in lipopolysaccharide-activated rat primary microglia. NF-κB may be activated by oxidative stress, which in turn regulates the expressions of many genes including pro-inflammatory cytokines, particularly, TNF-α, IL-1 β, iNOS, IL-6, and IL-10.[72] Pro-inflammatory cytokines degraded the inhibitor kappa B by phosphorylation and activated the NF-κB signaling cascade.[73] Activated NF-κB promotes the induction of iNOS from activated microglia. NO derived from iNOS-expressing microglia partially accounted for the loss of dopaminergic neurons in PD patients.[74]

Enhanced expressions of inflammatory indices including NF-κB and iNOS were demonstrated in rotenone-injected rats, and on the other hand, treatment with DMC not only diminished expression of iNOS and NF-κB significantly but also diminished IL-6, IL-1 β, and TNF-α expression. This indicated that microglial activation has been alleviated by DMC administration. Activated microglia upregulated the levels of COX-2, a key enzyme responsible for the synthesis of inflammation-related prostaglandins.[75] The prostaglandins can be directly toxic to neurons through activation of caspase 3 or indirectly through the release of glutamate by astrocytes leading to excitotoxicity.[76] Subsequently, the expression of its products such as prostaglandin E2, which is cytotoxic, is also increased during times of microglial activation. COX-2 expression is normally limited to the forebrain; however, under pathological conditions involving inflammation, COX-2 expression is significantly increased throughout the brain. It is unsurprising, therefore, that increased COX-2 expression has been found to be localized to the SNpc in the postmortem brains of PD patients. Moreover, the inhibition of COX-2 has been shown to attenuate the toxic effects of MPTP in mice, further suggesting that microglial-mediated COX-2 expression is harmful to DA neurons of the SNpc.[77]

Our study found significant enhancement in COX-2 expression after rotenone administration, which is concordant with previous studies.[40] Inhibition of COX-2 activity improves behavioral impairment

and shields dopaminergic neuronal loss against various PD inducing neurotoxins.[78,79] Astrocytes have diverse and critical functions in the central nervous system that includes providing energetic,[80] antioxidant,[81] and other trophic support essential for the survival and function of neurons. Several reports have suggested that the activation of astrocytes is due to secretion of inflammatory cytokines, such as TNF-α and IFN-γ, by the surrounding microglial cells.[68,82]

The astrocytic reaction is another well-known neuropathological characteristic of the SN in PD. Damier *et al*.[83] showed that astrocytes are heterogeneously distributed within the mesencephalon in healthy individuals and their density of astrocytes is low in the SNpc, which is severely affected in PD. One of the earlier studies reporting neuroinflammation in PD involved a quantitative confirmation of the astroglial reaction using glial fibrillary acidic protein (GFAP) immune-staining in the SN of patients with PD. Reactive astrocytes have been demonstrated in regions of dopaminergic nerve cell loss in PD.[84] Guo *et al*.[23] reported that DMC and BDMC inhibited NO production and COX-2 and iNOS expression and suppressed LPS-induced IB-phosphorylation and degradation in a dose-dependent manner. Astrogliosis induced by cytokines such as IL-1 β and IL-6 could accelerate GFAP release, which favored neuroinflammatory response and neuronal loss.[85] In contrast, it is observed that treatment of DMC significantly diminished GFAP expression, which indicates that astrogliosis activation has been mitigated.

CONCLUSION

Our study demonstrated that DMC treatment could be able to provide protection against the neurotoxic effects of rotenone in rats and the mechanisms of protection induced by this agent possibly due to its anti-inflammatory and antioxidant activities.

Acknowledgements

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Jenner P. Oxidative stress in Parkinson's disease. Ann Neurol 2003;53 Suppl 3:S26-36.

2. Przedborski S, Goldman JE. Pathogenic role of glial cells in Parkinson's disease. In: Hertz L, editor. Non-neuronal Cells of the Nervous System: Function and Dysfunction. New York: Elsevier; 2004. p. 967-82.

3. Dhanalakshmi C, Janakiraman U, Manivasagam T, Justin Thenmozhi A, Essa MM, Kalandar A, *et al.* Vanillin attenuated behavioural impairments, neurochemical deficts, oxidative stress and apoptosis against rotenone induced rat model of Parkinson's disease. Neurochem Res 2016;41:1899-910.

4. Moore DJ, Dawson TM. Value of genetic models in understanding the cause and mechanisms of Parkinson's disease. Curr Neurol Neurosci Rep 2008;8:288-96.

5. Di Monte DA. The environment and Parkinson's disease: Is the nigrostriatal system preferentially targeted by neurotoxins? Lancet Neurol 2003;2:531-8.

6. Dhanalakshmi C, Manivasagam T, Nataraj J, Justin Thenmozhi A, Essa MM. Neurosupportive role of vanillin, a natural phenolic compound, on rotenone induced neurotoxicity in SH-SY5Y neuroblastoma cells. Evid Based Complement Alternat Med 2015;2015:626028.

7. Alam M, Schmidt WJ. Rotenone destroys dopaminergic neurons and induces parkinsonian symptoms in rats. Behav Brain Res 2002;136:317-24.

8. Betarbet R, Sherer TB, MacKenzie G, Garcia-Osuna M, Panov AV, Greenamyre JT, *et al.* Chronic systemic pesticide exposure reproduces features of Parkinson's disease. Nat Neurosci 2000;3:1301-6.

9. Sherer TB, Betarbet R, Testa CM, Seo BB, Richardson JR, Kim JH, *et al.* Mechanism of toxicity in rotenone models of Parkinson's disease. J Neurosci 2003;23:10756-64.

10. Chen L, Ding Y, Cagniard B, Van Laar AD, Mortimer A, Chi W, *et al.* Unregulated cytosolic dopamine causes neurodegeneration associated with oxidative stress in mice. J Neurosci 2008;28:425-33.

11. Gao HM, Liu B, Hong JS. Critical role for microglial NADPH oxidase in rotenone-induced degeneration of dopaminergic neurons. J Neurosci 2003;23:6181-7.

12. Hirsch EC, Hunot S. Neuroinflammation in Parkinson's disease: A target for neuroprotection? Lancet Neurol 2009;8:382-97.

13. Bezard E, Brotchie JM, Gross CE. Pathophysiology of levodopa-induced dyskinesia: Potential for new therapies. Nat Rev Neurosci 2001;2:577-88.

14. Nutt JG, Woodward WR, Carter JH, Gancher ST. Effect of long-term therapy on the pharmacodynamics of levodopa. Relation to on-off phenomenon. Arch Neurol 1992;49:1123-30.

15. Tamilselvam K, Nataraj J, Janakiraman U, Manivasagam T, Essa MM. Antioxidant and anti-inflammatory potential of hesperidin against 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine-induced experimental Parkinson's disease in mice. Int J Nutr Pharmacol Neurol Dis 2013;3:294-302.

16. Khatri DK, Juvekar AR. Abrogation of locomotor impairment in a rotenone-induced *Drosophila melanogaster* and zebrafish model of Parkinson's disease by ellagic acid and curcumin. Int J Nutr Pharmacol Neurol Dis 2014;4:4-10.

17. Sharma OP. Antioxidant activity of curcumin and related compounds. Biochem Pharmacol 1976;25:1811-2.

18. Wang J, Du XX, Jiang H, Xie JX. Curcumin attenuates 6-hydroxydopamine-induced cytotoxicity by anti-oxidation and nuclear factor-kappa B modulation in MES23.5 cells. Biochem Pharmacol 2009;78:178-83.

19. Fetoni AR, Paciello F, Mezzogori D, Rolesi R, Eramo SL, Paludetti G, *et al.* Molecular targets for anticancer redox chemotherapy and cisplatin-induced ototoxicity: The role of curcumin on pSTAT3 and nrf-2 signalling. Br J Cancer 2015;113:1434-44.

20. Srivastava R, Dikshit M, Srimal RC, Dhawan BN. Anti-thrombotic effect of curcumin. Thromb Res 1985;40:413-7.

21. Abe Y, Hashimoto S, Horie T. Curcumin inhibition of inflammatory cytokine production by human peripheral blood monocytes and alveolar macrophages. Pharmacol Res 1999;39:41-7.

22. Adams BK, Ferstl EM, Davis MC, Herold M, Kurtkaya S, Camalier RF, *et al.* Synthesis and biological evaluation of novel curcumin analogs as anti-cancer and anti-angiogenesis agents. Bioorg Med Chem 2004;12:3871-83.

23. Guo LY, Cai XF, Lee JJ, Kang SS, Shin EM, Zhou HY, *et al.* Comparison of suppressive effects of demethoxycurcumin and bisdemethoxycurcumin on expressions of inflammatory mediators *in vitro* and *in vivo*. Arch Pharm Res 2008;31:490-6.

24. Kim DS, Park SY, Kim JK. Curcuminoids from *Curcuma longa* L. (*Zingiberaceae*) that protect PC12 rat pheochromocytoma and normal human umbilical vein endothelial cells from betaA (1-42) insult. Neurosci Lett 2001;303:57-61.

25. Sandur SK, Pandey MK, Sung B, Ahn KS, Murakami A, Sethi G, *et al.* Curcumin, demethoxycurcumin, bisdemethoxycurcumin, tetrahydrocurcumin and turmerones differentially regulate anti-inflammatory and anti-proliferative responses through a ROS-independent mechanism. Carcinogenesis 2007;28:1765-73.

26. Simon A, Allais DP, Duroux JL, Basly JP, Durand-Fontanier S, Delage C, *et al.* Inhibitory effect of curcuminoids on MCF-7 cell proliferation and structure-activity relationships. Cancer Lett 1998;129:111-6.

27. Zhang LJ, Wu CF, Meng XL, Yuan D, Cai XD, Wang QL, *et al.* Comparison of inhibitory potency of three different curcuminoid pigments on nitric oxide and tumor necrosis factor production of rat primary microglia induced by lipopolysaccharide. Neurosci Lett 2008;447:48-53.

28. Lee JW, Hong HM, Kwon DD, Pae HO, Jeong HJ. Dimethoxycurcumin, a structural analogue of curcumin, induces apoptosis in human renal carcinoma caki cells through the production of reactive oxygen species, the release of cytochrome C, and the activation of caspase-3. Korean J Urol 2010;51:870-8.

29. Kim H, Park BS, Lee KG, Choi CY, Jang SS, Kim YH, *et al.* Effects of naturally occurring compounds on fibril formation and oxidative stress of beta-amyloid. J Agric Food Chem 2005;53:8537-41.

30. Rajasankar S, Manivasagam T, Surendran S. Ashwagandha leaf extract: A potential agent in treating oxidative damage and physiological abnormalities seen in a mouse model of Parkinson's disease. Neurosci Lett 2009;454:11-5.

31. Rozas G, López-Martín E, Guerra MJ, Labandeira-García JL. The overall rod performance test in the MPTP-treated-mouse model of Parkinsonism. J Neurosci Methods 1998;83:165-75.

32. Janakiraman U, Manivasagam T, Justin Thenmozhi A, Dhanalakshmi C, Essa MM, Song BJ, *et al.* Chronic mild stress augments MPTP induced neurotoxicity in a murine model of Parkinson's disease. Physiol Behav 2017;173:132-43.

33. Muralikrishnan D, Mohanakumar KP. Neuroprtotection by bromocriptine against 1- methyl 4-phenyl 1,2,3,6 tetrahydropyridine induced neurotoxicity in mice. FASEB J 1998;12:905-12.

34. Utley HC, Bernheim F, Hochslein P. Effect of sulfhydryl reagent on peroxidation in microsome. Arch Biochem Biophys 1967;118:29-32.

35. Jollow DJ, Mitchell JR, Zampagloine N, Gillette JR. Bromobenzene-induced liver necrosis: Protective role of glutathione and evidence for 3, 4 bromobenzeneoxide as the hepatotoxic intermediate. Pharmacol 1974;11:151-69.

36. Mohandas J, Marshall JJ, Duggin GG, Horvath JS, Tiller DJ. Differential distribution of glutathione and glutathione-related enzymes in rabbit kidney. Possible implications in analgesic nephropathy. Biochem Pharmacol 1984;33:1801-7.

37. Claiborne A. Catalase activity. In: Greenwald RA, editor. CRC Handbook of Methods for Oxygen Radical Research. Boca Raton, FL: CRC Press; 1985. p. 283-84.

38. Oberley LW, Spitz DR. Assay of superoxide dismutase activity in tumor tissue. Methods Enzymol 1984;105:457-64.

39. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-75.

40. Anandhan A, Tamilselvam K, Radhiga T, Rao S, Essa MM, Manivasagam T, *et al.* Theaflavin, a black tea polyphenol, protects nigral dopaminergic neurons against chronic MPTP/probenecid induced Parkinson's disease. Brain Res 2012;1433:104-13.

41. Sedelis M, Schwarting RK, Huston JP. Behavioral phenotyping of the MPTP mouse model of Parkinson's disease. Behav Brain Res 2001;125:109-25.

42. Wächter T, Röhrich S, Frank A, Molina-Luna K, Pekanovic A, Hertler B, *et al.* Motor skill learning depends on protein synthesis in the dorsal striatum after training. Exp Brain Res 2010;200:319-23.

43. Andersen JK. Iron dysregulation and Parkinson's disease. J Alzheimers Dis 2004;6:S47-52.

44. Barnham KJ, Masters CL, Bush AI. Neurodegenerative diseases and oxidative stress. Nat Rev Drug Discov 2004;3:205-14.

45. Dexter DT, Carter CJ, Wells FR, Javoy-Agid F, Agid Y, Lees A, *et al.* Basal lipid peroxidation in substantia nigra is increased in Parkinson's disease. J Neurochem 1989;52:381-9.

46. Good PF, Olanow CW, Perl DP. Neuromelanin-containing neurons of the substantia nigra accumulate iron and aluminum in Parkinson's disease: A LAMMA study. Brain Res 1992;593:343-6.

47. Sian J, Dexter DT, Lees AJ, Daniel S, Agid Y, Javoy-Agid F, *et al.* Alterations in glutathione levels in Parkinson's disease and other neurodegenerative disorders affecting basal ganglia. Ann Neurol 1994;36:348-55.

48. Sofic E, Lange KW, Jellinger K, Riederer P. Reduced and oxidized glutathione in the substantia nigra of patients with Parkinson's disease. Neurosci Lett 1992;142:128-30.

49. Dexter DT, Holley AE, Flitter WD, Slater TF, Wells FR, Daniel SE, *et al.* Increased levels of lipid hydroperoxides in the parkinsonian substantia nigra: An HPLC and ESR study. Mov Disord 1994;9:92-7.

50. Yoritaka A, Hattori N, Uchida K, Tanaka M, Stadtman ER, Mizuno Y, *et al.* Immunohistochemical detection of 4-hydroxynonenal protein adducts in Parkinson disease. Proc Natl Acad Sci U S A 1996;93:2696-701.

51. Bashkatova V, Alam M, Vanin A, Schmidt WJ. Chronic administration of rotenone increases levels of nitric oxide and lipid peroxidation products in rat brain. Exp Neurol 2004;186:235-41.

52. Sánchez-Reus MI, Gómez del Rio MA, Iglesias I, Elorza M, Slowing K, Benedí J, *et al.* Standardized hypericum perforatum reduces oxidative stress and increases gene expression of antioxidant enzymes on rotenone-exposed rats. Neuropharmacology 2007;52:606-16.

53. Sharma N, Nehru B. Characterization of the lipopolysaccharide induced model of Parkinson's disease: Role of oxidative stress and neuroinflammation. Neurochem Int 2015;87:92-105.

54. Sherer TB, Betarbet R, Stout AK, Lund S, Baptista M, Panov AV, *et al.* An *in vitro* model of Parkinson's disease: Linking mitochondrial impairment to altered alpha-synuclein metabolism and oxidative damage. J Neurosci 2002;22:7006-15.

55. Xiong N, Huang J, Zhang Z, Zhang Z, Xiong J, Liu X, *et al.* Stereotaxical infusion of rotenone: A reliable rodent model for Parkinson's disease. PLoS One 2009;4:e7878.

56. Dairam A, Limson JL, Watkins GM, Antunes E, Daya S. Curcuminoids, curcumin, and demethoxycurcumin reduce lead-induced memory deficits in male wistar rats. J Agric Food Chem 2007;55:1039-44.

57. Jenner P, Olanow CW. Oxidative stress and the pathogenesis of Parkinson's disease. Neurology 1996;47:S161-70.

58. Wei H, Meng Z. Epigallocatechin-3-gallate protects Na+channels in rat ventricular myocytes against sulfite. Cardiovasc Toxicol 2010;10:166-73.

59. Agrawal SS, Gullaiya S, Dubey V, Singh V, Kumar A, Nagar A, *et al.* Neurodegenerative shielding by curcumin and its derivatives on brain lesions induced by 6-OHDA model of Parkinson's disease in albino wistar rats. Cardiovasc Psychiatry Neurol 2012;2012:942981.

60. Ahmad N, Umar S, Ashafaq M, Akhtar M, Iqbal Z, Samim M, *et al.* A comparative study of PNIPAM nanoparticles of curcumin, demethoxycurcumin, and bisdemethoxycurcumin and their effects on oxidative stress markers in experimental stroke. Protoplasma 2013;250:1327-38.

61. Septembre-Malaterre A, Le Sage F, Hatia S, Catan A, Janci L, Gonthier MP, *et al.* *Curcuma longa* polyphenols improve insulin-mediated lipid accumulation and attenuate proinflammatory response of 3T3-L1 adipose cells during oxidative stress through regulation of key adipokines and antioxidant enzymes. Biofactors 2016;42:418-30.

62. Liu JP, Feng L, Zhu MM, Wang RS, Zhang MH, Hu SY, *et al.* The *in vitro* protective effects of curcumin and demethoxycurcumin in *Curcuma longa* extract on advanced glycation end products-induced mesangial cell apoptosis and oxidative stress. Planta Med 2012;78:1757-60.

63. Jellinger KA. Neuropathology of sporadic Parkinson's disease: Evaluation and changes of concepts. Mov Disord 2012;27:8-30.

64. Tansey MG, McCoy MK, Frank-Cannon TC. Neuroinflammatory mechanisms in Parkinson's disease: Potential environmental triggers, pathways, and targets for early therapeutic intervention. Exp Neurol 2007;208:1-25.

65. Nagatsu T, Mogi M, Ichinose H, Togari A. Cytokines in Parkinson's disease. J Neural Transm Suppl 2000;58:143-51.

66. Allan SM, Rothwell NJ. Cytokines and acute neurodegeneration. Nat Rev Neurosci 2001;2:734-44.

67. Clarke DJ, Branton RL. A role for tumor necrosis factor alpha in death of dopaminergic neurons following neural transplantation. Exp Neurol 2002;176:154-62.

68. Hunot S, Dugas N, Faucheux B, Hartmann A, Tardieu M, Debré P, *et al.* FcepsilonRII/CD23 is expressed in Parkinson's disease and induces, *in vitro*, production of nitric oxide and tumor necrosis factor-alpha in glial cells. J Neurosci 1999;19:3440-7.

69. Possel H, Noack H, Putzke J, Wolf G, Sies H. Selective upregulation of inducible nitric oxide synthase (iNOS) by lipopolysaccharide (LPS) and cytokines in microglia: *In vitro* and *in vivo* studies. Glia 2000;32:51-9.

70. Mogi M, Togari A, Kondo T, Mizuno Y, Komure O, Kuno S, *et al.* Caspase activities and tumor necrosis factor receptor R1 (p55) level are elevated in the substantia nigra from Parkinsonian brain. J Neural Transm (Vienna) 2000;107:335-41.

71. Zhang L, Wu C, Zhao S, Yuan D, Lian G, Wang X, *et al.* Demethoxycurcumin, a natural derivative of curcumin attenuates LPS-induced pro-inflammatory responses through down-regulation of intracellular ROS-related MAPK/NF-kappaB signaling pathways in N9 microglia induced by lipopolysaccharide. Int Immunopharmacol 2010;10:331-8.

72. Carbone DL, Moreno JA, Tjalkens RB. Nuclear factor kappa-B mediates selective induction of neuronal nitric oxide synthase in astrocytes during low-level inflammatory stimulation with MPTP. Brain Res 2008;1217:1-9.

73. Litteljohn D, Mangano E, Clarke M, Bobyn J, Moloney K, Hayley S, *et al.* Inflammatory mechanisms of neurodegeneration in toxin-based models of Parkinson's disease. Parkinsons Dis 2010;2011:713517.

74. Selvakumar GP, Janakiraman U, Essa MM, Justin Thenmozhi A, Manivasagam T. Escin attenuates behavioral impairments, oxidative stress and inflammation in a chronic MPTP/probenecid mouse model of Parkinson's disease. Brain Res 2014;1585:23-36.

75. Hoozemans JJ, Veerhuis R, Janssen I, van Elk EJ, Rozemuller AJ, Eikelenboom P, *et al.* The role of cyclo-oxygenase 1 and 2 activity in prostaglandin E (2) secretion by cultured human adult microglia: Implications for Alzheimer's disease. Brain Res 2002;951:218-26.

76. Consilvio C, Vincent AM, Feldman EL. Neuroinflammation, COX-2, and ALS – A dual role? Exp Neurol 2004;187:1-0.

77. Przedborski S. Neuroinflammation and Parkinson's disease. In: Vinken PJ, Bruyn GW, editors. Handbook of Clinical Neurology. Vol. 83. Netherlands: Elsevier; 2007. p. 535-51.

78. Feng ZH, Wang TG, Li DD, Fung P, Wilson BC, Liu B, *et al.* Cyclooxygenase-2-deficient mice are resistant to 1-methyl-4-phenyl1, 2, 3, 6-tetrahydropyridine-induced damage of dopaminergic neurons in the substantia nigra. Neurosci Lett 2002;329:354-8.

79. Vijitruth R, Liu M, Choi DY, Nguyen XV, Hunter RL, Bing G, *et al.* Cyclooxygenase-2 mediates microglial activation and secondary dopaminergic cell death in the mouse MPTP model of Parkinson's disease. J Neuroinflammation 2006;3:6.

80. Pellerin L, Magistretti PJ. Neuroenergetics: Calling upon astrocytes to satisfy hungry neurons. Neuroscientist 2004;10:53-62.

81. Pekny M, Nilsson M. Astrocyte activation and reactive gliosis. Glia 2005;50:427-34.

82. Liberatore GT, Jackson-Lewis V, Vukosavic S, Mandir AS, Vila M, McAuliffe WG, *et al.* Inducible nitric oxide synthase stimulates dopaminergic neurodegeneration in the MPTP model of Parkinson disease. Nat Med 1999;5:1403-9.

83. Damier P, Hirsch EC, Zhang P, Agid Y, Javoy-Agid F. Glutathione peroxidase, glial cells and Parkinson's disease. Neuroscience 1993;52:1-6.

84. McGeer PL, Itagaki S, McGeer EG. Expression of the histocompatibility glycoprotein HLA-DR in neurological disease. Acta Neuropathol 1988;76:550-7.

85. Woiciechowsky C, Schöning B, Stoltenburg-Didinger G, Stockhammer F, Volk HD. Brain-IL-1 beta triggers astrogliosis through induction of IL-6: Inhibition by propranolol and IL-10. Med Sci Monit 2004;10:BR325-30.