Neuroprotective Effect of Capsaicin Against Rotenone-Induced Parkinson's Disease in Mice

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ABSTRACT

Capsaicin, the active ingredient of hot pepper exerts neuroprotective effects. In this study, the effect of capsaicin on rotenone-induced Parkinson's disease in mice was investigated. Mice were given subcutaneous rotenone injections (1.5 mg/kg, every other day) and at the same time treated with the vehicle, L-dopa (25 mg/kg) or capsaicin at doses of 0.5 or 1.0 mg/kg orally once a day for two weeks. Biochemical indices of oxidative stress, malondialdehyde, reduced glutathione and nitric oxide were determined in brain tissue and histopathological study of the brain was done. Behavioral tests included stair, wire hanging and wood walking tests. Results showed that rotenone treatment led to significant increases in brain malondialdehyde and nitric oxide contents parallel with marked depletion of reduced glutathione. Rotenone induced degeneration of pigmented neurons in substantia nigra and of cerebral cortex and hippocampus neurons. Rotenone impaired neuromuscular strength, motor balance and coordination. Treatment with capsaicin significantly ameliorated the neuronal degeneration caused by rotenone and improved motor function. Capsaicin alleviated the increase in lipid peroxidation (malondialdehyde) and nitric oxide and prevented the depletion of reduced glutathione in brain of rotenone-treated animals. These data indicate that capsaicin protects against rotenone-induced neuronal damage and this involves decreased level of oxidative stress. Capsaicin therefore might prevent cell death in the brain of Parkinson's disease patients.

1. Introduction

Parkinson's disease is a common neurological movement disorder affecting about 1% of individuals above 65 years of age (Wirdefeldt et al. 2011). Classically, the disease manifests as bradykinesia or difficulty in initiation and slowness of voluntary movements, muscle rigidity, resting tremor and postural instability (Jankovic, 2008). These symptoms result from a progressive death of dopaminergic cells in midbrain substantia nigra pars compacta (SNc) and consequent dopamine depletion in SNc and striatum (Hughes et al. 1992). It is this loss of dopamine which leads to inability of basal ganglia motor circuits to exert control over motor action via its connections to the thalamus (which in turn projects to motor cortex, cortical supplementary motor area and other premotor cortical areas) or other brainstem nuclei (De Long and Wichmann, 2007). In addition to motor symptoms, cognitive decline, apathy, depression and autonomic dysfunction occur during the course of illness. The disease occurs in a predominantly sporadic form (idiopathic PD) in ~ 95% of cases (Kumar et al., 2011). The exact cause of the preferential dopaminergic cell death in PD is not yet

clear, but interaction between environmental factors (probably toxins) and genetic susceptibility is a widely accepted hypothesis (Ritz et al., 2016). In support of this notion, a number of epidemiological studies have suggested a link between exposure to pesticides and an increased risk for developing PD (Dhillon et al., 2008).

Increased level of oxidative stress is a key event that underlies cell death in PD (Jenner, 2003). Reactive oxygen species are produced within the cell as byproducts of normal cellular metabolism, the most important source being the mitochondrial respiratory chain where leakage of electrons onto molecular oxygen results in the formation of superoxide ($O_2^{\bullet -}$). Other sources are the autoxidation or metabolism of monoamine neurotransmitters by the enzyme monoamine oxidase, activated phagocytes that release superoxide, hydrogen peroxide and hypochlorous acid, activated lipoxygenase and cyclooxygenase and nitric oxide synthases (Halliwell, 2001, 2009). In face of ROS, the cell is equipped by a number of antioxidant defenses that includes the enzymes superoxide dismutase and glutathione peroxidase, reduced glutathione (GSH), ascorbate, and α-tocopherol (Sies, 1997). Oxidative stress develops when the cell's antioxidants are overwhelmed by the increased production of reactive oxygen species (ROS), resulting in oxidative damage to membrane lipids, nucleic acids and proteins (Halliwell, 2001). The abundance of highly polyunsaturated fatty acids coupled with the paucity of antioxidants makes the brain particularly susceptible to oxidative stress (Floyd, 1999). Moreover, mitochondrial complex I damage in the brain of PD patients can lead to increased production of superoxide (Schapira et al., 1990; Zorov et al., 2006).

Currently, there are no treatments which can cure or halt the progression of PD. Dopamine replacement therapy with the dopamine precursor levodopa (L-dopa) is accepted as the most effective in alleviating symptoms (LeWitt and Fahn, 2016). However, several years after initiation of L-dopa, complications such as motor fluctuations and dyskinesias arise (Encarnacion and Hauser, 2008; Abdel-Salam, 2015a). This highlights the need of finding novel therapies for PD.

Capsaicin (8-methyl-N-vanillyl-6-nonenamide), the pungent principle of hot chilli pepper of the plant genus Capsicum is a selective sensory excitotoxin that in low doses excites while in high doses desensitizes a subset of mammalian primary afferents with unmyelinated C fibers and thinly myelinated Aδ fibres that express the transient receptor potential vanilloid-1 (TRPV1). Capsaicin is a selective agonist at the TRPV1 or capsaicin receptor (Szolcsányi, 2014). TRPV1 channel is also expressed in the cerebral cortex, hippocampus and substantia nigra in neuronal cell bodies and dendrites, microglia, astrocytes and pericytes (Steenland et al., 2006). In previous studies, we have shown that treatment with extracts of red hot pepper were able to prevent brain neuron damage in experimentally-induced PD (Abdel-Salam et al., 2019) and during insulin-induced hypoglycemia via inhibition of oxidative stress and 5-lipoxygenase (Abdel-Salam et al., 2018).

In this study, we aimed to examine the effect of capsaicin administration on oxidative stress and the neurodegeneration in brain of rotenone-treated mice. Rotenone is a botanical insecticide and pesticide derived from the roots of tropical plants of the *Leguminosae* family. It is used in home gardens for insects, for lice and ticks on pets, and to kill fish populations (Hien et al., 2003). Rotenone has been shown to result in the neuropathologic and motor changes similar to that of PD when injected into rodents (Betarbet et al., 2000). Rotenone is thus widely used to investigate the pathogenetic mechanisms underlying nigrostriatal degeneration and possible therapeutic targets (Abdel-Salam, 2015b).

2. Materials and methods

2.1. Animals

Swiss male albino mice, weighing 25-26 g obtained from the breeding colony at the animal house of National Research Center were used in the study. Mice were kept under standard laboratory conditions (20–22 ◦C and 12 h/12 h light/dark cycle) and standard diet and water ad libitum. Animal procedures followed the guidelines of the Institute ethics committee for the use of animals in experimental studies and the Guide for Care and Use of Laboratory Animals by the U.S. National Institutes of Health (Publication No. 85-23, revised 1996).

2.2. Drugs and chemicals

Rotenone and capsaicin were purchased from Sigma-Aldrich (St Louis, MO, USA). Rotenone was freshly prepared in 100% dimethyl sulfoxide. Stock solutions of capsaicin (4 mg/ml) contained 10% ethanol, 10% Tween 80, and 80% saline solution. Capsaicin was diluted in saline obtain the necessary doses. All the used chemicals and reagents in the present study were of analytical grade and obtained from Sigma-Aldrich.

2.3. Experimental design

Mice were randomly assigned to different treatment groups (6 animals each). Group 1 received the vehicle (DEMSO) three times a week and served as control -ve. Groups 2, 3, 4 & 5 were given rotenone at 1.5 mg/kg, subcutaneously every other day for two weeks and treated at the same time with the vehicle (group 2; control +ve), L-dopa at 25 mg/kg orally once a day (group 3) or capsaicin at 0.5 or 1 mg/kg, orally once a day (groups 4 & 5).

Treatments were continued for two weeks. Behavioral tests were done 24 hrs after last injection of rotenone. After that, mice were euthanized by cervical dislocation and each brain was quickly removed, washed with ice-cold phosphate-buffered saline (PBS, pH 7.4), placed on ice-cold plate, dissected, weighed, and stored at −80°C for the biochemical study. Tissues were homogenized in 0.1 M phosphate-buffered saline at pH 7.4 to give a final concentration of 10 % w/v. Homogenization was performed using a homogenizer (ULTRA-TURAX, IKA T10 basic, Germany) at speed 5000 rpm for 30 seconds. The histopathological study was carried out on separate groups of rats (n = 4 per group).

2.4. Biochemical assays

2.4.1. Determination of lipid peroxidation

Lipid peroxidation was measured in brain homogenates by determining malondialdehyde (MDA) according to Nair and Turne (1984). In this assay 2-thiobarbituric acid reacts with MDA at 25°C to yield a red colored complex with a peak absorbance at 532 nm.

2.4.2. Determination of reduced glutathione

Reduced glutathione was determined in brain homogenates according to Ellman (1959). In this assay, Ellman´s reagent (DTNB; 5, 5'-dithiobis (2-nitrobenzoic acid)) reacts with the free thiol group of GSH to form 2-nitro-s-mercaptobenzoic acid. The chromophore has yellow color and is determined with spectrophotometer at 412 nm.

2.4.3. Determination of nitric oxide

Nitric oxide production was determined by measuring nitrite in the supernatant with the Griess reagent. Nitrate is converted to nitrite with by the enzyme nitrate reductase. Nitrite then reacts with the Griess reagent to form a purple azo compound, and its absorbance is measured at 540 nm with spectrophotometer (Archer, 1993).

2.5. Behavioral testing

2.5.1. Wire hanging test

This test is used for the measurement of neuromuscular strength where mice were allowed to hang by their forelimbs from a steel rod (25 cm long, 0.2 cm in diameter), 0.25 m above the bench. The latency to fall was counted for three trials with a cutoff time of 180 s (Crawley, 2007).

2.5.2. Wood walking test

In order to assess motor coordination and balance, mice were made to cross over a wooden stick (~1 m in length, 1 cm in width and elevated 30 cm from the ground) and the time each mouse spent to reach the end is recorded (Rogers et al., 1997).

2.5.3. Stair test

This is a test for skilled reaching in which mice were placed at the bottom of a stair (30 cm in length), placed at an angle of 55° above the bench, and the latency to climb the stair is recorded for each mouse (Baird et al., 2001).

2.6. Statistical analysis

The experimental data are expressed as mean ± SE. Differences between vehicle control and treatment groups were tested using one-way ANOVA followed by Tukey's multiple comparisons test. Statistical analysis was performed using GraphPad Prism 6 for Windows (GraphPad Prism Software Inc., San Diego, CA, USA) was used. Statistical significance was considered at a probability value of less than 0.05.

2.7. Histopathology

Brain sections were fixed in freshly prepared 10% neutral buffered formalin, processed routinely, and embedded in paraffin. Paraffin sections 5 µm thick were prepared and stained with hematoxylin and eosin for histopathological examination. Images were examined and photographed under a digital camera (Microscope Digital Camera DP70, Tokyo, Japan), and processed using Adobe Photoshop version 8.0 (San Jose, CA, USA).

3. Results and Discussion

3.1. Biochemical results

3.1.1. Lipid peroxidation

The level of MDA, an indicator of lipid peroxidation, was significantly higher in the rotenone-treated group by 84.7% compared with the vehicle control (31.19 ± 0.8 vs. 16.89 ± 0.71 nmol/g.tissue). MDA levels were significantly decreased by 38.7% and 44.3% in the rotenone + capsaicin groups, respectively, compared with those of the rotenone control group. It also decreased by 29.5% in the rotenone + l-dopa group compared with the rotenone control. Values were 21.98 ± 0.62 nmol/g.tissue in the rotenone + l-dopa group and 19.12 ± 1.24 and 17.36 ± 1.11 nmol/g.tissue in the rotenone + capsaicin groups, respectively.

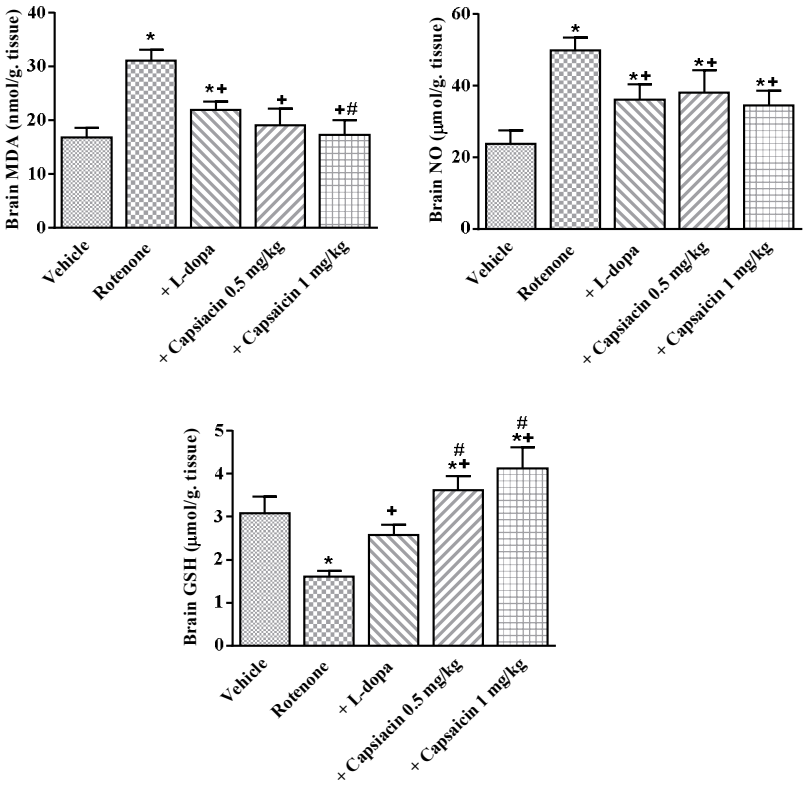

Fig. 1. Effect of capsaicin and L-dopa on oxidative stress biomarkers in brain of mice treated with rotenone. Values represent means ± SEM. *: P<0.05 vs. vehicle and between different groups as indicated in the graph. +: P<0.05 vs. rotenone control. #: P<0.05 vs. L-dopa-treated group.

3.1.2. Nitric oxide

In the group treated with only rotenone, nitric oxide level was significantly increased by 110% compared with the vehicle control value (49.93 ± 1.47 vs. 23.77 ± 1.57 µmol/g. tissue). The level of nitric oxide fell by 23.7% and 30.1% in the rotenone + capsaicin (0.5 and 1 mg/kg)-treated groups, respectively, compared with the rotenone only group (38.10 ± 2.5, 34.54 ± 1.68 vs. 49.93 ±1.47 µmol/g. tissue). There was also significant decrease in the level of nitric oxide by 27.7% in mice treated with rotenone + l-dopa (36.10 ± 1.77 vs. 49.93 ± 1.47 µmol/g. tissue).

3.1.3. Reduced glutathione

Mice treated with only rotenone had significantly lower GSH levels by 48.1% compared with the vehicle control (1.61 ± 0.05 vs. 3.1 ± 0.20 µmol/g. tissue). Meanwhile, GSH levels increased by 124.8% and 155.9% by capsaicin administration compared to the rotenone only group (3.62 ± 0.13, 4.12 ± 0.2 vs. ± 1.61 ± 0.05 µmol/g. tissue). In rotenone + l-dopa group, GSH levels were increased by 60.2% compared with that of rotenone only group (2.58 ± 0.10 vs. 1.61 ± 0.05 µmol/g. tissue).

3.2. Behavioral results

3.2.1. Stair test

In the rotenone group, the time spent in ascending the stair was significantly increased by 58.4% compared to the vehicle group (19.44 ± 0.68 vs. 12.27 ± 0.56 sec). In the groups treated with rotenone and capsaicin (0.5 and 1 mg/kg) or rotenone + L-dopa, the time taken by mice to ascend the stair was significantly shorter by 47%, 64.1% and 57.8%, compared to the rotenone only group (10.30 ± 0.95, 6.98 ± 0.74, 8.21 ± 0.57 vs.19.44 ± 0.68 sec).

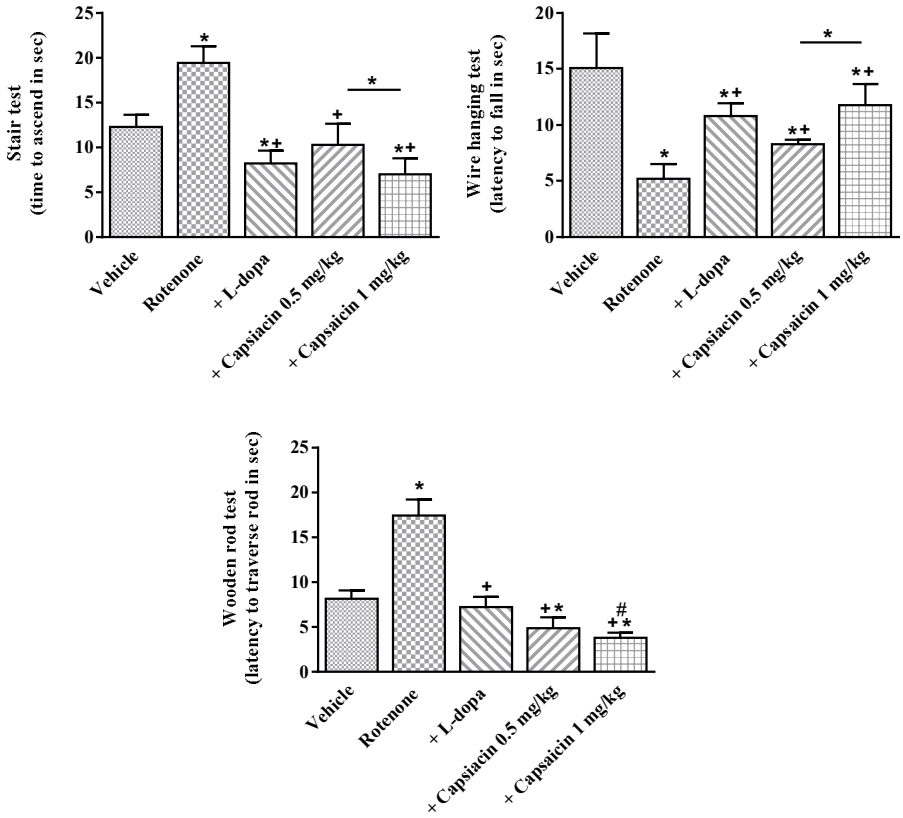

Fig. 2. Effect of capsaicin and L-dopa on motor strength and coordination in mice treated with rotenone. Values represent means ± SEM. *: P<0.05 vs. vehicle and between different groups as indicated in the graph. +: P<0.05 vs. rotenone control. #: P<0.05 vs. L-dopa-treated group.

3.2.2. Wire hanging test

Mice receiving rotenone showed decreased ability to hang suspended from the steel rod by 65.7% compared to their vehicle controls (5.18 ± 0.49 vs. 15.10 ± 1.25 sec). When treated with capsaicin, the latency to fall increased by 59.6% and 126.8% as compared with rotenone only group (8.27 ± 0.15, 11.75 ± 0.77 vs. 5.18 ± 0.49 sec). Mice treated with rotenone + L-dopa exhibited 108.9% increase in heir latency to fall (10.82 ± 0.46 vs. 5.18 ± 0.49 sec).

3.2.3. Wood walking test

Compared with the vehicle control group, rotenone-treated mice were significantly slower to traverse a wooden stick by 114.2% (17.46 ± 0.66 vs. 8.15 ± 0.39 sec). In the groups treated with capsaicin, the latency to traverse the stick was significantly decreased by 72.1% and 78.1% compared with the rotenone only group (4.88 ± 0.51, 3.82 ± 0.22 vs. 17.46 ± 0.66 sec). Meanwhile, mice given rotenone + L-dopa showed 58.6% decrease in latency (7.22 ± 0.48 vs. 17.46 ± 0.66 sec).

3.3. Histopathological results

Following rotenone injections, neurodegenerative changes could be observed in the substantia nigra, cerebral cortex and hippocampus brain regions. The pigmented cells in the substantia nigra were markedly reduced in size and number. In the cerebral cortex and hippocampus, many deeply stained neurons with shrunken cytoplasm and damaged nuclei could be seen. The pathological changes caused by rotenone were attenuated by capsaicin and L-dopa (Figs. 3,4 & 5).

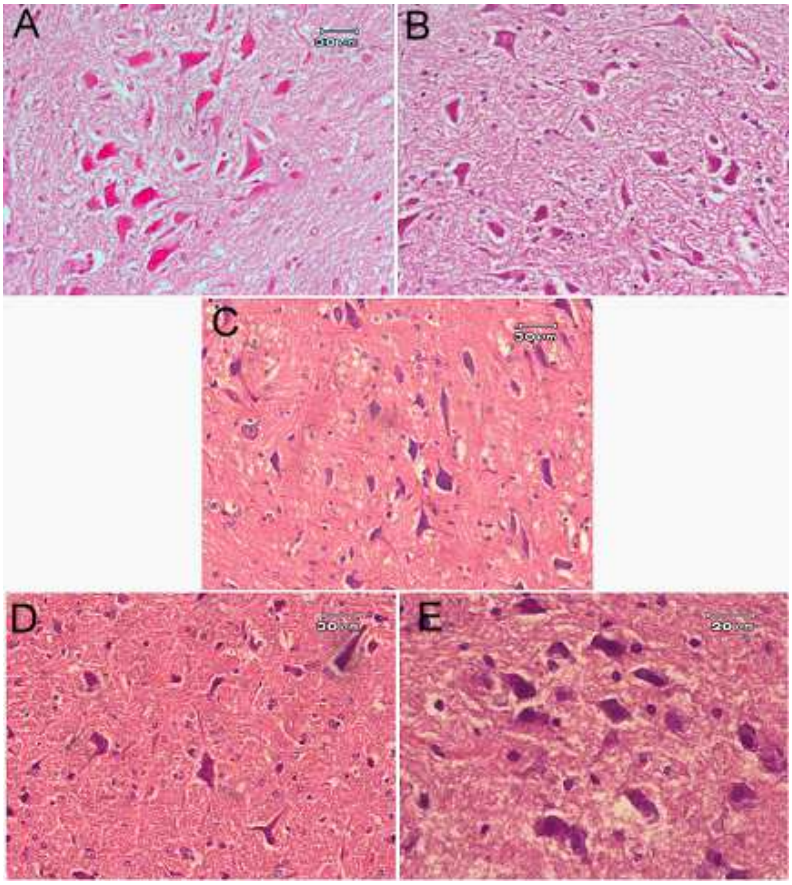

Fig. 3. Representative photomicrographs of Hx & E stained sections of the substantia nigra area after treatment with: **(A)** Vehicle showing the normal structure of the pigmented neurons in this area. **(B)** Rotenone showing decrease in size and number of pigmented cells. **(C)** Rotenone + L-dopa showing increase of pigmented neurons in number, but many of them are still smaller in size than normal. **(D)** Rotenone + capsaicin 0.5 mg/kg showing marked decrease of size and number of pigmented neurons. **(E)** Rotenone + capsaicin 1 mg/kg showing noticeable increase in number and size of pigmented cells.

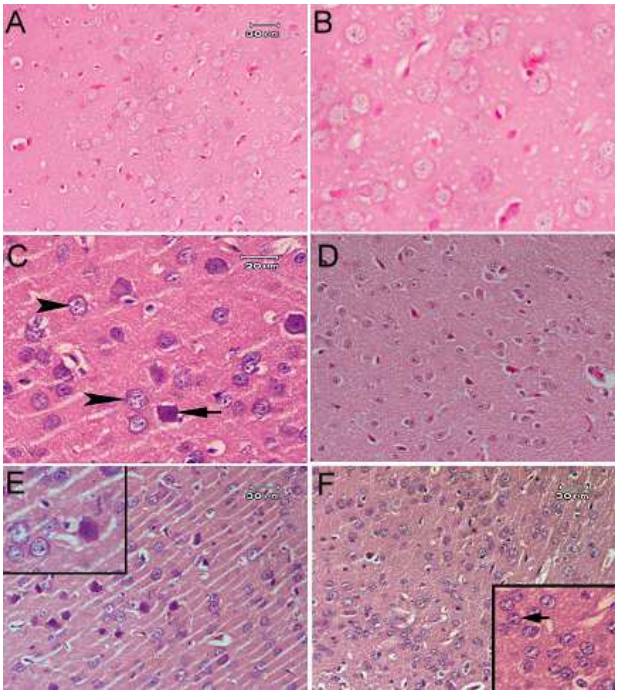

Fig. 4. Representative photomicrographs of Hx & E stained sections of the cerebral cortex tissue from mice treated with: **(A)** Vehicle: showing the normal structure of cerebral cortex tissue. **(B)** A higher magnification of the previous section shows the same result. **(C)** Rotenone showing deeply stained neurons (arrow) and karyorrhexia in others (arrowhead). **(D)** Rotenone + L-dopa showing shows marked decrease of deeply stained neurons, only a few deeply stained cells are noticed among normal neurons. **(E)** Rotenone + capsaicin 0.5 mg/kg showing a slight decrease of darkly stained and karyorrhectic neurons. **(F)** Rotenone capsaicin 1 mg/kg showing marked decrease of darkly stained nuclei, although neurons with karyorrhectic nuclei are still noticed (arrow).

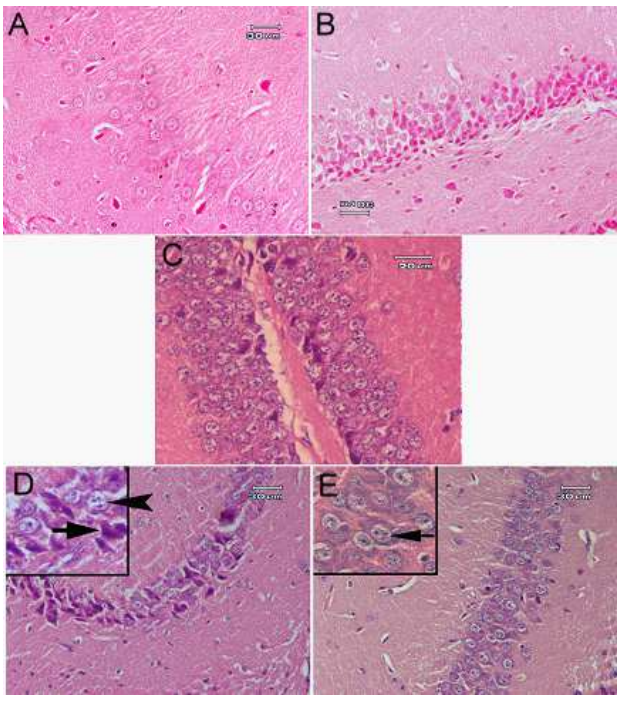

Fig. 5. Representative photomicrographs of Hx & E stained sections of the hippocampus area from mice treated with: **(A)** Vehicle: showing the normal structure of this tissue. **(B)** Rotenone showing many deeply stained neurons (arrow). A slight decrease of the granular cell layer thickness is observed. **(C)** Rotenone + L-dopa showing decrease of deeply stained neurons. **(D)** Rotenone + capsaicin 0.5 mg/kg showing many darkly stained (arrowhead) and karyorrhectic (arrow) neurons. A noticeable decrease of the granular cell layer thickness is seen. **(E)** Rotenone + capsaicin 1 mg/kg showing no darkly stained neurons are observed, although neurons with karyorrhectic nuclei are still noticed (arrow).

This study demonstrates a neuroprotective effect for theTPRV1 agonist capsaicin on neuronal cell degeneration in brain after systemic rotenone administration in mice. The number of degenerated neurons in substantia nigra, cerebral cortex and hippocampus after rotenone was reduced by capsaicin. This was accompanied by a decrease in oxidative stress and improved motor strength and coordination.

The neuroprotective potential of capsaicin reported in the present study is supported by previous findings. Capsaicin (0.01–0.6 mg/kg, s.c.) protected against global cerebral ischemia in Mongolian gerbils (Pegorini et al., 2005). Capsaicin (0.5 mg/kg) was also reported to prevent dopaminergic neurons in experimentally-induced PD (Chung et al., 2017). Capsaicin (0.15 or 1.5 mg/kg, i.p.) showed antioxidant effects increasing brain reduced glutathione content and decreasing serum nitric oxide levels (Abdel-Salam et al, 2012) and when given at 1 mg/kg, s.c., it decreased plasma nitric oxide, interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α) during endotoxaemia in rats (Demirbilek et al, 2004). It also alleviated brain oxidative stress protected against neuronal damage in experimental status epilepticus in rats (Abdel-Salam et al., 2020).

Rotenone is a known mitochondrial complex I inhibitor which can lead to oxidative stress and results in dopaminergic cell death. Because of its lipophilic properties, rotenone readily permeates the blood–brain barrier and cell membranes and accumulates in mitochondria, where it inhibits oxidative phosphorylation by blocking the activity of complex I of the electron transport chain (Sherer et al., 2003). Inhibition of complex I results in the increased production of reactive oxygen species leading to the development of oxidative stress, depletion in cellular ATP and consequent neuronal death (Li et al., 2003; Han et al., 2014). Rotenone was found to activate microglia cells causing the increased production of superoxide and hypochlorous acid (Gao et al., 2003; Chang et al., 2011). The development of oxidative stress in the brain following rotenone injection is evidenced by the increased brain lipid peroxidation, depletion of reduced glutathione. The latter is an essential antioxidant capable of scavenging ROS in both cytosol and mitochondria (Townsend et al., 2003). Rotenone was also found to decrease total antioxidant capacity (Abdel-Salam et al., 2018, 2020) and the activity of the antioxidant enzymes superoxide dismutase (Abdel-Salam et al., 2018) and catalase (Abdel-Salam et al., 2015c). Neuronal death caused by rotenone could be prevented by administering antioxidants such as glutathione, *N*-acetylcysteine, ascorbate, and α-tocopherol, thereby, lending further support to the important contribution of oxidative stress in rotenone neurotoxicity (Li et al., 2003; Testa et al., 2005; Ibrahim et al., 2017; Abdel-Salam et al., 2019).

Our results also indicated a significant in the level of brain nitric oxide following rotenone treatment which is consistent with previous studies (Bashkatova et al., 2004; Xiong et al., 2015; Abdel-Salam et al., 2017). Nitric oxide is synthesized from L-arginine by the action of nitric oxide synthase (NOS). The constitutive endothelial (eNOS) and neuronal (nNOS) isoforms produce small amounts of nitric oxide for short period. In contrast, high concentrations result from inducible NOS (iNOS) in resident brain immune cells, microglia and astrocytes following their activation by ROS, cytokines, and bacterial lipopolysaccharide (Pacher et al., 2007). High levels of nitric oxide can be damaging to neurons. This is largely thought to be caused by the strong oxidant peroxynitrite generated from the reaction of nitric oxide and superoxide. The result is oxidation of membrane lipids, proteins, and DNA, nitrosylation of thiol residues in proteins or glutathione, and nitrotyrosination of proteins (Moncada and Bolanos, 2006; Weidinger and Kozlov, 2015). Studies indicated increased iNOS expression in substantia nigra and striatum in rotenone-treated animals (Abdel-Salam et al., 2015d). Rotenone neurotoxicity is reduced iNOS or nNOS inhibitors, thereby, suggesting that both isoenzymes contribute to the production of the nitric oxide that mediates the rotenone-induced dopaminergic neuron apoptosis and degeneration (He et al., 2003; Gao et al., 2015).

The presence of bradykinetic motor function is the most important feature of human PD. Therefore, in the present study, the effect of rotenone treatment on motor functions was evaluated using stair, wire hanging and wood walking tests. We also found that administration of rotenone induced motor dysfunction which can be alleviated by treating animals with capsaicin or l-dopa. Previous studies using the present regimen of rotenone have shown depletion of striatal dopamine and tyrosine hydroxylase (Abdel-Salam et al., 2014). It is likely, therefore, that the improvement of motor

function by capsaicin or l-dopa reflects interference with the rotenone-induced degeneration of nigrostriatal neurons and the consequent depletion of striatal dopamine levels. This conclusion is supported by the histpathological study which showed noticeable increase in the number and size of dopaminergic pigmented cells in the substantia nigra.

4. Conclusion

In summary, our findings indicate that capsaicin was capable of preventing neuronal loss and motor dysfunction in experimental model of PD induced by rotenone in mice, possibly via decreased oxidative stress.

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Conflict of interest

The authors declare no conflicts of interest.

References

[1] Wirdefeldt, K., Adami, H.O., Cole, P., Trichopoulos, D., & Mandel, J. (2011). Epidemiology and etiology of Parkinson's disease: a review of the evidence. *European Journal of Epidemiology, 26 (Suppl 1),* S1–58.

[2] Jankovic, J. (2008). Parkinson's disease: clinical features and diagnosis. *Journal of Neurology, Neurosurgery and Psychiatry, 79,* 368–376.

[3] Hughes, A.J., Daniel, S.E., Kilford, L., & Lees, A.J. (1992). Accuracy of clinical diagnosis of idiopathic Parkinson's disease: A clinico-pathological study of 100 cases. *Journal of Neurology, Neurosurgery and Psychiatry, 55(3),* 181–184.

[4] DeLong, M.R., & Wichmann, T. (2007). Circuits and circuit disorders of the basal ganglia. *Archives of Neurology. 64(1),* 20-24..

[5] Kumar, K.R., Djarmati-Westenberger, A., & Grunewald, A. (2011). Genetics of Parkinson's disease. *Seminars in Neurology, 31(5),* 433–440.

[6] Ritz, B.R., Paul, K.C., & Bronstein, J.M. (2016). Of pesticides and men: a California story of genes and environment in Parkinson's disease. *Current Environmental Health Reports, 3,* 40-52.

[7] Dhillon, A.S., Tarbutton, G.L., Levin, J.L., Plotkin, G.M., Lowry, L.K., Nalbone, J.T., et al. (2008). Pesticide/environmental exposures and Parkinson's disease in East Texas. *Journal of Agromedicine, 13(1),*37–48.

[8] Jenner, P. (2003). Oxidative stress in Parkinson's disease. *Annals of Neurology, 53(Suppl 3),* S26–36.

[9] Halliwell, B. (2001). Role of free radicals in the neurodegenerative diseases: therapeutic implications for antioxidant treatment. *Drugs & Aging, 18(9),* 685–716.

[10] Halliwell, B. (2009).The wanderings of a free radical. *Free Radical Biology and Medicine, 46(5),* 531–542.

[11] Sies, H. (1997). Oxidative stress: oxidants and antioxidants. *Experimental Physiology, 82(2),* 291-295.

[12] Floyd, R.A. (1999). Antioxidants, oxidative stress, and degenerative neurological disorders. *Proceedings of the Society for Experimental Biology and Medicine, 222(3),* 236–245.

[13] Schapira, A.H., Cooper, J.M., Dexter, D., Clark, J.B., Jenner, P., & Marsden CD. (1990). Mitochondrial complex I deficiency in Parkinson's disease. *Journal of Neurochemistry, 54(3),* 823–827.

[14] Zorov, D.B., Juhaszova, M., & Sollott, S.J. (2006). Mitochondrial ROS-induced ROS release: an update and review. *Biochimica et Biophysica Acta. 1757(5–6),* 509–517.

[15] LeWitt, P.A., & Fahn, S. (2016). Levodopa therapy for Parkinson disease. A look backward and forward. *Neurology, 86(14 Suppl 1),* S3-12.

[16] Encarnacion, E.V., & Hauser, R.A. (2008). Levodopa-induced dyskinesias in Parkinson's disease: etiology, impact on quality of life, and treatments. *European Neurology, 60,* 57–66.

[17] Abdel-Salam, O.M.E. (2015a). Drug therapy for Parkinson's disease: an update. *World Journal of Pharmacology, 4(1),*117-143.

[18] Szolcsányi, J. (2014). Capsaicin and sensory neurones: a historical perspective. In: Capsaicin as a Therapeutic Molecule (Progress in Drug Research 68) (Abdel-Salam, O.M.E.). Springer, Berlin, Germany. pp. 1–37.

[19] Steenland, H.W., Ko, S.W., Wu, L.J., & Zhuo, M. (2006). Hot receptors in the brain. *Molecular Pain, 2,* 34.

[20] Abdel-Salam, O.M.E., Sleem, A,A., Youness, E.R., Yassen, N.N., Shaffie, N., & El-Toumy, S.A. (2018). Capsicum protects against rotenone-induced toxicity in mice brain via reduced oxidative stress and 5-lipoxygenase activation. *Journal of Pharmacy and Pharmacology Research, 2 (3),* 060-77.

[21] Abdel-Salam, O.M.E., Sleem, A.A., Youness, E.R., Morsy, F.A., Shaffie, N.M., & Souleman, A.M.A. (2019). Hot Pepper extract protects against hypoglycemia-induced brain and liver injury in mice. *Reactive Oxygen Species, 8(23),* 297–311.

[22] Hien, P.P., Gortnizka, H., & Kraemer, R. (2003) Rotenone-potential and prospect for sustainable agriculture. *Omonrice, 11,* 83–92.

[23] Betarbet, R., Sherer, T.B., MacKenzie, G., Garcia-Osuna, M., Panov, A.V., & Greenamyre, J.T. (2000). Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nature Neuroscience, 3(12),* 1301–6.

[24] Abdel-Salam, OME. (2015b). The rotenone model of parkinson's disease in studying the mechanisms of nigrostriatal cell death. *Jacobs Journal of Pharmacology and Pharmacovigilance, 1(1),* 007.

[25] Nair, V., & Turner, G.A. (1984). The thiobarbituric acid test for lipid peroxidation: structure of the adduct with malondialdehyde. *Lipids, 19(10),* 804-805.

[26] Ellman, G.L. (1959). Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics, 82(1),* 70–77.

[27] Archer, S. (1993). Measurement of nitric oxide in biological models. *The FASEB Journal, 7(2),* 349–60.

[28] Crawley, J.N. (2017). What's wrong with my mouse? Behavioral phenotyping of transgenic and knockout mice. Second edition ed. Hoboken: Wiley.

[29] Rogers, D.C., Campbell, C.A., Stretton, J.L., & Mackay, K.B. (1997). Correlation between motor impairment and infarct volume after permanent and transient middle cerebral artery occlusion in the rat. *Stroke, 28(10),* 2060–2065.

[30] Baird, A.L., Meldrum, A., & Dunnett, S.B. (2001). The staircase test of skilled reaching in mice. *Brain Research Bulletin, 54(2),* 243–250.

[31] Pegorini, S., Braida, D., Verzoni, C., Guerini-Rocco, C., Consalez, G.G., Croci, L., et al. (2005). Capsaicin exhibits neuroprotective effects in a model of transient global cerebral ischemia in Mongolian gerbils. *British Journal of Pharmacology, 144(5),* 727–735.

[32] Chung, Y.C., Baek, J.Y., Kim, S.R., Ko, H.W., Bok, E., Shin, W.H., et al. (2017). Capsaicin prevents degeneration of dopamine neurons by inhibiting glial activation and oxidative stress in the MPTP model of Parkinson's disease. Experimental & Molecular Medicine*, 49(3)*, e298.

[33] Abdel-Salam, O.M.E., Abdel-Rahman, R.F., Sleem, A.A., & Farrag, A.R. (2012). Modulation of lipopolysaccharide-induced oxidative stress by capsaicin. *Inflammopharmacology, 20(4),* 207–217.

[34] Demirbilek, S., Ersoy, M.O., Demirbilek, S., Karaman, A., Gürbüz, N., Bayraktar, N., et al (2004). Small-dose capsaicin reduces systemic inflammatory responses in septic rats. *Anesthesia & Analgesia, 99(5),* 1501–1507.

[35] Abdel-Salam, O.M.E., Sleem, A,A., Sayed, MAEM.,Youness, E.R., & Shaffie, N. (2020). Capsaicin exerts anti-convulsant and neuroprotective effects in pentylenetetrazole-induced seizures. *Neurochemical Research, 45(5),*1045-1061.

[36] Sherer, T.B., Betarbet, R., Testa, C.M., Seo, B.B., Richardson, J.R., & Kim, J.H. (2003). Mechanism of toxicity in rotenone models of Parkinson's disease. *Journal of Neuroscience, 23(34),*10756–64.

[37] Li, N., Ragheb, K., Lawler, G., Sturgis, J., Rajwa, B., & Melendez, J.A. (2003). Mitochondrial complex I inhibitor rotenone induces apoptosis through enhancing mitochondrial reactive oxygen species production. *Journal of Biological Chemistry, 278(10),* 8516-25.

[38] Han, G., Casson, R.J., Chidlow, G., & Wood, J.P.M. (2014). The mitochondrial complex I inhibitor rotenone induces endoplasmic reticulum stress and activation of GSK-3b in cultured rat retinal cells. *Investigative Ophthalmology & Visual Science, 55,*5616–5628.

[39] Gao, H.M., Liu, B., & Hong, J.S. (2003). Critical role for microglial NADPH oxidase in rotenone-induced degeneration of dopaminergic neurons. *Journal of Neuroscience, 23(15),* 6181–7.

[40] Chang, C.Y., Song, M.J., Jeon, S.B., Yoon, H.J., Lee, D.K., & Kim, I.H. (2011). Dual functionality of myeloperoxidase in rotenone-exposed brain-resident immune cells. *American Journal of Pathology, 179(2),* 964–79.

[41] Townsend, D.M., Tew, K.D., & Tapiero, H. (2003). The importance of glutathione in human disease. *Biomed Pharmacother, 57(3–4),* 145–155.

[42] Abdel-Salam, O.M.E., Medhat, D., Sleem, A.A., & Shaffie, N. (2018). Neuroprotection by montelukast against rotenone-induced rat brain damage. *Reactive Oxygen Species, 5(15),* 209–219.

[43] Abdel-Salam, O.M.E., Morsy, S.M.Y., Youness, E.R., Yassen, N.N., Shaffie, N., & Sleem, A.A. (2020). Citric acid protects dopaminergic cells against rotenone-induced neurodegeneration. *Reactive Oxygen Species 9(27),*118–135.

[44] Abdel-Salam, O.M.E, Youness, E.R., Khadrawy, Y.A., Mohammed, N.A., Abdel-Rahman, R.F., & Omara, E.A. (2015c).The effect of cannabis on oxidative stress and neurodegeneration induced by intrastriatal rotenone injection in rats. *Comparative Clinical Pathology, 24,* 359–378.

[45] Testa, C.M., Sherer, T.B., & Greenamyre, J.T. (2005). Rotenone induces oxidative stress and dopaminergic neuron damage in organotypic substantia nigra cultures. *Brain Research. Molecular Brain Research, 134(1),* 109–118.

[46] Ibrahim, N.A., Abdel-Salam, O.M., Khadrawy, Y.A., Hashem, A.M., & Sameer, E.M. (2017). Non-steroidal anti-inflammatory drugs and vitamin C in the rotenone induced nigrostriatal damage in mice. *European Journal of Clinical and Biomedical Sciences, 3(4),* 67–79.

[47] Abdel-Salam, O.M.E., Sleem, A.A., Youness, E.R., Mohammed, N.A., Omara, E.A., & Shabana, M.E. (2019). Neuroprotective effects of the glutathione precursor N-acetylcysteine against rotenone-induced neurodegeneration. *Reactive Oxygen Species, 8(22),* 231–244.

[48] Bashkatova, V., Alam, M., Vanin, A., & Schmidt, WJ. (2004). Chronic administration of rotenone increases levels of nitric oxide and lipid peroxidation products in rat brain. *Experimental Neurology, 186(2)*, 235-241.

[49] Xiong, Z.K., Lang, J., Xu, G., Li, H.Y., Zhang, Y., Wang, L., et al. (2015). Excessive levels of nitric oxide in rat model of Parkinson's disease induced by rotenone. *Experimental and Therapeutic Medicine, 9(2),* 553–558.

[50] Abdel-Salam, O.M.E., Youness, E.R., Ahmed, N.A., El-Toumy, S.A., Souleman, A.M.A., Shaffie, N., et al. (2017). Bougainvillea spectabilis flowers extract protects against the rotenone-induced toxicity. *Asian Pacific Journal of Tropical Medicine, 10(5),* 478–490.

[51] Pacher, P., Beckman, J.S., & Liaudet, L. (2007). Nitric oxide and peroxynitrite in health and disease. *Physiological Reviews, 87(1),* 315–424.

[52] Moncada, S., & Bolanos, J.P. (2006). Nitric oxide, cell bioenergetics and neurodegeneration. *Journal of Neurochemistry, 97(6),*1676–89.

[53] Weidinger, A., & Kozlov, A.V. (2015). Biological activities of reactive oxygen and nitrogen species: oxidative stress versus signal transduction. *Biomolecules, 5(2),* 472–484.

[54] Abdel-Salam, O.M.E., Youness, E.R., Khadrawy, Y.A., Mohammed, N.A., Abdel-Rahman, R.F., Omara, E.A., et al. (2015d). The effect of cannabis on oxidative stress and neurodegeneration induced by intrastriatal rotenone injection in rats. *Comparative Clinical Pathology*, 24:359–378.

[55] He, Y., Imam, S.Z., Dong, Z., Jankovic, J., Ali, S.F., & Appel, S.H. (2003). Role of nitric oxide in rotenone induced nigrostriatal injury. *Journal of Neurochemistry, 86(6),* 1338–45.

[56] Gao, H.M., Liu, B., & Hong, J.S. (2003). Critical role for microglial NADPH oxidase in rotenone-induced degeneration of dopaminergic neurons. *Journal of Neuroscience, 23(15),* 6181–7.

[57] Abdel-Salam, O.M.E., Omara, E.A., Youness, E.R., Khadrawy, Y.A., Mohammed, N.A., & Sleem, A.A. (2014). Rotenone-induced nigrostriatal toxicity is reduced by methylene blue. *Journal of Neurorestoratology, 2(1),* 65–80.