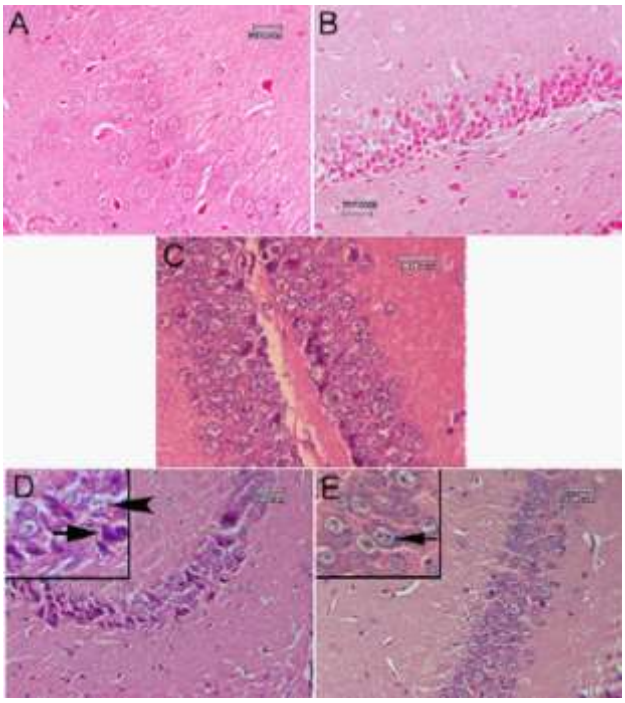

Fig. 5 Representative photomicrographs of Hx & E stained sections of the hippocampus area from mice treated with: **(A)** Vehicle: showing the normal structure of this tissue. **(B)** Rotenone showing many deeply stained neurons (arrow). A slight decrease of the granular cell layer thickness is observed. **(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).

4 Discussion

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 [30]. Capsaicin (0.5 mg/kg) was also reported to prevent dopaminergic neurons in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) -induced PD in mice [31]. Capsaicin (0.15 or 1.5 mg/kg, i.p.) showed antioxidant effects increasing brain reduced glutathione content and decreasing serum nitric oxide levels after systemic administration of the endotoxin lipopolysaccharide (100 μg/kg, i.p.) in rats [32] 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 [33].

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 [34]. 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 [35],[36]. Rotenone was found to activate microglia cells causing the increased production of superoxide and hypochlorous acid [37],[38].

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 [39]. Rotenone was also found to decrease total antioxidant capacity and the activity of the antioxidant enzymes superoxide dismutase [40]. Neuronal death caused by rotenone could be prevented by administering antioxidants such as glutathione, *N*-acetylcysteine, and α-tocopherol, thereby, lending further support to the important contribution of oxidative stress in rotenone neurotoxicity [35],[41].

Our results also indicated a significant in the level of brain nitric oxide following rotenone treatment which is consistent with previous studies [42],[43]. 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 [44]. 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 [45],[46]. Studies indicated increased iNOS expression in substantia nigra and striatum in rotenone-treated animals [47]. Rotenone neurotoxicity is reduced by 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 [37],[48],[49].

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 [50]. 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.

5 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. By interfering with oxidative-mediated neuronal injury and death, capsaicin may also prove beneficial in Parkinson's disease.

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Contribution of Individual Authors to the Creation of a Scientific Article (Ghostwriting Policy)

Marwa El-Shamarka and Omar Abdel-Salam designed the study and conducted the experiments. Nermeen Shaffie performed the histological studies and its interpretation. Omar Abdel-Salam prepared the manuscript. Marwa El-Shamarka, Omar Abdel-Salam, and Nermeen Shaffie approved the final version of the manuscript.

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Conflict of Interest

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