Hydroxychloroquine Attenuated Motor Impairment and Oxidative Stress in a Rat 6-hydroxydopamine Model of Parkinson's Disease

Seyed Zanyar Athari

Tabriz University of Medical Sciences

Fereshteh Farajdokht

Tabriz University of Medical Sciences

Saeed Sadigh Eteghad

Tabriz University of Medical Sciences

Daryoush Mohajeri

Islamic Azad University Tabriz Branch

Mir Alireza Nourazar

Islamic Azad University Tabriz Branch

Gisou Mohaddes (✉ gmohades@yahoo.com)

Tabriz University of Medical Sciences

Research Article

Keywords: Parkinson's disease, hydroxychloroquine, levodopa, 6-OHDA, oxidative stress

Posted Date: September 15th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-890394/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: Parkinson's disease (PD) is associated with the destruction of dopaminergic neurons in the substantia nigra (SN). Hydroxychloroquine (HCQ) has the capability to cross the blood-brain barrier and promote a neuroprotective potential. This study evaluated the effects of HCQ on the 6-hydroxydopamine (6-OHDA)-induced PD model in rats.

Methods: Wistar rats were randomly divided into sham, PD, PD+levodopa, and PD+HCQ groups. The PD model was induced by a stereotactic administration of 6-OHDA into the left SN pars compacta (SNpc) and confirmed by rotation and the Murprogo's tests. HCQ (100 mg/kg, p.o.) and levodopa (12 mg/kg, p.o.) were administered once a day for 21 days. Three weeks after surgery, the behavioral tests were performed. Brain lipid peroxidation index (MDA), glutathione peroxidase activity (GPx), total antioxidant capacity (TAC) levels, and α -synuclein protein expression in the SN were also measured.

Results: The behavioral tests demonstrated that induction of PD increased the muscle rigidity and the number of rotations, which were reversed by HCQ treatment. Also, induction of PD was associated with an increase in α -synuclein protein levels and MDA and decreased TAC levels and GPx activity. However, HCQ decreased α -synuclein and MDA levels while increased TAC levels and GPx activity. Additionally, histopathological data showed that HCQ protects dopaminergic neurons against 6-OHDA-induced toxicity.

Conclusion: According to the results, HCQ has a beneficial effect in improving PD-related pathophysiology, in part, by mitigating oxidative stress and protecting the dopaminergic neurons in the SN.

Introduction

Neurological diseases are the main cause of disability and premature death among aged people worldwide, owing to the gradual destruction of neurons and age-associated motor and cognitive impairments [1]. Parkinson's disease (PD) is a neurodegenerative disease, which is characterized by accumulation of α -synuclein (called Lewy Bodies) and progressive loss of dopaminergic neurons in the midbrain [2, 4]. It is estimated that 60% of dopaminergic neurons are destroyed at the beginning of the disease preceding diagnosis [4].

6-hydroxydopamine (6-OHDA), a hydroxylated analog of dopamine, is easily absorbed by dopaminergic neurons and oxidized by reactive oxygen species (ROS). It induces neurotoxicity by inhibiting the mitochondrial electron transport chain and provoking oxidative stress [5]. 6-OHDA lesion is widely used to test new treatment strategies in PD [6]. 6-OHDA administration into the substantia nigra pars compacta (SNpc) or the striatum results in specific biochemical and motor dysfunctions, which mimic those of human PD [7].

Current available PD treatments include dopamine analogs, dopamine-degrading enzyme inhibitors, and deep brain stimulation, all aimed at improving secondary motor symptoms, but no solution has been reported to stop progressive neuronal death [8]. Management of moderate to severe stages of PD is challenging because in the later stages of the disease, response to levodopa is progressively declined, and some levodopa-resistant symptoms, such as falling and freezing, dysarthria, dysphagia, and dementia are elicited [4].

Hydroxychloroquine (HCQ), a chloroquine hydroxyl-derivative, crosses the blood-brain barrier [9] and exerts anti-malarial, anti-rheumatic, anti-inflammatory, and neuroprotective effects [10, 11]. Kim et al. reported that some anti-malarial drugs, including chloroquine, improved motor disturbances in 6-OHDA-lesioned rats [12]. Evidence also shows that HCQ alleviates neurological symptoms in sarcoidosis patients [13]. Recently, Heyda et al. demonstrated that HCQ ameliorated motor deficits induced in an animal model of PD induced by rotenone through suppression of striatal inflammatory cytokines, restoring tyrosine hydroxylase (TH) levels, GSK-3 β inhibition, and up-regulation of the orphan nuclear receptor mRNA expression [11].

Hence, this study investigated the effects of HCQ administration on motor function, oxidative stress markers, and histopathological changes in a rat 6-OHDA model of PD.

Materials And Methods

Materials

HCQ was purchased from Tehran-Darou company (Tehran, Iran), 6-OHDA (Lot: STBH3207V), apomorphine (Batch: SLBF5500V), and ascorbic acid (Lot: MKCM8021) were purchased from Sigma-Aldrich Company (St. Louis, MO, USA).

Animals and experimental design

Forty-eight adult male Wistar rats, weighing 250 ± 20 g, were used in this study. Animals were obtained from Pasteur Institute (Tehran, Iran) and housed 4 rats per cage under a standard laboratory condition (12 h light/dark cycles starting at 8:00 h, room temperature $23 \pm 2^\circ\text{C}$). The diet and water were freely provided. All the experimental procedures were carried out in accordance with the guidelines of Ethical Committee of Tabriz University of Medical Sciences for Care and Use of Laboratory Animals (IR.TBZMED.VCR.REC.1400.024), and all efforts were made to decrease the number of animals used and minimize the suffering of rats during the study.

Following a week of adaptation to the new conditions, the animals were randomly divided into four groups ($n=12$) as follows: sham, PD, PD+levodopa, and PD+HCQ groups. Animals in the sham group underwent stereotaxic surgery, and the vehicle of 6-OHDA was injected into the SNpc, and then treated with normal saline (2 ml/kg, p.o.) for 21 days. Animals in the PD, PD+levodopa, and PD+HCQ were received unilateral intra SNpc injection of 6-OHDA. Three weeks following neurotoxin administration, the

model was confirmed by behavioral tests. Animals with rotation more than 7 times/min were considered as an established PD model [14]. Then, the PD animals were treated with normal saline (2 ml/kg, p.o.), levodopa (12 mg/kg, p.o., twice daily) [15], and HCQ (100 mg/kg, p.o.) [11], for 21 consecutive days, respectively.

PD model

Animals were anesthetized by injection of ketamine (80 mg/kg, i.p.) and xylazine (8 mg/kg, i.p.), and fixed in the stereotaxic frame symmetrically. 6-OHDA toxin (12.5 µg in 5 µl of a solution containing % 0.2 ascorbic acid) was injected into the left SNpc according to the corresponding area in Paxinos atlas coordination (bregma: AP=-5.5 mm, DV=-7.3 mm, ML=-2.6 mm) [16]. In the sham group, the same volume of normal saline containing ascorbic acid was injected. Five minutes after the solution infusion (1 µl/min), the needle was removed. Vehicle and toxin were administered slowly at a rate of 1 µl/min using a Hamilton syringe.

Behavioral tests

The behavioral tests, including the Murprogo's test and apomorphine-induced rotational behavior, were carried out 21 days after 6-OHDA injection, for confirmation of the model, and at the end of the treatments, by a blinded experimenter.

Rotational behavior

Apomorphine-induced rotation is a common test for assessment the extent of motor impairment induced by 6-OHDA. In this study, all animals were tested for rotational behavior 21 days after 6-OHDA injection (before treatment) and 21 days after lesion (after treatment). Animals were individually positioned in a circular cage, and after at least 10 min of habituation, they received a single injection of apomorphine hydrochloride (0.5 mg/kg, s.c.). Following one min of injection, full numbers of rotations were counted for 30 min at 10-min intervals. The number of contralateral turns to the lesion side was considered as positive scores for apomorphine, and the mean rotational numbers were calculated by the positive scores minus the negative scores [17].

Murprogo's test

To study muscle stiffness, the animals were individually placed on a flat surface. The animal received a score of zero if the animal's standing and walking were normal. If the animal remained immobile due to muscle stiffness or had difficulty in moving the arms and legs, it received a score of 0.5. Then the right hand of the animals was placed on a wooden platform with 3 cm height; if the animal held its hand on the platform for at least 10 seconds, it received a score of 0.5. This step was repeated for the opposite hand, and if the hand was held steady, they received another 0.5 points. Then, the animal's right hand was placed on a 9 cm platform, and they were given a score of 1 if they held their hand for 10 seconds. This step was repeated for the opposite hand, and if the hand was held steady after 10 seconds, they

received another 1 point. The PD animal received a score of 3.5, indicating full rigidity, and the healthy animal (the sham group) received a score of zero [18].

Tissue collection

At the end of the behavioral tests, all rats were anesthetized by a mixture of ketamine (100 mg/kg, i.p) and xylazine (10 mg/kg, i.p) and sacrificed by decapitation. The whole brain was extracted from the skull carefully. For biochemical assessments and Western blotting, brain samples were sliced using a tissue chopper, and the SN was dissected on a cold plate and stored in a -80 °C freezer. For histological examination, the brain was immersed in 4% buffered paraformaldehyde fixation solution for 48 h.

Assessment of oxidative stress status

Brain samples were homogenized in ice-cold 1.15% KCl solution and then centrifuged at 12000 rpm for 15 min at 4 °C to obtain supernatant. The Bradford method was used to estimate protein concentration in the supernatant.

Malondialdehyde (MDA) levels

Thiobarbituric acid reaction (TBAR) colorimetric assay was used to evaluate the concentration of MDA, as a lipid peroxidation marker. Briefly, 200 µl of the supernatant was mixed with 1 ml of thiobarbituric acid, trichloroacetic acid, and 0.9 ml of Tris-HCl (pH 7.4) and incubated for 20 min at 100 °C. Following centrifugation of the samples at 3,000 rpm for 10 min optical density of the supernatant was read at 540 nm in a plate reader, and the results were presented as nmol/mg protein [19].

Glutathione peroxidase (GPx)

Enzyme activity of GPx was assessed spectrophotometrically using the RANSEL kit (Randox Laboratories Ltd.) based on the method of Paglia and Valentine. The absorbance was read at 340 nm at 37°C, and the results were presented as nmol/mg protein.

Total antioxidant capacity (TAC) levels

To determine brain TAC levels, the 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) method was applied using a Randox total antioxidant status kit (Randox Laboratories Ltd, Crumlin, United Kingdom). A spectrophotometer was used to measure the absorbance at 600 nm, and the results were expressed as µmol/mg protein.

Western blotting

SN samples were homogenized (1:10, w:v) in 20 mM Tris-HCl (pH 7.4) buffer containing 1 mM NaF, 150 mM NaCl, 1% Triton-100, and protease inhibitor cocktail. The homogenate was centrifuged at 12,000 g for 10 min at 4 °C to obtain supernatant. Protein concentrations were determined in the supernatant using the Bradford method. An equal amount of protein (40 µg) was subjected to electrophoresis on 12.5%

SDS-polyacrylamide gel and then transferred onto a PVDF (Roche, UK) membrane. Membranes were blocked in 5% dried milk in 15 mM Tris–HCl (pH 7.4), 150 mM NaCl, and 0.05% Tween 20 (TBST) for 1 h. Subsequently, the membranes were incubated with the primary antibodies (Santa Cruz, CA, USA) against α -synuclein (sc-12767) and β -actin (sc-47778) as an internal loading control overnight at 4 °C. After washing three times with TBST, the membranes were incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG secondary antibody (1:5000, sc-2004) for 2 h. Following washing the membranes with PBS, the membranes were placed in enhanced chemiluminescence (ECL) detection solution (Amersham, UK) and exposed to X-ray film to visualize the signals. The density of protein bands was evaluated by Image J software [20].

Histological examination

The blocks of SN were embedded in paraffin, and slides were prepared by dehydration in ascending alcohol series and then cleared in xylene. Subsequently, coronal sections (40 μ m) were serially collected (-4.52 mm to -6.04 mm from bregma) from midbrains [21] using a microtome and stained with 0.1% cresyl violet. The representative images of the left hemisphere SNpc were captured, and the numbers of Nissl-stained neurons of SNpc were counted manually under light microscopy (magnification $\times$ 200) for three equivalent sections per brain by a blinded person to the study groups.

Statistical analysis

GraphPad Prism 6.01 (GraphPad Software Inc., La Jolla, CA, USA) was used to analyze the data. Values were presented as mean $\pm$ S.E.M. One-way ANOVA followed by Tukey *post-hoc* test was performed for comparison of the differences among the groups. The criterion for statistical significance was set at a *p*-value <0.05 .

Results

Behavioral test

1. Rotational behaviors with apomorphine

There was no significant difference in the number of rotations before the induction of the PD among groups. The mean number of rotations were significantly increased in the normal saline-received PD group compared to the sham animals ($p<0.001$). Besides, treatment with levodopa and HCQ markedly reduced the number of rotations compared to the PD animal treating with normal saline (Fig. 1B, $p<0.001$ for both). Interestingly, the average number of rotations in the PD+HCQ group was significantly lower than the PD+levodopa group ($p<0.05$).

2. Muscle stiffness

The result of this test indicated that PD induction significantly increased muscle rigidity in the 6-OHDA-lesioned groups compared to the sham group. However, rigidity was markedly decreased following

treatment with levodopa and HCQ compared to the PD animals (Fig. 2B, $p<0.001$ for both). There was no significant difference between the PD+levodopa and the PD+HCQ groups regarding the mean scores obtained from the Murprogo's test after treatment.

Brain MDA levels and antioxidant status

We found that 6-OHDA injection in the PD groups significantly increased brain MDA production compared to the sham group (Fig. 3A, $p<0.001$). However, administration of levodopa or HCQ had no significant effect on the brain MDA levels in the PD animals ($p>0.05$).

Moreover, PD animals demonstrated lower GPx enzyme ((Fig. 3B) activity and brain TAC ((Fig. 3C) than the sham group ($p<0.001$ for all comparisons). On the other hand, levodopa ($p<0.001$) and HCQ ($p<0.05$) significantly increased GPx activity in the PD animals. Nevertheless, levodopa or HCQ had no significant effects on the brain TAC levels compared to the PD group ($p>0.05$)

Levels of α -synuclein in the SNpc

Western blot analysis demonstrated a significant increase in levels of α -synuclein in the SNpc of the PD groups compared to the sham group (Fig. 4, $p<0.001$ for all comparisons). However, protein levels significantly decreased in the PD+levodopa group compared to the PD group ($p<0.001$). Also, animals in the PD+HCQ showed lower protein levels of α -synuclein compared to the PD group ($p<0.001$). On the other hand, the PD+HCQ group showed a significant reduction in protein levels of α -synuclein compared to the PD+levodopa group ($p<0.001$).

Number of neurons in the SNpc

The results of histological examination (Table 1 and Fig. 5) revealed no significant difference in the number of dopaminergic neurons on the right and left sides of SN in the sham group. However, the PD group had lower numbers of Nissl-stained neurons in the left side of SN than the sham group ($p<0.001$). Meanwhile, treatment with levodopa and HCQ markedly increased the number of neurons on the lesion side of the SN compared to the PD group ($p<0.001$ for both).

Table 1 Comparison of the mean number of dopaminergic neurons on the lesion side in the SN in the experimental groups.

Groups	Neuron Count
Sham	157.50±7.84
PD	67.66±5.04 ***
PD+levodopa	108.33±6.77 ###
PD+HCQ	121.00±8.69 ###

Data are expressed as mean±SEM. *** p <0.001 vs. Sham group; ### p <0.001 vs. PD animals. [PD, Parkinson's disease; HCQ, Hydroxychloroquine; SN, Substantia nigra pars compacta].

Discussion

The main findings of this study revealed that treatment of PD animals with HCQ decreased apomorphine-induced rotational behavior and muscle rigidity. These results were accompanied by the reduction in oxidative stress and α -synuclein protein levels and the increased number of Nissl-stained neurons in the SN.

According to the dopaminergic theory, reduction in the midbrain and striatal dopamine levels causes motor impairment, including resting tremor, muscle stiffness, bradykinesia, and postural instability [22, 23]. Pathologically the underlying mechanisms leading to the degeneration of dopaminergic neurons in PD are not fully understood. Though levodopa, a dopamine precursor, is the main treatment of PD, upon long-term administration (after 5 years), its efficacy wears off, and abnormal involuntary movements or dyskinesia are developed that limit its utility [24]. Hence, identifying new therapeutic interventions which can prevent or reverse the neuronal loss and dampen motor deficits are highly demanded.

Unilateral 6-OHDA injection into different parts of the nigrostriatal pathway is a widely utilized and reproducible method to develop the hemiparkinsonian model in animals [25]. This neurotoxin results in asymmetric motor behaviors and dopaminergic neural loss corresponding to the degree of the lesion [25, 26]. 6-OHDA enters dopaminergic neurons through dopamine transporters and accumulates in the cytosol, resulting in the degradation of dopaminergic neurons by disrupting the mitochondrial respiratory chain (I and IV complexes) and augmentation of oxidative stress [27, 28]. In line with previous reports [29–31], our results showed that unilateral 6-OHDA microinjection into the left SNpc increased rotational behavior contralateral to the lesion side and muscle stiffness. However, treatment with levodopa reduced the number of rotations induced by apomorphine and decreased rigidity in the Murprogo's test. Interestingly, the effect of HCQ on abolishing these motor deficits was superior to the effect of levodopa in the lesioned animals.

HCQ is a chloroquine derivative in which one of the *N*-ethyl groups is β -hydroxylated [13]. Evidence shows that HCQ has less toxicity than chloroquine [32]. Interestingly, amodiaquine and chloroquine significantly improved behavioral deficits in the 6-OHDA-lesioned rat PD model without any noticeable signs of

dyskinesia-like behavior [12], which is consistent with the present study. Moreover, HCQ has been shown to improve motor performance and coordination in the rotenone-induced PD model [11].

Accumulating preclinical and clinical evidence supported the causative role of oxidative stress and mitochondrial dysfunction in mediating the cascade of events that result in PD-associated dopaminergic neuron loss and motor deficits [33–36]. Cells usually protect themselves against free radical damage through a variety of mechanisms. Superoxide dismutase (SOD), catalase (CAT), GPx, and glutathione reductase are endogenous antioxidant enzymes that play an essential role in cellular protection [37]. In this regard, the effects of antioxidants in preventing or reducing damage caused by free radicals have been investigated in PD [38].

Alongside inducing ROS accumulation, 6-OHDA diminishes intracellular antioxidant enzymes, leading to abnormalities in the cell structure and metabolism, and hence neuronal deterioration [39]. In this study, MDA levels, a lipid peroxidation marker, were increased while TAC levels and GPx activity were decreased in the brain of 6-lesioned animals, suggesting 6-OHDA neurotoxicity. In line with our study, previous studies demonstrated that injection of 6-OHDA into the SN causes a significant increase in the production of free radicals and deteriorates the antioxidant defensive system in the midbrain and striatum [40–42]. These results suggest that the neurotoxicity of 6-OHDA occurs through the induction of severe oxidative stress in dopaminergic neurons [43].

Although levodopa and HCQ treatments for 21 days failed to increase brain TAC levels, these treatments increased brain GPx activity in the unilateral 6-OHDA injected animals. Few studies investigated the effect of HCQ treatment on oxidative stress. Farombi et al. reported that HCQ treatment in patients with malaria induces systemic oxidative stress [44]. Animal studies also showed that HCQ induces oxidative stress and does not improve the body's antioxidant status [45–47]. Another study reported that acute and chronic chloroquine administration increased NADPH-induced lipid peroxidation in the rat retina [47].

Pathologically PD is associated with progressive loss of dopaminergic neurons in the midbrain and aggregation of α -synuclein protein, resulting in motor impairments [48]. Previous studies supported the contributing role of excessive production of ROS and decreased capacity of the antioxidant defense system to scavenge toxic free radicals in the deterioration of dopaminergic neurons and the aggregation of α -synuclein in PD [34, 49, 50]. Zeng et al. demonstrated that unilateral 6-OHDA increased the mRNA expression of α -synuclein in the SN, and levodopa had no effect on its mRNA expression [51]. Li et al. also reported that 6-OHDA up-regulated α -synuclein protein levels in PC12 cells [52]. The aggregation of α -synuclein is linked to impaired lipid metabolism and hence mitochondrial dysfunction in dopaminergic neurons [53–55]. Likewise, we found that intra-striatal 6-OHDA injection up-regulated α -synuclein in the SN. Nevertheless, levodopa and HCQ administration markedly down-regulated α -synuclein protein expression. Interestingly, HCQ treatment was more effective than levodopa in decreasing α -synuclein protein levels. Autophagy disruption results in the accumulation of abnormal proteins like α -synuclein contributing to the deterioration of PD prognosis [56]. Notably, HCQ accumulates within lysosomes, resulting in lysosome neutralization and the inhibition of autophagic flux [57].

Moreover, dopaminergic neurons are more susceptible to oxidative damage due to their high baseline ROS levels and limited antioxidant activity, namely GPx [58]. In agreement with previous reports [59–62], our histological examination revealed decreased Nissl-stained neurons in the ipsilateral SNpc of the 6-OHDA-lesioned animals compared to the sham group, while we did not observe decreased Nissl-stained neurons in the contralateral SNpc. Conversely, levodopa and HCQ significantly increased the number of Nissl-stained neurons, suggesting that these treatments protected dopaminergic neurons from neurotoxicity of 6-OHDA in the SN. HCQ protects striatal new DA neurons in the rotenone-induced PD model by up-regulation of the orphan nuclear receptor (Nurr1) gene, a crucial factor involved in the preservation and differentiation of dopaminergic neurons and attenuation of striatal inflammatory mediators [11].

Conclusion

HCQ treatment ameliorated 6-OHDA induced motor impairment and neuronal loss in the SN possibly by enhancing GPx activity and down-regulation of α -synuclein protein levels. More studies are needed to clarify the exact mechanism of the neuroprotective effect of HCQ in PD models.

Declarations

Ethics Approval

Ethical consent was given by Ethics Committee at Tabriz University of Medical Sciences, Tabriz, Iran with Ethic code No: IR.TBZMED.VCR.REC.1400.024.

Declaration of conflict of interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The research protocol was approved and financially supported by a grant from Student Research Committee, Tabriz University of Medical Sciences (grant number: 67457) awarded to Seyed Zanyar Athari.

References

1. Savica R, Grossardt BR, Bower JH et al (2013) Incidence of Dementia With Lewy Bodies and Parkinson Disease Dementia. *JAMA Neurol* 70:1396. <https://doi.org/10.1001/jamaneurol.2013.3579>
2. Wright Willis A, Evanoff BA, Lian M et al (2010) Geographic and Ethnic Variation in Parkinson Disease: A Population-Based Study of US Medicare Beneficiaries. *Neuroepidemiology* 34:143–151. <https://doi.org/10.1159/000275491>

3. Brundin P, Ma J, Kordower JH (2016) How strong is the evidence that Parkinson's disease is a prion disorder? *Curr Opin Neurol* 29:459–466. <https://doi.org/10.1097/WCO.0000000000000349>
4. Balestrino R, Schapira AHV (2020) Parkinson disease. *Eur J Neurol* 27:27–42. <https://doi.org/10.1111/ene.14108>
5. Mazziro EA, Reams RR, Soliman KFA (2004) The role of oxidative stress, impaired glycolysis and mitochondrial respiratory redox failure in the cytotoxic effects of 6-hydroxydopamine in vitro. *Brain Res* 1004:29–44. <https://doi.org/10.1016/j.brainres.2003.12.034>
6. Colpo GD, Ribeiro FM, Rocha NP, Teixeira AL (2017) Animal Models for the Study of Human Neurodegenerative Diseases. In: *Animal Models for the Study of Human Disease*. Elsevier, pp 1109–1129
7. Duty S, Jenner P (2011) Animal models of Parkinson's disease: a source of novel treatments and clues to the cause of the disease. *Br J Pharmacol* 164:1357–1391. <https://doi.org/10.1111/j.1476-5381.2011.01426.x>
8. Kalia LV, Lang AE (2015) Parkinson's disease. *Lancet* 386:896–912. [https://doi.org/10.1016/S0140-6736\(14\)61393-3](https://doi.org/10.1016/S0140-6736(14)61393-3)
9. Adjene J, Adenowo T (2009) Histological studies of the effects of chronic administration of chloroquine on the inferior colliculus of the adult wistar rat. *J Med Biomed Res* 4:. <https://doi.org/10.4314/jmbr.v4i1.10673>
10. Silva J, Mariz H, Rocha L Jr et al (2013) Hydroxychloroquine decreases Th17-related cytokines in systemic lupus erythematosus and rheumatoid arthritis patients. *Clinics* 68:766–771. [https://doi.org/10.6061/clinics/2013\(06\)07](https://doi.org/10.6061/clinics/2013(06)07)
11. Hedya SA, Safar MM, Bahgat AK (2019) Hydroxychloroquine antiparkinsonian potential: Nurr1 modulation versus autophagy inhibition. *Behav Brain Res* 365:82–88. <https://doi.org/10.1016/j.bbr.2019.02.033>
12. Kim C-H, Han B-S, Moon J et al (2015) Nuclear receptor Nurr1 agonists enhance its dual functions and improve behavioral deficits in an animal model of Parkinson's disease. *Proc Natl Acad Sci* 112:8756–8761. <https://doi.org/10.1073/pnas.1509742112>
13. Sharma OP (1998) Effectiveness of Chloroquine and Hydroxychloroquine in Treating Selected Patients With Sarcoidosis With Neurological Involvement. *Arch Neurol* 55:1248–1254. <https://doi.org/10.1001/archneur.55.9.1248>
14. Ye M, Wang X-J, Zhang Y-H et al (2007) Therapeutic effects of differentiated bone marrow stromal cell transplantation on rat models of Parkinson's disease. *Parkinsonism Relat Disord* 13:44–49. <https://doi.org/10.1016/j.parkreldis.2006.07.013>
15. Carvalho MM, Campos FL, Marques M et al (2017) Effect of Levodopa on Reward and Impulsivity in a Rat Model of Parkinson's Disease. *Front Behav Neurosci* 11:145. <https://doi.org/10.3389/fnbeh.2017.00145>
16. Roghani M, Behzadi G, Baluchnejadmojarad T (2002) Efficacy of elevated body swing test in the early model of Parkinson's disease in rat. *Physiol Behav* 76:507–510.

[https://doi.org/10.1016/S0031-9384\(02\)00753-9](https://doi.org/10.1016/S0031-9384(02)00753-9)

17. Wei X, He S, Wang Z et al (2014) Fibroblast growth factor 1 attenuates 6-hydroxydopamine-induced neurotoxicity: an in vitro and in vivo investigation in experimental models of parkinson's disease. *Am J Transl Res* 6:664–677
18. MORPURGO C (1962) Effects of antiparkinson drugs on a phenothiazine-induced catatonic reaction. *Arch Int Pharmacodyn Ther* 137:84–90
19. Ozdemir E, Cetinkaya S, Ersan S et al (2009) Serum selenium and plasma malondialdehyde levels and antioxidant enzyme activities in patients with obsessive–compulsive disorder. *Prog Neuro-Psychopharmacology Biol Psychiatry* 33:62–65. <https://doi.org/10.1016/j.pnpbp.2008.10.004>
20. Jin F, Wu Q, Lu Y-F et al (2008) Neuroprotective effect of resveratrol on 6-OHDA-induced Parkinson's disease in rats. *Eur J Pharmacol* 600:78–82. <https://doi.org/10.1016/j.ejphar.2008.10.005>
21. Paxinos G, Watson C (2014) *The Rat Brain in Stereotaxic Coordinates Seventh Edition*. Elsevier Acad Press
22. Prasad EM, Hung S-Y (2020) Behavioral Tests in Neurotoxin-Induced Animal Models of Parkinson's Disease. *Antioxidants* 9:1007. <https://doi.org/10.3390/antiox9101007>
23. Amano S, Kegelmeyer D, Hong SL (2015) Rethinking energy in parkinsonian motor symptoms: a potential role for neural metabolic deficits. *Front Syst Neurosci* 8:. <https://doi.org/10.3389/fnsys.2014.00242>
24. Jenner P (2008) Molecular mechanisms of L-DOPA-induced dyskinesia. *Nat Rev Neurosci* 9:665–677. <https://doi.org/10.1038/nrn2471>
25. Deumens R, Blokland A, Prickaerts J (2002) Modeling Parkinson's Disease in Rats: An Evaluation of 6-OHDA Lesions of the Nigrostriatal Pathway. *Exp Neurol* 175:303–317. <https://doi.org/10.1006/exnr.2002.7891>
26. Jolicoeur FB, Rivest R, Drumheller A (1991) Hypokinesia, rigidity, and tremor induced by hypothalamic 6-OHDA lesions in the rat. *Brain Res Bull* 26:317–320. [https://doi.org/10.1016/0361-9230\(91\)90245-F](https://doi.org/10.1016/0361-9230(91)90245-F)
27. Glinka Y, Gassen M, Youdim MBH (1997) Mechanism of 6-hydroxydopamine neurotoxicity. In: *Journal of Neural Transmission, Supplement*, pp 55–66
28. Varešlija D, Tipton KF, Davey GP, McDonald AG (2020) 6-Hydroxydopamine: a far from simple neurotoxin. *J Neural Transm* 127:213–230. <https://doi.org/10.1007/s00702-019-02133-6>
29. Ma Y, Zhan M, OuYang L et al (2014) The effects of unilateral 6-OHDA lesion in medial forebrain bundle on the motor, cognitive dysfunctions and vulnerability of different striatal interneuron types in rats. *Behav Brain Res* 266:37–45. <https://doi.org/10.1016/j.bbr.2014.02.039>
30. Ziai SA, Niknami Z, Nasri S, Roghani M (2018) Protective Effects of Water Extract of Morus Nigra L. on 6-Hydroxydopamine Induced Parkinson's Disease in Male Rats. *Nov Biomed* 6:43–50. <https://doi.org/10.22037/nbm.v6i1.19910>

31. Carvalho MM, Campos FL, Coimbra B et al (2013) Behavioral characterization of the 6-hydroxydopamine model of Parkinson's disease and pharmacological rescuing of non-motor deficits. *Mol Neurodegener* 8:14. <https://doi.org/10.1186/1750-1326-8-14>
32. Song W, Muste JC, Greenlee TE, Singh RP (2020) Chloroquine and Hydroxychloroquine Toxicity. *Am J Ophthalmic Clin Trials* 2:8. https://doi.org/10.25259/AJOCT_2_2020
33. Filograna R, Beltramini M, Bubacco L, Bisaglia M (2016) Anti-Oxidants in Parkinson's Disease Therapy: A Critical Point of View. *Curr Neuropharmacol* 14:260–271. <https://doi.org/10.2174/1570159X13666151030102718>
34. Guo J, Zhao X, Li Y et al (2018) Damage to dopaminergic neurons by oxidative stress in Parkinson's disease. *Int J Mol Med* 41:1817–1825. <https://doi.org/10.3892/ijmm.2018.3406>
35. Dias V, Junn E, Mouradian MM (2013) The Role of Oxidative Stress in Parkinson's Disease. *J Parkinsons Dis* 3:461–491. <https://doi.org/10.3233/JPD-130230>
36. Jenner P (2003) Oxidative stress in Parkinson's disease. *Ann Neurol* 53:S26–S38. <https://doi.org/10.1002/ana.10483>
37. Butler R, Morris AD, Belch JJ et al (2000) Allopurinol normalizes endothelial dysfunction in type 2 diabetics with mild hypertension. *Hypertens (Dallas, Tex 1979)* 35:746–51. <https://doi.org/10.1161/01.hyp.35.3.746>
38. Park H-A, Ellis AC (2020) Dietary Antioxidants and Parkinson's Disease. *Antioxidants* 9:570. <https://doi.org/10.3390/antiox9070570>
39. Blum D, Torch S, Lambeng N et al (2001) Molecular pathways involved in the neurotoxicity of 6-OHDA, dopamine and MPTP: contribution to the apoptotic theory in Parkinson's disease. *Prog Neurobiol* 65:135–172. [https://doi.org/10.1016/S0301-0082\(01\)00003-X](https://doi.org/10.1016/S0301-0082(01)00003-X)
40. Massari CM, Castro AA, Dal-Cim T et al (2016) In vitro 6-hydroxydopamine-induced toxicity in striatal, cerebrocortical and hippocampal slices is attenuated by atorvastatin and MK-801. *Toxicol Vitro* 37:162–168. <https://doi.org/10.1016/j.tiv.2016.09.015>
41. Blandini F, Armentero M-T, Martignoni E (2008) The 6-hydroxydopamine model: News from the past. *Parkinsonism Relat Disord* 14:S124–S129. <https://doi.org/10.1016/j.parkreldis.2008.04.015>
42. Haddadi R, Nayebi AM, Farajniya S et al (2014) Silymarin improved 6-OHDA-induced motor impairment in hemi-parkinsonian rats: behavioral and molecular study. *DARU J Pharm Sci* 22:38. <https://doi.org/10.1186/2008-2231-22-38>
43. Inden M, Abe M, Minamino H, et al (2012) Effect of Selective Serotonin Reuptake Inhibitors via 5-HT < SUB > 1A</SUB > Receptors on L-DOPA-Induced Rotational Behavior in a Hemiparkinsonian Rat Model. *J Pharmacol Sci* 119:10–19. <https://doi.org/10.1254/jphs.12003FP>
44. Olatunde Farombi E, Shyntum YY, Emerole GO (2003) Influence of Chloroquine Treatment and Plasmodium falciparum Malaria Infection on Some Enzymatic and Non-enzymatic Antioxidant Defense Indices in Humans. *Drug Chem Toxicol* 26:59–71. <https://doi.org/10.1081/DCT-120017558>
45. Ogunbayo OA, Adisa RA, Ademowo OG, Olorunsogo OO (2005) Incidence of Chloroquine Induced Oxidative Stress in the Blood of Rabbit. *Int J Pharmacol* 2:121–125.

<https://doi.org/10.3923/ijp.2006.121.125>

46. GIOVANELLA F, FERREIRA GK, PRÁ SDT DE et al (2015) Effects of primaquine and chloroquine on oxidative stress parameters in rats. *An Acad Bras Cienc* 87:1487–1496.
<https://doi.org/10.1590/0001-3765201520140637>
47. Klouda CB, Stone WL (2020) Oxidative Stress, Proton Fluxes, and Chloroquine/Hydroxychloroquine Treatment for COVID-19. *Antioxidants* 9:894. <https://doi.org/10.3390/antiox9090894>
48. Stefanis L (2012) α -Synuclein in Parkinson's Disease. *Cold Spring Harb Perspect Med* 2:a009399–a009399. <https://doi.org/10.1101/cshperspect.a009399>
49. Masoud ST, Vecchio LM, Bergeron Y et al (2015) Increased expression of the dopamine transporter leads to loss of dopamine neurons, oxidative stress and L-DOPA reversible motor deficits. *Neurobiol Dis* 74:66–75. <https://doi.org/10.1016/j.nbd.2014.10.016>
50. Needham L-M, Weber J, Fyfe JWB et al (2018) Bifunctional fluorescent probes for detection of amyloid aggregates and reactive oxygen species. *R Soc Open Sci* 5:171399.
<https://doi.org/10.1098/rsos.171399>
51. Zeng B-Y, Dass B, Owen A et al (2002) 6-Hydroxydopamine lesioning differentially affects α -synuclein mRNA expression in the nucleus accumbens, striatum and substantia nigra of adult rats. *Neurosci Lett* 322:33–36. [https://doi.org/10.1016/S0304-3940\(02\)00083-6](https://doi.org/10.1016/S0304-3940(02)00083-6)
52. Li X, Jiang X, Chu H et al (2021) Neuroprotective effects of kukoamine A on 6-OHDA-induced Parkinson's model through apoptosis and iron accumulation inhibition. *Chinese Herb Med* 13:105–115. <https://doi.org/10.1016/j.chmed.2020.12.004>
53. Ruipérez V, Darios F, Davletov B (2010) Alpha-synuclein, lipids and Parkinson's disease. *Prog Lipid Res* 49:420–428. <https://doi.org/10.1016/j.plipres.2010.05.004>
54. Yakunin E, Moser A, Loeb V et al (2009) α -Synuclein abnormalities in mouse models of peroxisome biogenesis disorders. *J Neurosci Res* NA-NA. <https://doi.org/10.1002/jnr.22246>
55. Proft J, Faraji J, Robbins JC et al (2011) Identification of Bilateral Changes in TID1 Expression in the 6-OHDA Rat Model of Parkinson's Disease. *PLoS One* 6:e26045.
<https://doi.org/10.1371/journal.pone.0026045>
56. Lynch-Day MA, Mao K, Wang K et al (2012) The Role of Autophagy in Parkinson's Disease. *Cold Spring Harb Perspect Med* 2:a009357–a009357. <https://doi.org/10.1101/cshperspect.a009357>
57. Cook KL, Warri A, Soto-Pantoja DR et al (2014) Chloroquine Inhibits Autophagy to Potentiate Antiestrogen Responsiveness in ER + Breast Cancer. *Clin Cancer Res* 20:3222–3232.
<https://doi.org/10.1158/1078-0432.CCR-13-3227>
58. Soto-Otero R, Méndez-Álvarez E, Hermida-Ameijeiras Á et al (2000) Autoxidation and neurotoxicity of 6-hydroxydopamine in the presence of some antioxidants: Potential implication in relation to the pathogenesis of Parkinson's disease. *J Neurochem*. <https://doi.org/10.1046/j.1471-4159.2000.0741605.x>
59. Ichitani Y, Okamura H, Nakahara D et al (1994) Biochemical and Immunocytochemical Changes Induced by Intrastriatal 6-Hydroxydopamine Injection in the Rat Nigrostriatal Dopamine Neuron

60. Stott SRW, Barker RA (2014) Time course of dopamine neuron loss and glial response in the 6-OHDA striatal mouse model of Parkinson's disease. Eur J Neurosci 39:1042–1056.
<https://doi.org/10.1111/ejn.12459>
61. Callio J, Oury TD, Chu CT (2005) Manganese Superoxide Dismutase Protects against 6-Hydroxydopamine Injury in Mouse Brains. J Biol Chem 280:18536–18542.
<https://doi.org/10.1074/jbc.M413224200>
62. Pang Y, Lin S, Wright C et al (2016) Intranasal insulin protects against substantia nigra dopaminergic neuronal loss and alleviates motor deficits induced by 6-OHDA in rats. Neuroscience 318:157–165.
<https://doi.org/10.1016/j.neuroscience.2016.01.020>

Figures

Figure 1

The number of apomorphine-induced rotations A) after induction of PD model and B) after treatment with levodopa or HCQ in the experimental groups. Data are expressed as mean±SEM (n=12). *** p<0.001 vs. Sham group. ###p<0.001 vs. PD animals. @p<0.05 vs. PD+Levodopa group. [PD, Parkinson's disease; HCQ, Hydroxychloroquine].

Figure 2

Evaluation of muscle stiffness by the Murprogo's test A) after PD induction and B) following treatment with levodopa or HCQ in the experimental groups. Data are expressed as mean $\pm$ SEM (n=12). *p<0.05, *** p<0.001 vs. Sham group; ###p<0.001 vs. PD animals. [PD, Parkinson's disease; HCQ, Hydroxychloroquine].

Figure 3

Effect of HCQ on brain A) MDA levels, B) GPx activity, and C) TAC levels in the experimental groups. Data are expressed as mean±SEM (n=6). ** p<0.01, *** p<0.001 vs. Sham group; #p<0.05, ###p<0.001 vs. PD animals. [PD, Parkinson’s disease; HCQ, Hydroxychloroquine; MDA, Malondialdehyde; GPx, Glutathione peroxidase; TAC, Total antioxidant capacity].

Figure 4

A) Quantitative densitometric analysis of the α -synuclein in the experimental groups. B) Immunoblotting images of the expression of α -synuclein and β -Actin in the SNpc of the experimental groups. Data are expressed as mean $\pm$ SEM (n=4). ***p<0.001 vs. Sham group; ###p<0.001 vs. PD animals. @@@p<0.001 vs. PD+Levodopa group. [PD, Parkinson's disease; HCQ, Hydroxychloroquine; SNpc: Substantia nigra pars compacta].

Figure 5

Microscopic view of the SNpc area in the I: Sham, II: PD, III: PD+Levodopa, and IV: PD+HCQ groups. In Crystal violet staining, blue parts represent the Nissl-stained active neurons. [PD, Parkinson's disease; HCQ, Hydroxychloroquine; SNpc, substantia nigra pars compacta]