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Research report

Hydroxychloroquine antiparkinsonian potential: Nurr1 modulation versus autophagy inhibition

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ABSTRACT

The nuclear orphan receptor (Nurr1) has recently received a perceivable solicitude as a target for the therapeutic intervention against PD. Meanwhile, the dysregulation of autophagy, along with other processes is believed to contribute massively to PD pathophysiology. Hydroxychloroquine, a hydroxy derivative of chloroquine, is an antimalarial agent which is also used as an anti-rheumatic drug. The neuroprotective potential of hydroxychloroquine and chloroquine remained controversial until recently a study showed that chloroquine exhibited an antiparkinsonian activity through Nurr1 modulation. The aim of this work is to identify whether the less toxic derivative, hydroxychloroquine, could show a similar pattern. In rat rotenone model, hydroxychloroquine effectively boosted Nurr-1 expression, exhibited an anti-inflammatory effect as verified by hindering certain pro-inflammatory cytokines and successfully reduced GSK-3β activity. Consequently, an increase in the striatal tyrosine hydroxylase content, as well as improved locomotion and muscle coordination was shown. However, this improvement was opposed by hydroxychloroquine induced autophagic inhibition as manifested by enhancing both LC3-II and P62 levels possibly through the prominent decline in sirtuin 1 level and elevated apoptotic biomarkers. In conclusion, hydroxychloroquine successfully ameliorated PD motor dysfunction in spite of the fact that both autophagy and apoptosis were deregulated through Nurr1 modulation.

1. Introduction

Parkinson's disease (PD) is a chronic progressive, yet incurable neurological disorder affecting mainly the elder population [1]. The most prominent hallmark of the disorder is dopaminergic neurons degeneration in the substantia nigra pars compacta (sNpc) and subsequent exhaustion of the dopamine content (DA) in the striatum [2]. This depletion in DA results in the cardinal motor manifestations afflicting PD patient including tremors, rigidity and bradykinesia among others [3]. The pathogenesis of the disorder is intricate as several interconnected factors influence the disease including; apoptosis, abnormal protein aggregates, inflammation and mitochondrial dysfunction [4]. Thus, finding a suitable agent that amends these complex interrelated factors remains a challenge.

The orphan nuclear receptor (Nurr1) up-regulation is proved to preserve the dopaminergic neurons functionality and integrity in the sNpc thus, it can be considered as a pivotal target for the intervention against PD [5]. This receptor was shown to trigger tyrosine hydroxylase (TH) transcription along with other genes crucial for dopamine synthesis and normal development of dopaminergic neurons [6]. Moreover, pro-inflammatory cytokines release was mitigated by Nurr1 expression in astrocytes and microglia, an effect that could protect the dopaminergic neurons against inflammation induced neuronal death [7]. Noteworthy [8], showed that treatment with rotenone, a known pesticide used to induce symptoms resembling PD, was associated with a heightened inflammatory response and release of inflammatory cytokines such as interleukin 1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) mediated by nuclear factor-kappa B (NF-κB), an effect that was associated with Nurr1 down-regulation. Thus, drugs activating Nurr1 over- expression have received a perceivable interest as potential candidates providing protection against PD [9].

Lately, activated glycogen synthase kinase-3 beta (GSK-3β) has been claimed to participate significantly in pathogenic mechanisms of neurodegenerative diseases including, PD and Alzheimer's disease [10]. It was shown that GSK-3β regulates various components of the complex network involved in PD such as, inflammation and abnormal protein aggregates formation [11,12]. Hence, GSK-3β inhibition can exhibit a beneficial outcome in PD treatment.

Normal turnover of proteins and cells within an organism is controlled by various processes including, apoptosis and autophagy

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[13,14]. PD is characterized by the accumulation of abnormal protein aggregates preceding the neurological damage [15]. One of the natural physiological procedures responsible for removing those aggregates is autophagy [16]. Accordingly, autophagy dysregulation is believed to contribute massively to PD pathogenesis [17]. The neurotoxic pesticide rotenone was found to result in autophagic flux inhibition through lysosomal dysfunction, an effect that could be reversed by autophagic activators providing a protective effect against rotenone cytotoxicity [18,19]. On the other hand, dopaminergic cell death in PD is proved to be a direct result of an enhanced apoptosis [20]. Previous reports showed that dopaminergic neurons may survive and their function and morphology could be preserved after using apoptosis inhibitors [21]. Therefore, it is of an utmost importance to keep normal balanced cell death mechanisms to maintain the normal homeostasis without causing irreparable damage.

Hydroxychloroquine (HCQ), a chloroquine hydroxy-derivative, is an anti-malarial and anti-rheumatic drug [22]. HCQ neuroprotective potential is still questionable. A previous study failed to prove HCQ efficacy against Alzheimer's disease [23]. On the other hand, a recent study suggested that some anti-malarial drugs including chloroquine succeeded in ameliorating PD motor disturbances in 6-OHDA-treated rats [24]. Moreover, HCQ managed to alleviate neurological sarcoidosis manifestations [25], an effect that could support the claims around its neuroprotective potential.

Hence, this study aims to unveil the discrepancy regarding the possible Nurr1 mediated neuroprotective effects of hydroxychloroquine versus autophagy and apoptosis dysregulation, in a rat model of PD.

2. Materials and methods

2.1. Animals

All the investigational procedures abided by the published US National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 2011) and was carried out conforming to ethical procedures and policies stipulated by the Ethics Committee of Faculty of Pharmacy, Cairo University PT (1600). All procedures adhere to the ARRIVE Guidelines for reporting animal research. A completed ARRIVE guidelines checklist is included at the end of the manuscript. All efforts were made to minimize the suffering of rats during the experimental period.

Adult male Wistar albino rats weighing (200–250) g were housed under standard environmental conditions; constant humidity (60 ± 10%), temperature (25 ± 2 °C), and a12/12-h light/dark cycle. Standard chow diet and water were allowed ad libitum. Behavioral testing was conducted in a Proper and secluded room. All animals used were purchased from Faculty of pharmacy Cairo University animal facility, Egypt.

2.2. Chemicals and drugs

All chemicals used during the study were of the highest purity and analytical grade. HCQ was kindly provided by The National Organization for Drug Control and Research "NODCAR" (Cairo, Egypt). Dimethyl sulfoxide (DMSO) and rotenone were purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich (St. Louis, MO, USA), respectively.

2.3. Experimental design

Seventy two rats were scattered randomly using randomization table amongst three groups, 24 rats each (4 rats per cage). Group I received a total of 11 subcutaneous injections of 10% DMSO every other day (0.2 ml/kg) and served as control group for rotenone. Groups II and III received rotenone every other day at a dose of 1.5 mg/kg dissolved in 10% DMSO, a total of 11 subcutaneous injections for twenty one days [26,27]. In the meantime, for 3 weeks, HCQ was added to normal saline and orally administered daily and on the days of rotenone administration, HCQ was given one hour before rotenone injection to rats of group III at a dose of 100 mg/kg [28].

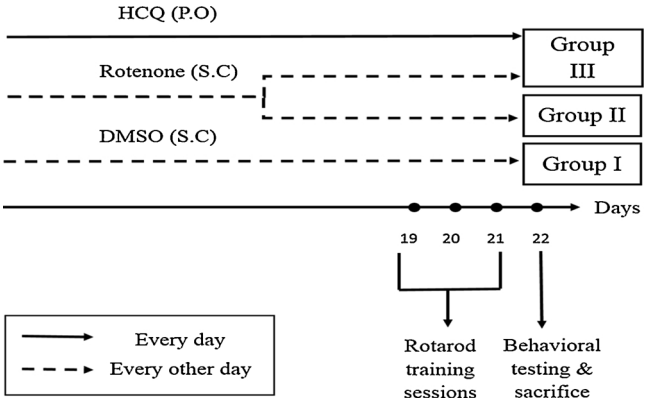

Fig. 1. Time line diagram illustrating the experimental procedures.

Open field and rotarod were used to test animals' motor functions. They were performed 24 h after the last injection of rotenone (Fig. 1). Animals in each group were further divided into 3 sets. Finally, animals were sacrificed by decapitation whilst under anesthesia to eliminate the pain. Brains were excised rapidly, rinsed with ice-cold saline. Each brain was dissected on an ice-cold glass plate to separate both striata in the first three sets and substantia nigra in the third set. Afterwards, in the first set both striata were homogenized and used for the assessment of tyrosine hydroxylase (TH), nuclear factor NF-κB, IL-1β and TNF-α levels. In the second set, both striata were homogenized for the evaluation of, cytochrome C, caspase-3 and GSK3-β (pS9). The third set was conducted to estimate autophagy biomarkers LC3-II, p62 and SIRT-1 in the striatum and Nurr1 expression in the SN.

2.4. Behavioral assessment

2.4.1. Open field

The movement and behavior of each animal namely ambulation was recorded using a fixed video camera installed on a square wooden box measuring 80 × 80 × 40 cm with red walls and white smooth polished floor divided by black lines into 16 equal squares 4 × 4 for later off-line analysis. Each rat was allowed to freely and individually explore the area for 3 min after gently placing them at the central zone of the wooden box. After testing each rat the apparatus was thoroughly wiped to eliminate any odors left by preceding rats abolishing probable bias [29].

2.4.2. Rotarod

A rotarod apparatus was used to test both balance and motor coordination (3 cm in diameter and rotating at a constant speed of 20 rpm). Three training sessions were given to animals of 5 min each on three successive days before the sacrifice day to be acclimatized to sustain their posture on the rotarod. On the sacrifice day, after conducting the open field test, the falling time was recorded after allowing them to move over the rotarod using a cutoff limit of 300 s [30].

2.5. Biochemical measurements

2.5.1. Striatal TH, inflammatory mediators, and apoptotic biomarkers

TH (Cloud-clone, Wuhan, PRC), NF-κB and cytochrome C (EIab ELISA kit, Wuhan, PRC), TNF-α, GSK-3β (pS9) and IL-1β (RayBio ELISA kit, Ray Biotech, Norcross, GA), caspase-3 (Cusabio ELISA kit, PRC) were quantified in the striatal tissue using the commercially available ELISA kits.

2.5.2. Autophagy biomarkers and SIRT-1

Western blot assay was used to estimate the expression of striatal LC3-II, p62 proteins as well as SIRT-1. Initially, striatal tissues were subjected to an extraction procedure of the protein solutions then; the proteins were separated according to their molecular weight via loading equal amount of them onto a sodium dodecylsulfate polyacrylamide gel electrophoresis. Thereafter, a nitrocellulose membrane (Amersham Bioscience, Piscataway, NJ, USA) received the isolated proteins via the aid of a semidry transfer apparatus (Bio-Rad, Hercules, CA, USA). Then, non-specific binding sites were blocked by putting the membranes in a 5% skim milk.

Membranes were then incubated overnight on a roller shaker at 4 °C with the antiLC3-II, antip62 or antiSIRT-1 primary antibody solution (1:1000; thermofisher scientific, MA, USA). Afterward they were washed and incubated with the horseradish peroxidase-conjugated secondary antibody (1:2000; Fluka, St. Louis, MO, USA). Finally, the blots were developed with enhanced chemiluminescence detection reagents (Amersham Biosciences, Arlington Heights, IL, USA). By the aid of a scanning laser densitometer (GS-800system, Bio-Rad, Hercules, CA, USA), the amount of LC3-II, p62 and SIRT-1 proteins was quantified by densitometric analysis. Results were expressed as arbitrary units after normalization with ß-actin protein expression.

2.5.3. Determination of Nurr1 expression

Real time-PCR (RT-PCR) was carried out to assess Nurr1 gene expression. Initially, SN tissue was used to extract the total RNA By means of SV Total RNA Isolation system (Promega, Madison, WI, USA) and the obtained RNA purity was established spectrophotometrically at OD 260/280 nm. Thereafter, the extracted RNA was used to obtain the complementary DNA through reverse transcription using RT-PCR kit (Strata gene, La Jolla, CA, USA). Eventually, SYBR Green Jump Start Taq Ready Mix (Sigma-Aldrich, St. Louis, MO, USA) was used to perform Quantitative RT-PCR. The total reaction volume was 25 µl where, 5 µl of complementary DNA was added to 12.5 µl SYBR Green mixture, 5.5 µl RNase free water, and 2 µl of each primer (5 pmol/µl). A list of used primers is provided in Table 1. The PCR amplifications were performed with 40 cycles of denaturation for 15 s at 95 °C, annealing 60 s at 60 °C, and extension 60 s at 72 °C. After the quantitative RT-PCR run, the relative expression of target gene was obtained using the $2^{-\Delta\Delta CT}$ formula using ß-actin as a house keeping gene [31].

2.6. Statistical analysis

Prior to ANOVA analyses, all data were tested for normality as well as homogeneity of variance using Kolmogorov-Smirnov and Bartlett's tests, respectively. One-way ANOVA followed by Tukey-Kramer multiple comparisons were used to analyze data sets that met the assumptions for parametric analysis and were expressed as mean ± S.E.M. On the other hand, behavioral experiments data was analyzed using Kruskal-Wallis nonparametric one-way ANOVA followed by Dunn's multiple comparisons test and were expressed as median and range as their data failed to attain the assumption for normality and homogeneity of variance. A probability level of less than 0.05 ($p < 0.05$) was accepted as statistically significant. Statistical analysis was performed using GraphPad Prism software version 6 (San Diego, CA, USA).

Table 1
The sequence of primers used for real-time PCR analysis.

Gene	Primer sequence
Nurr1	F: 5'-CGCGTCGCAGTTGCTTGACAC-3' R: 5'-TTGCCTGGAACCTGGAATAGT-3'
ß-actin	F: 5'-CGTTGACATCCGTAAAGACCTC-3' R: 5'-TAGGAGCCAGGGCAGTAATCT-3'

Table 2
Effects of HCQ on rotenone-induced alterations in rats' behavior in the open-field and rotarod tests.

Parameters/group	Control	Rotenone	HCQ
Open field test			
Ambulation (No.)	49.5(19-80)	8.5 (2-16) [a]	22 (4-28) [ab]
Rotarod test			
Latency till fall (s)	265 (200-295)	65 (10-105) [a]	125 (60-197) [ab]

HCQ amended behavioral changes induced by rotenone. HCQ (100 mg/kg,orally) was administered daily for 3 weeks, meanwhile rats received 11 subcutaneous injections of rotenone (1.5 mg/kg) every other day. Values are expressed as median and range20-24 animals per group; a *vs* control, b *vs* rotenone Kruskal-Wallis non parametric One-Way ANOVA followed by Dunn's multiple comparisons test; $p < 0.05$).

3. Results

3.1. HCQ mitigated rotenone-inflicted behavioral changes in open field and rotarod testing

Motor performance and coordination of rats subjected to rotenone administration incurred marked deterioration as compared with the control group (Table 2). Pretreatment with HCQ managed to reverse the deterioration in ambulation 2.5 fold and falling time 1.9 fold as compared with the rotenone group. Thus, HCQ might ameliorate the dysfunctional motor co-ordination and functions in parkinsonian animals.

3.2. HCQ attenuated rotenone-induced alterations in striatal inflammatory mediators in rats

Rotenone produced a marked increase in striatal NF-κB by about 5.6 times its normal value. This effect was attenuated by HCQ which succeeded in hampering NF-κB by 39%. In addition both TNF-αand IL-1β were increased after repetitive rotenone injections versus the control by 3.24 and 3.42 fold, respectively. HCQ suppressed these inflammatory cytokines by 48.43% for TNF-α, and 22.85% for IL-1β, as compared to the parkinsonian rats (Table 3). Therefore, HCQ managed to reduce the inflammatory response witnessed in the rotenone group.

3.3. HCQ attenuated rotenone-induced alterations in GSK-3β, Nurr1 expression and TH content

Repeated subcutaneous injection of rotenone caused a marked decline in striatal GSK-3β (pS9) (48%) along with SN Nurr1 content (78.73%) as compared to the control group (Fig. 2A and B). Pretreatment with HCQ produced a 5 fold increase in GSK-3β (pS9) content together with a 1.85 elevation of Nurr1 when compared with the rotenone group. In parallel with these findings, the diminished TH level after rotenone administration (62%) was partially reversed by HCQ showing a 1.4 fold boost in striatal TH content when compared with the rotenone group (Fig. 2C). Consequently, the reduced Nurr1 expression

Table 3
Effects of HCQ on rotenone-induced alterations in striatal downstream inflammatory mediators.

Parameters/group	Control	Rotenone	HCQ
NF-κβ (ng/g tissue)	0.85 ± 0.072	4.75 ± 0.14[a]	2.898 ± 0.13[ab]
TNF-α (pg/g tissue)	13.03 ± 1.16	42.22 ± 1.31[a]	21.77 ± 0.49[ab]
IL-1β (pg/g tissue)	11.87 ± 0.9	40.7 ± 2.37[a]	31.4 ± 1.08[ab]

HCQ attenuated rotenone induced inflammatory changes. HCQ (100 mg/kg,orally) was administered daily for 3 weeks, meanwhile rats received 11 subcutaneous injections of rotenone (1.5 mg/kg) every other day. Values are expressed as mean ± S.E.M of 6–8 animals; a *vs* control, b *vs* rotenone (One-Way ANOVA followed by Tukey-Kramer multiple comparisons test; $p < 0.05$).

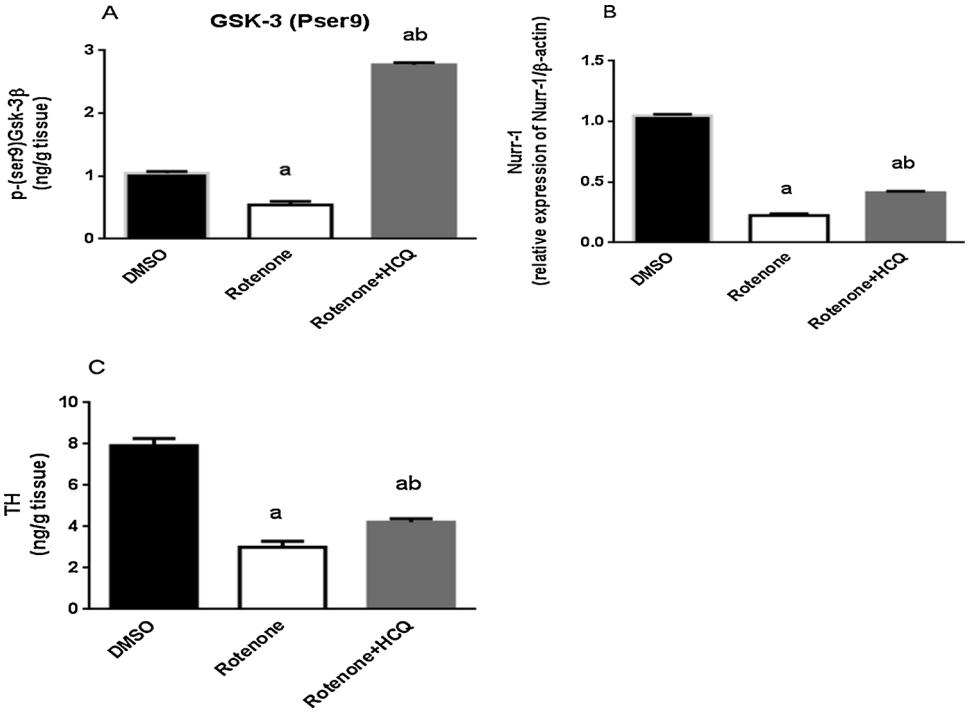

Fig. 2. Effects of HCQ on rotenone-induced alterations in GSK-3β (pS9) (A), Nurr1(B) and TH (C) content in rats.
HCQ administration increased GSK-3β (pS9) level, Nurr-1 expression and TH content. HCQ (100 mg/kg, orally) was administered daily for 3 weeks, meanwhile rats received 11 subcutaneous injections of rotenone (1.5 mg/kg) every other day. Each bar with vertical line represents the mean ± S.E.M of 6–8 rats per group. a *vs* control, b *vs* rotenone (One-Way ANOVA followed by Tukey-Kramer multiple comparisons test; $p < 0.05$).

as well as TH and phosphorylated GSK-3β content in rotenone treated rats were elevated significantly by HCQ.

3.4. HCQ failed to attenuate rotenone-induced alterations in autophagy and SIRT-1

Rotenone caused a marked elevation in both LC3-II and p62 levels by 3.56 and 6 fold, respectively, as compared to the control group. While HCQ administration dramatically escalated LC3-II and P62 levels by 37.3% and 73.27%, respectively, when compared to parkinsonian rats (Fig. 3A and B). Moreover, rotenone administration reduced SIRT-1 by 61% as compared to the control group. HCQ didn't show a significant difference from those results observed in the rotenone group (Fig. 3C). Thence, HCQ couldn't counteract the effect of rotenone upon autophagic flux and autophagy related protein.

3.5. HCQ failed to attenuate rotenone-induced alterations in striatal apoptotic biomarkers

Rotenone managed to spike up apoptotic biomarkers, where striatal caspase-3 was elevated by nearly 6 fold and cytochrome C was increased by 4.23 fold above its normal values. HCQ didn't show a

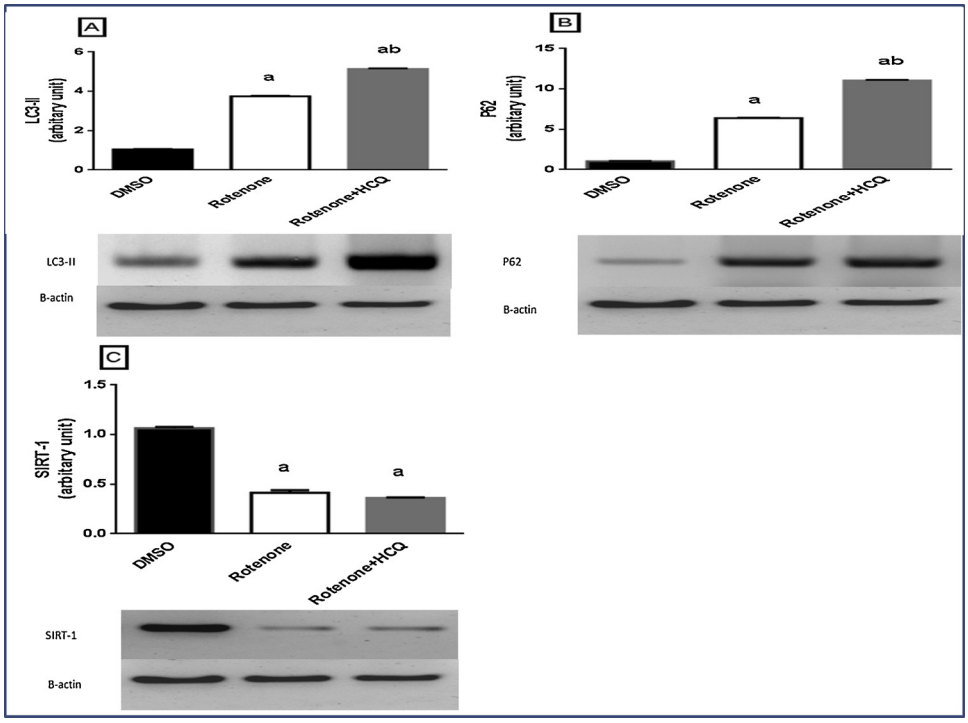

Fig. 3. Effects of HCQ on rotenone-induced alterations in striatal LC3-II (A), P62 (B) and SIRT-1 (C) in rats.
HCQ precipitated significant autophagic inhibition. HCQ (100 mg/kg, orally) was administered daily for 3 weeks, meanwhile rats received 11 subcutaneous injections of rotenone (1.5 mg/kg) every other day. Each bar with vertical line represents the mean ± S.E.M of 6–8 rats per group. a *vs* control, b *vs* rotenone (One-Way ANOVA followed by Tukey-Kramer multiple comparisons test; $p < 0.05$).

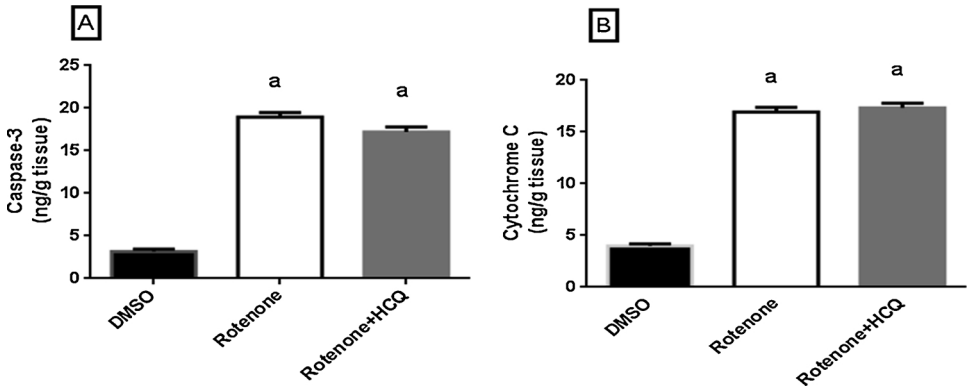

Fig. 4. Effects of HCQ on rotenone-induced alterations in striatal caspase-3(A) and cytochrome c (B) in rats.
HCQ failed to reverse rotenone induced apoptotic cell death. HCQ (100 mg/kg, orally) was administered daily for 3 weeks, meanwhile rats received 11 subcutaneous injections of rotenone (1.5 mg/kg) every other day. Each bar with vertical line represents the mean ± S.E.M of 6–8 rats per group. a *vs* control, b *vs* rotenone (One-Way ANOVA followed by Tukey-Kramer multiple comparisons test; $p < 0.05$).

significant difference from those results observed in the rotenone group (Fig. 4A and B). Subsequently, HCQ is unable to prevent the apoptotic damage afflicting the rotenone induced parkinsonian rats.

4. Discussion

This study attempted to settle the ambiguity regarding the neuroprotective potential of HCQ. This uncertainty emerged from two opposing factors, the potential ability of HCQ to modulate Nurr1 expression which stood up recently as a promising target for PD treatment versus the inhibitory action exerted by HCQ on autophagy and enhanced apoptosis which may result in neuronal cell death.

In the present study, HCQ resulted in elevated Nurr1 expression and reduced GSK-3β activity as manifested by increased phospho-Ser9 of GSK-3β which could explicate the improved Nurr1 expression and subsequent improvement in TH level and motor functions evident herein after HCQ injection. Nurr1 was found to be expressed in the dopaminergic neurons of the SN to control variety of functions including dopamine synthesis and reuptake [32]. Moreover, Nurr1 is proved to be a crucial requirement for dopaminergic neurons differentiation and maintenance through regulating TH expression together with other genes [6]. Therefore, integrity of dopaminergic neurons and normal motor functions can be restored with enhanced Nurr1 expression [24].

Remarkably, it has been proposed that a tight association between GSK-3β inhibition and Nurr1 expression is present, where GSK-3β inhibition is associated with increased Nurr1 expression through enhancing β-catenin binding on the upstream promoter region of Nurr1 [33]. Moreover, rotenone toxicity was successfully reversed after β-catenin, which is modulated directly by GSK-3β, interacted with AF1 region of Nurr1 [34]. Several reports revealed that GSK-3β activity was enhanced after rotenone injection [35–37]. Additionally, it was previously reported that the parkinsonian rats showed reduced Nurr1 expression [8]. Notably, it has been shown previously that agents which increase protein kinase C (PKC) activity could suppress GSK-3β by phosphorylating this kinase (Ser9) [38]. HCQ is proved to activate PKC [39]. Consequently, HCQ mediated PKC activation could explain the raise in the inhibited form P-ser9-GSK-3β, which may provide neuronal protection from GSK-3β induced neuronal damage.

The rotenone group showed a boost in the master regulator of inflammation NF-κB with its downstream inflammatory biomarkers including striatal TNF-α and IL-1β. These findings were found to be in line with the results showed by previous studies [8,24]. Whereas HCQ showed an anti-inflammatory action evidenced by the marked decrease in striatal inflammatory cytokines TNF-α, IL-1β and NF-κB. Given that, a heightened inflammatory state and increased pro-inflammatory cytokines secretion could be attributed to GSK-3β activation through NF-κB regulation [11]. Furthermore, Nurr1 expression in microglia and astrocytes could repress pro-inflammatory genes [7]. Therefore, HCQ mediated GSK-3β inhibition as well as increased Nurr1 expression may explain the anti-inflammatory effect exerted by HCQ.

On the other hand, the present study showed that HCQ resulted in inhibition of autophagic flux as depicted by increase in both LC3-II and p62, those results hold up with a previous report [40]. Moreover, HCQ failed to reverse apoptosis enhancement exerted by rotenone which was manifested by enhanced cytochrome c release and caspase-3 activation. This disruption seen in both processes in HCQ treated group may be attributed to its lysomotropic behavior. HCQ has the ability to penetrate through the lysosomes and alter its PH. Thereby, autophagosome will not merge with the lysosomes and the autophagic flux will be inhibited [40]. Concerning apoptosis, it was shown that, the enhanced permeability of the lysosomal membrane exerted by HCQ is associated with increased lysosomal protease release, an effect which will result in mitochondrial cytochrome c release and subsequent activation of the apoptotic cascade [41]. Furthermore [42] showed that, HCQ induced lysosomal membrane enhanced permeability was followed by caspase activation advocating enhanced apoptosis. Noteworthy, apoptosis activation might be associated with autophagic inhibition. Caspase activation during the apoptotic cell death was found to block out the autophagic procedure, since numerous vital proteins involved in the autophagic pathway activation are believed to be digested by the caspases [43].

Apoptosis and autophagy are normal physiological processes that maintain normal cellular functions and homeostasis [44], yet their dysregulation may promote the neuronal cell death observed in PD [45]. In this context, it was reported that rotenone-induced apoptosis was successfully mitigated using autophagy activator, while this effect was plugged partially when autophagy related gene 5 was suppressed [46]. On the other hand, apoptosis is believed to be one of the main cell death mechanisms precipitating neurodegeneration in PD [47] and that rotenone induced cytotoxicity to SK-N-SH neuroblastoma cells was reversed through apoptosis suppression [48].

Interestingly, HCQ failed to reverse SIRT-1 reduction after rotenone administration which could also clarify both enhanced apoptosis and inhibited autophagy. SIRT-1 is proved to be critical in autophagy enhancement via autophagy related proteins deacetylation including FoxO, Atg5, Atg8, [49,50]. Moreover, it was demonstrated previously that, using an autophagy enhancer in an in-vitro model with elevated β-amyloid peptide resulted in enhanced cell viability, an effect which was blocked after using SIRT-1 gene silenced cells [51]. Furthermore, it was formerly reported that, SIRT-1 activation resulted in autophagic enhancement and subsequent apoptotic inhibition supporting the firm link between them [52]. Thus, hampering SIRT-1 may consolidate autophagic and apoptotic deregulation and subsequent cell death.

In conclusion, this study reveals beneficial potentials of HCQ in a parkinsonian model through Nurr1 modulation, anti-inflammatory effect and GSK-3β inhibition which may defy certain deleterious effects precipitating neurological damage, *viz.* autophagy inhibition and apoptotic enhancement.

Conflict of interest disclosure

The authors declare that there are no conflicts of interest that could prejudice the impartiality of this scientific work.

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