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MitoQ alleviates hippocampal damage after cerebral ischemia: The potential role of SIRT6 in regulating mitochondrial dysfunction and neuroinflammation

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ABSTRACT

Aims: Mitochondrial perturbations are the major culprit of the inflammatory response during the initial phase of cerebral ischemia. The present study explored the neuroprotective effect of the mitochondrial-targeted antioxidant, Mitoquinol (MitoQ), against hippocampal neuronal loss in an experimental model of brain ischemia/reperfusion (I/R) injury.

Main methods: Rats were subjected to common carotid artery occlusion for 45 min, followed by reperfusion for 24 h. MitoQ (2 mg/kg; i.p daily) was administered for 7 successive days prior to the induction of brain ischemia.

Key findings: I/R rats exhibited hippocampal damage evidenced by aggravated mitochondrial oxidative stress, thereby enhancing mtROS and oxidized mtDNA, together with inhibiting mtGSH. Mitochondrial biogenesis and function were also affected, as reflected by the reduction of PGC-1α, TFAM, and NRF-1 levels, as well as loss of mitochondrial membrane potential (△Ψm (. These changes were associated with neuroinflammation, apoptosis, impairment of cognitive function as well as hippocampal neurodegenerative changes in histopathological examination. Notably, SIRT6 was suppressed. Pretreatment with MitoQ markedly potentiated SIRT6, modulated mitochondrial oxidative status and restored mitochondrial biogenesis and function. In addition, MitoQ alleviated the inflammatory mediators, TNF-α, IL-18, and IL-1β and dampened GFAB immunoexpression along with downregulation of cleaved caspase-3 expression. Reversal of hippocampal function by MitoQ was accompanied by improved cognitive function and hippocampal morphological aberrations.

Significance: This study suggests that MitoQ preserved rats' hippocampi from I/R insults via maintenance of mitochondrial redox status, biogenesis, and activity along with mitigation of neuroinflammation and apoptosis, thereby regulating SIRT6.

1. Introduction

Cerebral ischemia is one of the most destructive diseases associated with increased morbidity, disability, and mortality worldwide [1]. Various pathophysiological processes are involved in ischemic brain injury including oxidative stress, neuroinflammation, calcium homeostasis, excitotoxicity, and mitochondrial failure [2,3]. Although rapid restoration of cerebral blood flow is the first therapeutic approach to overcome ischemic stroke, ischemia/ reperfusion (I/R) may worsen the cerebral damage, causing I/R injury [1].

Increasing evidence suggests that mitochondrial mutilation is among the most important players that reinforce neuronal cell damage following cerebral ischemia [4]. Mitochondrial impairment induced by oxygen and glucose deprivation during brain ischemia leads to ATP depletion and reactive oxygen species (ROS) overproduction, resulting in oxidative stress. Indeed, neurons are primarily dependent on

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mitochondria for calcium homeostasis and energy production, rendering mitochondrial defects serious conditions on neuronal function in the setting of cerebral ischemia and hypoxia [5]. Consequently, therapeutic strategies targeting mitochondria are crucial for neuronal protection against ischemic stroke [6].

Among sirtuins (SIRT), SIRT6 is a member of NAD^+-dependent enzymes possessing deacetylation and ribosylation catalytic activities [7]. Deacetylation mechanisms associated with SIRT6 play an essential role in several physiological and pathophysiological events such as DNA repair, cell metabolism, inflammation, and apoptosis [1]. It has been reported that SIRT6 stimulation alleviates I/R injury affecting heart [8], liver [9], and brain [10]. A recent study indicated that SIRT6 down-regulation contributes to the production of inflammatory mediators in mice subjected to ischemic brain damage [1]. In addition, SIRT6 over-expression has been found to protect human vascular endothelial cells from inflammation mediated by TNF-α [11]. Regulation of oxidative stress and mitochondrial dysfunction might be a potential mechanism for the anti-inflammatory effect of SIRT6 [9]. Previous studies have identified the fundamental role of SIRT6 in maintaining mitochondrial function in skeletal and cardiac muscles [12,13] and renal podocytes [7]. Although SIRT6 has been proven to impede brain I/R injury in mice [14], the impact of SIRT6 on mitochondrial dysfunction of the hippocampus under brain ischemia remains to be elucidated.

It is now well-established that antioxidants dealing with mitochondria can delay the progression of brain ischemia complications and reduce the associated mortality [15]. The mitochondrial-targeted antioxidant, Mitoquinol (MitoQ), has been reported to exert beneficial effects in different diseases such as liver fibrosis [16], cardiac remodeling [17], and neurodegenerative diseases [18]. Mitoquinol consists of a lipophilic triphenylphosphonium (TTP) cation in conjugation with coenzyme Q10, enabling it to accumulate within the mitochondrial inner membrane [19] highly. It has been shown that MitoQ attenuated epithelial cell damage in a rodent model of intestinal I/R injury [19] and improved kidney function in response to renal I/R [20]. Recently, MitoQ has been observed to ameliorate brain microvascular endothelial cell injury following glucose overload [21]. In the clinical setting, MitoQ supplementation has been reported to attenuate mitochondrial oxidative status and improve the vascular and skeletal muscle functions [22–24]. These findings raised the impetus to explore the neuroprotective effect of MitoQ against hippocampal damage in a rat model of cerebral I/R injury via targeting SIRT6-mediated regulation of mitochondrial impairment and neuroinflammation.

2. Materials & methods

2.1. Animals

Adult male albino rats of the Wistar strain weighing 200-250 g were used in the current investigation. Rats were supplied from the Animal House of the Faculty of Pharmacy, Badr University in Cairo (Cairo, Egypt). They were housed in plastic cages under standard laboratory conditions (23 ± 1 °C, 40–60 % humidity; 12 h light/dark alternating cycles) and were given chow diet and water ad libitum. Prior to the experiment, rats were maintained for one week as an acclimation period. The Research Ethics Committee of the Faculty of Pharmacy, Badr University in Cairo, has approved the experimental procedures (PO-109-A), which complied with the guidelines submitted by the US National Institutes of Health for the proper care and use of laboratory animals (NIH Publication No. 85-23, revised 2011).

2.2. Chemicals

Mitoquinol was purchased from Antipodean Pharmaceuticals (Auckland, New Zealand). Thiopental sodium was obtained from Sigma-Aldrich (MO, USA). Penicillin G (Pencitard®) and buprenorphine hydrochloride (Buprenex®) were supplied from Acdima pharmaceuticals Inc. (6th October City, Egypt) and Hospira, Inc. (Wake Forest, USA), respectively. The source of reagents, kits, and antibodies used in this study was specified in the biological assay.

2.3. Induction of experimental brain ischemia

Rats were anesthetized with thiopental sodium (50 mg/kg; *i.p*) [25,26]. A midline ventral incision was made in the neck of rats and the two common carotid arteries were unveiled and precisely freed from the surrounding tissues and autonomic innervation. Using artery clamps, the exposed bilateral common carotid arteries were then occluded for 45 min to produce global brain ischemia. Clamps were afterward removed, and the neck incision was sutured, allowing reperfusion for 24 h [25,27]. Ischemic rats were given buprenorphine hydrochloride (30 μg/kg, S.C) and aqueous suspension of penicillin G (30,000 U; I.M) and individually housed. A sham-operated control group was included in which rats underwent the same previous events without carotid artery obstruction.

2.4. Experimental design

Four groups of experimental rats (9 rats each) were randomly set as follows: (i) sham-operated group (Sham), (ii) ischemia/reperfusion group (I/R), (iii) Sham + MitoQ (2 mg/kg; *i.p daily*) group, (iv) I/R + MitoQ (2 mg/kg; *i.p daily*) group. The MitoQ was dissolved in distilled water and injected 7 days and 2 h before the induction of brain ischemia. The selection of MitoQ dose was in accordance with a previous study [28].

2.5. Tissue collection

Following 24 h reperfusion, the behavioral test was conducted. The rats were afterward sacrificed by decapitation under anesthesia with thiopental (50 mg/kg) which accords with AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. Brain tissues were collected, and hippocampi were isolated and subdivided into 2 parts; the 1st part (n = 3) was treated with 10 % neutral-buffered formalin for histopathological and immunohistochemical examination. The 2nd part (n = 6) was then subdivided into both left and right hippocampi and rapidly transferred into liquid nitrogen and kept at −80 °C for proteins analysis and mitochondrial isolation, respectively. Frozen hippocampal tissues were used to assess nuclear respiratory factor 1 (NRF-1) and interleukin-1β (IL-1β) gene expression using polymerase chain reaction and SIRT6 levels, mitochondrial reactive oxygen species (mtROS), oxidized mitochondrial DNA (mtDNA), cytochrome *c*, peroxisome proliferator-activated receptor gamma coactivator 1α (PGC-1α), mitochondrial transcription factor A (TFAM), tumor necrosis factor-alpha (TNF-α), and interleukin-18 (IL-18), by ELISA technique in addition to mitochondrial glutathione (mtGSH) by a colorimetric assay and mitochondrial membrane potential)△Ψm) by fluorometric assay. All the investigations were conducted in triplicate. Throughout the examination, the samples' identities were kept hidden from the investigators.

2.6. Mitochondrial isolation

Mitochondria were isolated from hippocampal tissues according to previously described method [29]. In brief, hippocampal tissues were homogenized in 5-ml ice-cold hypertonic buffer containing 10 mmol/l NaCl, 2.5 mmol/l $MgCl_2$, and 10 mmol/l Tris base at pH 7.5 using a glass homogenizer (Thermo Fischer scientific). Homogenates were afterward centrifugated for 5 min. At 1300 g and 4 °C, and supernatants were centrifugated for 15 min at 17000 g and 4 °C. After that, an isotonic buffer (100 μl) containing mannitol, sucrose, Tris base, and EDTA (pH 7.5) was added to the final mitochondrial pellets which were stored at −80 °C for assessment of mitochondrial indices.

2.7. Behavioral experiment

For estimation of spatial learning and memory efficiency in I/R rats, the Morris water maze (MWM) was used. The MWM consisted of a 140-cm circular pool in which water (26 °C) was filled to 45 cm deep. To make the water opaque, a water-soluble, non-toxic pigment was utilized. The pool was split into 4 compartments in which a submerged platform made of transparent Plexiglas (8 cm in diameter) was set 1 cm below the surface of the water in the target quadrant to afford a movable escape zone. The pool was placed in a slightly lit room supplied with a fixed distal visual clue which provides navigational aids for rats in locating the target. In the acquisition trial, every rat was allowed 3 trials per day for four days, each lasting 120 s and separated by a 5-min interval. Rats were dropped into one of the four compartments of the pool, facing the tank's wall, and allowed to find and escape onto the hidden platform where they were permitted to stay on it for 10 s. Animals that did not attain the platform within 120 s were put on it for 30 s, and the latency for this trial was recorded as 120 s. The average time needed to identify the platform in all trials on each day of the acquisition phase was used to calculate the escape latency time (ELT), which was considered an indication of spatial learning. Following the final training session, a surgical procedure was conducted, and a retrieval test was done on day 5 (probe) session in which the platform was taken away. Every rat was put in the opposite quadrant to the platform quadrant and given 60 min to navigate the maze. The time spent searching for the hidden platform while swimming in the target compartment was monitored by an overhead camera [27].

2.8. Colorimetric analysis

Hippocampal mtGSH was measured by colorimetric assay kits (Cat#: K464–100; Bio Vision Incorporated, Milpitas, CA, USA) following the manufacturer's instructions.

2.9. Fluorometric analysis

Hippocampal mitochondrial membrane potential)△Ψm) was detected using a fluorometric assay kit (Cat#:10009172; Cayman Chemical Company, Ann Arbor, USA) which was estimated by Fluoroskan™ Microplate Fluorometer (Thermo Fisher Scientific, USA).

2.10. ELISA technique

According to the manufacturer's guidelines, the following hippocampal biomarkers were analyzed (i) mtROS (Cat#: LS-F9759; LS Bio, Seattle, USA), (ii) oxidized mtDNA (Cat#: CAY589320; Cayman Chemical Company, Ann Arbor, United States), (iii) SIRT6 (Cat#: MBS1600516; My BioSource, USA), (iv) cytochrome *c* (Cat#: EAMT001; Merck KGaA, Darmstadt, Germany), (v) PGC-1α (Cat#: SEH337Ra; Cloud-Clone Corp., USA), (vi) TFAM (Cat#: E1616Ra; BT Lab, Shanghai, China), (vii) TNF-α (Cat#: abx050220; Abbexa Ltd., Cambridge, UK), and (viii) IL-18 (Cat#: ER0036; Wuhan Fine Biotech Co., Ltd., Wuhan, China).

2.11. Quantitative real-time polymerase chain reaction (RT-PCR)

Tissues were homogenized, and total RNA was extracted from the hippocampi using a commercially available kit (Direct-zol RNA Miniprep Plus, Cat#: R2072, Zymo Research Corp. USA). The total RNA was estimated (quality & quantity) by Beckman spectrophotometer (USA) and then utilized for reverse transcription and cDNA synthesis. The RT-PCR was performed using a SuperScript IV One-Step kit (Cat#: 12594100, ThermoFisher Scientific, Waltham, USA). A thermal cycle using StepOne equipment (Applied Biosystem, USA) was conducted based on the following conditions: 15 s at 95 °C, 1 min at 60 °C, and 1 min at 72 °C (40 cycles). Data from the RT-PCR runs were presented as relative mRNA expression of the targeted genes, IL-1β and NRF-1, after normalization referring to the endogenous control gene, GAPDH. According to the $2^{-\Delta\Delta Ct}$ method, the relative quantitation (RQ) of each studied gene was calculated [30]. Primers pairs of the studied genes were prepared using Primer Premier 5.0 software (Premier Biosoft, USA) and their sequences are described in Table 1.

2.12. Histopathological investigation

Hippocampal tissues were flushed and fixed in 10 % phosphate-buffered formalin for 72 h. Samples were trimmed, processed in serial grades of ethanol, cleared in xylene, infiltrated, and embedded into Paraplast® tissue embedding media. About 4-μm sagittal hippocampal sections were stained with hematoxylin and eosin (H & E) for morphological inspection of hippocampi and toluidine blue for damaged and intact neurons determination. Intact neurons numbers were investigated in CA1 and CA3 subregions through random selection of 6 different fields for each tissue section. Hippocampi images were captured using light microscope (magnification, ×400) and calculated using full HD microscopic imaging system powered by Leica Application Suite (Leica Microsystems GmbH, Wetzlar, Germany).

2.13. Immunohistochemistry analysis

In compliance with the manufacturer's protocol, the paraffin-embedded hippocampi slices were dewaxed and used for immunohistochemical analysis. Deparaffinized retrieved tissue sections were treated with 0.3 % H_2O_2 for 20 min followed by incubation at 4 °C overnight with anti-cleaved caspase-3 (Cat#: Asp175; 1:200; Cell signaling Tech. USA), as well as anti-Glial Fibrillary Acidic Protein (GFAP) (Cat#: MS-280-P1; 1:100; Thermo Fisher Scientific Inc. USA). Slides were afterward washed out by PBS and incubated with a secondary antibody (HRP Envision kit; DAKO) for 20 min followed by incubation with diaminobenzidine (DAB) for 15 min. Thereafter, specimens were washed with PBS and counterstained with hematoxylin for microscopic examination. For estimation of the percentage of GFAP and cleaved caspase-3 immunopositive area in hippocampal tissues, 6 non-interfering fields (mean ± S·D of 6 fields) were randomly selected and analyzed using full HD microscopic imaging system powered by Leica Application module (Leica Microsystems GmbH, Wetzlar, Germany).

2.14. Statistical analysis

All data were evidenced as mean ± standard deviation (S.D). The Shapiro-Wilks and the Fisher F tests were used to determine normality and homoscedasticity, respectively. When available, Welch's adjustment for variance inhomogeneity was used. For all parameters, One-way analysis of variance (One-way ANOVA) was used, followed by Tukey's multiple comparisons test. Statistical analysis was carried out using GraphPad Prism software version 8 (San Diego, CA, USA) and the significance of all statistical tests was fixed at $p < 0.05$.

Table 1
Primer sequence of GAPDH, IL-1β and NRF-1 mRNA.

Gene	Primer sequence (5′-3′)	Accession no
GAPDH	F: 5′- CCTCGTCTCATAGACAAGATGGT -3' R: 5′- GGGTAGAGTCATACTGGAACATG -3'	NM_017008.4
IL-1β	F: 5′-ATTGTGGCTGTGGAGAAG-3′ R: 5′- AAGATGAAGGAAAAGAAGGTG-3′	NM_031512.2
NRF-1	F: 5′ TGTCACCATGGCCCTTAACAGTGA-3′ R: 5′ TGAACTCCATCTGGGCCATTAGCA-3′	NM_001100708.1

3. Results

3.1. MitoQ alleviates spatial learning and memory deficits induced by I/R

The MWM test was performed to estimate memory efficiency. Before executing the I/R surgery and probe test, all animals underwent training sessions for 4 days. At this stage, all rats behaved similarly and exhibited a notable decline in ELT, indicating normal learning ability (Fig. 1A). On day 5 probe test, I/R rats depicted a dramatic reduction of the time spent seeking the lost platform in the target quadrant (53 %) relative to sham rats, reflecting memory impairment. Administration of MitoQ improved memory impairment by increasing the time spent in the target compartment by 79 % versus I/R rats (Fig. 1B).

3.2. MitoQ alleviates hippocampal SIRT6 and inflammatory mediators, TNF-α and IL-1β following cerebral I/R injury

As presented in Fig. 2A, rats subjected to brain I/R damage revealed a profound suppression in hippocampal SIRT6 levels by approximately 71 % compared to sham-operated ones. Pretreatment of I/R rats with MitoQ induced a significant increase in SIRT6 by 130 % compared to untreated ones.

Neuroinflammation has been suggested to mediate neuronal cell damage following the early episode of brain ischemia [31]. The inflammatory mediators, TNF-α and IL-18 protein levels as well as IL-1β mRNA expression were dramatically increased in the hippocampi tissues of I/R rats by 5-fold, 4.3-fold, and 3.9-fold, respectively, relative to the sham ones. Pretreatment of I/R rats with MitoQ significantly attenuated TNF-α, IL-18, and IL-1β levels compared to the untreated ones (52 %, 62 % and 61 %, respectively) (Fig. 2B-D).

3.3. MitoQ alleviates mitochondrial oxidative stress mediated by I/R

Impaired mitochondrial oxidative status plays a fundamental role in the development of cerebral ischemia [5]. A prominent increment of hippocampal mtROS and oxidized mtDNA (331 % and 135 %, respectively) together with a marked reduction of hippocampal mtGSH was demonstrated in I/R rats by 66 % compared to the sham group. A noticeable suppression in hippocampal mtROS and oxidized mtDNA by 57 % and 45 %, respectively, coincident with a profound surge of mtGSH (2.6-fold) was observed in I/R rats pretreated with MitoQ relative to the untreated ones. In addition, MitoQ reverted mtGSH and oxidized mtDNA back to their normal levels (Fig. 3A-C).

3.4. MitoQ alleviates mitochondrial biogenesis markers, PGC-1α and TFAM protein levels, and NRF-1 mRNA as well as mitochondrial dysfunction following cerebral I/R injury

Induction of hippocampal neuronal injury in rats produced an obvious decline in PGC-1α and TFAM levels in addition to the gene expression of NRF-1 by 69 %, 69 %, and about 78 %, respectively, relative to the sham group. However, I/R rats that received MitoQ for 7 successive days exhibited a patent elevation in the protein contents of PGC-1α (2.5-fold) and TFAM (2.8-fold) and NRF-1 mRNA (3.7-fold) as compared to untreated I/R ones. Noteworthy, MitoQ normalized PGC-1α, TFAM and NRF-1 (Fig. 4A-C).

To further elucidate the impact of MitoQ on mitochondrial activity, △Ψm was analyzed. Reduced △Ψm was depicted in I/R rats relative to the control sham, and MitoQ pretreatment markedly reversed this effect (Fig. 4D).

3.5. MitoQ alleviates hippocampal GFAP immunoreactivity following cerebral I/R injury

To further demonstrate the anti-inflammatory potential of MitoQ, GFAP was immunohistochemically examined in the hippocampi tissues of I/R rats as a main sign for astrocytes activation. As shown in Fig. 5a-e, brain I/R increased astrocyte activation as evidenced by significant increase in hippocampal GFAP immunopositive areas (3.7-fold) versus the sham rats. Otherwise, MitoQ markedly reinstated I/R-induced astrocyte activation (57 %).

3.6. MitoQ alleviates hippocampal cell apoptosis induced by cerebral I/R injury

To clarify the ameliorative effect of MitoQ against hippocampal neuronal death, cytochrome C protein content and cleaved caspase-3 immunoexpression were analyzed. Cytochrome C was evidently

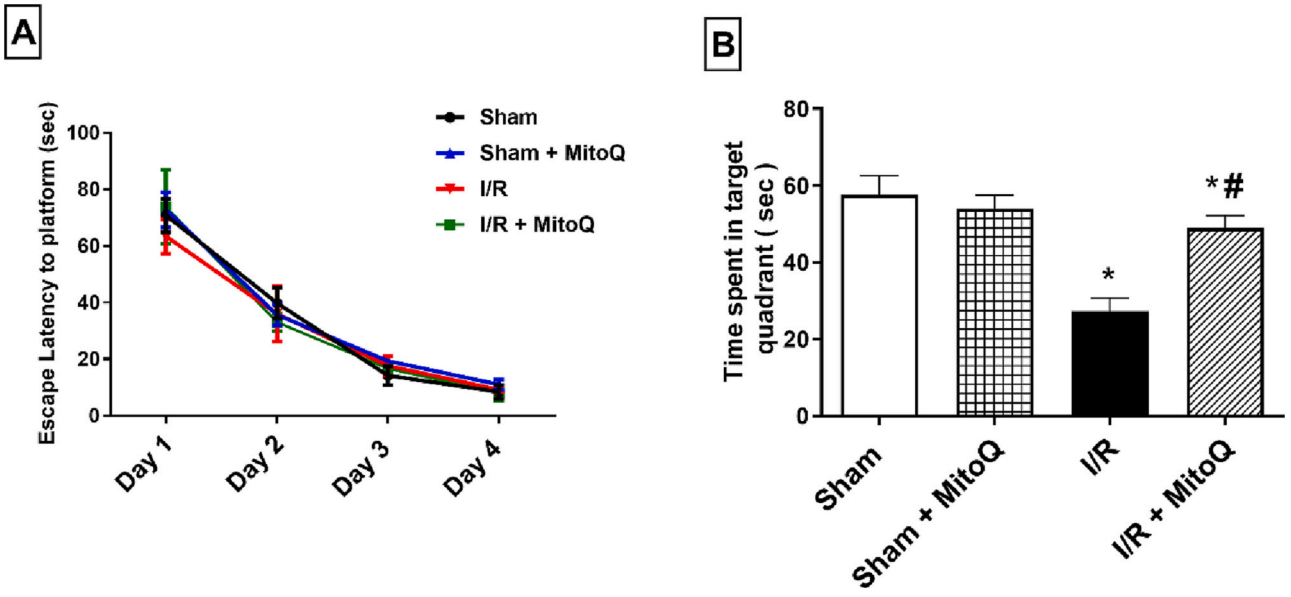

Fig. 1. Effects of MitoQ on 4-days MWM latency time (A), probe test of water maze (B). Rats were administered MitoQ (2 mg/kg; *i.p* daily) for 7 consecutive days then subjected to bilateral carotid artery occlusion for 45 min followed by 24 h reperfusion period. Data were represented as the mean ± SD (n = 6). *, # significant difference from the Sham and I/R group, respectively, Two-way ANOVA followed by Bonferroni post-hoc test and One-way ANOVA followed the Tukey's multiple comparisons test for 4-days MWM latency time and probe test of water maze, respectively, $p < 0.05$.

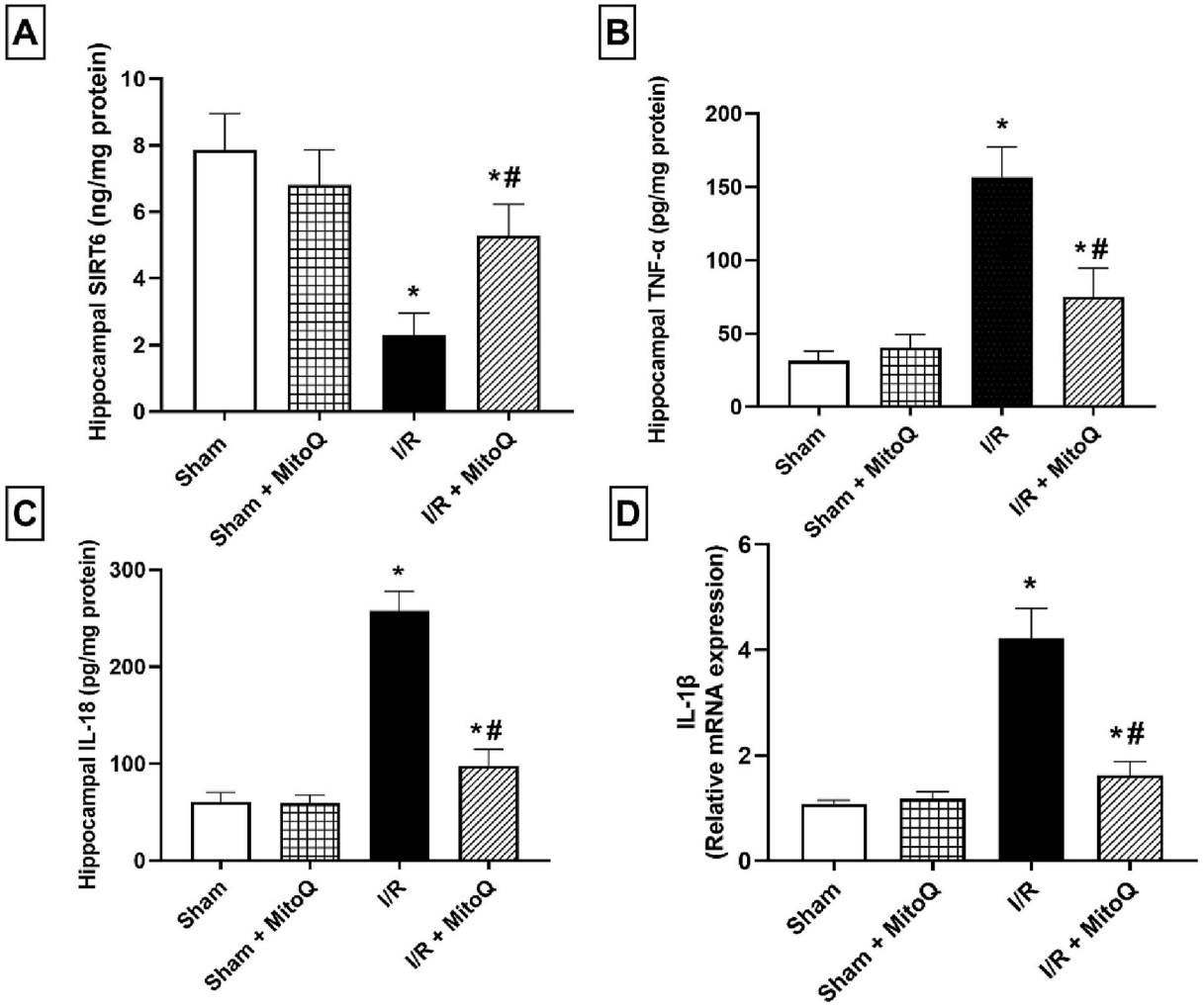

Fig. 2. Effects of MitoQ on hippocampal Sirt6 (A) and inflammatory mediators, TNF-α (B), IL-18 (C), and IL-1β mRNA (D). Rats were administered MitoQ (2 mg/kg; *i. p* daily) for 7 consecutive days then subjected to bilateral carotid artery occlusion for 45 min followed by 24 h reperfusion period. Data were represented as mean ± SD (n = 6). *, # significant difference from the Sham and I/R group, respectively, One-way ANOVA, followed by the Tukey's multiple comparisons test; $p < 0.05$.

increased in I/R rats (4-fold) versus the sham ones, which was markedly reduced by MitoQ pretreatment (44.5 %) (Fig. 6a). As compared to the sham group, hippocampi sections of I/R rats depicted a prominent increment in active caspase-3 immunoreactive areas by 8.8-fold. In contrast, pretreatment with MitoQ prevented hippocampal neuronal apoptosis by drastically reducing caspase-3 immunoexpression by 68 % in comparison to the untreated I/R group (Fig. 6b-f).

3.7. MitoQ alleviates histopathological abnormalities and maintains neuronal viability following I/R Injury

The sham group revealed normal histological findings throughout the field, with intact pyramidal neurons and minimal glial cell infiltrate (Fig. 7a & e). The sham-MitoQ treated group showed almost the same normal histological features as the sham group (Fig. 7b & f). meanwhile, in the CA1 and CA3 areas, the I/R group depicted significant neuronal degeneration with many karyopyknotic nuclei, glial cell infiltrate, and moderate to severe edema (Fig. 7c & g). Pretreatment with MitoQ significantly reduced neuronal degeneration, resulting in nearly normal histological characteristics in the CA1 and CA3 regions, with only few scattered degenerated neurons. (Fig. 7d & h).

Similarly, histopathological investigation showed that both the sham (Fig. 8a & e) and Sham-MitoQ (Fig. 8b & f) groups had integrated Nissl bodies and rounded nuclei. However, the I/R rats exhibited a remarkable decrease in the count of intact cells by 91 % and 89 % in the CA1 and CA3 regions, respectively, as related to the sham group (Fig. 8c & g). Nevertheless, MitoQ significantly preserved neuronal loss in CA1 and CA3 by 11-fold and 9-fold, respectively (Fig. 8d & h).

4. Discussion

Mitochondrial mutilation has been suggested to exacerbate neuronal cell damage; hence targeting mitochondria by antioxidant therapies is an appealing prospect for attenuation of brain ischemia [3]. The current study demonstrated that the targeted mitochondrial antioxidant, MitoQ, could bestow neuronal protection against I/R-induced hippocampal cerebral injury in rats. Alleviation of hippocampal SIRT6 with subsequent mitigation of mtROS and mtGSH as well as oxidized mtDNA together with inhibition of neuronal inflammatory and apoptotic reactions verified the neuroprotection of MitoQ during brain I/R insult. In addition, MitoQ reinstated mitochondrial biogenesis proteins thereby enhancing PGC-1α and TEAM levels as well as NRF-1 mRNA expression in hippocampi tissues and restored $\triangle\Psi m$, contributing to stabilization of mitochondrial function following cerebral ischemia. These events were associated with significant improvement of cognitive function and restoration of normal hippocampal characteristics in morphological examination.

Increasing evidence has suggested that sirtuins are involved in a

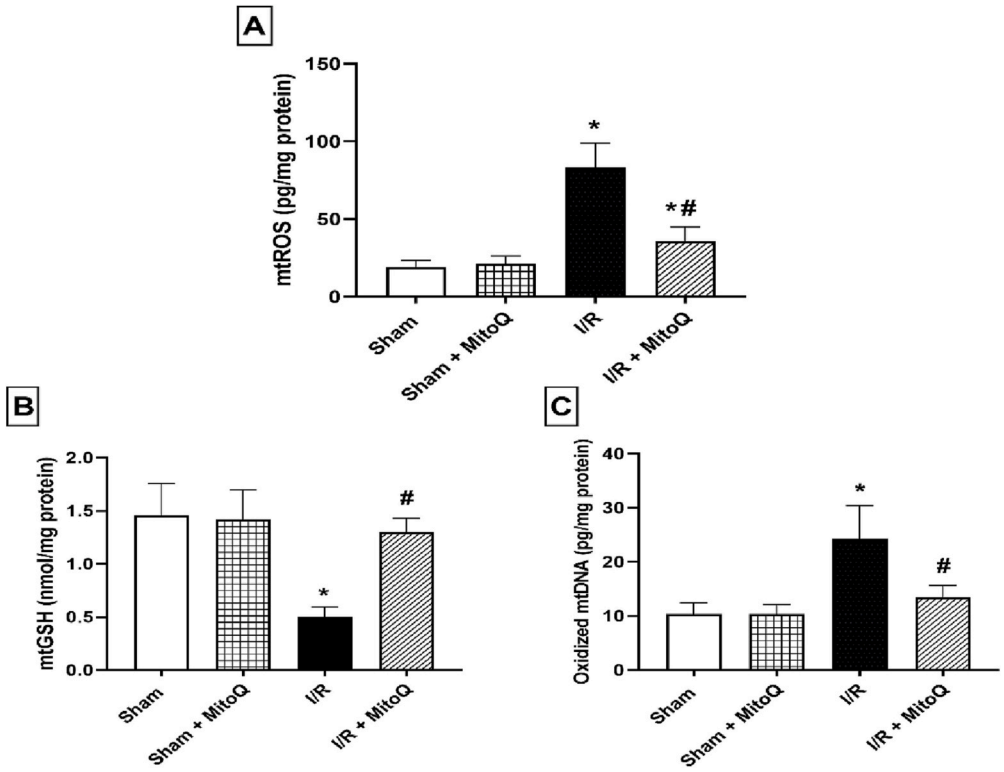

Fig. 3. Effects of MitoQ on hippocampal mitochondrial oxidative stress, mtROS (A), mtGSH (B), and oxidized mtDNA (C). Rats were administered MitoQ (2 mg/kg; *i.p* daily) for 7 consecutive days then subjected to bilateral carotid artery occlusion for 45 min followed by 24 h reperfusion period. Data were represented as mean ± SD (n = 6). *, # significant difference from the Sham and I/R group, respectively, One-way ANOVA, followed by the Tukey's multiple comparisons test; $p < 0.05$.

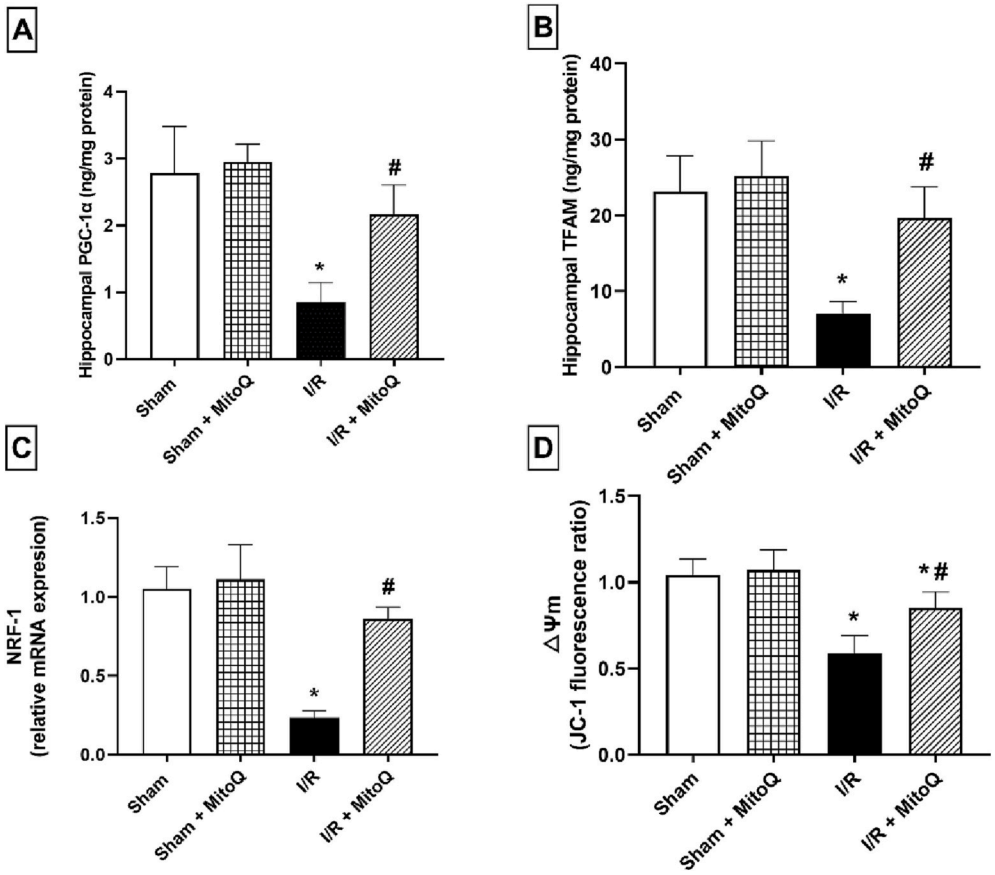

Fig. 4. Effects of MitoQ on mitochondrial biogenesis proteins, PGC-1α (A), TFAM (B), and NRF-1 mRNA (C) and mitochondrial function, △Ψm (D). Rats were administered MitoQ (2 mg/kg; *i.p* daily) for 7 consecutive days then subjected to bilateral carotid artery occlusion for 45 min followed by 24 h reperfusion period. Data were represented as mean ± SD (n = 6). *, # significant difference from the Sham and I/R group, respectively, One-way ANOVA, followed by the Tukey's multiple comparisons test; $p < 0.05$.

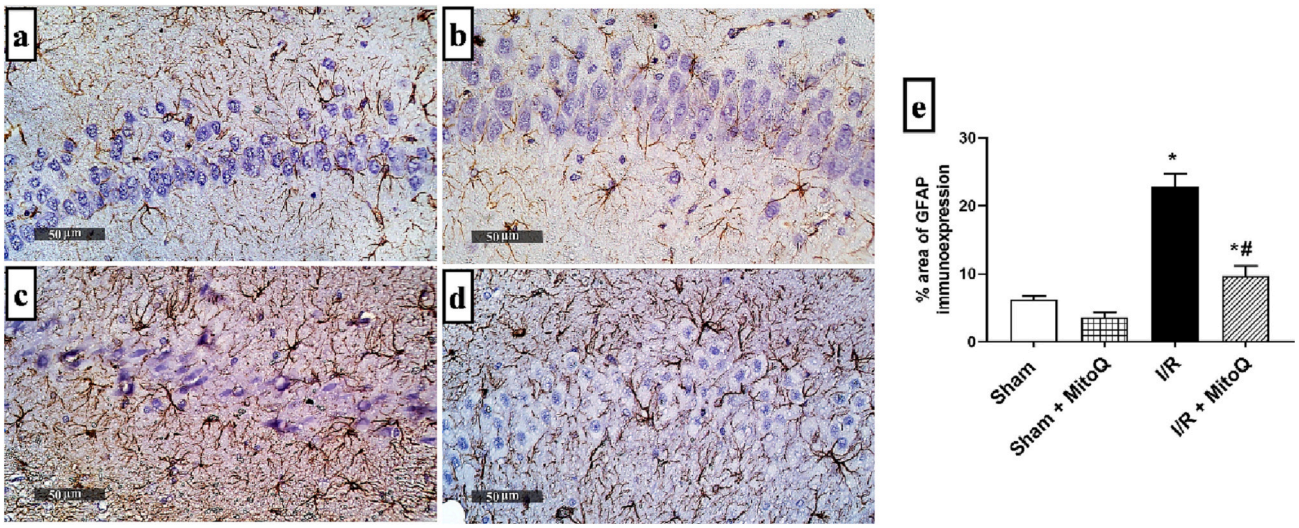

Fig. 5. Representative photomicrographs of immunohistochemical staining of GFAP in the hippocampi sections of the different groups (Magnification: ×400). (a) Sham, (b) Sham + MitoQ, (c) I/R, (d) I/R + MitoQ, and (e) GFAP optical density of % area of GFAP immunoexpression in the hippocampal sections of the four groups. Each bar with a vertical line represents a mean ± SD. of 6 fields. *, # significant difference from the Sham and I/R group, respectively, One-way ANOVA, followed by the Tukey's multiple comparisons test; $p < 0.05$.

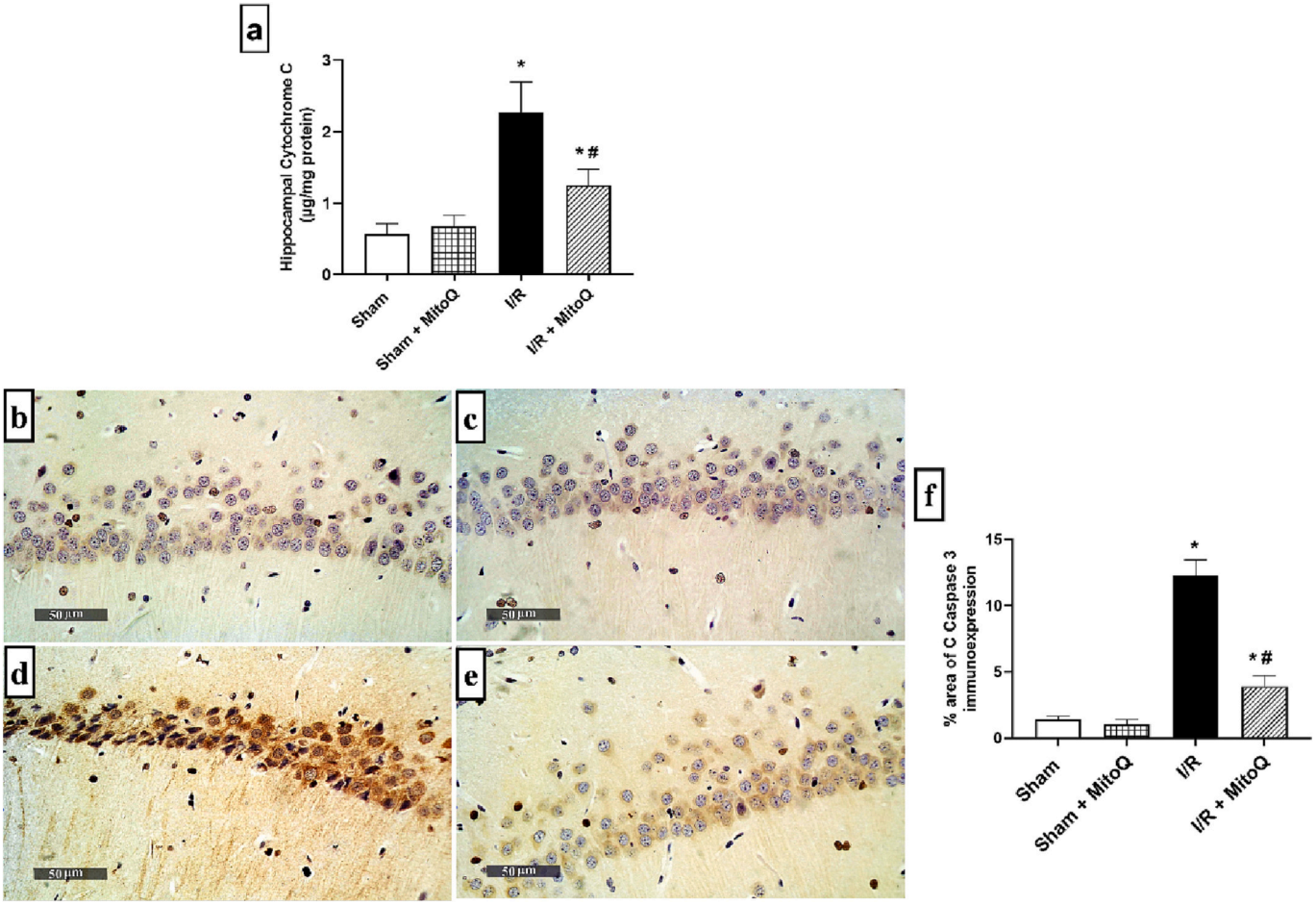

Fig. 6. Effects of MitoQ on hippocampal cell apoptosis, (a) cytochrome C protein content and (b-f) cleaved caspase-3 immunohistochemical expression (Magnification: ×400). (b) Sham, (c) Sham + MitoQ, (d) I/R, (e) I/R + MitoQ, and (f) caspase-3 optical density of % area of caspase-3 expression in the hippocampal section of the four groups. Each bar with a vertical line represents a mean ± SD. of 6 fields. *, # significant difference from the Sham and I/R group, respectively, One-way ANOVA, followed by the Tukey's multiple comparisons test; $p < 0.05$.

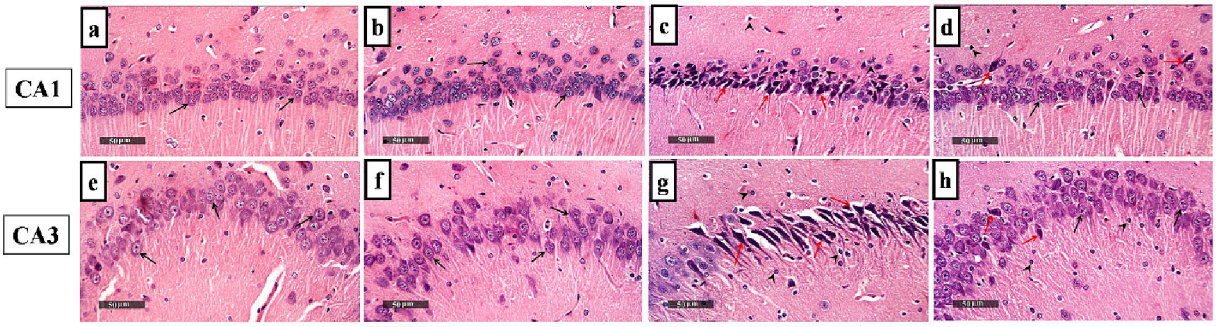

Fig. 7. Representative photomicrographs illustrating H&E staining of the CA1, and CA3 regions of hippocampus (Magnifications: ×400). (a,e) Sham, (b,f) Sham + MitoQ, (c,g) I/R, and (d,h) I/R + MitoQ. Black arrows indicate intact well-organized neurons; Red arrows indicate pyknosis. Arrowheads indicate glial cells infiltration.

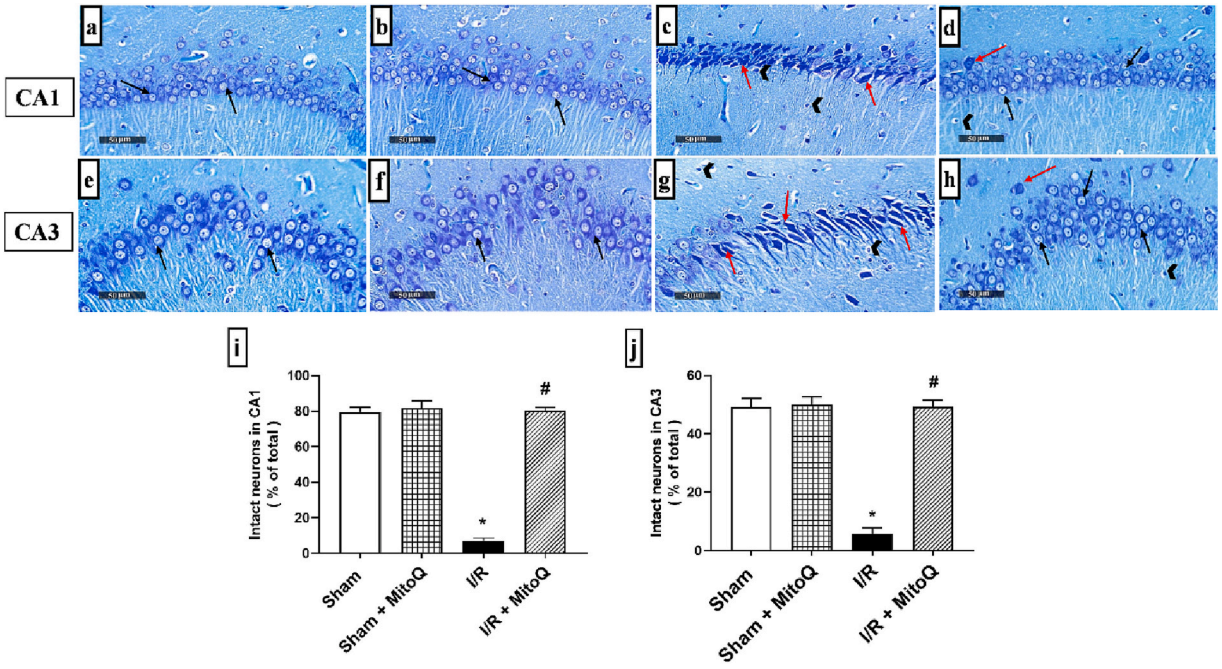

Fig. 8. Representative photomicrographs illustrating Nissl staining of the CA1 and CA3 regions of hippocampus (Magnifications: ×400). (a,e) Sham, (b,f) Sham + MitoQ, (c,g) I/R, and (d,h) I/R + MitoQ. Panels (i) and (j) depicts the number of intact neurons in CA1 and CA3 regions, respectively, of the hippocampal sections of the four groups as % of the total area per each section. Black arrows indicate intact well-organized neurons, red arrows indicate pyknosis, and arrowheads indicate glial cells infiltration. Each bar with a vertical line represents a mean ± SD. of 6 fields. * $p < 0.05$ versus Sham and # $p < 0.05$ versus I/R, One-way ANOVA, followed by the Tukey's multiple comparisons test; $p < 0.05$.

variety of brain conditions, including brain aging [32] and neurodegenerative disorders such as Huntington's disease and ischemic brain injury [33]. In this study, MitoQ modulated SIRT6 in the brain tissues of I/R rats, resulting in profound neuroprotection. A previous study reported that endothelial SIRT6 overexpression blunted stroke volumes, protected blood-brain barrier and improved neurological functions in mice subjected to transient middle cerebral artery occlusion [34]. Upregulation of SIRT6 has also been demonstrated to attenuate neuronal deficits in mice subjected to cerebral I/R injury and preserve the neurons which underwent glucose-deprivation/reoxygenation-induced neuronal cell death in vitro thereby curbing oxidative stress [33]. Moreover, administration of the SIRT6 activator, MDL-811, ameliorated brain damage and dampened neuronal injury via interfering with inflammatory response in mice models of cerebral I/R injury and lipopolysaccharide-induced neuroinflammation [1]. Therefore, targeting SIRT6 by MitoQ herein could halt cerebral hippocampal damage in I/R rats. To the best of authors' knowledge, this the first study evaluating the impact of MitoQ on hippocampal SIRT6 levels during experimental cerebral ischemia.

In the current investigation, MitoQ abrogated mitochondrial oxidative stress via suppressing mtROS and oxidized mtDNA together with enhancing the mitochondrial antioxidant machinery, GSH in the hippocampi tissues of I/R rats. These observations extend those of an earlier study in which MitoQ hampered mtROS, prevented DNA oxidation, and restored SOD and GSH activity in an experimental model of toxin-driven brain cell death [35,36]. Herein, MitoQ might have improved hippocampal mitochondrial oxidative status through triggering SIRT6. It has been explored that downregulation of SIRT6 contributed to mitochondrial dysfunction thereby increasing mitochondrial SOD production and impairing mitochondrial structure. However, mitigation of SIRT6 expression suppressed mitochondrial SOD and maintained normal mitochondrial morphology in high glucose-induced mitochondrial malfunction and apoptosis in cultured podocytes [7]. In addition, activation of SIRT6 incited the overexpression of antioxidant factors, protecting the cardiac myocytes from I/R-induced oxidative stress [8]. Importantly, MitoQ has been detected to exert its

antioxidant effect and improve mitochondrial abnormalities via regulating intermediate transcription mediators in different experimental models including diabetic nephropathy [33], alcoholic liver disease [37] and intestinal I/R [19], observations that align with results reported in this study. Therefore, the neuroprotection donated by MitoQ, might be mediated by promoting SIRT6 involved in regulation of hippocampal mitochondrial oxidative stress.

Ample evidence demonstrates that enhanced mitochondrial biogenesis is associated with neuronal protection following cerebral ischemia [38,39]. In this regard, MitoQ pretreatment reinforced mitochondrial biogenesis proteins including PGC-1α and TFAM as well as NRF-1 gene expression in the hippocampi tissues of I/R rats. These results corroborated the idea of Hu et al., who indicated that MitoQ stabilized mitochondrial TFAM involved in mtDNA replication with subsequent reduction of mtROS, contributing to protection of intestinal mucosa from I/R injury [19]. In a mouse model of Parkinson's disease, MitoQ was also found to rescue dopaminergic neurons from loss via maintaining mitochondrial function thereby activating the mitochondrial biogenesis protein, PGC-1α [40]. Moreover, MitoQ has been reported to preserve striatal neurons expressed mutant huntingtin by attenuating PGC-1α, NRF-1, and TFAM in an in vitro model of Huntington's disease [41]. Noteworthy, TFAM over-expression has been noticed to protect intestinal barrier and cardiac myocytes against experimental I/R injury-induced mitochondrial oxidative damage [19,42]. In addition, PGC-1α upregulation was observed to enhance mtDNA and antioxidative enzymes in mice model of muscle atrophy [43]. Several studies have confirmed the fundamental role of TFAM, PGC-1α and NRF-1 as protective mechanisms against neuronal deficits and oxidative stress in cerebral I/R injury [44,45], providing evidence that stabilizing mitochondrial biogenesis by MitoQ participated in protecting hippocampi tissues from injury following cerebral I/R.

It is interesting to highlight that SIRT6 activation has been shown to improve mitochondrial biogenesis, thereby stimulating PGC-1α, NRF-1, and TFAM expression, resulting in attenuation of cardiomyopathy in diabetic rats underwent I/R injury. Meanwhile, silencing SIRT6 reduced PGC-1α and exacerbated mitochondrial dysfunction [46]. Based on the previous report, our results speculate that regulation of SIRT6 by MitoQ could provoke hippocampal mitochondrial biogenesis, leading to profound neuronal protection against brain I/R injury. The current findings extend and verify those of earlier evidence, in which SIRT6 upregulation instigated the mitochondrial biogenesis proteins, PGC-1α, NRF-1, and TFAM and ensured renal podocyte against hyperglycemia-induced mitochondrial dysfunction and apoptosis [7].

Neuroinflammation has been recognized to worsen clinical outcomes after ischemic stroke [31]. In the present work, MitoQ impeded hippocampal neuronal inflammation as confirmed by curtailing hippocampal TNF-α and IL-18 levels and IL-1β mRNA as well as the protein expression of GFAB in I/R rats, another indication for the neuroprotection afforded by MitoQ. These observations accord with recent literature which revealed that MitoQ effectively rescinded hippocampal inflammation via inhibiting TNF-α with subsequent downregulation of GFAP in D-galactose-induced aging rats [47]. This study further supports the data obtained from neuronal cell line where MitoQ blocked the inflammatory cascade and prevented the proinflammatory cytokines, TNF-α and IL-1β [47]. Furthermore, a recent study reported that MitoQ modulated brain inflammatory response in an animal model of intracerebral hemorrhage, thereby inhibiting mtROS [48]. Inevitably, mtROS is involved in the activation of proinflammatory pathways during experimental cerebral I/R injury [49]. Accordingly, maintaining mitochondrial oxidative status and function by MitoQ might play a pivotal role in hindering hippocampal inflammation after I/R injury.

In this study, the anti-inflammatory potential of MitoQ might be correlated with SIRT6 potentiation. Indeed, SIRT6 plays a key role in the pathogenesis of systemic inflammation [50] and has been demonstrated to exhibit anti-neuroinflammatory effects in different experimental models of ischemic stroke [1,14,34]. In addition, the study of Jiang et al. proved that SIRT6 exerted anti-inflammatory effects via regulation of TNF-α synthesis in mouse embryonic fibroblast cells [51]. Furthermore, SIRT6 ablation in mice subjected to hepatic I/R injury aggravated oxidative stress and mitochondrial malfunction, followed by activation of proinflammatory cytokines. Nevertheless, restoring SIRT6 expression in hepatocytes attenuated excessive ROS and mitochondrial dysfunction with succeeding inhibition of inflammatory mediators such as TNF-α and IL-1β, culminating in suppressing apoptotic signals, including caspase-3 [9]. Noticeably, MitoQ dampened hippocampal neuronal death via inhibiting mitochondrial apoptotic signals, thereby preventing cytochrome *c*-mediated downstream activation of caspase-3 in I/R rats. In the same context, SIRT6 overexpression alleviated caspase-3 with subsequent curtailing of podocyte cell death via repressing mitochondrial mutilation in a mouse model of diabetic nephropathy [7]. Taken together, this study suggests that SIRT6 maintenance by MitoQ could be associated with the amendment of hippocampal inflammation and apoptosis caused by cerebral I/R in rats, thereby regulating mitochondrial oxidative milieu and function.

Conclusively, this study indicated that MitoQ exhibited apparent neuroprotection in cerebral I/R-induced hippocampal injury in rats. Restoring hippocampal SIRT6 accompanied with stabilization of mitochondrial redox status, biogenesis, and function and mitigation of hippocampal inflammation and apoptosis might underlie the neuronal protection of MitoQ. Further investigations are warranted to clarify and confirm whether SIRT6 modulation is a key factor in the mitochondrial effects of MitoQ in different experimental and clinical settings.

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Declaration of competing interest

The authors declare that they have no conflicts of interest.

References

[1] T. He, J. Shang, C. Gao, X. Guan, J. Zhang, T. Pang, A Novel SIRT6 Activator Ameliorates Neuroinflammation and Ischemic Brain Injury Via EZH2 / FOXC1 Axis, 2020, https://doi.org/10.1016/j.apsb.2020.11.002.

[2] J. Song, W. Zhang, J. Wang, H. Yang, Inhibition of FOXO3a / BIM signaling pathway contributes to the protective effect of salvianolic acid A against cerebral ischemia / reperfusion injury 9 (2019) 505–515, https://doi.org/10.1016/j.apsb.2019.01.010.

[3] P. Bakthavachalam, P.S.T. Shanmugam, Mitochondrial dysfunction – silent killer in cerebral ischemia, J. Neurol. Sci. 375 (2017) 417–423, https://doi.org/10.1016/j.jns.2017.02.043.

[4] J. Ruszkiewicz, J. Albrecht, Changes in the mitochondrial antioxidant systems in neurodegenerative diseases and acute brain disorders, Neurochem. Int. 88 (2015), https://doi.org/10.1016/j.neuint.2014.12.012.

[5] F. Liu, J. Lu, A. Manaenko, J. Tang, Q. Hu, Mitochondria in Ischemic Stroke : New Insight and Implications 9, 2018, pp. 924–937.

[6] J.L. Huang, A. Manaenko, Z.H. Ye, X.J. Sun, Q. Hu, Hypoxia therapy-a new hope for the treatment of mitochondrial dysfunctions, Med. Gas Res. 6 (2016) 174–176, https://doi.org/10.4103/2045-9912.191365.

[7] Y. Fan, Q. Yang, Y. Yang, Z. Gao, Y. Ma, L. Zhang, W. Liang, G. Ding, Sirt6 suppresses high glucose-induced mitochondrial dysfunction and apoptosis in podocytes through AMPK activation, Int. J. Biol. Sci. 15 (2019) 701–713, https://doi.org/10.7150/ijbs.29323.

[8] X.-X. Wang, X.-L. Wang, M.-M. Tong, Lu Gan, Huali Chen, S.-S. Wu, J.-X. Chen, R.-L. Li, Y. Wu, Heng-Yu Zhang, Y. Zhu, Y.-X. Li, J.-H. He, M. Wang, W. Jiang, SIRT6 protects cardiomyocytes against ischemia/reperfusion injury by augmenting FoxO3a-dependent antioxidant defense mechanisms, (n.d.). doi:https://doi.org/10.1007/s00395-016-0531-z.

[9] S. Zhang, S. Jiang, H. Wang, W. Di, C. Deng, Z. Jin, W. Yi, X. Xiao, Y. Nie, Y. Yang, SIRT6 protects against hepatic ischemia/reperfusion injury by inhibiting apoptosis and autophagy related cell death, Free Radic. Biol. Med. 115 (2018) 18–30, https://doi.org/10.1016/j.freeradbiomed.2017.11.005.

[10] Y. Hu, R. Li, H. Yang, H. Luo, Z. Chen, Sirtuin 6 is essential for sodium sulfide-mediated cytoprotective effect in ischemia/reperfusion-stimulated brain endothelial cells, J. Stroke Cerebrovasc. Dis. 24 (2015) 601–609, https://doi.org/10.1016/j.jstrokecerebrovasdis.2014.10.006.

[11] Y. He, G. Yang, F. Yao, Y. Xian, G. Wang, L. Chen, X. Lv, H. Gao, Z. Zheng, L. Sun, W. Wang, R. Lin, Sitagliptin inhibits vascular in fl ammation via the SIRT6-dependent signaling pathway, Int. Immunopharmacol. 75 (2019) 1–10, https://doi.org/10.1016/j.intimp.2019.105805.
[12] X. Cui, L. Yao, X. Yang, Y. Gao, F. Fang, J. Zhang, Q. Wang, Y. Chang, SIRT6 regulates metabolic homeostasis in skeletal muscle through activation of AMPK, Am. J. Physiol. Endocrinol. Metab. 313 (2017) 493–505, https://doi.org/10.1152/ajpendo.00122.2017.-Because.
[13] M.-Y. Cheng, Y.-W. Cheng, J. Yan, X.-Q. Hu, H. Zhang, Z.-R. Wang, Q. Yin, W. Cheng, SIRT6 suppresses mitochondrial defects and cell death via the NF-κB pathway in myocardial hypoxia/reoxygenation induced injury, Am. J. Transl. Res. 8 (2016) 5005–5015. https://europepmc.org/articles/PMC5126343.
[14] W. Zhang, R. Wei, L. Zhang, Y. Tan, C. Qian, Sirtuin 6 Protects the Brain from Cerebral Ischemia/Reperfusion Injury through NRF2 Activation, 2017, https://doi.org/10.1016/j.neuroscience.2017.09.035.
[15] E. Ahmed, T. Donovan, L. Yujiao, Q. Zhang, Mitochondrial targeted antioxidant in cerebral ischemia, J. Neurol. Neurosci. 6 (2016) 1, https://doi.org/10.21767/2171-6625.100017.
[16] H. Rehman, Q. Liu, Y. Krishnasamy, Z. Shi, V.K. Ramshesh, K. Haque, R. G. Schnellmann, M.P. Murphy, J.J. Lemasters, D.C. Rockey, Z. Zhong, The Mitochondria-targeted Antioxidant MitoQ Attenuates Liver Fibrosis in Mice. www.ijppp.org, 2016.
[17] D. Graham, N.N. Huynh, C.A. Hamilton, E. Beattie, R.A.J. Smith, H.M. Cochemé, M.P. Murphy, A.F. Dominiczak, Mitochondria Mitochondria-targeted Antioxidant MitoQ 10 Improves Endothelial Function and Attenuates Cardiac Hypertrophy, 2009, https://doi.org/10.1161/HYPERTENSIONAHA.109.130351.
[18] M. Manczak, P. Mao, M.J. Calkins, A. Cornea, A.P. Reddy, M.P. Murphy, H.H. Szeto, B. Park, P. Hemachandra Reddy, Mitochondria-targeted Antioxidants Protect Against Amyloid-β Toxicity in Alzheimer's Disease Neurons, (n.d.). doi: https://doi.org/10.3233/JAD-2010-100564.
[19] Q. Hu, J. Ren, G. Li, J. Wu, X. Wu, G. Wang, G. Gu, H. Ren, The mitochondrially targeted antioxidant MitoQ protects the intestinal barrier by ameliorating mitochondrial DNA damage via the Nrf2 / ARE signaling pathway, Cell Death Dis. (2018), https://doi.org/10.1038/s41419-018-0436-x.
[20] A.J. Dare, E.A. Bolton, G.J. Pettigrew, J.A. Bradley, K. Saeb-parsy, M.P. Murphy, Protection against renal ischemia – reperfusion injury in vivo by the mitochondria targeted antioxidant MitoQ, Redox Biol. 5 (2015) 163–168, https://doi.org/10.1016/j.redox.2015.04.008.
[21] M. Yang, Z. Fan, Z. Zhang, J. Fan, MitoQ Protects Against High Glucose-induced Brain Microvascular Endothelial Cells Injury Via the Nrf2 / HO-1 Pathway 145, 2021, https://doi.org/10.1016/j.jphs.2020.10.007.
[22] S.C. Broome, T. Pham, A.J. Braakhuis, R. Narang, H.W. Wang, A.J.R. Hickey, C. J. Mitchell, T.L. Merry, MitoQ supplementation augments acute exercise-induced increases in muscle PGC1α mRNA and improves training-induced increases in peak power independent of mitochondrial content and function in untrained middle-aged men, Redox Biol. 53 (2022), https://doi.org/10.1016/j.redox.2022.102341.
[23] M.J. Rossman, J.R. Santos-Parker, C.A.C. Steward, N.Z. Bispham, L.M. Cuevas, H. L. Rosenberg, K.A. Woodward, M. Chonchol, R.A. Gioscia-Ryan, M.P. Murphy, D. R. Seals, Chronic supplementation with a mitochondrial antioxidant (MitoQ) improves vascular function in healthy older adults, Hypertension 71 (2018), https://doi.org/10.1161/HYPERTENSIONAHA.117.10787.
[24] T. Pham, C.L. MacRae, S.C. Broome, R.F. D'souza, R. Narang, H.W. Wang, T. A. Mori, A.J.R. Hickey, C.J. Mitchell, T.L. Merry, MitoQ and CoQ10 supplementation mildly suppresses skeletal muscle mitochondrial hydrogen peroxide levels without impacting mitochondrial function in middle-aged men, Eur. J. Appl. Physiol. 120 (2020), https://doi.org/10.1007/s00421-020-04396-4.
[25] R.M. Atef, A.M. Agha, The Ying and Yang of adenosine a 1 and a 2A receptors on ERK1 / 2 activation in a rat model of global cerebral ischemia reperfusion injury, Mol. Neurobiol. (2017), https://doi.org/10.1007/s12035-017-0401-1.
[26] M.A. Saad, R.M. Abdel Salam, S.A. Kenawy, A.S. Attia, Pinocembrin attenuates hippocampal inflammation, oxidative perturbations and apoptosis in a rat model of global cerebral ischemia reperfusion, Pharmacol. Rep. 67 (2015) 115–122, https://doi.org/10.1016/j.pharep.2014.08.014.
[27] S.S. Abdel Mageed, R.M. Ammar, N.N. Nassar, H. Moawad, A.S. Kamel, Role of PI3K/Akt axis in mitigating hippocampal ischemia-reperfusion injury via CB1 receptor stimulation by paracetamol and FAAH inhibitor in rat, Neuropharmacology. 207 (2022), https://doi.org/10.1016/J.NEUROPHARM.2021.108935.
[28] A.A. Ibrahim, H.M. Karam, E.A. Shaaban, M.M. Safar, M.F. El-Yamany, MitoQ ameliorates testicular damage induced by gamma irradiation in rats: modulation of mitochondrial apoptosis and steroidogenesis, Life Sci. 232 (2019), https://doi.org/10.1016/j.lfs.2019.116655.
[29] J. Hao, W. Shen, G. Yu, H. Jia, X. Li, Z. Feng, Y. Wang, P. Weber, K. Wertz, E. Sharman, J. Liu, Hydroxytyrosol promotes mitochondrial biogenesis and mitochondrial function in 3T3-L1 adipocytes, J. Nutr. Biochem. 21 (2010), https://doi.org/10.1016/j.jnutbio.2009.03.012.
[30] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method, Methods 25 (2001) 402–408, https://doi.org/10.1006/meth.2001.1262.
[31] C.V.B. Connor Stonesifer, Sydney Corey, Shaila Ghanekar, Zachary Diamandis, A. Sandra, Acosta1, stem cell therapy for abrogating stroke-induced neuroinflammation and relevant secondary cell death mechanisms, Prog. Neurobiol. 158 (2017) 94–131, https://doi.org/10.1016/j.pneurobio.2017.07.004. Stem.
[32] H. Jęśko, Przemysław Wencel, P. Strosznajder Robert, J.B. Strosznajder, Sirtuins and their roles in brain aging and neurodegenerative disorders, Neurochem. Res. 42 (2017) 876–890, https://doi.org/10.1007/s11064-016-2110-y.
[33] W. Zhang, R. Wei, L. Zhang, Y. Tan, C. Qian, Sirtuin 6 protects the brain from cerebral ischemia/reperfusion injury through NRF2 activation, Neuroscience 366 (2017) 95–104, https://doi.org/10.1016/j.neuroscience.2017.09.035.
[34] L. Liberale, D.S. Gaul, A. Akhmedov, N.R. Bonetti, J. Weber, V. Nageswaran, S. Costantino, V. Fehr, D. Vdovenko, A. Semerano, G. Giacalone, G.A. Kullak-ublick, M. Sessa, U. Eriksson, F. Paneni, H. Beer, F. Ruschitzka, F. Montecucco, T. F. Lu, C.M. Matter, G.G. Camici, Endothelial SIRT6 Blunts Stroke Size and Neurological Deficit by Preserving Blood – Brain Barrier Integrity: A Translational Study, 2019, pp. 1–13, https://doi.org/10.1093/eurheartj/ehz712.
[35] W.Y. Wani, S. Gudup, A. Sunkaria, A. Bal, P.P. Singh, R.J.L. Kandimalla, D. R. Sharma, K.D. Gill, Protective efficacy of mitochondrial targeted antioxidant MitoQ against dichlorvos induced oxidative stress and cell death in rat brain, Neuropharmacology 61 (2011) 1193–1201, https://doi.org/10.1016/j.neuropharm.2011.07.008.
[36] L. Xiao, X. Xu, F. Zhang, M. Wang, Y. Xu, D. Tang, J. Wang, Y. Qin, Y. Liu, C. Tang, L. He, A. Greka, Z. Zhou, F. Liu, Z. Dong, L. Sun, The mitochondria-targeted antioxidant MitoQ ameliorated tubular injury mediated by mitophagy in diabetic kidney disease via Nrf2/PINK1, Redox Biol. 11 (2017) 297–311, https://doi.org/10.1016/j.redox.2016.12.022.
[37] L. Hao, Q. Sun, W. Zhong, W. Zhang, X. Sun, Z. Zhou, Mitochondria-targeted ubiquinone (MitoQ) enhances acetaldehyde clearance by reversing alcohol-induced posttranslational modification of aldehyde dehydrogenase 2: a molecular mechanism of protection against alcoholic liver disease, Redox Biol. 14 (2018) 626–636, https://doi.org/10.1016/j.redox.2017.11.005.
[38] J.C.R. Anne Stetler, Rehana K. Leak, Wei Yin, Lili Zhang, Suping Wang, Yanqin Gao, Mitochondrial biogenesis contributes to ischemic neuroprotection afforded by LPS preconditioning, J. Neurochem. 123 (2012) 125–137, https://doi.org/10.1111/j.1471-4159.2012.07951.x.
[39] L. Yang, Y.M. Ma, X.L. Shen, Y.C. Fan, J.Z. Zhang, P.A. Li, L. Jing, The involvement of mitochondrial biogenesis in selenium reduced hyperglycemia-aggravated cerebral ischemia injury, Neurochem. Res. 45 (2020) 1888–1901, https://doi.org/10.1007/s11064-020-03055-6.
[40] Y. Xi, D. Feng, K. Tao, R. Wang, Y. Shi, H. Qin, M.P. Murphy, Q. Yang, G. Zhao, MitoQ protects dopaminergic neurons in a 6-OHDA induced PD model by enhancing Mfn2-dependent mitochondrial fusion via activation of PGC-1α, Biochim. Biophys. Acta Mol. basis Dis. 2018 (1864) 2859–2870, https://doi.org/10.1016/j.bbadis.2018.05.018.
[41] X. Yin, M. Manczak, P.H. Reddy, Mitochondria-targeted molecules MitoQ and SS31 reduce mutant huntingtin-induced mitochondrial toxicity and synaptic damage in Huntington's disease, Hum. Mol. Genet. 25 (2016) 1739–1753, https://doi.org/10.1093/hmg/ddw045.
[42] R. Yue, X. Xia, J. Jiang, D. Yang, Y. Han, X. Chen, Y. Cai, L. Li, W.E. Wang, C. Zeng, Mitochondrial DNA oxidative damage contributes to cardiomyocyte ischemia/reperfusion-injury in rats: cardioprotective role of lycopene, J. Cell. Physiol. 230 (2015) 2128–2141, https://doi.org/10.1002/jcp.24941.
[43] C. Kang, L.L. Ji, PGC-1α overexpression via local transfection attenuates mitophagy pathway in muscle disuse atrophy, Free Radic. Biol. Med. 93 (2016) 32–40, https://doi.org/10.1016/j.freeradbiomed.2015.12.032.
[44] L. Li, L. Xiao, Y. Hou, Q. He, J. Zhu, Y. Li, J. Wu, J. Zhao, S. Yu, Y. Zhao, Sestrin2 silencing exacerbates cerebral ischemia/reperfusion injury by decreasing mitochondrial biogenesis through the AMPK/PGC-1α pathway in rats, Sci. Rep. 6 (2016) 1–11, https://doi.org/10.1038/srep30272.
[45] Y. Xie, J. Li, G. Fan, S. Qi, B. Li, Reperfusion promotes mitochondrial biogenesis following focal cerebral ischemia in rats, PLoS One 9 (2014) 1–12, https://doi.org/10.1371/journal.pone.0092443.
[46] L.M. Yu, X. Dong, X.D. Xue, S. Xu, X. Zhang, Y.L. Xu, Z.S. Wang, Y. Wang, H. Gao, Y.X. Liang, Y. Yang, H.S. Wang, Melatonin attenuates diabetic cardiomyopathy and reduces myocardial vulnerability to ischemia-reperfusion injury by improving mitochondrial quality control: role of SIRT6, J. Pineal Res. 70 (2021) 1–21, https://doi.org/10.1111/jpi.12698.
[47] J. Jeong, J. Koo, J.S. Yook, J. Cho, E. Kang, Neuroprotective benefits of exercise and MitoQ on memory function, mitochondrial dynamics, oxidative stress, and neuroinflammation in D-galactose-induced aging rats, Brain Sci. 11 (2021) 1–15, https://doi.org/10.3390/brainsci11020164.
[48] W. Chen, C. Guo, S. Huang, Z. Jia, J. Wang, J. Zhong, H. Ge, J. Yuan, T. Chen, X. Liu, R. Hu, Y. Yin, H. Feng, MitoQ attenuates brain damage by polarizing microglia towards the M2 phenotype through inhibition of the NLRP3 in fl ammasome after ICH, Pharmacol. Res. 161 (2020), 105122, https://doi.org/10.1016/j.phrs.2020.105122.
[49] Z. Gong, J. Pan, Q. Shen, M. Li, Y. Peng, Mitochondrial dysfunction induces NLRP3 inflammasome activation during cerebral ischemia/reperfusion injury, J. Neuroinflammation 15 (2018) 1–17, https://doi.org/10.1186/s12974-018-1282-6.
[50] J.H. Koo, H.Y. Jang, Y. Lee, Y.J. Moon, E.J. Bae, S.K. Yun, B.H. Park, Myeloid cell-specific sirtuin 6 deficiency delays wound healing in mice by modulating inflammation and macrophage phenotypes, Exp. Mol. Med. 51 (2019), https://doi.org/10.1038/s12276-019-0248-9.
[51] H. Jiang, S. Khan, Y. Wang, G. Charron, B. He, C. Sebastian, J. Du, R. Kim, E. Ge, R. Mostoslavsky, H.C. Hang, Q. Hao, H. Lin, SIRT6 regulates TNF-α secretion through hydrolysis of long-chain fatty acyl lysine, Nature 496 (2013) 110–113, https://doi.org/10.1038/nature12038.