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Chrysin attenuates global cerebral ischemic reperfusion injury via suppression of oxidative stress, inflammation and apoptosis

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ABSTRACT

Global cerebral ischemia is a leading cause of mortality worldwide. Several biomechanisms play a role in the pathology of cerebral ischemia reperfusion damage, such as oxidative stress, inflammation, apoptosis and excitotoxicity. Chrysin, a natural flavonoid with many important biological activities, was investigated in the present study for its possible neuroprotective properties in a rat model of global ischemia reperfusion. Male Wistar rats were allocated into three groups: sham-operated, ischemia/reperfusion, and chrysin (30 mg/kg) groups. All animals were subjected to ischemia for 15 min followed by reperfusion for 60 min, except for the sham-operated group. Rats were decapitated, then both hippocampi were rapidly excised to evaluate several biomarkers that reflect ischemic injury. The obtained results showed that pre-treatment with chrysin attenuated ischemia-induced oxidative stress by: (i) restoring the glutathione level; and (ii) depressing the levels/activities of thiobarbituric acid reactive substances, the hippocampal NADPH, as well as the xanthine oxidase. Exposure to chrysin also suppressed the inflammation accompanying the ischemia/reperfusion (I/R) damage, through increasing the interleukin-10 level, while decreasing the levels of both interleukin-6 and tumour necrosis factor-alpha. Moreover, an increase in Bcl2 and a decrease in both BAX and Hsp90 levels were recorded following chrysin exposure, which was also accompanied with elevated glutamate and aspartate levels. In conclusion, chrysin has demonstrated an anti-ischemic potential, through attenuation of the mechanisms underlying I/R injury. These data add to the knowledge on the significance of natural flavonoids as neuroprotective agents.

1. Introduction

Cerebral ischemia, also called stroke, is one of the leading causes of mortality worldwide [1]. This cerebrovascular disease occurs at the brain secondary to a pathological disorder of blood vessels (usually of arterial origin) or blood supply [2]. While a number of aetiological factors may contribute to this condition, the most common of these factors include vascular occlusion by thrombi or emboli, loss of vascular integrity (either due to vessel wall rupture or to a disease), and disturbance of normal blood physiology [3].

While stroke can be classified according to the cause of injury into hemorrhage and ischemia [4], ischemic stroke can be further subdivided into global and focal ischemia [5]. Elucidation of the underlying mechanisms of each of these subsets has been aided by the availability of several animal models [6]. In global cerebral ischemia, there is no cerebral blood flow to any area of the brain, which causes neuronal damage that first starts with injuryprone brain areas, but eventually all neurons would die if global ischemia continued indefinitely. On the other hand, incomplete global ischemia model is characterized by a more simple surgical preparation, readily accomplished reperfusion, and suitability for chronic survival studies [7].

The brain injury following cerebral ischemia emerges from a complicated sequence of events that develop in spatiotemporal pattern to a certain extent. In this regard, excitotoxicity events that develop in the earlier stages could contribute to a rapid necrotic cell death that induces the infarction core. At this stage, the ischemic penumbra that

Abbreviations: ANOVA, analysis of variance; BAX, the Bcl2-associated X protein; BBB, blood brain barrier; Bcl2, B-cell lymphoma 2 protein; CCAs, common carotid arteries; COX-2, cyclooxygenase-2; GSH, reduced glutathione; Hsp90, heat shock protein 90; ICAM-1, intercellular adhesion molecule-1; IL, interleukin; iNOS, inducible nitric oxide synthase; ip, intraperitoneal; I/R, ischemia/reperfusion; MAPK, mitogen-activated protein kinase; MDA, malondialdehye; MHC, major histocompatibility complex; NADPH, nicotinamide adenine dinucleotide phosphate; NF-IL-6, nuclear factor for interleukin-6; NO, nitric oxide; OD, optical density; *P*, probability values; PI3K, phosphatidylinositol 3-kinase; PPAR-γ, peroxisome proliferator-activated receptor-γ; po, per oral; ROS, reactive oxygen species; SEM, standard error of the mean; SO, sham-operated; TBARS, thiobarbituric acid reactive substances; TNF-α, necrosis factor-alpha; XO, xanthine oxidase

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envelops the infarct core is prone to encounter milder insults. In such region, mild inflammatory and excitotoxic mechanisms contribute to delayed cell death, which indicates the apoptosis and the associated biochemical characteristics [8]. These deleterious events mark the challenging situations encountered by the brain cells. Because of this, brain cells stimulate innate protective brain programs that could in turn be investigated by ischemic preconditioning. Therefore, cerebral ischemia develops into such a state where extracranial systems and the brain parenchyma get affected. Hence, it appears that a complex signaling cascade plays an influential role in determining the cell survival, neurological deficit, and the mortality following the stroke [8].

The hippocampus, which plays vital roles in learning and memory, is selectively susceptible to global ischemic insults. Distinct populations of hippocampal neurons are targeted by ischemia and multiple factors, including excitotoxicity, oxidative stress, and inflammation, are accountable for their damage and death [9]. Studies propose that the hippocampus is a central site of pathological alteration in cerebral ischemia, and that injury limited to the hippocampus is sufficient to impair memory. Histological examination of memory impairment associated with bilateral damage revealed a confined bilateral lesion involving the entire CA1 field of the hippocampus. Minimal pathology was detected elsewhere, and that the damage that could merely be associated with the memory deficit was in the hippocampus [10,11].

Chrysin, a flavonoid extracted mainly from honey and bee propolis [12], is known to be associated with several biological activities. For example, it has been shown to possess: (i) antiallergic properties through the reduction of serum histamine production; (ii) antiinflammatory activity, by controlling the exchange of macrophage phenotype (M1/M2) through the peroxisome proliferator-activated receptor-γ (PPAR-γ) network [13]; (iii) an HIV inhibitory function, as well as its antihypertensive and anti-cancer potentials [14]; an antihypertensive effect, by downregulating the cyclooxygenase-2 (COX-2) mRNA and protein expression via the suppression of DNA binding activity of nuclear factor for interleukin-6 (NF-IL-6) [15]; (iv) sex driveenhancing agent, capable of promoting virility and enhancing sexual stamina [16].

Because of these diverse biological activities, the current study aimed to investigate the possible neuroprotective potential of chrysin in global cerebral ischemia-exposed rats, as well as the role of oxidative stress biomarkers, inflammatory mediators, apoptotic mediators and neurotransmitters, if any.

While a number of studies have reported the effects of chrysin on cerebral ischemia [17,18], yet, the therapeutic potential of chrysin in I/R injury has not been explored. Thus, we attempt to address this gap by specifically examining the neuroprotective capacity of chrysin pretreatment on global cerebral ischemia induced by bilateral carotid artery occlusion in a rat model. While the main methodological differences between the current study and previous work are summarized in Table 2, still no clear conclusion can be reached from these studies concerning the anti-ischemic efficacy of this drug in humans. This highlights the need for further studies to assess the mechanisms underlying the neuroprotective effects of chrysin and its dosage requirements.

2. Material and methods

2.1. Chemicals

Chrysin, purchased from Alfa Aesar (Lancashire, United Kingdom), was freshly suspended before each experiment in 1% tween 80 solution in water. This suspension was administered to rats via oral route in a dose of 30 mg/kg, which was selected based on a previous related study [19].

2.2. Animals and experimental design

A total of 45 male Wistar rats, each weighing 200–250 g, were purchased from the Egyptian Organization for Biological Products and Vaccines (VACSERA; Cairo, Egypt), and housed in the animal house of Modern Sciences and Arts University (MSA), Egypt. The animals were fed on standard pellet chow (El-Nasr chemical company, Cairo, Egypt) and allowed free access to water. They were kept under controlled environmental conditions as humidity (50 ± 10%) and temperature (25 ± 3 °C), maintained on a 12/12 h dark cycle, and were acclimatized to the environment for one week prior to experiment start-up.

Animals were divided randomly to three groups (each contained 15 rats) as follows: group 1: sham-operated (SO), group 2: ischemia/reperfusion (I/R) group, and group 3: chrysin-treated. While animals in the first two groups received the vehicle (1% tween 80) per oral (po) once daily for 14 days, each rat in the chrysin group was given chrysin (30 mg/kg, po) for the same time period (14 consecutive days). For induction of ischemia, rats from all groups were anesthetized on the 15th day (after 24 h from the last oral dose) of the experiment by intraperitoneal (ip) injection of thiopental (30 mg/kg), then the animal's head was fixed, and both common carotid arteries (CCAs) were exposed through a midline ventral incision in the neck [20]. After carefully separating the CCAs from adjacent tissues, both CCAs were occluded with hemostatic clips for 15 min. Following clips removal, reperfusion was permitted for one hour [21]. This procedure was applied for all the tested rats, except in the sham-operated animals, in which arterial occlusion was not performed. After this 1-hour period, animals were sacrificed by cervical decapitation under light ether anesthesia. The brains were subsequently removed and washed with ice-cold saline, then dried between two filter papers. Afterwards, both hippocampi were dissected and homogenized in ice-cold saline for further assessment of multiple hippocampal biomarkers, as detailed below.

It is important to highlight that all animal experiments were carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, Egypt, and to the guidelines of the Ethics Committee, Faculty of Pharmacy, Cairo University (Serial No: PT-924, 2013). All surgeries were performed under thiopental anesthesia and all efforts were made to minimize animal suffering.

2.3. Biochemical estimations

2.3.1. Estimation of hippocampal oxidative stress biomarkers

The assessed oxidative stress biomarkers included the reduced glutathione (GSH), thiobarbituric acid reactive substances (TBARS), xanthine oxidase (XO), and nicotinamide adenine dinucleotide phosphate (NADPH). The levels of GSH were measured spectrophotometrically at 412 nm using the colorimetric Glutathione Assay Kit [20,21]. The levels of TBARS were assessed based on the reactivity of thiobarbituric acid toward malondialdehye (MDA) to yield 1:2 MDA-TBA adduct, which was measured colorimetrically at 535 nm [22]. Meanwhile, XO assay was conducted based on the principle that XO oxidizes xanthine to hydrogen peroxide, which reacts with 4aminoantipyrine to generate a color. The color intensity, which was proportional to XO content, was measured at 550 nm [22,23]. The NADPH assay applied involved doubleantibody sandwich technique [23], in which the concentration of the NADPH was positively correlated with the intensity of the formed color, which was measured at 450 nm.

2.3.2. Measurement of hippocampal inflammatory mediators

Tumor necrosis factor- alpha (TNF-α) was determined using an ELISA kit in which monoclonal antibody specific for rat TNF-α was used [19]. The intensity of colour is directly proportional to the concentration of TNF-α present in the samples. As described by Ida et al. [20], a standard sandwich ELISA has been used to measure rat interleukin-6 (IL-6). The intensity of the yellow colour produced is proportional to

the concentration of IL-6. Measurment of interleukin-10 (IL-10) was done according to the method described by Mosmann et al. [21], rat IL-10 ELISA Kit is based on standard sandwich enzyme-linked immunesorbent assay technology. The intensity of yellow colour is proportional to the amount of IL-10 in the sample, measured at 450 nm.

2.3.3. Determination of hippocampal apoptotic mediators

The apoptosis or apoptotic-related biomarkers investigated in the current study included the B-cell lymphoma 2 (Bcl2) protein, the Bcl2-associated X protein (BAX), and the heat shock protein 90 (Hsp90). Estimation of the Bcl2 protein involved a double-antibody sandwich technique [24]. The yellow color produced, was read at 450 nm, which was proportional to Bcl2 concentration. Furthermore, BAX was estimated according to Nass et al. [25], using an immunoassay kit that engages a polyclonal antibody specific for BAX. The color produced was read at 450 nm and was proportional to BAX concentration. As described by Moineau et al. [26], a standard sandwich ELISA has been used in the current study to measure the levels of heat shock protein 90 (Hsp90).

2.3.4. Measurement of hippocampal excitatory neurotransmitters

The excitatory neurotransmitters studied in the present study included the aspartate and the glutamate. Starting with the aspartate, the levels of this transmitter were determined in accordance to Sundaram et al. [27]. Briefly, aspartate was first converted to pyruvate, which was then subjected to an oxidation–reduction step that generated a highly colored product (that can be measured at 570 nm) proportional to the amount of aspartate present. Moving to the glutamate, its concentration was measured according to the method of Danninger et al. [28]. Briefly, glutamate dehydrogenase catalyzed a reaction leading to the formation of NADH, which subsequently reduced a tetrazolium dye (MTT) reagent. The color intensity of the formed product, measured at 565 nm, was proportional to the glutamate concentration.

2.4. Statistical analyses

Significant differences among the subgroups were determined by univariate analysis of variance (ANOVA), followed by Tukey Kramer's test for multiple comparisons, whenever appropriate. For optical density (OD) measurements, samples were run in triplicates and the data were expressed as means ± standard error of the mean (SEM). All statistical analyses were performed using the Statistical Package for Social Science (SPSS) program, version 16.0 (SPSS Inc, Chicago, IL, USA), with probability (P) values of < 0.05 as the level of significance.

3. Results

3.1. Effect of chrysin on hippocampal oxidative stress biomarkers

Rats subjected to I/R showed a marked reduction in hippocampal GSH content to 50% of that in the SO group, while these rats demonstrated an increase in TBARS and NADPH contents to 144% and 243% of the values in the SO group, respectively. Moreover, XO activity was shown to be increased by 2.5-fold compared to the SO group.

Pre-treatment with chrysin seem to have protected the rats against oxidative stress following I/R, as observed by the elevation of GSH level to 235.76% of that in the I/R group, as well as the reduction in TBARS, NADPH and XO to 60.59%, 36.92% and 34.27% of the values in the I/R group, respectively (Table 1).

3.2. Effect of chrysin on hippocampal inflammatory mediators

Exposure to I/R was coupled with an acute inflammation, manifested as an increase in TNF-α and IL-6 contents to 145% and 162% of the values in the SO group, respectively. This elevation in pro-inflammatory mediators was shown to be associated with a decrease in the level of the anti-inflammatory cytokine IL-10, to 43% of that in the SO group.

The anti-inflammatory potential of chrysin was noted through reduction of the pro-inflammatory cytokines TNF-α (Fig. 1a) and IL-6 (Fig. 1b) to 60.31% and 48.72% of the values in the I/R group, respectively, together with the elevation of IL-10 level to 257.19% of that in the I/R group (Fig. 1c).

3.3. Effect of chrysin on I/R-induced hippocampal apoptosis

Following exposure to I/R, we detected a reduction in Bcl2 level accompanied with an increase in both BAX and Hsp90 contents. Following ischemia and reperfusion, hippocampal Bcl2, BAX and Hsp90 were 47%, 205% and 209% of those in the SO group, respectively.

Pre-administration of chrysin caused an increase in Bcl2 level (Fig. 2a) to almost 2.5 folds of that in I/R group (Fig. 2). Furthermore, a decline in BAX (Fig. 2b) and Hsp90 (Fig. 2c) contents was also prominent in the chrysin-treated group, in which they reached 37.51% and 38.29% of the values in the I/R group.

3.4. Effect of chrysin on hippocampal excitatory neurotransmitters

A marked decline in both aspartate and glutamate contents occurred following I/R exposure, in which they were reduced to 47% and 67% of those in the SO group, respectively. On the other hand, pre-treatment with chrysin resulted in an increase in aspartate (Fig. 3a) and glutamate (Fig. 3b) contents to 194.76% and 160.79% of those in the I/R group, respectively.

4. Discussion

Cerebral ischemia reperfusion is a multifactor injury, in which oxidative stress, inflammation, apoptosis, and excitotoxicity play major roles. Following I/R exposure, we noticed a decrease in GSH content, which is in agreement with previous studies [29–31]. Song et al. [32] suggested that GSH is involved in: (i) attenuation of cerebral infarct volume; (ii) maintenance of blood brain barrier (BBB) disruption after ischemic injury; and (iii) improvement of the survival of brain endothelial cells by promoting the phosphatidylinositol 3-kinase (PI3K)/Akt pathway. The increase in NADPH levels observed in the current study following I/R exposure is in harmony with former studies [33,34]. These elevated NADPH oxidase levels have been previously shown to be associated with the generation of oxygen radicals and consequently, the direct oxidative injury to the brain, in addition to exerting their excitotoxicity and inflammatory effects [35]. Furthermore, the observed increase in XO activity following I/R injury has been previously reported [29]. More specifically, XO has been previously shown to be phosphorylated in ischemic microvascular endothelial cells through a mechanism involving p38 MAPK (mitogen-activated protein kinase) signaling pathway, which is a stressactivated PK that has been shown to be mostly associated with reperfusion injury and casein kinase II. This phosphorylation appears to be necessary for ischemia-induced enzyme activation [36]. Moreover, the exacerbated TBARS levels detected after I/R exposure are in agreement with earlier studies [29,30,37]. An imbalance between TBARS levels and antioxidant defense systems is expected to play an important role in ischemic stroke injury [17].

Ischemic injury is known to be accompanied with inflammation, which is arising from an inequity between antiinflammatory and proinflammatory mediators. The decrease in IL-10 level observed in the current study has been described previously in several studies [38,39]. The anti-inflammatory properties of IL-10 may be mediated through attenuating the potency of mononuclear phagocytes to elicit inflammatory responses, by inhibiting the secretion of many inflammatory cytokines and chemokines, or by opposing the effects of TNF-α [40,41]. In line with previous studies [31,42], TNF-α

Table 1
Effect of chrysin on hippocampal oxidative stress biomarkers in global cerebral ischemia/reperfusion induced in rats.

Groups	GSH	TBARS	XO	NADPH
Sham-operated (SO) group	1.52 ± 0.02	16.43 ± 0.35	9.86 ± 0.09	11.69 ± 0.25
Ischemia/reperfusion (I/R) group	0.76 ± 0.03*	23.60 ± 0.53*	24.26 ± 0.97*	28.56 ± 0.61*
Chrysin (30 mg/kg, po) group	1.78 ± 0.03*#	14.30 ± 0.21*#	8.31 ± 0.21#	10.54 ± 0.18#

Statistical analysis was performed using the one-way analysis of variance (ANOVA), followed by Tukey-Kramer's multiple comparison test. Values are expressed as mean ± SEM (n = 8–10, after omitting outliers).
* Significant difference from the sham-operated group at $P < 0.05$.
# Significant difference from the I/R group at $P < 0.05$.

hippocampal content was found to be elevated in the current investigation. TNF-α has been shown to exert its neurotoxic effects by inducing apoptotic neuronal cell death and enhancing the expression of major histocompatibility complex (MHC) class II molecules and intercellular adhesion molecule-1 (ICAM-1) in astrocytes, leading to leukocyte infiltration and BBB breakdown. Blockade of TNF-α receptors has been previously shown to reduce brain infarct volume and cerebral edema after transient focal ischemia in rats [43]. IL-6 is particularly remarkable because it is an interleukin that, on one hand, acts as a proinflammatory cytokine and, on the other hand, as an anti-inflammatory myokine [44]. Cerebral overexpression of IL-6 in astrocytes, or the systemic administration of IL-6 in combination with its soluble receptor sIL6Rα has been shown leading to neurodegeneration, gliosis, microglial activation, and vascular proliferation [45].

Reperfusion has been reported to boost nitric oxide (NO) production, while the apoptosis likely to be induced by these relatively high levels of NO has been shown to be accompanied by down-regulation of Bcl2, which, in turn, has been shown to be linked to the release of apoptotic factors such as cytochrome c from mitochondria into the cytosol [46]. The observed increase in the ratio of BAX/Bcl2 expression after ischemia reperfusion could be related to reactive oxygen species (ROS) production, which is reflected as an increase in MDA level, and a decrease in antioxidant enzyme activities, including superoxide dismutase and catalase [47]. Phosphorylation of Hsps has been shown to occur rapidly in response to a variety of stresses, including hydrogen peroxide and other oxidants, and the phosphorylation status of these proteins appears to be related to their capacity to interact with different apoptotic stimuli [48]. Our current results demonstrated that the aspartate and glutamate levels were decreased following I/R exposure as compared to the shamoperated group. This result is in agreement with those reported by several studies [49–51], but in contrast to some other [52–54]. In the present study, the decrease in glutamate and aspartate levels may be due to the short duration of ischemia. It is postulated that if the duration of severe ischemia is short enough (5–20 min), cell death tends to be delayed and is selective for neurons, whereas longer durations lead to broader and more rapid cellular destruction, resulting in infarct in the core of focal ischemia or patient death for global ischemia [55]. The concentrations of glutamate and aspartate returns to baseline levels soon after reperfusion [50].

In the present study, chrysin was examined for its anti-oxidant, anti-inflammatory, antiapoptotic effects, and its effect on excitatory neurotransmitters. According to He et al. [19], the cognitive deficits in rats subjected to bilateral common carotid arteries occlusion have been markedly ameliorated by treatment with 30 mg/kg chrysin, with no significant difference from the results obtained when the drug was administered at a dose of 100 mg/kg. Keeping in mind the possible toxicity of the high doses, the authors emphasized that a successful treatment of ischemia may not always require a high dosage of the drug. These findings support the need for further studies to assess the optimal dosing regimens of putative neuroprotective drugs, including the intervals between administrations, from preclinical trial data.

In another study, Kilic et al. [56] found that pretreatment with chrysin (50 mg/kg) can reverse GSH depletion following lung injury. The ability of chrysin to prevent depletion of lung glutathione stores in the bleomycin-induced lung fibrosis model was mediated, at least in part, by its antioxidant features. Chrysin may save intracellular reduced GSH by acting as ROS scavenger. In parallel with findings of a previous study, it was established that chrysin prevented NADPH-induced rise in mesenteric vascular bed by about 42% [57]. Results suggest that the functional inhibition of NADPH oxidase activity by flavonols is mainly a result of scavenging of superoxide by these compounds [58]. In the current study, chrysin inhibited the activity of XO, which is consistent with previous studies [59–61]. Chrysin interacts with several residues located within the active cavity of XO. The binding induces conformational changes of the enzyme [59]. Several studies conducted to test the efficacy of chrysin indicated a reduction in TBARS levels when compared to the I/R group [17,62]. It was reflected that prolonged exposure to chrysin could result in a decrease in MDA level, and in return decreasing TBARS levels, supporting the result of the current study.

Table 2
Comparison of the methodological differences between the current study and previous studies that have examined the anti-ischemic potential of chrysin on cerebral ischemia.

Experimental set-up	Current study	Yao *et al.* [18]	Durak *et al.* [17]
Hypoxic/ischaemic stimulus	Bilateral carotid artery occlusion	Middle cerebral artery occlusion	Bilateral carotid artery occlusion
Type of I/R	Global	Focal	Global
Model	Rat	Mice	Mice
Ischaemia duration	15 min	1 h	15 min on the 1st day of the experiment
Reperfusion duration	1 h	24 h	
Chrysin			
Dose	30 mg/kg	75 or 100 mg/kg	50 mg/kg
Administered as	Pre-ischemia treatment for 14 days	Pre-ischemia treatment for 7 days	Post-ischemia treatment for 10 days
Parameters assessed	TBARS, GSH, NADPH, XO, IL-6, IL-10, TNF-α Glutamate, aspartate, Hsp90, Bcl2 and BAX	Glial cell numbers, proinflammatory cytokines, nuclear factor-kappa B (NF-κB), COX-2, and iNOS.	TBARS, reduced GSH, catalase, superoxide dismutase, and glutathione peroxidase

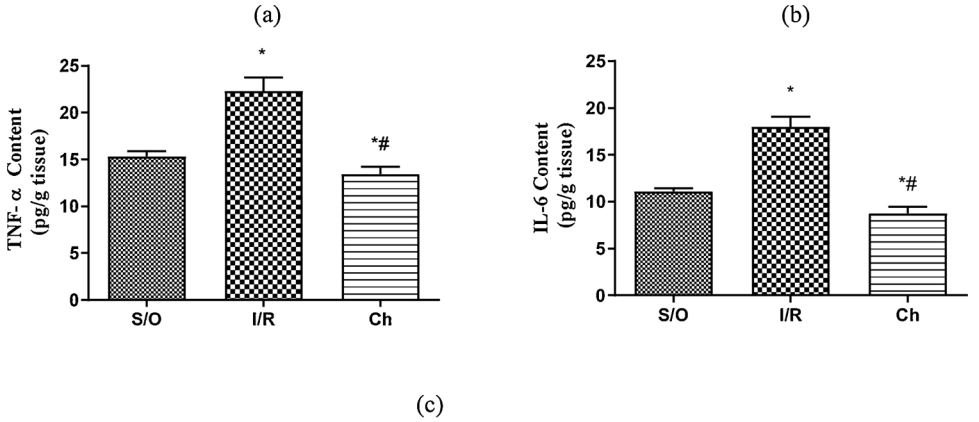

Fig. 1. Effect of chrysin on hippocampal levels of TNF-α (a), IL-6 (b), and IL-10 (c) in a rat model of global cerebral ischemia/reperfusion. Statistical analysis was performed using the univariate ANOVA, followed by Tukey-Kramer's multiple comparison test. All values are expressed as mean ± SEM (n = 8–10, after omitting outliers).
* Significant difference from the sham-operated group at $P < 0.05$.
# Significant difference from the I/R group at $P < 0.05$.

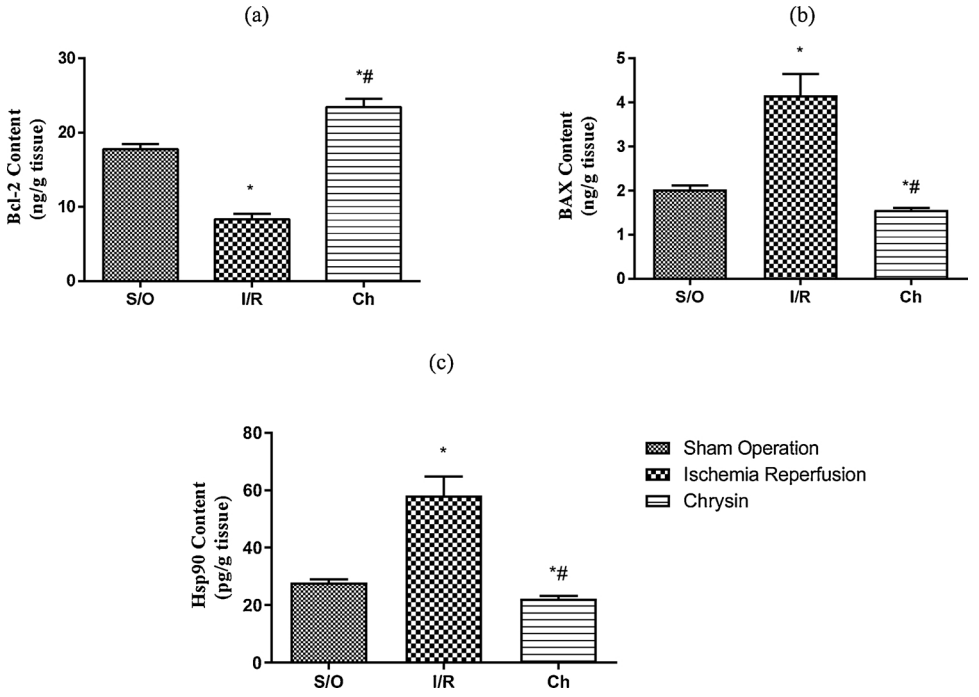

Fig. 2. Effect of chrysin on hippocampal levels of Bcl2 (a), BAX (b) and Hsp90 (c) in a rat model of global cerebral ischemia/reperfusion. Statistical analysis was performed using the univariate ANOVA, followed by Tukey-Kramer's multiple comparison test. All values are expressed as mean ± SEM (n = 8–10, after omitting outliers).
* Significant difference from the sham-operated group at $P < 0.05$.

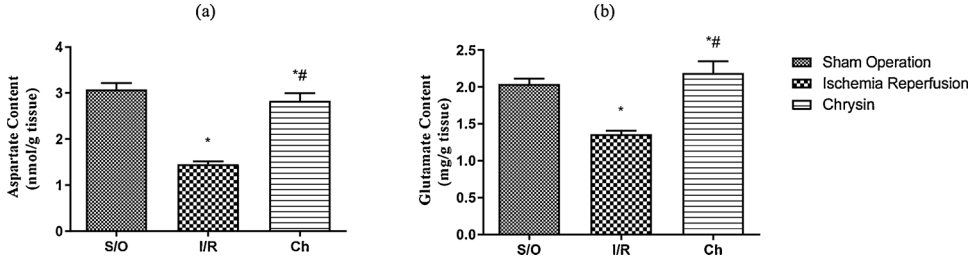

Fig. 3. Effect of chrysin on hippocampal levels of aspartate (a) and glutamate (b) in a rat model of global cerebral ischemia/reperfusion. Statistical analysis was performed using the univariate ANOVA, followed by TukeyKramer's multiple comparison test. All values are expressed as mean ± SEM (n = 8–10, after omitting outliers).
* Significant difference from the sham-operated group at $P < 0.05$.
# Significant difference from the I/R group at $P < 0.05$.

Results of the current study illustrated an increase in IL-10 levels against the I/R group. Through regulating macrophage polarization, chrysin increases the anti-inflammatory factor IL-10 (M2 marker gene), which is involved in the anti-inflammation, repair or remodeling of tissues [13]. The decrease in IL-6 observed in the present work is in line with previous studies [63–65]. It was believed that chrysin exerted its protective effects against spinal cord impairment via suppressing inducible nitric oxide synthase (iNOS) and concomitantly decreasing NO bioavailability, thus decreasing IL6 levels [64]. In the present study, a decrease in TNF-α levels was observed. This is in harmony with former studies [18,64,66]. It was observed that chrysin treatment suppressed TNF-α induced adhesion molecule expression [67].

Chrysin has a potent protective effect against doxorubicin-induced acute cardiotoxicity in rats via suppressing oxidative stress, inflammation, and apoptotic tissue damage, by decreasing BAX and cytochrome c expressions and caspase-3 activity while increasing the expression of Bcl2 [68]. The level of excitatory neurotransmitters, aspartate and glutamate, was increased through the administration of chrysin in comparison to the I/R group. Considerable evidence shows that flavonoids have specific effects on the glutamate system and that this contributes to their ability to reduce excitotoxicity. Some reports indicate that they interact with the dopamine transporter [69,70].

The underlying mechanisms involved in I/R insult are various and include oxidative stress, inflammation, apoptosis, and excitotoxicity. Chrysin demonstrated a potential for ischemic control, through attenuation of oxidative stress, inflammation, apoptosis, and neuroexcitatory effects, therefore adding to the knowledge on the significance of natural flavonoids as neuroprotective agents. Further studies are needed to investigate the mechanisms underlying the anti-ischemic effects of chrysin and its potential application in the clinical setting.

Author contributions

I.K., R.A., A.A. and A.E. planned and designed the study. I.K. performed the experiments, carried out the statistical analysis and wrote early draft manuscripts and contributed to data interpretation. All authors read and approved the final manuscript.

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The authors declare no conflict of interest.

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