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Chrysin prevents cognitive and hippocampal long-term potentiation deficits and inflammation in rat with cerebral hypoperfusion and reperfusion injury

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ABSTRACT

Introduction: Ischemic stroke is one of the leading causes of death worldwide, and extensive efforts have focused on the neuroprotective strategies to minimize complications due to ischemia. This study aimed to examine neuroprotective potential of chrysin, as a natural potent antioxidative and anti-inflammatory agent in an animal model of bilateral common carotid artery occlusion and reperfusion (BCCAO/R).

Methods: Adult male Wistar rats (250–300 g) were randomly divided into 6 groups and submitted to either sham surgery or BCCAO/R after pretreatment with chrysin (10, 30 and 100 mg/kg, once daily, for 21 consecutive days) or saline containing %5 DMSO. To make the animal model of BCCAO/R, bilateral common carotid arteries were occluded for 20 min, followed by reperfusion. Subsequently, spatial cognitive performance was evaluated in a Morris water maze (MWM), hippocampal long-term potentiation (LTP) was recorded from hippocampal dentate gyrus region, after then the hippocampal tissue content of IL-1β and TNF-α were assayed using ELISA kits.

Results: The results showed that pretreatment with chrysin significantly prevented BCCAO/R-induced cognitive and hippocampal LTP impairments ($p < 0.001$). Additionally, BCCAO/R- induced elevation in hippocampal content of IL-1β and TNF-α significantly ($p < 0.01$, $p < 0.01$ respectively) while pre-treatment with chrysin restored them ($p < 0.01$).

Conclusion: Our data confirm that chrysin could prevent brain inflammation and thereby prevents cognitive and LTP impairments due to cerebral ischemia. So it could be a promising neuroprotective agent against cerebrovascular insufficiency states.

1. Introduction

Cerebral ischemia is one of the leading causes of physical disability and death worldwide [1,2], which results in insufficient oxygen and glucose delivery to support cellular homeostasis followed by oxidative stress, inflammation and cell death in the brain tissue. Cerebral ischemia followed by reperfusion is known to cause learning and memory impairments in the animal model of ischemic stroke [3].

It was reported that synaptic plasticity and long term potentiation (LTP) are disrupted by cerebral ischemia which changes cognition ability [4,5]. Ischemia induces electrophysiological changes in pyramidal cells following hypoxic conditions [6–8]. Recent studies have demonstrated that global cerebral ischemia resulted in apoptotic cell death in the hippocampus [9], which closely correlated with ischemia-induced cognitive impairments [10].

Previous studies have demonstrated that cerebral hypoperfusion induces inflammatory processes [11], and increase brain damage and poor outcome in stroke patients [12]. It was reported that inflammation contributes to cognitive impairments, and has a strong association with brain tissue injuries following cerebral hypoperfusion [13,14]. Inflammation is a key factor in the pathobiology of cerebral ischemia [15–17], and caused by inflammatory cytokines such as interleukin 1-beta (IL-1β), interleukin-6 (IL-6) and alpha tumor necrosis factor (TNF-α) [18–20].

Recently, a number of natural flavonoids exert innumerable pharmacological effects in humans [21]. Chrysin (5,7-dihydroxyflavone) is a

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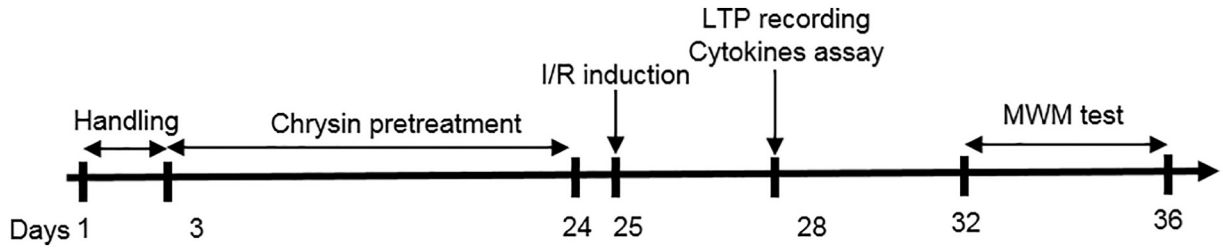

Fig. 1. Schematic diagram of the experimental schedule.

natural flavonoid found largely in honey and propolis [22,23], which has several biological activities including anti-inflammatory, anti-oxidative and vasorelaxant effects [24,25]. It was shown that chrysin could inhibit expression of pro-inflammatory enzymes such as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (cox2) [26]. Moreover, chrysin could protect neurons from oxidative insults and apoptosis [27,28].

Reconstruction of the pathophysiological condition in the animal model is essential to understand mechanisms that underlie neuronal injuries. Therefore, two-vessel occlusion (2VO) also known as bilateral common carotid arteries occlusion (BCCAO) is a well-documented ischemic model that used to investigate mechanisms that link hypoperfusion condition to neurodegenerative processes [29,30].

Given that BCCAO/R is known to cause hippocampal electrical activity deficits and cognitive impairments [31], we decided to examine the efficacy of chrysin pretreatment on the cognitive deficits, LTP impairments and hippocampal inflammation induced by BCCAO/R in adult male rats. Therefore, in the present study spatial cognitive function was assessed by using Morris water maze (MWM) test, and hippocampal LTP was evaluated by electrophysiological procedures. In addition, hippocampal inflammation was evaluated using ELISA kits for pro- and anti-inflammatory cytokines such as TNF-α and IL-1β respectively.

2. Materials and methods

2.1. Animals

Seventy-two adult male Wistar rats (six months old, weighing 250–300 g, at beginning of experiments) were single-housed at 22 ± 2 °C, with a 12-h light/dark cycle, and were allowed free access to a standard rodent pellet diet and drinking tap water ad libitum. All experiments were performed according to the institutional guidelines of the Experimental Animal Ethics Committee of the Jundishapur University of Medical Sciences (Ahvaz, Iran) and in accordance with the principles outlined in the NIH Guide for the Care and Use of Laboratory Animals (with ethics code IR.AJUMS.REC.1395.680). Pretreatment with different doses did not alter the mortality rate during the ischemic surgery (mortality rate was 30% in all ischemia groups).

2.2. 2VO ischemia/reperfusion model induction

Surgical procedure for BCCAO/R (cerebral 2VO-I/R) induction was adapted from previous studies [3], and the surgery was conducted in all rats between 9:00 and 17 P.M. After 21 consecutive days of pretreatment with different doses of chrysin (Sigma Chemical Co., USA) or saline containing %5 DMSO in saline (gavage, once daily), rats were anesthetized using ketamine-xylazine (90/10 mg/kg, i.p.). Following anesthesia, a ventral midline cervical incision was performed, and BCCAs were identified and separated from the adjacent vagus nerves. In order to induction the hypoperfusion, BCCAs were occluded using microvascular clips for 20 min, after then the clamps were removed to allow reperfusion. Subsequently, assessments of different behavioral, electrophysiological and inflammatory parameters were done 3 days and one week after I/R induction, respectively. Sham-operated surgery was conducted to show the effects of anesthesia and surgical manipulation on the results and involved exposure of BCCA without any occlusion.

2.3. Experimental design

Animals were divided in simple random order into six groups with 12 in each; 1) Veh + Sham; sham-operated group received DMSO 5% in saline as vehicle 2) Veh + I/R; BCCAO/reperfusion group received vehicle 3) CH10 + I/R; I/R rats received chrysin (10 mg/kg, gavage) as pretreated; 4) CH30 + I/R; I/R rats received chrysin (30 mg/kg, gavage) as pretreated 5) CH100 + I/R; I/R rats received chrysin (100 mg/kg, gavage) a pretreated 6) CH30 + Sham; healthy rats received the most effective dose of chrysin (30 mg/kg, gavage) as a positive control group. Chrysin or vehicle was administered by gavage once daily for 21 consecutive days before I/R induction as pretreatment. The timeline of the study has been presented in Fig. 1.

2.4. Morris water maze (MWM) test

One week after cerebral I/R induction, spatial learning and memory were evaluated using an MWM. The MWM apparatus (a pool of water with 150 cm diameter, 60 cm heights) was filled with water (25 ± 1 °C) to a depth of 40 cm. The hidden escape platform (13 cm diameter) was located in one of the four quadrants and submerged in a permanent position at 2 cm below the water surface. The apparatus was located inside a room with proper lighting with extra maze cues. The pool was divided into four quadrants and four starting positions that were equally spread out around the perimeter of the pool. The rats were given four training trials per day for 4 consecutive days, with one-minute inter-trial intervals.

Each trial lasted until the animal found the hidden platform or until a maximum of 60 s had passed, and each trial was started at one of the four starting locations in a different order each day. If the rat failed to find the hidden platform, then the animal was guided gently to the platform by the experimenter. If the animal could find the hidden platform, allowed to stay on the platform for 30 s. Escape latency to rat find the hidden platform was measured as acquisition. Twenty-four hours after the last trial (at 5th day), a probe trial was conducted by removing the hidden platform to evaluate spatial memory, and the rats were allowed to freely swim for 60 s. The escape latencies during spatial learning and percentage of the time spent in target quadrant were measured by a video-tracking software (Ethvision software ver.7, Noldus Co., Netherland). After the trials, a visible platform test was done to detect possible deficits in visual acuity and motor ability, which the platform fixed in a new quadrant 1 cm above the water level [32,33]. To determine whether the group differences in escape latency and swimming distance were due to their motor ability differences during swimming, the swimming speed was also measured.

2.5. Electrophysiological studies

Three days following cerebral hypoperfusion and reperfusion,

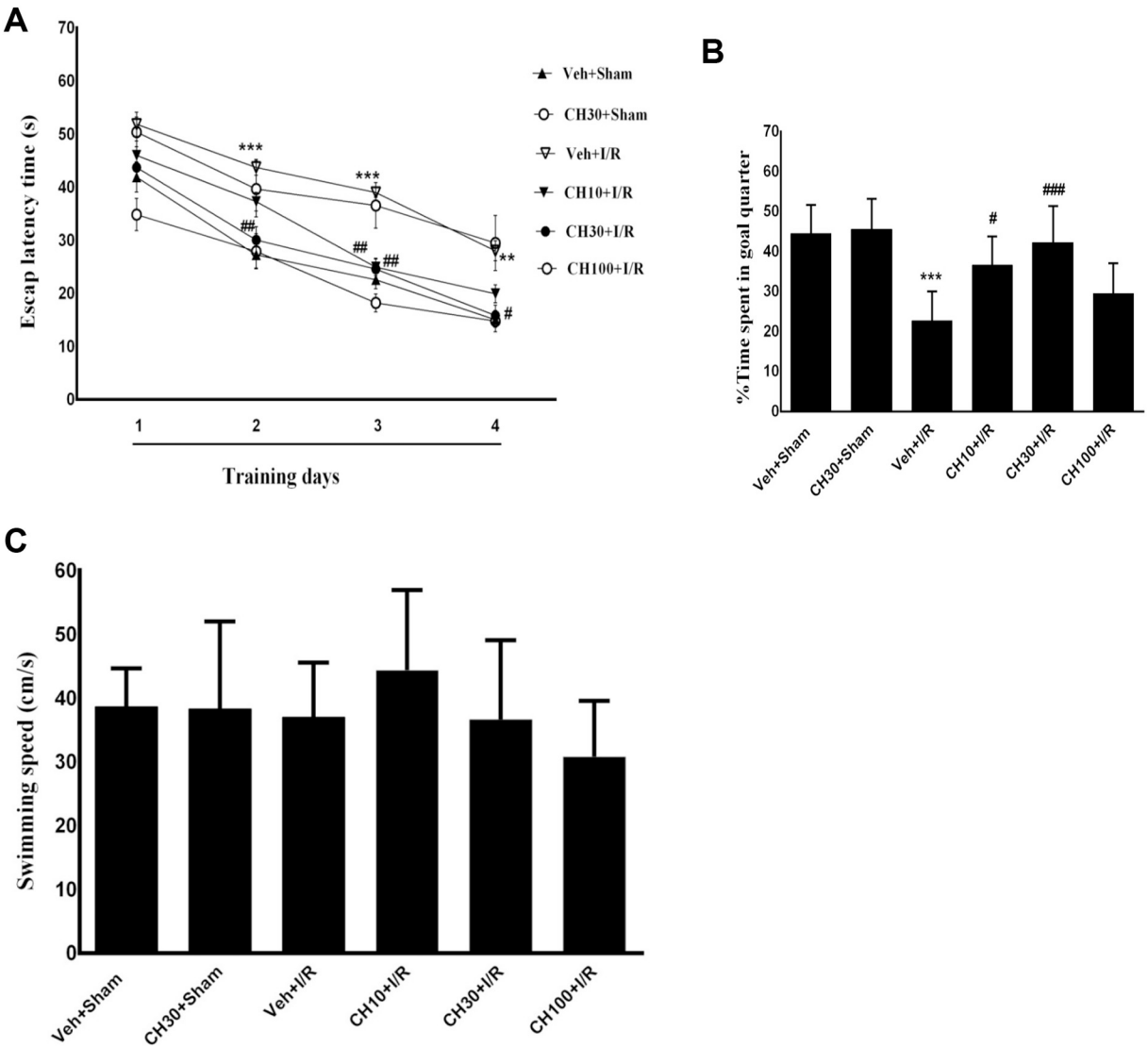

Fig. 2. Spatial learning and memory of all rats were evaluation in Morris water maze. Acquisition trials last for 4 consecutive days, with hidden platform training, followed by a probe trial. (A) Escape latency of each group to find the hidden platform during the 4 consecutive days of the acquisition trial; Analyzed by repeated measures two-way ANOVA, followed by Tukey's post-hoc test. (B) Percentage of time spent in the target quadrant during the probe trials; Analyzed by one-way ANOVA followed by Tukey's post-hoc test. (C) Swimming speed in the Morris water maze test; Analyzed by one-way ANOVA followed by Tukey's post-hoc test. Data were expressed as mean ± SEM (n = 7), I/R: bilateral common carotid artery occlusion and reperfusion, CH: chrysin. $^{**}p < 0.01$, $^{***}p < 0.001$ vs. Veh + Sham group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. Veh + I/R group.

animals were prepared for electrophysiology surgery and hippocampal LTP recording. After anesthesia with an intraperitoneal injection of ketamine-xylazine (90/10 mg/kg), the head of the rat was fixed in a stereotaxic apparatus for implantation of the electrodes and field excitatory postsynaptic potential (fEPSP) recording. To maintain body temperature at 36.5 ± 0.5 °C, a non-electric heating pad was used. The skull of the rat was drilled where the recording and stimulating metal wire microelectrodes would be located. A bipolar metal wire stimulating microelectrode (stainless steel, 100 µm in diameter, tip separation 500 µm, CFW, USA) were implanted into the left perforant path (PP) at AP: −7.5 [from bregma]; ML: −4; DV: 3.9 mm from dura, and electrodes were positioned in the high fEPSP location. A bipolar metal wire recording microelectrode (tungsten, 50 µm in diameter, tip separation 1 mm, CFW, USA) were implanted into the ipsilateral hippocampal dentate gyrus region (DG) at AP: −3.8 [from the bregma]; ML: −2.2; DV: 3.5 mm from dura consistent with the atlas of Paxinos and Watson, respectively [34]. The microelectrodes were lowered gradually (0.1 mm/30 s) from dura to the PP to minimize traumatic injuries to the brain tissue [35].

2.5.1. LTP induction

After stimulation of the PP region for 30 s, the field potential recordings were monitored in the DG area. The difference in voltage between the peak of the first positive wave and the peak of the first negative deflection was measured as the post-tetanic stimulation population spike (PS) amplitude. Furthermore, the fEPSP slope was obtained as the maximum slope between the initial point of the fEPSP and the first positive peak of the wave in order to measure synaptic efficacy. Extracellular field potentials were amplified (100×), filtered (0.1 Hz–3 kHz) and analyzed using eProbe software (version 1.53, Science Beam Co., Iran). After the baseline trace recording, a high-frequency stimulation (HFS) protocol was used to LTP induction. HFS protocol involved 10 bursts of 20 stimuli (50 µs) at 400 Hz, and a 20 s inter-burst interval [35]. The HFS stimulus intensity was assessed as the stimulus intensity that evoked a fEPSP slope and PS amplitude of approximately 80% of the maximum response. To recording LTP, all baseline potentials were selected at a stimulus intensity which produces 40% of its maximum amplitude on the input/output (I/O) curve with different intensities [36,37]. In order to determine any alterations in

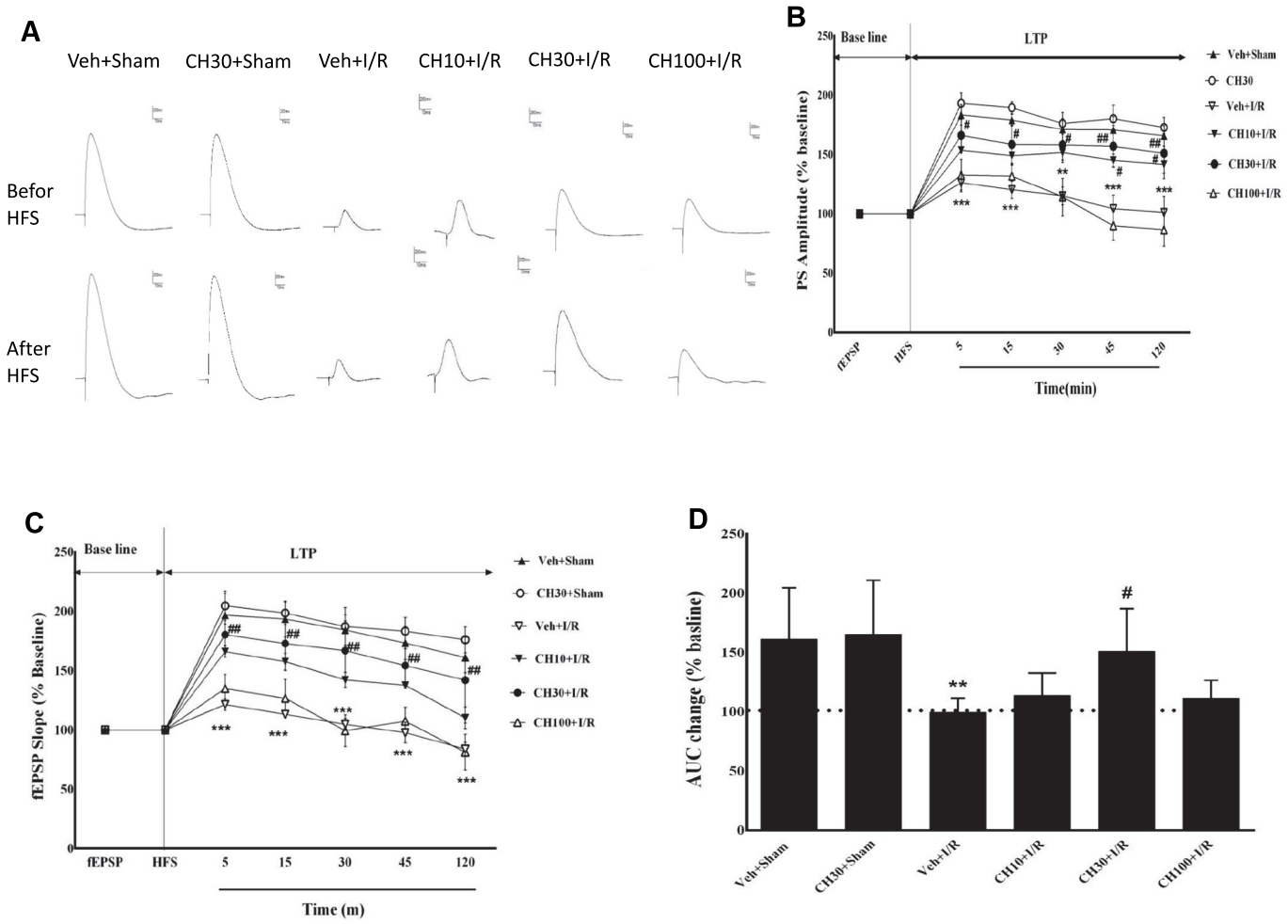

Fig. 3. LTP recording after HFS induction in all experimental groups. A) Sample traces recorded. B) Population spikes (PS) amplitude during 5, 15, 30, 45 and 120 min after the HFS; Analyzed by repeated measures two-way ANOVA, followed by Tukey's post-hoc test. C) fEPSP slope during 5, 15, 30, 45 and 120 min after the HFS; Analyzed by repeated measures two-way ANOVA, followed by Tukey's post-hoc test. D) Area under curve (AUC) for PSs during 5, 15, 30, 45 and 120 min after the HFS; analyzed by one-way ANOVA, followed by Tukey's post-hoc test. Data were represented as mean ± SEM (n = 5), I/R: bilateral common carotid artery occlusion and reperfusion, CH: chrysin, AUC: area under curve, HFS: high frequency stimulation. $^{**}p < 0.01$, $^{***}p < 0.001$ vs. Veh + Sham group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. Veh + I/R group.

the synaptic response of DG pyramidal neurons, LTP was recorded for the periods of 5, 15, 30, 45, 120 min after HFS [36,38].

2.6. Hippocampi samples collection and ELISA assay

Three days after cerebral I/R, selected animals were deeply anesthetized with an overdose of sodium pentobarbital (Nembutal) and perfused transcardially with normal saline [39]. After animal decapitation, brains were rapidly taken out, and hippocampi tissues were immediately removed on the ice, rinsed with saline, and frozen at −80 °C. The hippocampi tissues were homogenized in a cold PBS with pH 7.4, which contains a protease inhibitor cocktail (100 mg tissue/1.2 ml of the buffer). The hippocampi samples were centrifuged at 10,000 rpm for 20 min at 4 °C, and the supernatants were aliquot and stored at −80 °C until used. The protein content of the supernatant was estimated using Bio-Rad protein assay kit (based on the Bradford dye-binding method) to ensure an equal amount of protein from each sample was used [36,40]. ELISA kit for IL-1β was purchased from ZellBio GmbH (Cat. No: ZB-10119S-R96, Germany), and for TNFα was purchased from Diaclone SAS (Cat. No: 865.000.096, France) and according to the manufacturer's guidelines, the assays were performed. The concentrations of the hippocampal level of cytokines were quantified as picograms per milligrams of protein (pg/mg) [41].

2.7. Statistical analysis

The results were expressed as mean ± SEM and acquisition data from the MWM test and LTP data were analyzed using repeated measure two-way ANOVA followed by Tukey's post-hoc test.

Other data were analyzed by one-way ANOVA followed by Tukey's post-hoc test. p-Values < 0.05 were assigned to be statistically significant. Statistical analysis was performed using GraphPad Prism 6 software (GraphPad Software Inc. version 6, San Diego, USA).

3. Results

3.1. Effect of chrysin on the memory

As shown in Fig. 2A, the latency time during four-days training trials in the MWM task was reduced in all experimental groups, indicating that all groups learned to find the hidden platform. The result of two way repeated measure ANOVA analysis of the latency time to find the hidden platform showed significant effects of pretreatment with chrysin ($F_{5,30} = 18.40$; $p < 0.001$) and days ($F_{3,18} = 124.6$; $p < 0.001$). However, there were no significant effects of day × pretreatment interaction ($F_{15,90} = 0.706$; $p > 0.05$) for it in all training days. The analyzed by Tukey's post hoc test showed that the rats

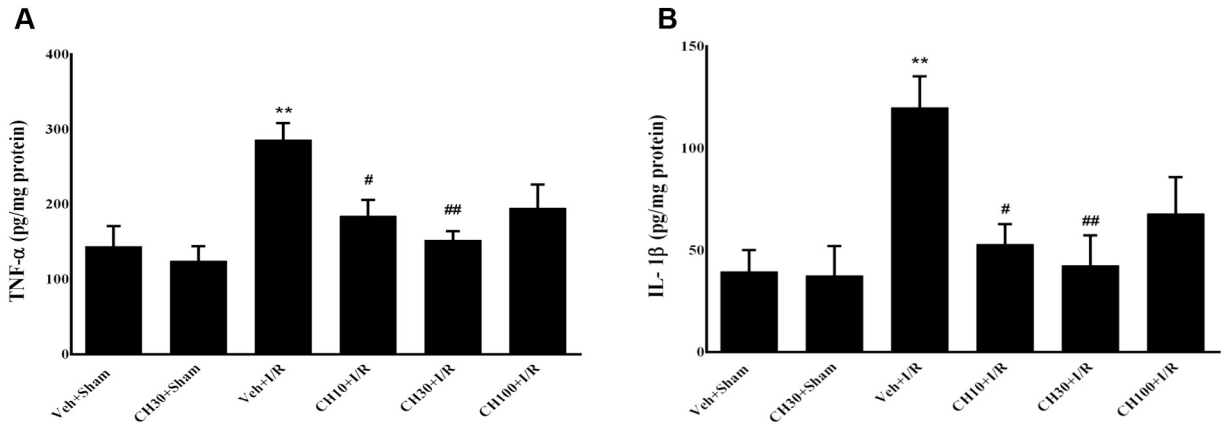

Fig. 4. The hippocampal tissue levels of TNF-α and IL-1β in all tested groups. A) Hippocampal TNF-α contents in different experimental groups with and without chrysin pretreatment. B) Hippocampal IL-1β contents in different experimental groups with and without chrysin pretreatment, Data were represented as mean ± SEM (n = 5). I/R: bilateral common carotid artery occlusion and reperfusion, CH: chrysin. **p < 0.01 vs. Veh + Sham group, #p < 0.05, ##p < 0.01 vs. Veh + I/R group. Analyzed by one-way ANOVA, followed by Tukey's post-hoc test.

subjected to I/R showed prolonged latency to find the hidden platform compared with the sham group in days 2, 3, 4 of training days ($p < 0.001$, $p < 0.001$, $p < 0.01$ for each comparison respectively), suggesting learning deficits in the I/R rats. While, pretreatment with chrysin (30 mg/kg, once daily) for three consecutive weeks significantly decreased the escape latency in the CH30 + IR group compared with the Veh + I/R ($p < 0.01$), showing that spatial learning was improved in pretreated I/R rats. Furthermore, pretreatment with chrysin (10 mg/kg) in the CH10 + I/R group changed basal learning performance of rats only at the 3rd day of training with compare to the Veh + I/R group ($p < 0.01$). However, there were not any significant changes in all training days between CH100 + I/R and Veh + I/R group ($p > 0.05$ for each comparison). Furthermore, pretreatment with chrysin in CH30 + Sham group did not change basal learning performance of rats during the 4th day of training as compared to the Veh + Sham group ($p > 0.05$).

Memory retrieval of rats in all groups was evaluated using a probe trial test, which conducted twenty-four hours after the last trial and without the hidden platform. The percentage of time spent in the target quadrant was used to evaluate memory of trained rats. As shown in Fig. 2B, rats in the Veh + I/R group spent lesser time in the target quadrant versus to the Veh + Sham group ($F_{5,36} = 9.902$, $p < 0.001$), while, pretreatment with chrysin in the CH10 + I/R and CH30 + I/R groups showed significantly increased percentage of time spent in the target quadrant when compared with Veh + I/R group ($p < 0.05$ and $p < 0.001$, respectively). There was no significant difference in time spent in target quadrant between CH100 + I/R and Veh + I/R groups ($p > 0.05$). Furthermore, pretreatment with chrysin in the CH30 + Sham group did not affect the tested parameters as compared to the Veh + Sham group ($p > 0.05$). As shown in Fig. 2C, there was no difference in swimming speed among all groups ($F_{5,36} = 1.164$ $p > 0.05$). These results showed that pretreatment with chrysin (10 mg/kg and 30 mg/kg) ameliorate memory impairment in the I/R rats.

3.2. Hippocampus electrophysiological activity

A typical sample traces recorded from the hippocampal DG area before and after HFS are shown in Fig. 3A. As shown in Fig. 3B, the result of two way repeated measure ANOVA analysis of PS amplitude showed significant effects of pretreatment with chrysin ($F_{5,168} = 37.20$; $p < 0.001$) and time ($F_{6,168} = 41.82$; $p < 0.001$). Also, there were significant effects of time × pretreatment interaction ($F_{30,168} = 2.875$; $p < 0.001$) for PS amplitude at different times. After analysis of data by Tukey's post hoc test, PS amplitude significantly decreased in the rats with I/R when compared with the sham group ($p < 0.001$). Whereas its amplitude significantly restored in the CH30 + I/R and CH10 + I/R group compared with Veh + I/R ($p < 0.01$ and $p < 0.05$, respectively). However, there were not any significant changes in the PS amplitude between CH100 + I/R and Veh + I/R group ($p > 0.05$). Furthermore, pretreatment of healthy rats with chrysin in the CH30 + Sham group did not affect PS amplitude as compared to the Veh + Sham ($p > 0.05$).

According to Fig. 3C, the result of two way repeated measure ANOVA analysis of fEPSP slope showed significant effects of pretreatment with chrysin ($F_{5,168} = 33.28$; $p < 0.001$) and time ($F_{6,168} = 31.64$; $p < 0.001$). Also, there were significant effects of time × pretreatment interaction ($F_{30,168} = 2.345$; $p < 0.001$) for fEPSP slope in different time. After analysis of data by Tukey's post hoc test, the fEPSP slope was significantly decreased in I/R group compared with the sham ($p < 0.001$), while, it was reversed significantly toward to normal state in the CH30 + I/R group ($p < 0.01$). However, there were not any significant changes in the fEPSP slope between CH10 + I/R and CH100 + I/R versus Veh + I/R group ($p > 0.05$). Furthermore, pretreatment with chrysin in the CH30 + Sham group did not affect fEPSP slope as compared to the sham group ($p > 0.05$). As shown in Fig. 3D, the effects of chrysin pretreatment on the AUC of PS ($F_{5,36} = 5.80$ $p < 0.001$) was significantly decreased in the Veh + I/R rats compared with sham group ($p < 0.01$), while it was restored significantly due to pretreatment with chrysin in CH30 + I/R group ($p < 0.05$). However, data showed that there were not any significant changes in AUC for PSs between CH10 + I/R and CH100 + I/R and Veh + I/R groups ($p > 0.05$). Furthermore, pretreatment with chrysin in the CH30 + Sham group did not affect the AUC for PS as compared to the sham group ($p > 0.05$).

3.3. Hippocampal tissue IL-1β and TNF-α

Fig. 4A revealed the effects of chrysin pretreatment (once daily, for 21 consecutive days) on the hippocampal levels of TNF-α after I/R ($F_{5,24} = 6.3$ $p < 0.001$). These data showed that hippocampal level of TNF-α was significantly increased in the Veh + I/R group compared with the sham ($p < 0.01$), while, it was reversed significantly in both pretreated groups with doses 10 and30 chrysin (CH10 + I/R and CH30 + I/R) ($p < 0.05$ and $p < 0.01$, respectively). While data showed that there were not any significant changes in the hippocampal level of TNF-α between CH100 + I/R and Veh + I/R groups ($p > 0.05$).

Fig. 4B demonstrates the effects of chrysin pretreatment (once daily, for 21 consecutive days) on the hippocampal content of IL-1β following

I/R ($F_{5,24} = 4.999$; $p < 0.01$). Data showed that hippocampal content of IL-1β was significantly increased in the Veh + I/R rats compared with Veh + Sham group ($p < 0.01$), while, it was restored in both pretreated groups with chrysin (CH10 + I/R and CH30 + I/R) significantly when compared with Veh + I/R group ($p < 0.05$ and $p < 0.01$, respectively). However, data showed that there were not any significant changes in the hippocampal level of IL-1β between CH100 + I/R and Veh + I/R groups ($p > 0.05$).

4. Discussion

The present study intended to evaluate the preventive effects of chrysin pretreatment on physiological functions such as cognitive performance, induction and persistence of LTP and hippocampal tissue inflammation following cerebral hypoperfusion and reperfusion (I/R) with using bilateral common carotid arteries occlusion and reperfusion (BCCAO/R) in rats. We showed that BCCAO/R produced a marked impairment in spatial learning and memory, which was associated with a significant decline in hippocampal LTP properties, as well as elevated content of TNF-α and IL-1β in hippocampal tissue. The main findings of this study indicate that pretreatment with chrysin could improve cognitive impairment, ameliorate LTP deficit, and recovered hippocampal content of IL-1β and TNF-α toward normal levels.

It was well documented that cerebral hypoperfusion is a critical cause of vascular dementia and neurodegenerative disease [42–44], and causing learning and memory impairments in the animal models [18,45,46]. Recently it was demonstrated that BCCAO/R leading to spatial memory impairments in the MWM task [47]. In the present study, we showed that memory function of rats with BCCAO/R was significantly reduced in the MWM performance, which supports other studies concerning to memory loss due to cerebral hypoperfusion in animal models [48]. We also showed that pretreatment with chrysin restores memory deficit, as shown by the increase of the time spent in the target quadrant during the probe trial test in the MWM task, which is related to the brain hippocampal functions [49].

Recently, it was recognized that cerebral hypoperfusion resulted in LTP deficits and memory impairments [3]. Furthermore, the BCCAO/R ischemic model that likes a part to heart arrest pathological condition was used to investigate the effects of I/R on memory performance and synaptic plasticity [50], and the level of LTP induction in the hippocampus is a good index in studies of learning and memory [51]. The present study provides new findings that pretreatment with chrysin ameliorated synaptic plasticity impairment in I/R rats. Our electrophysiological findings showed that chrysin could increase PS amplitude, fEPSP slope and AUC following HFS in the ischemic rats. These results indicated that chrysin could increase synaptic plasticity in the DG region of the hippocampus with pyramidal neurons, and also showing the beneficial effect of chrysin pretreatment on memory formation in the BCCAO/R animals. Our data support previous studies of LTP deficits and memory impairments due to I/R in rats [47]. It was established that cerebral ischemia results in different injuries in several brain regions such as the hippocampus, prefrontal cortex and thalamus [52]. It has been suggested that impairments in learning and memory induced by cerebral ischemia show a close correlation with neuronal damage in the hippocampus [3,47,53]. Khoshnam and his colleagues reported that cerebral hypoperfusion causes LTP impairment and serious memory deficits [47], which was consistent with the results of the present study. Therefore, our data suggest that pretreatment with chrysin in the BCCAO/R rats may affect ischemic-induced cognition impairments via synaptic plasticity in the hippocampus. This is the first report showed that pretreatment with chrysin can ameliorate synaptic plasticity deficits in 2-VO ischemic and reperfusion model.

Inflammatory mechanisms in the stroke subjects causing an increase in brain damage and poor outcome following ischemic conditions [12]. Cytokines are found at low concentrations in the nervous system, but they rapidly increased following ischemic stroke [54]. An increase in the production of pro-inflammatory cytokines such as IL-1β and TNF-α is correlated with a worse clinical outcome and a larger infarct size in animal models [55]. Previous studies showed that inflammatory cytokines are increased in the hippocampal tissue after ischemia and reperfusion [47]. Our data in the present study showed that 2VO ischemic state followed by reperfusion could trigger inflammatory processes in the hippocampal area, which was established by increasing the inflammatory cytokines such as IL-1β and TNF-α. These results are in line with other studies [18,47]. In the present study, we showed that chrysin could attenuate hippocampal content of IL-1β and TNF-α in the ischemic rats, suggesting its protective action through anti-inflammatory activity. A growing body of literature suggests the involvement of IL-1β and TNF-α in the pathophysiology of cerebral ischemic injury [56,57]. It was established that increasing tissue levels of the IL-1β [58] and TNF-α [47] cause memory impairment. It has been suggested that IL-1β could affect the magnitude of LTP by influencing the function of glutamatergic *N*-methyl-D-aspartic acid receptors (NMDARs) and calcium channel influx [59]. Our observations indicated that a significant increase in hippocampal content of IL-1β and TNF-α were associated with inhibition of hippocampal LTP. Furthermore, our results in the current study have shown that chrysin pretreatment before I/R induction maintained the hippocampal LTP due to the prevention of IL-1β and TNF-α content in the hippocampus. Together with other parts of our results, the effect of chrysin on LTP recovery may be associated with decreasing the elevated levels of IL-1β and TNF-α through its anti-inflammatory effects.

To our knowledge, it is a study using chrysin to attenuate synaptic plasticity impairment following cerebral I/R. Our results suggest that chrysin may act as a potent neuroprotective component against BCCAO/R in the I/R rats, the decreased fEPSP slope and PS amplitude were observed, which significantly prevented in chrysin-pretreated animals. According to MWM data, chrysin pretreatment improves learning and memory impairments. Furthermore, in chrysin-pretreated rats, the hippocampal content of IL-1β and TNF-α were significantly decreased in I/R animal which may lead to restoring hippocampal LTP and improvement of learning and memory deficits. On the other hand, it was well established that protective effect of chrysin may is related to its anti-inflammatory effect, which mediated by inhibition of astrocytic activation, and tended to reduce proliferation of astrocytes in the hippocampus. Therefore, these results may show the effects of chrysin on astrocyte recovery due to its anti-inflammatory properties. The exact mechanisms by which chrysin induces these effects needs still to more investigations [60]. In this study, pretreatment with 30 mg/kg chrysin markedly ameliorated cognitive deficits, improved LTP properties and decreased the hippocampal content of IL-1β and TNF-α in the I/R rats. However, 100 mg/kg chrysin had no significant effects. Thus, it has shown that the high dose of chrysin may reverse its beneficial. It can't be assumed that increasing the dosage of a putative neuroprotective agent will lead to improved effect.

5. Conclusion

Our results suggest that chrysin is able to prevent cognitive impairment, LTP deficits, and inflammatory cytokines following cerebral hypoperfusion and reperfusion injury in rats. The reduced inflammatory cytokine and the improved the spatial memory and long term potentiation may all due to the neuroprotective effect of chrysin. The exact mechanisms by which chrysin induces these effects have not been fully understood.

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