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ORIGINAL ARTICLE

Pretreatment with bisoprolol and vitamin E alone or in combination provides neuroprotection against cerebral ischemia/reperfusion injury in rats

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Abstract

Global cerebral ischemia/reperfusion (I/R) induces selective neuronal injury in the hippocampus, leading to severe impairment in behavior, learning, and memory functions. This study aimed to evaluate the neuroprotective effects of bisoprolol (biso) and vitamin E (vit E) treatment alone or in combination on cerebral ischemia/reperfusion (I/R) injury. A total of 30 male rats were divided randomly into five groups ($n = 6$), sham, I/R, I/R + biso, I/R + vit E, and I/R + biso+vit E. Cerebral I/R group underwent global ischemia by bilateral common carotid artery occlusion for 20 min. Treatment groups received drugs once daily intraperitoneally for 7 days before the I/R induction. Locomotive and cognitive behaviors were utilized by open-field and Morris water maze tests. After behavioral testing, the brain was removed and processed to evaluate cerebral infarct size, histopathologic changes, myeloperoxidase (MPO) activity, and malondialdehyde (MDA) level. In I/R group tissue MDA and MPO levels and cerebral infarct size were significantly increased in comparison with the sham group. Furthermore, significant deficits were observed in locomotion and spatial memory after I/R. The areas of cerebral infarction, MPO, and MDA levels in biso, vit E, and combination group were significantly reduced compared with I/R group. Histopathological analysis demonstrated a significant reduction in leukocyte infiltration in all treated groups with the most profound reduction in the combination group. According to the behavioral tests, administration of biso and/or vit E protected locomotive ability and improved spatial memory after cerebral I/R. Our findings show that biso and vit E have beneficial effects against the I/R injury and due to their synergistic effects when administered in combination, may have a more pronounced protective effect on the cerebral I/R injury.

Keywords Bisoprolol · Infarct size · Ischemia/reperfusion · Myeloperoxidase · Malondialdehyde · Vitamin E

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Introduction

Ischemic stroke is the third leading cause of death all over the world and is associated with a high incidence of permanent disability in surviving individuals (Abd-Elsameea et al. 2014; Sun et al. 2018). This pathologic condition results from a permanent or transient decrease in cerebral blood flow which leads to activation of pathophysiological events such as oxidative stress, inflammation, and apoptosis of neurons in the brain (Pithadia et al. 2017). Therefore, restoring the blood flow by recanalization of occluded arteries via thrombectomy or thrombolysis is the primary treatment; however, this reperfusion can also cause serious brain injuries such as neuronal death, cerebral edema, and brain hemorrhage. This detrimental process is termed as cerebral I/R injury (Pithadia et al. 2017). During cerebral I/R, a large number of reactive oxygen species (ROS) are produced, and oxidative stress plays a

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crucial role in neuron injury (Aabdallah and Eid 2004). Clinically and experimentally, ischemic injury accompanies acute inflammatory responses, wherein leucocytes infiltration is a key initiating process (Matsuo et al. 1994). During reperfusion, activated leucocytes attach to endothelial cells, follow by the release of neutrophil-derived oxidant and proteolytic enzymes disrupting the blood–brain barrier, the leukocyte, in turn, extravasate from capillaries and infiltrate into brain tissue, releasing cytokines which mediate inflammation. It has been reported that the administration of anti-neutrophil monoclonal antibodies attenuated ischemic brain injury (Bednar et al. 1991; Kuroda and Siesjö 1997). It has been shown that oxidative stress and inflammation after I/R causes exploratory behavior changes and learning and memory impairment (Naderi et al. 2018). According to previous studies, selective β1-antagonists decreased neurological deficit scores after transient focal ischemia in rats (Goyagi et al. 2006; Goyagi et al. 2011). Iwata and colleagues demonstrated that post-treatment, but not pretreatment, with selective β1-antagonists provided neuroprotection in the hippocampus after transient forebrain ischemia in rats (Iwata et al. 2010). Since there are very few effective treatments that can save ischemic brain tissue, identification of new pharmacologic agents, administered as adjunctive therapy to common medication for rescuing the neurons from I/R injury, is a clinically important goal (Soares et al. 2019). Beta-blockers that reversibly block beta-adrenergic receptors and inhibit the effects of sympathetic stimulation are used for acute myocardial infarction (AMI), angina pectoris, hypertension, and glaucoma (OPIE 2019). Previous studies have reported their inhibitory effects on lipid peroxidation, free oxygen radical production, and inflammation (Gao et al. 2017; Ilhan et al. 2004; Nakamura et al. 2011; Savitz et al. 2000; So et al. 2019). They have also been demonstrated to reduce the infiltration of neutrophils in the myocardium in experimental studies (García-Prieto et al. 2017; Steffens and Montecucco 2009). Furthermore, numerous experimental studies have been demonstrated that beta-blockers have neuroprotective effects on cortical neurons and retinal neurons in both I/R and oxygen-free radical generating nervous system injuries (Chen et al. 2007; Gok et al. 2007; Osborne et al. 1999). For example, carvedilol and propranolol have been shown to provide brain protection in *in vivo* studies (Goyagi et al. 2010). The protective effects of nebivolol in the spinal cord I/R via its free radical scavenging and antioxidant properties and also neuroprotective effects of carvedilol in brain ischemia through its antioxidant and anti-apoptotic activity have been reported in previous studies (Ilhan et al. 2004; Savitz et al. 2000). Biso is a highly selective β1 adrenoceptor blocker. Previous reports have demonstrated that biso with antioxidant activity exerts a number of potentially beneficial pharmacological effects in some pathologic conditions such as arterial fibrillation, heart failure, and postural tachycardia syndrome (Nakamura et al. 2011; So et al. 2019). It has been demonstrated that biso reduces myocardial I/R injury by inhibiting oxidative stress and reducing pro-inflammatory cytokines such as IL-1β, TNF-α in the left ventricle of TO-2 hamsters (an animal model of dilated cardiomyopathy) (Ichihara et al. 2006). Strategies based on antioxidant supplementation have been reported to produce beneficial effects against neuron injury in experimental models of cerebral I/R. α-Tocopherol, as a powerful antioxidant, is the most important form of vit E. It has been demonstrated that α-tocopherol produces protective effects against cerebral I/R injury, most probably by the prevention of inflammation, ROS formation, and apoptosis (Aabdallah and Eid 2004). The protective effects of biso in cerebral I/R injury have not been previously studied and since ischemic stroke has complex pathophysiology and its treatment with single neuroprotective drugs has so far failed, therefore for the first time we examined the neuroprotective effects of biso and/or vit E against cerebral I/R.

Materials and methods

Animals

The experiments were performed on healthy adult male Wistar rats weighing 250 ± 20 g (age 8–10 weeks). Rats were housed in the animal house of Urmia University of Medical Sciences at a controlled ambient temperature of 22 ± 2 °C with 50 ± 10% relative humidity and a 12-h light/12-h dark cycle and free access to chow and water ad libitum. Our experimental protocol was approved by the institutional animal ethics committee of Urmia Medical Sciences University which follows the NIH guidelines for the care and use of animals (code: IR.UMSU.REC.1397.328).

Experimental design

Animals were randomly divided into five groups (n = 6 each group). Rats in bisoprolol and vitamin E (vit E) pretreated groups (I/R + biso and I/R + vit E) were given intraperitoneally (i.p.) bisoprolol (5 mg/kg) and vitamin E (200 mg/kg) respectively once a day, for 7 days prior to ischemia. Rats in the combination group (I/R + biso+vitE) were pretreated with bisoprolol (5 mg/kg) and vitamin E (200 mg/kg) as the same protocol. Animals in the sham-operated group were given saline for the whole period of the experiment (7 days). The last doses of drugs in pretreated groups were injected 1 h before ischemia induction. The post-ischemic behaviors were recorded 7 days after reperfusion in the Morris water maze and open field to assess spatial learning and memory and general locomotor activities (Fig. 1).

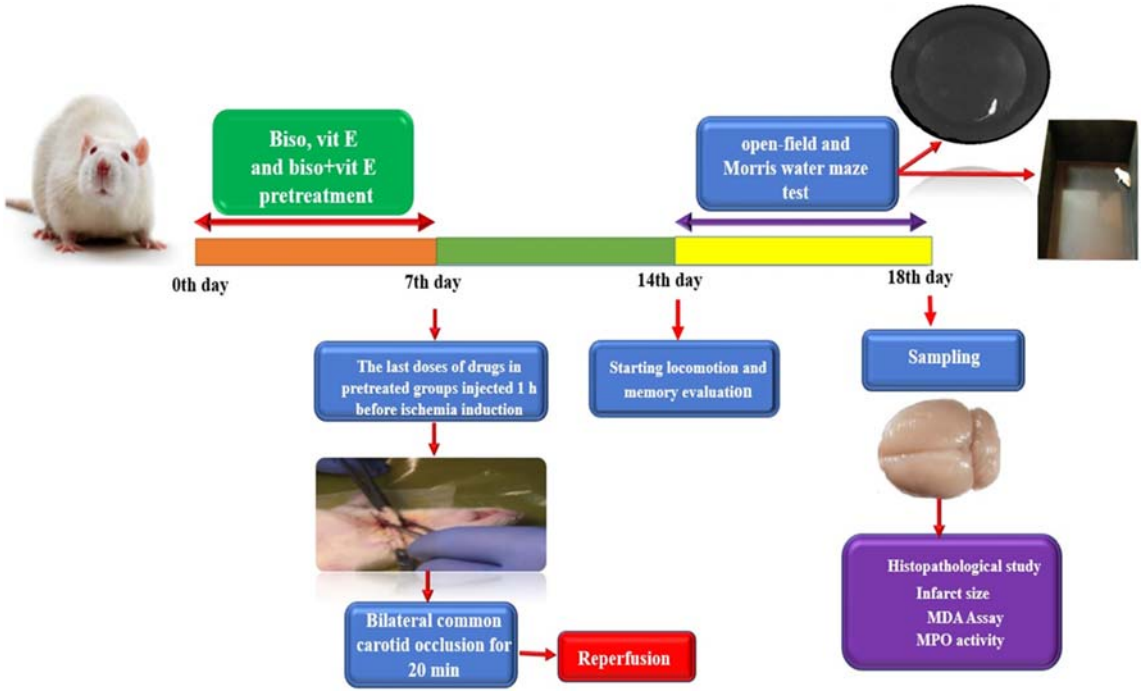

Fig. 1. Outline scheme of experimental designs. Rats were pretreated with biso, vit E, and biso+vit E once a day for 7 days prior to ischemia. The bilateral carotid occlusion was done for 20 min on day 7. Seven days after reperfusion, neurobehavioral tests were started. On day 18, rats were sacrificed for histopathological and molecular evaluation (biso: bisoprolol, vit E: vitamin E)

Surgery procedure

For induction of cerebral I/R, bilateral common carotid arteries occlusion was used. In brief, the animals were anesthetized with a mixture of ketamine (60 mg/kg, Alfasan, the Netherlands) and xylazine (10 mg/kg, ip). After deep anesthesia, when no longer responded to external stimuli, the animals were placed in a supine position and surgical sites were shaved and cleansed thoroughly by betadine. Then, a ventral midline skin incision was made from the lower mandible posterior to the sternum (~ 3 cm) to expose both common carotid arteries. The bilateral carotid was separated from vagal nerves and occluded by a bulldog clamp for 20 min. The clamp was removed to restore the circulation and skin was sutured (Zamani et al. 2013).

Histopathological examination

At the end of the experiment, the animals were anesthetized and blood samples were taken from the portal vein and then euthanized with pentobarbital, and the brains were rapidly removed and placed in 10% formalin. The brain tissues were embedded in paraffin then sliced at 5 μm thickness, and standard H&E staining was performed. The profile and degree of leukocyte infiltration into each section of the brain tissue were determined by morphometric point-counting procedure and two professional independent observers (double-blind) graded the histopathological changes from 1 to 4 showing low, moderate, high, and intensive pathological changes, respectively (Matsuo et al. 1994; Soraya et al. 2012).

Myeloperoxidase assay

MPO activity, an index of polymorphonuclear leukocyte accumulation, was measured as previously described by Bradley et al (Since 1982). Briefly, each brain sample was homogenized (1KA homogenizer, Staufen, Germany) in a solution containing 0.5% hexa-decyltrimethyl-ammonium bromide (HTAB) in 50 mM potassium phosphate buffer (pH = 6). The homogenate was centrifuged at 4500 rpm for 30 min at 4 °C and three times freeze/thaw cycles were then performed with sonication (10 s) between cycles. After that the suspensions were centrifuged at 4500 rpm for 30 min, then 0.1 ml of supernatant was allowed to react with 2.9 ML solution of 50 mM potassium phosphate buffer at PH = 6, containing 0.167 mg/ml o-dianisidine dihydrochloride and 0.0005% H_2O_2. After 5 min 0.1 ml of 1.2 M hydrochloric acid was used to stop the reaction. Then the change rate in absorbance of the mixture was measured at 450 nm with a spectrophotometer (Cecil 9000, Cambridge, UK). MPO activity was also measured in mU/g wet tissue.

Infarct size measurement

At the end of the experiment, the rats were anesthetized and sacrificed and the brains quickly removed and frozen 15 min at – 20 °C. Coronal brain sections of 2 mm thickness were cut. The slices were incubated with a 1% solution of 2, 3, 5-triphenyltetrazolium chloride (TTC) for 20 min at 37 °C in a darkroom. Then brain slice kept in 4% paraformaldehyde, to fix the stained area by TTC. The stained slices were digitally photographed and the percentage of infarction was measured using Image j software. Normal areas stain red whereas infarcted areas remain pale (Kamat et al. 2015; Karimipour et al. 2018).

Malondialdehyde Assay

Tissue MDA was measured using the thiobarbituric acid (TBA)-reactive substance assay, as described by KEI SATOH et al (Kei 1978). In brief, brain tissues were homogenized in 1.15% KCl to make a 10% homogenate. Then, samples were centrifuged at 3000 rpm for 15 min. 250 μl of supernatant was added to 3 ml of phosphoric acid 1%. Then 1 ml of thiobarbituric acid (TBA) was added and the mixture place at 90 °C for 45 min for performing the reaction. The samples were cooled and then 3 ml of *n*-butanol was added and centrifuged at 3000 rpm for 15 min, finally, the absorption changes were measured at 532 nm by spectrophotometer (Cecil 9000, UK). The level of lipid peroxides was expressed as nanomoles of MDA per mg protein

Open field test

The open field apparatus consisted of a square container, a video camera fixed 1 m above the arena, and a computerized tracking system for measuring the locomotive activity of the rats before the test, animals were placed in the dimly lit room for 1 h to accommodate to the new environment. The moving area was divided equally into nine parts to counting the number of crossing between different areas. The individual rat was placed in the center of the apparatus for each trial and the behavior of each rat was recorded for 5 min after 1 min adaptation. During the interval between trials, rats were returned to its home cage in the same room and the open field was wiped clean with a slightly damp cloth. To assess the locomotion of rats in the open field, the total distance, traveling speed, and number of crossings between different areas were measured (Ge et al. 2017).

Morris water maze testing

Learning and memory ability was evaluated by Morris water maze testing (MWM). The MWM consisted of a large circular black pool (diameter: 120 cm; height: 50 cm, filled with the depth of 30 cm water at 22 ± 2 °C), which was divided into four quadrants (*N*: north; S: south; E: east; W: west). In order to escape from the necessity of swimming a submerged platform was hidden in a fixed location, 55 cm away from the edge of the pool for climbing the animal. The pool was placed in an independent darkened room illuminated by dim light and cards with different patterns were attached to the wall of the room in order to distinguish the spatial orientation of the animal. The rats were given four swimming trials per day and lasted for 4 consecutive days. The starting position was different in each trial. The rat was given a maximum of 120 s to find the hidden platform and allowed to stay on it for 20 s. Rats that failed to locate the platform were put onto it by the experimenter for 20 s. When all tests were finished on day 5, the platform was removed and a 120 s probe trial was given. For each trial, a camera suspended above the maze was used to track each rat's path, and a tracking system (Maze Router Instruments) was used for analyzing each rat's tracking. Escape latency to the platform was measured to evaluate MWM performance. In the probe trial, the percentage of time spent in the target quadrant and the number of crossing previous platform location in the target quadrant was recorded (Wang et al. 2017).

Statistical analysis

All results were expressed as mean ± SD and the significance of differences was evaluated using one-way analysis of variance (ANOVA). If the ANOVA analysis indicated significant differences, a Student–Newman–Keuls post-test was performed to compare the mean values between the groups. Any differences between the groups were considered significant at $P < 0.05$.

Results

Effects of pre-treatment with bisoprolol and vit E on infarct size after cerebral ischemia reperfusion

TTC staining was performed to observe the effects of drugs on brain infarct size. As shown in Fig. 2A, the red region meant the non-ischemic area, and the white region was the ischemic area. The infarct size of the I/R group was significantly increased compared to the sham group ($P < 0.001$). pre-treatment with biso (5 mg/kg) and vit E (200 mg/kg) individually and in combination, that was started 7 days before induction of cerebral I/R remarkably reduced infarcted size in biso and vit E and combination group ($P < 0.01$, $P < 0.01$, $P < 0.001$ respectively). Infarct size was significantly decreased in the combination group compared to biso and vit E alone group (Fig. 2B).

Histopathological study

Histopathological examination of brain sections in I/R group revealed the infiltration of leukocyte (Fig.3). Leukocyte infiltration

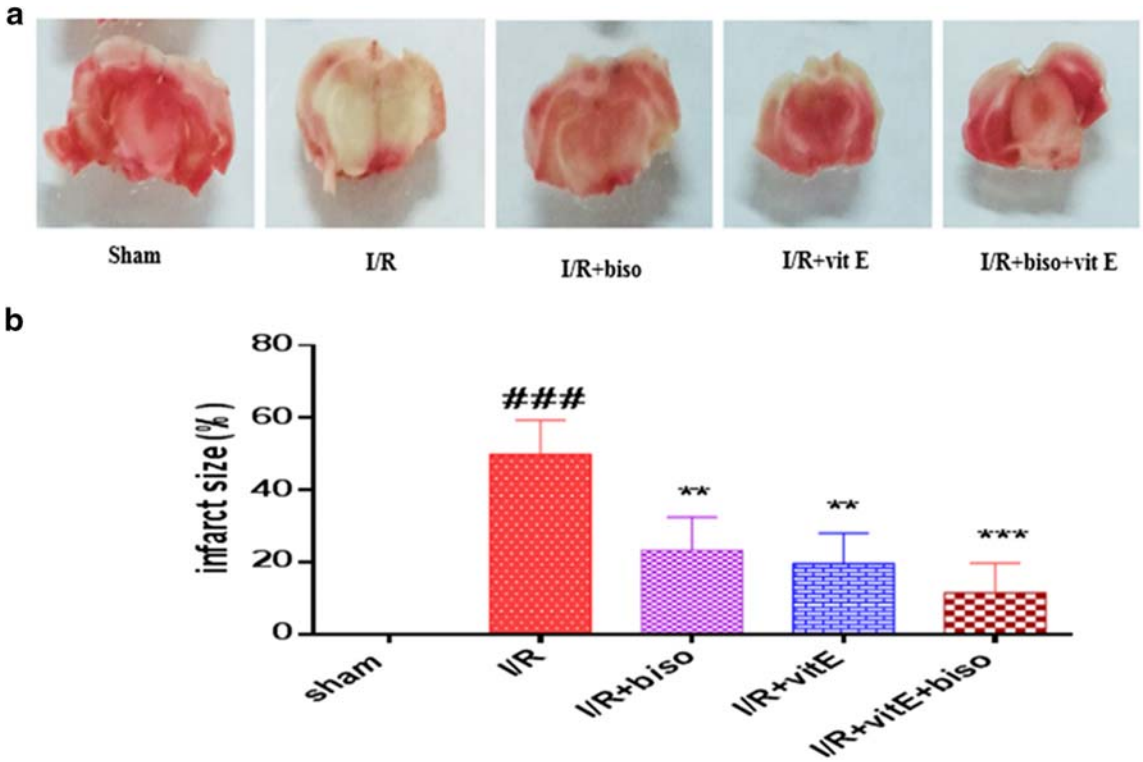

Fig. 2 **A** Representative brain slices stained with TTC in the studied groups. Ischemic regions are white and nonischemic regions are red. **B.** Effects of biso and vit E on infarct size. Rats were divided into sham group, I/R group, I/R plus biso group, I/R plus vit E group, I/R plus biso plus vit E group. Data were presented as mean ± SD. (###) $P < 0.001$ relative to sham group. (**) $P < 0.01$ relative to I/R. (***) $P < 0.001$ relative to I/R. $N = 6$ for each group. I/R: ischemia/reperfusion, biso: bisoprolol, vit E: vitamin E.

increased significantly in I/R group compared to the sham group ($P < 0.001$). Pre-treatment with biso (5 mg/kg) and vit E (200 mg/kg) individually and in combination, which was started 7 days before induction of cerebral I/R, decreased leukocyte infiltration significantly in all treated groups in comparison to I/R group ($P < 0.001$) after global cerebral ischemia as shown in Fig. 4.

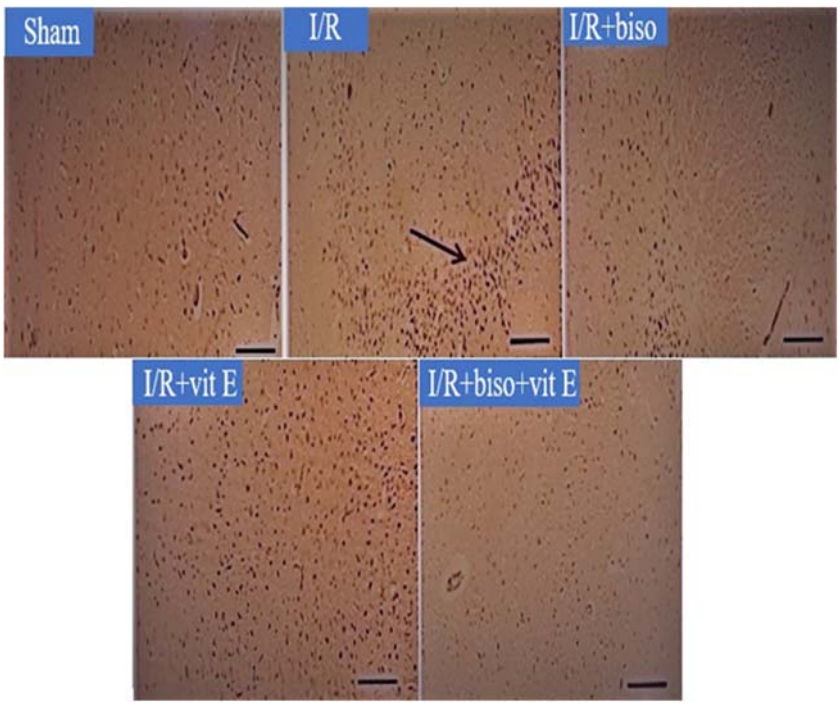

Fig. 3 Photomicrographs of brain stained with H&E. Pre-treatment with biso and vit E alone or in combination significantly reduced neutrophil accumulation. I/R: Ischemia/reperfusion, biso: bisoprolol, vit E: vitamin E. Scale bars represent 100 μm

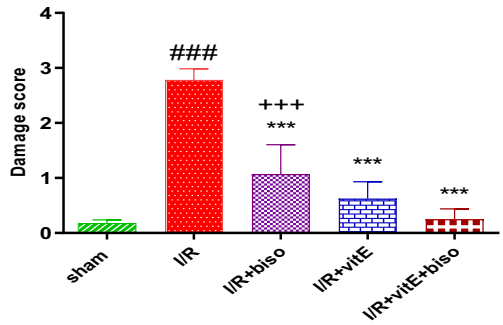

Fig. 4 Grading of leukocyte infiltration in the brain tissues. Rats were divided into sham group, I/R group, I/R plus biso group, I/R plus vit E group, I/R plus biso plus vit E group. Data were presented as mean ± SD. (###) $P < 0.001$ relative to sham group. (***) $P < 0.001$ relatives to I/R. (+++) $P < 0.001$ relative to I/R plus biso plus vit E group. $N = 6$ for each group. I/R: ischemia/reperfusion, biso: bisoprolol, vit E: vitamin E

Myeloperoxidase activity

Neutrophil infiltration into cerebral tissue was measured by MPO activity. It was found that MPO activity increased significantly in I/R group compared to the sham group ($P < 0.001$). Pre-treatment with biso (5 mg/kg) and vit E (200 mg/kg) individually and in combination, which was started 7 days before induction of cerebral I/R, reduced MPO activity in all treated groups in comparison to I/R group ($P < 0.001$) (Fig. 5).

MDA Assay

MDA levels were measured in brain tissue to determine lipid peroxidation. The induction of ischemia and reperfusion in the I/R group resulted in a significantly increased MDA level in comparison to the sham group ($P < 0.01$). Pre-treatment with biso (5 mg/kg) and vit E (200 mg/kg) individually and in combination, which was started 7 days before induction of cerebral I/R, decreased MDA levels in the biso-treated group ($P < 0.05$), vit E and combination group ($P < 0.01$) (Fig. 6).

Open field test

To evaluate the effects of biso and vit E on hippocampal-related behaviors, the open field test was utilized to examine changes of locomotion (Walker et al. 2011). We analyzed three parameters: number of crossing between different areas, total distance moved, and average speed (Fig. 7). The number of crossing, total distance moved and average speed could be indicators of locomotion (Ge et al. 2017). While the values of these three parameters were decreased in the I/R group (all $P < 0.001$), the addition of biso or vit E significantly ameliorated the locomotion caused by ischemia and the most improvement in the number of crossing and total distance were seen in combination group ($P < 0.001$).

Morris water maze testing

In order to investigate spatial learning and memory, MWM experiments were used. During the MWM test, we examined the performance of each rat for 4 days in the hidden-platform test of the MWM. In the sham group, the latency of escape was reduced with the increase of training time. This represents the spatial learning of rats (Fig. 8). As shown in Fig. 8, the escape latency in the I/R group significantly increased compared to sham group by day 4 ($P < 0.001$). Interestingly the escape latency in vit E or biso group was significantly shorter than that of I/R rats by day 4 (Fig. 8) (both $P < 0.05$). After 4 days of training, the probe trial was performed on the fifth day. As indicated by the number of crossing and the percentage of total time in the previous platform location, control rats showed a preference in the target quadrant, reflecting rats' reference memory. As shown in Fig. 8, the I/R group

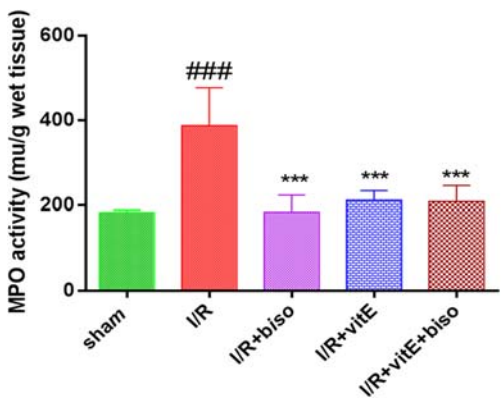

Fig. 5 Effect of biso and vit E on MPO levels. Rats were divided into sham group, I/R group, I/R plus biso group, I/R plus vit E group, I/R plus biso plus vit E group. Data were presented as mean ± SD. (###) $P < 0.001$ relative to sham group. (***) $P < 0.001$ relative to I/R. $N = 6$ for each group. I/R: ischemia/reperfusion, biso: bisoprolol, vit E: vitamin E

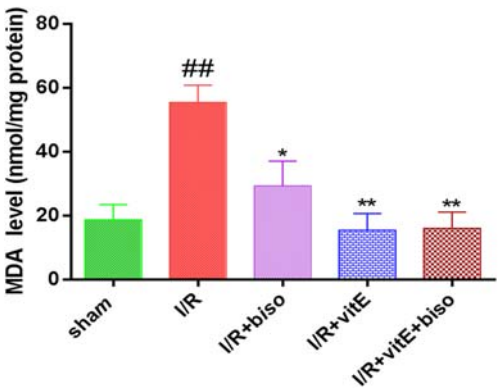

Fig. 6 Effect of biso and vit E on MDA levels. Rats were divided into sham group, I/R group, I/R plus biso group, I/R plus vit E group, I/R plus biso plus vit E group. Data were presented as mean ± SD. (##) $P < 0.01$ relative to sham group. (*) $P < 0.05$ relative to I/R. (**) $P < 0.01$ relative to I/R. $N = 6$ for each group. I/R: ischemia/reperfusion, biso: bisoprolol, vit E: vitamin E

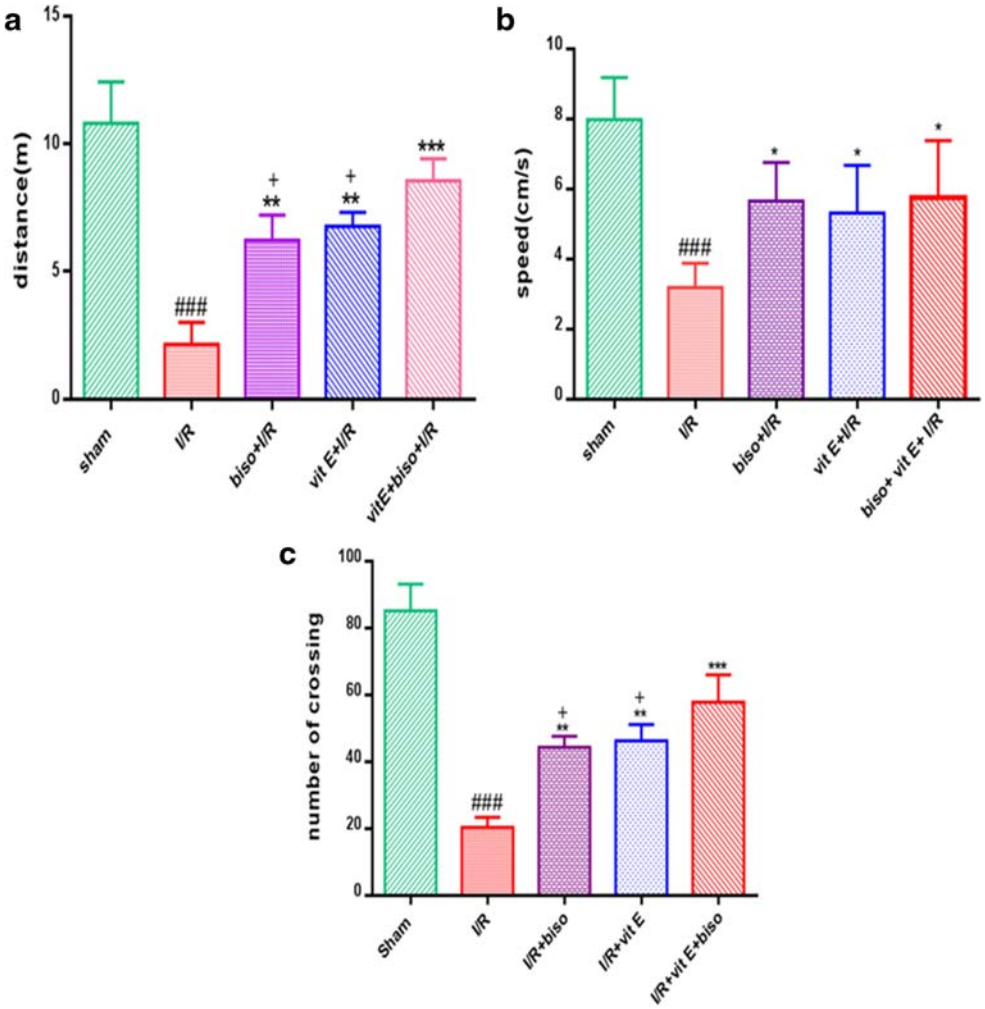

Fig. 7 Performance of rats in the open-field test. Rats were divided into sham group, I/R group, I/R plus biso group, I/R plus vit E group, I/R plus biso plus vit E group. Total distance (A), speed (B), and the number of crossing (C) of each group were measured for 5 min. Data were presented as mean ± SD. (###) $P < 0.001$ relative to sham group. (*) $P < 0.05$ relative to I/R. (**) $P < 0.01$ relative to I/R. (***) $P < 0.001$ relative to I/R. (+) $P < 0.05$ relative to I/R plus biso plus vit E group. $N = 6$ for each group. I/R: ischemia/reperfusion, biso: bisoprolol, vit E: vitamin E.

exhibited an obvious reduction in the target crossing numbers and the percentage of total time in the target quadrant compared with the sham group ($P < 0.001$). Pretreatment with vit E or biso before I/R significantly improved these two parameters and increased the preference for the target quadrant (Fig. 9) (all $P < 0.05$). Therefore, biso or vit E pre-treatment attenuates spatial learning and memory damage after I/R, and the best improvement was observed in the combination group.

Discussion

In the current study, the protective properties of biso and vit E and their combination in the cerebral I/R injury were evaluated. Our results indicated that pretreatment with biso and vit E alone or in combination have neuroprotective and ameliorative effects against cognitive impairments due to cerebral ischemia-reperfusion. Ischemic stroke is considered to be caused by impaired blood flow to brain tissue, followed by the activation of the ischemic cascade which leads to intensive neuronal injury and consequent cell death, which consist of inflammation, oxidative stress, autophagy, calcium overload, and apoptosis (Xing et al. 2012). This pathologic condition is a prevalent cause of severe disability and death worldwide and often results from thrombosis or embolism. Therefore, the prevention and recovery of stroke both are important issues. (Deb et al. 2010; Xing et al. 2012). During occlusion of blood flow in the ischemic period and subsequent reperfusion, excessive production of different types of free radicals occurs in the ischemic region (Ghasemnejad-Berenji et al. 2018; Jafari et al. 2020a; Yazdani et al. 2019). These oxidant radicals contribute to increased neuronal impairment by inducing the lipid peroxidation in cell membranes and damaging DNA (Gheibi et al. 2014). This is confirmed in our study by the enhancement of the MDA level after brain I/R, as one of the most prominent indicators of lipid peroxidation (Jafari et al. 2020b). As long as fatty acids, O2, and metal catalysts (Cu^{+}, Fe^{2+}) exist, lipid peroxidation leads to the genesis of new free radicals. Therefore, lipid peroxidation is intensively dependent on the period of reperfusion (Ghosh et al. 2013). Numerous researchers have reported the protective effect of drugs with anti-inflammatory or anti-oxidative properties in

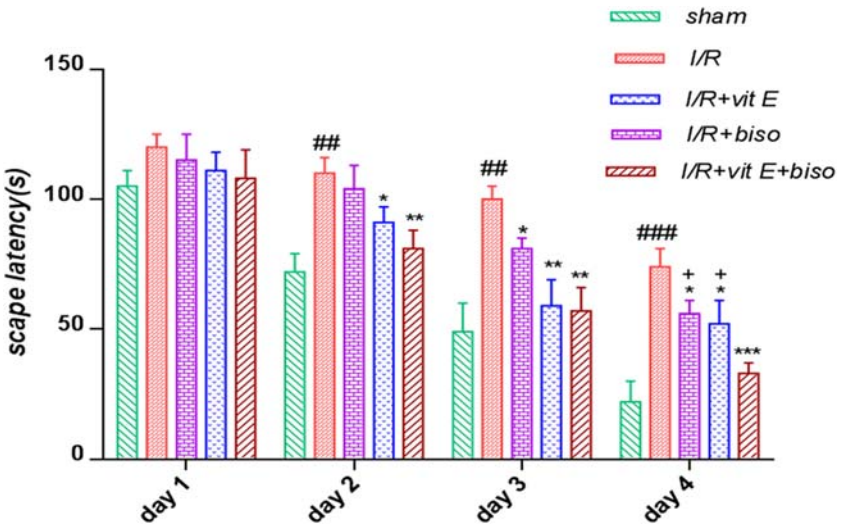

Fig. 8 Latency in the Morris water maze test. Rats were divided into sham group, I/R group, I/R plus biso group, I/R plus vit E group, I/R plus biso plus vit E group. (A) The time that each rat spent to reach the hidden platform (latency) during the 4 days was recorded and compared. Data were shown as mean ± SD. (###) $P < 0.001$, relative to sham group. . (##) $P < 0.01$, relative to sham group. (*) $P < 0.05$, relative to I/R. (**) $P < 0.01$, relative to I/R. (***) $P < 0.01$, relative to I/R. (+) $P < 0.05$, relative to I/R plus biso plus vit E group. $N = 6$for each group. I/R: ischemia/reperfusion, biso: bisoprolol, vit E: vitamin E

reducing brain injury after cerebral I/R (Aabdallah and Eid 2004; Goyagi et al. 2010; Ilhan et al. 2004; Karimipour et al. 2018), however, some of them have shown the beneficial effects of pretreatment with agents with such properties (Ghadernezhad et al. 2016; Patel et al. 2020; Sun et al. 2016). Previous studies reported the anti-oxidative effects of biso on the cardiovascular system by reducing infarct size and improving left ventricular functions after global ischemia and reperfusion injury (Toyoda et al. 2020; Xi et al. 2016). Treatment with vit E has also been reported to reduce brain injury during ischemia and reperfusion when given alone or in combination with other agents (Onem et al. 2006; Sato and Hall 1992). These data are inconsistent with the results of the present study, where vit E ameliorated the lipid peroxidation.

This property could be attributed to its capacity to scavenge free radicals, as seen by the restoration of the antioxidant enzyme activity and the prevention of I/R-induced neuronal death. It is well documented that blood–brain barrier breakdown is an important contributing factor to injury in stroke. Under ischemic stroke conditions, disruption of this barrier leads to increased paracellular permeability across cerebral vessels, and cerebral edema (Petty and Wettstein 2001). The Leucocyte infiltration in this pathologic condition leads to the subsequent production of inflammatory factors, enhances the blood–brain barrier permeability, and causes more intensive injury (Wang et al. 2014). There is a positive correlation between MPO-quantified neutrophil accumulation and ischemic brain injury in rats (Matsuo et al. 1994). Our results suggest that pretreatment with vit E or biso protects the blood–brain barrier from the hyperpermeability induced by cerebral I/R and reverses the induction of neutrophil infiltration in ischemic tissue. Furthermore, our results showed that both vit E and biso significantly decreased MPO and MDA levels after cerebral I/R. Previous studies have shown that biso has a variety of pharmacological properties, including anti-inflammatory and antioxidant effects. It has been reported that biso can inhibit myocardial cell apoptosis and reduce ROS production and has cardioprotective effects on cardiomyocytes under hypoxia/reoxygenation (Chin et al. 2003; Li et al. 2011; Sezai et al. 2012). Biso reduces myocardial I/R injury via improving post ischemic cardiac function, decreasing infarct size, reducing apoptotic index, suppressing TNF-α and IL-6 secretion and inhibiting unfolded protein response pathway (Zhang et al. 2017). In addition studies have indicated that biso can reduce the levels IL-1β, TNF-α, and anti-oxidative stress in an animal model of dilated cardiomyopathy (Ichihara et al. 2006). These observations lead to the hypothesis that pretreatment with biso may provide neuroprotective effects against ischemic-reperfusion injury. In the present study for the first time, we showed neuroprotective effects of pretreatment with biso in terms of neurological and histological outcomes. Furthermore, previous studies have demonstrated that treatment with vit E amplified the brain anti-oxidant capacity and reduced ischemic edema formation by protecting the blood–brain barrier integrity following focal cerebral ischemia (Azar et al. 2017). In the current study, the MDA level, as an oxidative stress marker, was reduced significantly in biso, vit E treated group, and combination group compared to the I/R group. MPO activity is frequently utilized to estimate neutrophil infiltration in inflamed tissues. In the presented study, as expected, I/R caused a significant increase in MPO activity, indicating inflammatory damage in rat brains. Our results showed that there was a significant decrease in MPO activity in all treated groups.

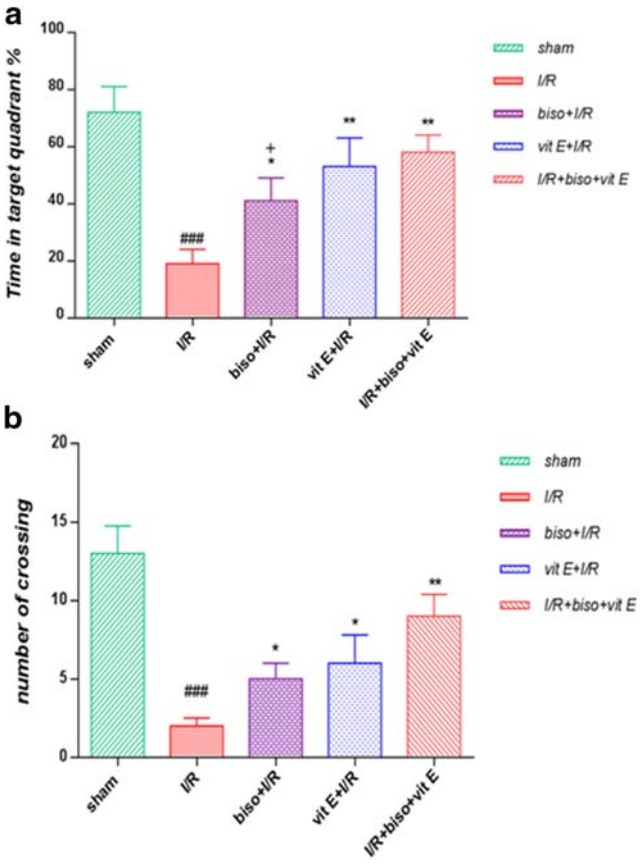

Fig. 9 Behaviors in the Morris water maze without a platform. Rats were divided into sham group, I/R group, I/R plus biso group, I/R plus vit E group, I/R plus biso plus vit E group. On the 5th day, the platform was removed for the memory test. The locus of rats swimming in the maze was recorded. (A) The percentage of time spent in the quadrant where the platform was located was calculated as mean ± SD. (B) The number that rats crossed the previous platform location in each group was presented as mean ± SD. Data were shown as mean ± SD. (###) $P < 0.001$, relative to sham group. (*) $P < 0.05$ relative to I/R. (**) $P < 0.051$ relative to I/R. (+) $P < 0.05$ relative to I/R plus biso plus vit E group. $N = 6$ for each group. I/R: ischemia/reperfusion, biso: bisoprolol, vit E: vitamin E

The area of cerebral infarction as observed in the and the vit E pretreated groups and combination group were significantly reduced compared with the I/R group. In the present study, we also find that both biso and vit E significantly decreased the infarct size; however, the combination of them could exert a much stronger effect on reducing these parameters. Furthermore, biso or vit E pretreatment decreased brain dysfunction and histopathological changes. The histopathological score was significantly decreased in all treated groups compared to the I/R group. Neuronal damage after I/R can cause disorders of memory and spatial learning, deficits of locomotive activity, as well as dysfunction of anxiety mediation (Ge et al. 2017). The hippocampus is crucial for cognition and memory formation, several evidences suggest that ischemia causes various neuronal damages in the hippocampus and leads to impairments of memory and learning (Kumral et al. 2004; Pu et al. 2009; Tao et al. 2017). To investigate whether pretreatment with biso and vit E improve spatial learning and memory as well as locomotor activity damaged by I/R, we performed MWM test and open field test experiments (Ge et al. 2017). Behavioral examination showed a significant improvement in memory and locomotor activity. The results showed that the escape latency of rats in the I/R group was longer than that of rats in the sham-operated group on the second, third, and fourth days (Fig. 7), which suggested that the learning capacity of the hippocampus was damaged after global cerebral I/R injury. Our results are consistent with previous studies showing that transient bilateral common carotid artery occlusion correlates with impaired learning performance in the MWM task (Hartman et al. 2005; Schiavon et al. 2014). The escape latencies of rats in the biso and vit E groups were shorter than that of rats in the I/R group, although there were no significant differences between the biso and vit E intervention groups on the fourth day. However, the best results were obtained in the combination group, which suggested that both biso and vit E improved the learning capacity of rats and their combination potentiated this effect. In the probe trial, time spent in the second quadrant was shorter in the I/R group compared with the sham-operated group (Fig. 8), which suggested that the I/R injury reduced the memory capacity of rats. According to our results, the number of crossing and the percentage of time in the target quadrant significantly increased in all of the treatment groups compared with I/R group in the probe trail. Our study showed that the administration of biso and vit E before I/R protected locomotive and cognitive ability in cerebral I/R injured rats. These results were in line with previous studies which have shown that pretreatment with antioxidant improved memory and locomotor activities after cerebral I/R (Ghadernezhad et al. 2016; Patel et al. 2020). However, post treatment by these agents should be examined to accept our working hypothesis in biso or vit E therapy. Our results were not consistent with the other previous study by Iwata et al which reported that post-treatment but not pretreatment with esmolol a selective adrenoreceptor 1 antagonist provides neuroprotection against cerebral ischemia. One possible cause of this inconsistency can be the difference in the duration of ischemia. The occlusion time in our study was longer (20 min versus 8 min). Therefore, the difference in experimental design may be the cause of this conflicting result. Furthermore, in addition to blocking of adrenoreceptor, the anti-inflammatory and anti-oxidative stress properties of bisoprolol may involve in the observed protective effects of this drug in the current study. the limitation of the present study is that we studied only the pretreatment effect of vit E and biso in our study. Therefore, we suggest that the protective effects of post treatment with these agents evaluated in future studies.

Conclusion

The present study indicates that pretreatment with biso and vit E each exert beneficial preventive effects against I/R injury. According to the behavioral tests pretreatment with these agents suppressed the memory and locomotion impairments induced by cerebral I/R by alleviating inflammation and oxidative stress with the most prominent effect in combination therapy However, in order to determine the efficacy of such therapies in patients with ischemic stroke, additional studies are required to verify the beneficial effects of these agents after cerebral I/R.

Author contributions The authors declare that all data were generated in-house and that no paper mill was used. M Ghasemnejad-Berenji and Hamid Soraya: study design, analysis of the data, revised the article, and funding acquisition. Chiman Salehi: investigation, literature review, and writing the original draft of the article. Monireh Seiiedy: literature review and writing manuscript. Farzaneh Fazli: performed the behavioral analyses. All authors contributed substantially to this work and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval All procedures were conducted in accordance with the accepted principles for the care and use of laboratory animals and were approved by the animal ethics committee of Urmia University of Medical Sciences (IR.UMSU.REC.1397.328).

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