

Original Research Article

## Beneficial effects of berberine and 6-gingerol against global cerebral ischemia: Insights from stereological, behavioral, and antioxidant enzyme activity

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### Abstract

**Objective:** Global cerebral ischemia (GCI) severely impacts brain function, leading to cognitive impairments and neuronal damage, particularly in the hippocampus. This study examined the neuroprotective and antioxidant properties of Berberine (BBR) and 6-Gingerol (GI), both separately and in combination, after inducing global cerebral ischemia (GCI) in Wistar rats.

**Materials and Methods:** Fifty rats were randomly assigned to five groups: Sham, GCI, GCI+BBR, GCI+GI, and GCI+BBR+GI. Treatments were given intragastrically 15 min after inducing GCI. Short-term spatial memory was evaluated through the Y-maze test, and hippocampal structure and antioxidant enzyme activity were analyzed using stereological and biochemical methods, respectively.

**Results:** GCI+BBR+GI showed better memory function ( $p<0.01$ ), antioxidant enzyme activities ( $p<0.01$ ), and stereological parameters than the other treatment groups.

**Conclusion:** BBR, GI, and their combination improved memory, preserved CA1 pyramidal neurons, and enhanced antioxidant enzyme activities compared to GCI alone. The combined treatment produced the most pronounced protective effects. These findings suggest that BBR and GI, particularly together, effectively mitigate hippocampal damage and cognitive deficits following GCI.

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### Introduction

Brain ischemia is a significant contributor to the second most common cause of death on a global scale (Li et al. 2017). Global cerebral ischemia (GCI) leads to a reduction in continuous blood flow during the aftermath, and the

hippocampus is particularly susceptible to the impact of this insult. Various subfields within the hippocampus exhibit varying degrees of vulnerability to cell death, with the CA1 pyramidal neurons being more susceptible (Simões Pires et al. 2014). The cognitive impairments observed in rodents

and humans after GCI are linked to damage in the hippocampus (Gulinello *et al.* 2006). In a cerebral stroke, the generation of reactive oxygen species (ROS) increases from the mitochondrial electron transport chain (ETC) at the cytochrome III level (Chen *et al.* 2003; Liu *et al.* 2002; Murphy 2009). The natural way for the body to protect itself against ROS is its inherent antioxidant capacity (Guo *et al.* 2017). However, the body's antioxidant capacity is not strong enough to avoid huge damages received from ROS because of cerebral ischemia in the brain. Studies indicate that taking medication can be crucial in protecting and preserving neurons. This is accomplished by maintaining or boosting the antioxidant level, such as glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD), in CA1 pyramidal neurons after experiencing GCI (Sadeghzadeh *et al.* 2023). Besides medication, several dietary strategies such as ketogenic and antioxidant-rich diets have also shown promise in easing the symptoms of progressive neurological disorders (Pistollato *et al.* 2018; Rusek *et al.* 2019).

Berberine is a quaternary ammonium alkaloid and a crucial component of Chinese herbal medicines like *Rhizoma Coptidis*, *Rhizoma Cyperus*, and *Rhizoma Rhei*. Research has demonstrated that berberine exhibits a range of biological activities including hypoglycemic, hypolipidemic, antimicrobial, antitumor (Gao *et al.* 2024), antioxidant, and anti-inflammatory (Kordestani *et al.* 2024) effects. On the other hand, *Zingiber officinale* Roscoe, commonly known as ginger, is a tropical monocotyledon plant with a perennial tuberous rhizome. It is widely used as a spice in African, American, and Chinese cuisines. Ginger has a long history of use in traditional medicine for treating various health issues such as nausea and inflammation. In recent times, ginger and its derivatives have been associated with a range of pharmacological properties including antioxidant and anti-inflammatory effects (Russo *et al.* 2023).

This study sought to evaluate the individual and combined protective effects of 6-gingerol (GI, Sigma Aldrich, USA, purity  $\geq 98\%$ ) and berberine (BBR, sigma aldrich-USA,  $\geq 98\%$ ) against GCI. It specifically focused on examining the hippocampal neural structure, assessing memory function, and evaluating the antioxidant response.

## Materials and Methods

### Animals

Fifty adult male Wistar rats, 6–8 weeks old, with body weights ranging from 160 to 210 g were housed under regulated conditions, featuring a 12-hr light/dark cycle and a temperature maintained at  $22\pm 2^\circ\text{C}$ , with continuous access to food and water.

### Study design:

The rats were randomly assigned to five groups, each consisting of ten animals: Sham (n=10), GCI (n=10), GCI with BBR treatment (n=10), GCI with GI treatment (n=10), and GCI with combined BBR and GI treatment (n=10).

### Sham group

The rats were anesthetized and underwent surgery without blocking the common carotid arteries. Fifteen minutes after the procedure, they received an intragastric dose of saline at 100 mg/kg.

### GCI group

Rats were subjected to surgery to induce GCI by occluding both common carotid arteries for 20 min, followed by intragastric administration of 100 mg/kg saline 15 min post-surgery.

### GCI + BBR group

Rats were subjected to surgery to induce GCI by temporarily blocking both common carotid arteries for 20 min, followed by intragastric administration (Liu *et al.* 2020) of 100 mg/kg of BBR 15

minutes after the surgery (Mehboodi et al. 2025a; Mehboodi et al. 2024b).

### **GCI + GI group**

Rats were subjected to surgery to induce GCI by blocking both common carotid arteries for 20 min, after which they received an intragastric dose of 100 mg/kg of GI (Endah Wulandari 2024) 15 min after the surgery.

### **GCI + BBR + GI group**

Rats underwent surgery to induce GCI by occluding both common carotid arteries for 20 min, followed by intragastric administration of 100 mg/kg of both BBR and GI 15 min after the surgery.

Twenty-four hours after surgery, every group was divided into two subgroups 5 rats from each group were used for a behavioral test and stereological analysis and 5 rats were used for antioxidant response evaluation

### **Induction of GCI**

The rats were anesthetized with a combination of 100 mg/kg ketamine and 10 mg/kg xylazine (Dehghani et al. 2025). After isolating the vagus nerve, the carotid arteries were temporarily clamped for 20 min. During this time, the rats' rectal temperatures were carefully monitored to stay within  $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ . Following the occlusion, the artery clamps were removed. To counteract any potential impact of hypothermia on the experimental outcomes, the rats were then placed in a heated enclosure set at  $30^{\circ}\text{C}$  for 3 hr before being returned to their cages (Mehboodi et al. 2025b).

### **Short-term spatial memory**

Twenty-four hours after the GCI, the rats' short-term spatial memory was assessed using a Y-maze. The Y-maze consists of three arms positioned at equal angles, each with dimensions of 20 cm in length, 5 cm in width, and 10 cm in height. The rats were placed at the center of the maze and given 8 min to explore freely. The

order and total number of arm entries were meticulously recorded, with an entry being counted once the rat's hind paws were completely inside the arm (Lee et al. 2012). The percentage of alternation was calculated using the formula

$$\left[ \frac{\text{(number of successive triplets)}}{\text{(total number of arm entries - 2)}} \right] \times 100$$

as described by Mori et al. in 2001 (Mori et al. 2001). The higher percentage of alternation shows better short-term spatial memory.

### **Preparation of hippocampus sections**

The rats were anesthetized with a mixture of 100 mg/kg ketamine and 20 mg/kg xylazine. Following this, their brains were extracted after performing transcardial perfusion with 0.9% saline and 10% formalin for stereological analysis. We prepared the samples using the same technique as described in our previous study (Mehboodi et al. 2024b). The brain hemispheres were thoroughly rinsed with running water, and the tissues were then processed through graded alcohol solutions, xylene, and finally paraffin embedding in specialized cassettes. For sectioning, serial slices of 25  $\mu\text{m}$  thickness were cut at 400  $\mu\text{m}$  intervals. Every 16th section was collected, resulting in a total of 10 sections per animal being selected then the tissues were stained by using the protocol of cresyl violet that we used in our previous study (Mehboodi et al. 2024b).

### **Antioxidant enzyme activity analysis (SOD, CAT and GPx)**

To assess the activity of biochemical factors such as superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT), five rats from each group were euthanized using an intraperitoneal injection of xylazine and ketamine (10 and 75 mg/kg, respectively; Sigma-Aldrich, Germany). Approximately 100 mg (Mehboodi et al. 2024a) of hippocampal tissue was combined with 1 ml of Phosphate-buffered saline (PBS), homogenized on ice, and centrifuged at

4,000 RPM for 20 min. The supernatant was then separated and analyzed according to the protocols provided with the respective kits (Zell BIO, Germany).

### **Stereological analysis**

The CA1 was examined using point-counting techniques by Cavalieri's principle. The reference volume ( $V_{ref}$ ) was determined using the formula:  $V_{ref} = d \times a(p) \times \Sigma P$ . In this formula, 'd' represents the distance between sections, 'a(p)' denotes the area per point on the grid, and ' $\Sigma P$ ' signifies the total number of points that intersect the rat hippocampus section (Mehboodi *et al.* 2025c; Namavar *et al.* 2012). Pyramidal neuron density and total neuron count in the pyramidal layer were determined using the optical disector technique. This involved a high-numerical-aperture (NA=1.30)  $\times 40$  oil-immersion objective linked to a video camera. Microscopic images were viewed on a monitor, while Z-direction movements were recorded with an electronic microcator (MT12, Heidenhain, Traunreut, Germany) featuring a digital readout. Neuronal density was calculated with the formula  $NV = \Sigma Q / [\Sigma P \times a(f) \times h]$ , where  $\Sigma Q$  represents the number of neurons within the sampling frame,  $\Sigma P$  denotes the number of directors,  $a(f)$  is the area of the sampling frame, and  $h$  is the height of the sampling frame in the Z-direction. (Owjfard *et al.* 2022). Brain cells were categorized as neurons or non-neurons. Neurons in Nissl-stained (Cresyl violet) sections were identified based on characteristics described by Gabbott and Stewart (Gabbott and Stewart 1987) and Ling *et al.* (Ling *et al.* 1973). In Cresyl Violet staining, a healthy neuron typically shows a large cell body (perikaryon) with well-defined dendrites and an axon. The perikaryon and dendrites are filled with Nissl substance, while the nucleus appears pale, euchromatic, and distinct, often with a visible nucleolus. Conversely, dead or dying neurons—whether apoptotic or necrotic exhibit dense, globular cytoplasmic material, fragmented

nuclei, a shrunken perikaryon, and darkly stained, smaller nuclei.

### **Statistical analysis**

Data analysis was conducted using SPSS software, version 27. To assess mean differences between groups at each time point, a one-way ANOVA test followed by Tukey's post hoc test was utilized. Statistical significance was determined with a threshold set at  $p < 0.05$ .

## **Results**

### **Effect of BBR, GI and their combination on the stereological parameters of the hippocampus**

The sham group showed significantly higher volume in the stereological parameters than the other groups. It was the closest to the GCI+BBR+GI group. Among the treatment groups, the GCI+BBR+GI group showed the largest improvement compared to the GCI group (Figure 1 and 2).

### **The results of short-term spatial memory analysis**

Based on the Y-maze test results, the sham group exhibited a higher alternation percentage compared to all other groups. The difference in alternation percentage between the GCI+BBR+GI group and the GCI group was notably greater than the differences observed between GCI and the other treatment groups (GCI+BBR and GCI+GI) (Figure 3).

### **Effect of BBR on the antioxidant enzyme activity in the hippocampus**

The sham group showed the highest levels of antioxidant activity. The sham group was closest to the GCI+BBR+GI groups in terms of CAT, GPX, and SOD activity. The most notable differences were found between the GCI group and the GCI+BBR+GI group. (Figure 4)

## Berberine and 6-Gingerol against GCI

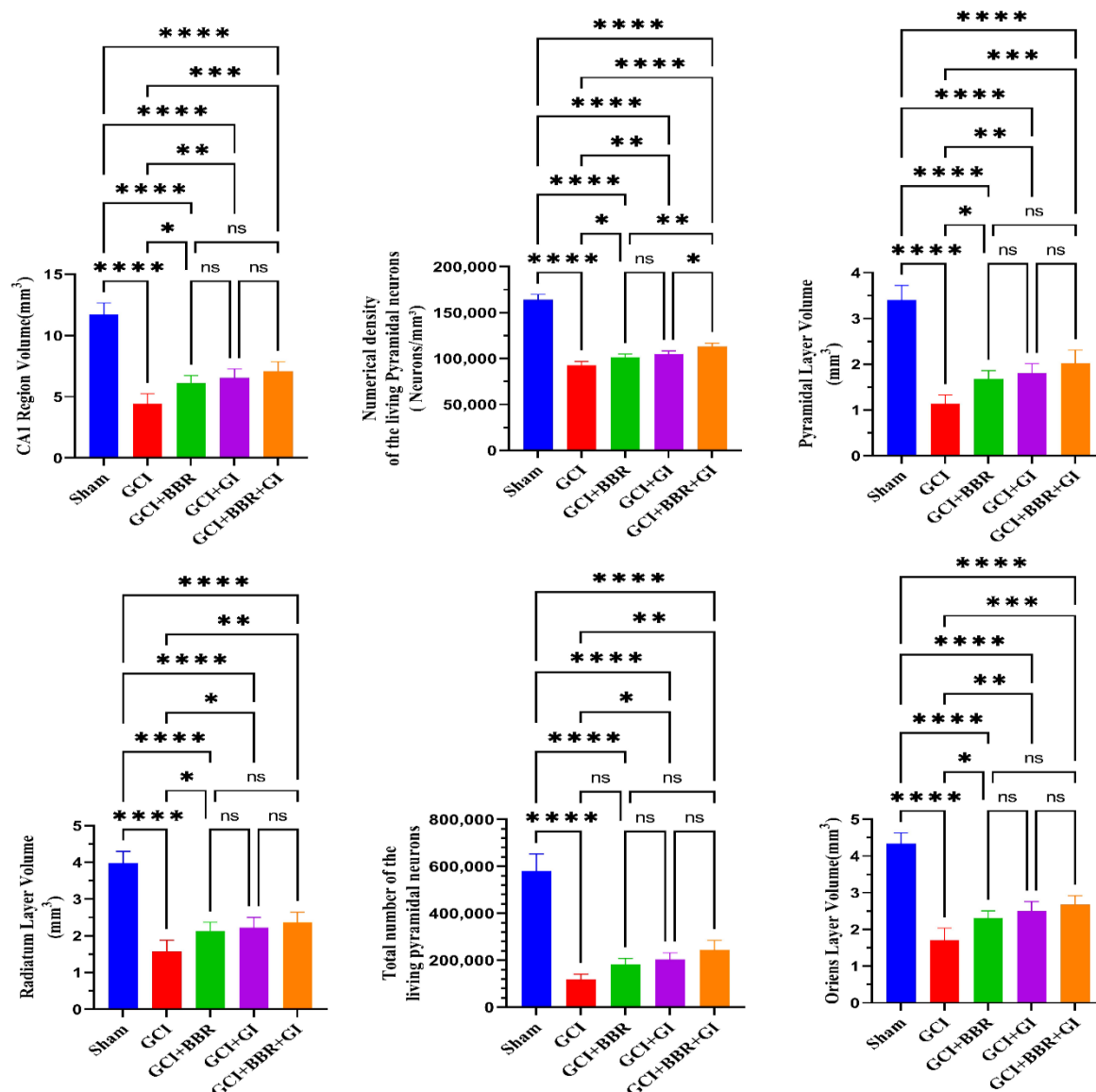


Figure 1. The stereological analysis results. The data are shown as Mean  $\pm$  SD (n = 5 rats per group). The study assessed the effect of 100 mg/kg of Berberine (BBR), 6-Gingerol (GI), and their combination, administered orally 15 min after the completion of Global Cerebral Ischemia (GCI) induction (20 min of bilateral common carotid occlusion), on the stereological parameters of the hippocampus. Significance levels were set at \* $p$ <0.05, \*\* $p$ <0.01, \*\*\* $p$ <0.001, and \*\*\*\* $p$ <0.0001.

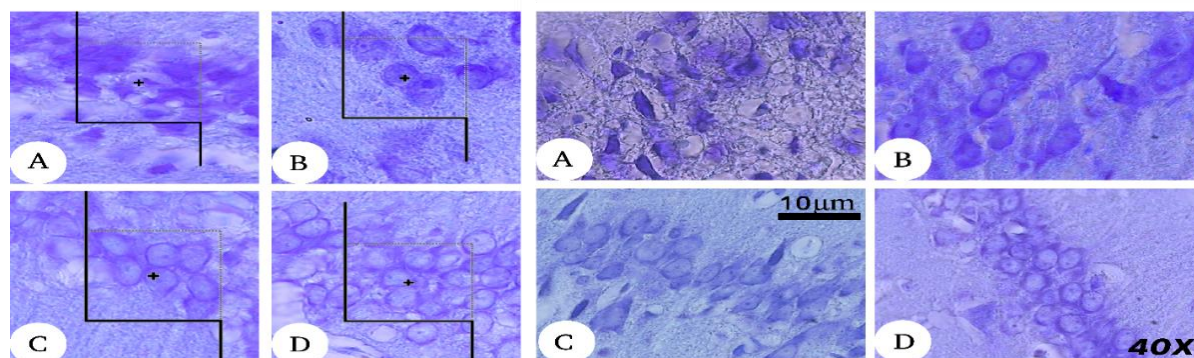


Figure 2. Histological images of the CA1 region in cresyl violet staining, As shown in these images, the density of living pyramidal neurons is highest in the following order: D: GCI+BBR+GI, C: GCI+GI, B: GCI+BBR, and A: GCI groups. GCI refers to Global Cerebral Ischemia, BBR stands for Berberine, and GI denotes 6-Gingerol. The reperfusion time was 24 hr post-GCI induction.

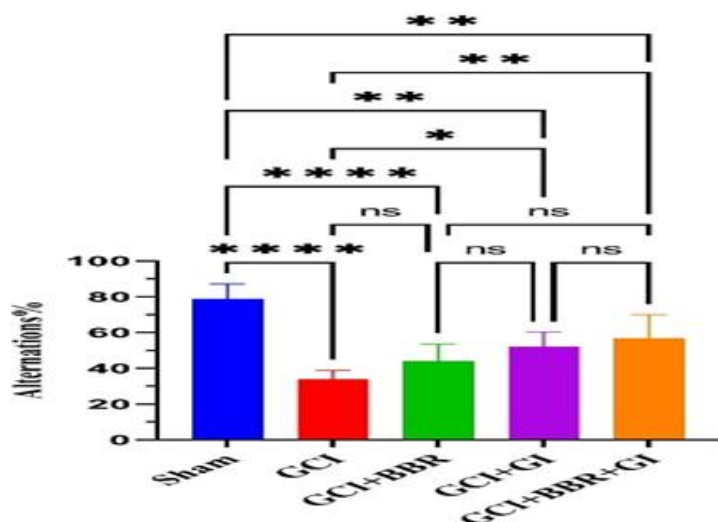


Figure 3. Short term spatial memory analysis result. Data are showed as Mean  $\pm$  SD (n = 5 rats per group). The effects of a 100 mg/kg dose of BBR, GI, and their combination, administered orally 15 minutes after the induction of Global Cerebral Ischemia (GCI) through 20 min of bilateral common carotid occlusion, on short-term spatial memory assessed by the Y-maze test. Statistical significance is indicated by \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ , with a significance threshold set at  $p < 0.05$ . GCI: Global Cerebral Ischemia, BBR: Berberine, GI: 6-Gingerol.

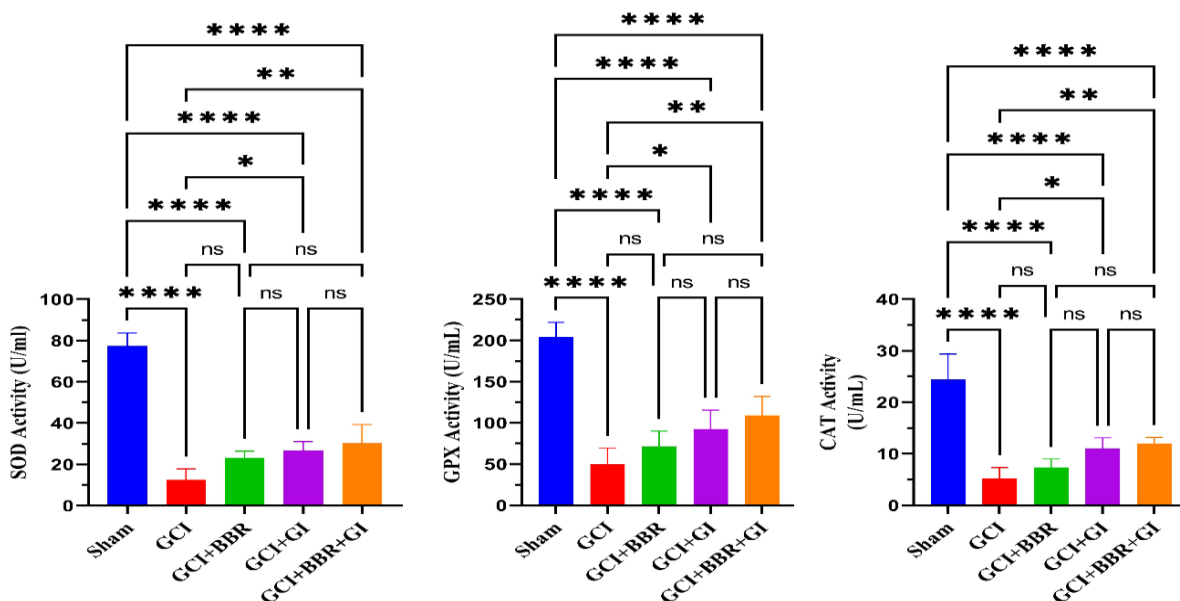


Figure 4. Antioxidant enzyme activity in the hippocampus analysis results. The data is presented as Mean  $\pm$  SD (n = 5 rats per group). The effect of a 100 mg/kg dose of Berberine (BBR), 6-Gingerol (GI), and their combination, administered orally 15 minutes after the induction of Global Cerebral Ischemia (GCI) through 20 min of bilateral common carotid occlusion, was assessed on short-term spatial memory using the Y maze test. Statistical significance was set at \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ . GCI: Global Cerebral Ischemia, BBR: Berberine, GI: 6-Gingerol. Reperfusion occurred 24 hr after GCI induction.

### Discussion

This study examined the effects of BBR, GI, and their combination in male Wistar rats using a GCI model. Stereological parameters, memory function, and antioxidant activity were evaluated. Treatment with BBR, GI, or their combination, each at a dose of 100 mg/kg, improved memory performance. Furthermore, the stereological analysis showed less neuronal damage in the hippocampus of the treated groups compared to the GCI group. The combination treatment was found to produce the most significant neuroprotective effect and antioxidant response among all protocols tested.

The subsequent reperfusion process leads to a marked rise in ROS in the brain, including hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), superoxide anions (O<sub>2</sub><sup>·-</sup>), and hydroxyl radicals (·OH). These ROS contribute to widespread nerve cell damage and significantly worsen brain injury (Kong et al. 2024). The results of the present study showed that administration of BBR, GI and their combination can increase the level of antioxidants and the combination of BBR and GI increased the antioxidant activities more than other treatment groups. As the results showed, GCI+GI increased antioxidants activity more than the GCI+BBR and it could be because of its higher bioavailability in oral administration in rats than the BBR (Bao et al. 2020; Liu et al. 2016). Several studies approved the positive role of BBR in increasing the antioxidant activity of SOD, GPX, and CAT; for example in a previous study we administered BBR for 7 days prior and 6 hr after GCI the activity of SOD, GPX, and CAT increased (Mehboodi et al. 2024c). Also some studies approved the ability of GI in decreasing oxidative stress in the tissue; for example, the research carried out by Jittiwat and Wattanathorn (Jittiwat and Wattanathorn 2012) showed its ability in decreasing oxidative stress in the rats model of right middle cerebral artery occlusion (Rt. MCAO). Also, the study that

conducted by Asuku et al (Asuku et al. 2024) approved the ability of GI in increasing SOD, GPX and CAT activities in rats brain against mercury chloride (HgCl<sub>2</sub>)-induced neurotoxicity.

Based on stereological analysis, the GCI+BBR+GI group exhibited less reduction in stereological parameters compared to the other treatment groups. The GCI+GI group showed a greater neuroprotective effect than GCI+BBR. In some stereological measures, GCI+BBR differed significantly from the GCI group, but a single dose of BBR in the GCI+BBR group was not sufficient to produce a significant improvement in pyramidal cell volume or their total number compared to the GCI group. The results of our previous study (Mehboodi et al. 2024b) about the neuroprotective effect of BBR against GCI showed that it could make a low significance difference with the GCI and these differences in the results could be because of the timetable of administration because that study we administered BBR two times after GCI to the rats. On the other hand, the study conducted by Wattanathorn and Jittiwat (Wattanathorn et al. 2011) showed ginger can decrease the loss in the density of neurons that happened in the rat model of MCAO, The results of the present study demonstrated that GI reduced neuronal density loss and improved other stereological parameters. The results of the stereological analysis approved the neuroprotective effect of GI and BBR against GCI, this protective effect had been seen in some other studies for example the study conducted by Luo et al (Luo et al. 2021) in 2021 approved this neuroprotective effect against MCAO. The Y-maze results supported the stereological findings. GCI impaired pyramidal neurons and caused poor short-term spatial memory. The GCI+BBR+GI group showed the highest alternation rate, indicating the best memory, followed by the GCI+GI group, which showed greater improvement compared to the GCI+BBR group. Several studies approved the memory protection

effect of GI against cerebral ischemia; the study conducted by Wattanathorn and Jittiwat (Wattanathorn et al. 2011) demonstrated a protective effect on memory impairment following MCAO. The results of our previous study (Mehboodi et al. 2024b) approved the memory protecting effect of BBR against GCI in different timetables and dosage administration. Our study is the first to examine the combined effects of GI and BBR against GCI. The combination improved all antioxidant, stereological, and behavioral outcomes compared to individual treatments.

In summary, our study confirmed the neuroprotective benefits of BBR, GI, and their combination against GCI. The greatest effect was observed with the GCI+BBR+GI treatment. The combined treatment enhanced SOD, GPX, and CAT activities, as well as stereological and behavioral outcomes, showing it is more effective in reducing GCI-related impairments than either treatment alone.

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### Conflicts of interest

The authors declared no conflict of interest.

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### Ethical Considerations

All experimental procedures were conducted in accordance with the guidelines for the care and use of laboratory

animals and were approved by Shahid Sadoughi University of Medical Sciences. Efforts were made to minimize animal suffering and reduce the number of animals used. All interventions, including GCI induction, drug administration, and behavioral tests, were carried out following ethical standards to ensure animal welfare.

### Code of Ethics

IR.SSU.AEC.1404.019

### Authors' Contributions

Conceptualization: Dariush Mehboodi; data curation: Dariush Mehboodi; formal analysis: Dariush Mehboodi; investigation: Dariush Mehboodi; methodology: all authors; project administration: Dariush Mehboodi; resources: Dariush Mehboodi, Abbas Shahedi, Mohammad Reza Namavar; writing—original draft: Dariush Mehboodi; validation: all authors; visualization: Dariush Mehboodi.

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