The increasing effect of pre-feeding with cumin extract on the permeability of the brain-blood barrier caused by stroke in rats

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Abstract:
Introduction:
Stroke is the most important neurological disease and the third leading cause of death after coronary heart diseases and cancer in old people. Despite the many attempts for treating or understanding the pathophysiology of strokes, no effective treatments have yet been found for this accident. With the scientific name Cuminum cyminum, cumin has great medicinal benefits and is used in the treatment of different diseases. The present study was conducted to investigate the effectiveness of cumin extract in mitigating brain-blood barrier damage caused by stroke.

Methods & Materials:
The present experimental study was conducted on five groups of six rats, including one sham, one control and three trial groups. The trial groups received 25, 50 or 100 mg/kg of body weight oral dose of cumin extract for 30 days. Two hours after the last gavage, the control and trial groups underwent middle cerebral artery occlusion surgery to induce ischemia and their brain-blood barrier integrity was then measured.

Results:
No significant differences were observed in the concentration of Evans blue in the damaged cerebral hemisphere (the right hemisphere) between the trial groups that received 25 and 50 mg/kg of body weight oral dose of cumin extract and the control group. Nevertheless, a significant difference was observed in this concentration between the control group and the trial group that received an 100 mg/kg dose of the extract, as the concentration of Evans blue reduced in the right hemisphere with the administration of this dose of the extract (P<0.05).

Conclusion:
Pre-treatment with 100 mg/kg of body weight oral dose of cumin extract reduces the brain-blood barrier damage caused by stroke.

Keywords: Stroke, Cumin, Blood-brain Barrier, Rat

Introduction
Stroke is considered as a major public health problem and one of the main causes of mortality (1). About a third of people die during the first few months after stroke and about 10% of people who survive will be dependent on others for life, and only about 50% of them are able to perform limited activities after stroke (2). There are two major mechanisms for stroke including ischemic and hemorrhagic strokes (3). In ischemic stroke, which can be observed in 85% to 90% of cases of stroke (4), blood flow is cut off due to blockage of blood vessels and as a result...
brain does not get enough oxygen or nutrients (5). Cerebral ischemia can lead to motor deficits, sensory deficits, visual impairment and speech impairment. After ischemia the permeability of the blood-brain barrier is increased that its intensity is directly related to the brain lesion (6). Disruption in the blood-brain barrier leads to disruption in tight junctions, disruption in the transport of molecules between the brain and blood, cerebral edema and inflammatory responses (7) which contribute to the creation of Alzheimer’s disease, Parkinson’s disease, multiple sclerosis and other diseases.

Cuminum cyminum is a flowering plant in the family Apiaceae, native from the east Mediterranean known as spices which also has many uses as a traditional medicinal plant (8). Therapeutic effects of Cuminum cyminum include digestive, carminative, diuretic and mild emmenagogic, anthelmintic, anti-seizure and galactagogue (9,10). Studies also show that this plant has other properties such as analgesic (11), anti-tumor (12), antioxidant (13), anti-diabetic (14), anti-bacterial (15), antifungal (16), hypolipidemic (17) and anti-thrombotic effects (18). Important components of this plant include Cuminol, Carvone, Apigenin and Luteolin (19,20). Flavonoids in Cuminum cyminum prevent cancer possibly by reducing free radicals (21). Given antioxidant compounds and beneficial effects of Cuminum cyminum and given that no study has been conducted yet on the possible effects of this plant in preventing ischemic stroke, this study aimed to investigate the effects of Cuminum cyminum to reduce the damage of blood-brain barrier caused by ischemic stroke.

**Materials and Methods**

In this study, Cuminum cyminum approved by the Herbarium of Research Center was used which was extracted by Soxhlet method. Male Wistar rats weighing 250-350 g were purchased from Razi Serum-Producing Institute. Rats were divided into five groups each with six. Control and sham groups were gavaged with distilled water and three experimental groups received Cuminum cyminum extract at a dose of 25, 50 and 100 mg/kg of body weight. Treatment period was considered thirty days.

**Middle cerebral artery occlusion (MCAO)**

Local brain ischemia was performed by middle cerebral artery occlusion according to guidelines of Lunga et al. to create ischemic stroke model (22). Two hours after the last gavage, rats were anesthetized by intraperitoneal injection of 400 mg/kg of body weight with chloral hydrate (Merck, Germany) and an incision was made under the loop in the anterior cervical region through surgery. Then common, external and internal carotid arteries were gently separated from surrounding tissues, especially the vagus nerve. External and common carotid arteries were temporarily and internal carotid artery was permanently blocked by a micro clamp and a small incision was made in the external carotid artery and a 0-3 nylon suture whose tip was rounded in front of the flame was entered the right arterial vessel through the external carotid artery trunk and continued until reaching the anterior cerebral artery through internal carotid artery. Suture yarn was directed toward the inside of the brain and the circle of Willis from the bifurcation of internal artery to reach a subtle resistance against the suture yarn and in front of the yarn with the size of about 20 mm. This feeling of resistance shows that the circle of Willis was blocked. Thus, blood flow was cut off in the middle cerebral artery and as a result ischemia occurred in areas of the brain that were perfused by this artery. One hour after middle cerebral artery occlusion, blood flow was restored by removing the filament. Body temperature was measured by a rectal and kept close to 37 ° C.
Strength of blood-brain barrier
The strength of blood-brain barrier was evaluated by the removal of Evans Blue (Merck, Germany). To do this, after thirty minutes of ischemia, rats received Evans Blue (2% solution 4 ml/kg of body weight) via tail vein. Twenty-four hours after restoration of blood flow, the chest of rats was open under anesthesia and Evans Blue was removed from circulation by 250 ml of normal saline through the left ventricle. This was continued until colorless profuse fluid was extracted from the right atrium. Then the brain was removed and its tissue was homogenized in 2.5 ml phosphate buffer to measure the extraction of Evans Blue and 2.5 ml Trichloroacetic acid 60% (Merck, Germany) was added for protein precipitation. Then, it was stirred by vertex (IKA, Germany) for three minutes and was kept in the refrigerator for thirty minutes. Then it was centrifuged for thirty minutes at 1000 rpm with a centrifuge (BOECO, Germany) and finally the absorbance of Evans Blue was measured at 610 nm by a spectrophotometer (TermoSpectronic, GENESYS 5, America) and its concentration was calculated according to standard curves (23).

Data analysis
Data were analyzed by SPSS-20 and using one-way ANOVA. LSD method was used for multiple comparisons. P<0.05 was considered as a statistical significance level.

Results:
Reduced concentration of Evans blue in the brain tissue indicates a reduction in permeability of the blood-brain barrier. The results showed that there is a significant difference between the concentration of Evans blue in the right hemisphere (damaged) in the sham group (6.23 ± 0.36) and the control group (7.79 ± 0.94) and the group with the dose of 25 mg (7.75 ± 0.51) (p=0.004, p=0.007) (Figure 1). On the other hand, no significant difference was observed between the concentration of Evans blue in the damaged hemisphere in the control group (7.79 ± 0.94) and the group with the dose of 25 mg (7.75 ± 0.51) and the group with the dose of 50 mg (7.75 ± 0.74) (p=0.838, p=0.371), but it was significantly reduced compared to the group with the dose of 100 mg (6.69 ± 0.49) (p=0.002) (Figure 1). In addition, there was a significant difference between the right and left hemispheres in the control group and the groups treated with doses of 25 and 50 mg Cuminum cyminum extract (p=0.00), but no significant difference was observed between the right and left hemispheres in the group treated with the Cuminum cyminum extract and the group treated with a dose of 100 mg (p=0.189) (Figure 1).

Figure 1: Comparison of Evans Blue concentration in the cerebral hemispheres in the groups

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Discussion

Evaluation of the effect of Cuminum cyminum on the strength of the blood-brain barrier in the middle cerebral artery occlusion model in this study showed that oral administration of the extract reduced the damage in the blood-brain barrier. The dose of 100 mg/kg aqueous extract of Cuminum cyminum caused a significant decrease in the permeability of the blood-brain barrier, but this effect was not observed in two doses of 25 and 50 mg/kg. The blood-brain barrier ensures proper control of homeostasis in the brain. The anatomical structure of blood-brain barrier includes glial-vascular complex and tight junctions between endothelial cells which make possible the transfer of selected substances from blood to the brain and from the brain to blood (24). An important part of ischemic brain edema is also related to increased permeability of the blood-brain barrier, which it called edema of vascular origin. This type of edema is associated with high leakage of fluid from damaged vessels and if is associated with bleeding it increases the risk of stroke (25). Blood-brain barrier consists of tight capillary walls and normally prevents the free exchange of solutes between the blood and the brain and vice versa. The barrier is completely different from neurovascular system that regulates Pericytes, Astrocytes and Microglia and isolates the blood circulation components from neuronal cells. In addition, blood-brain barrier protects the nervous environment which is necessary for the proper functioning of neural circuitry, synaptic transmission, synaptic regeneration, angiogenesis and neurogenesis in the brain of adults. In fact, the disruption of the blood-brain barrier is caused by the disruption of tight junctions, disruption in the transport of molecules between the brain and blood, and the brain, brain hypoperfusion and inflammatory responses which contribute to the creation of Alzheimer’s disease, Parkinson’s disease, multiple sclerosis and other diseases (7). Evans Blue after entering circulation is bound to plasma albumin and cannot be removed from healthy artery walls. The removal of Evans Blue with blood proteins from damaged cerebral vessels during ischemia and quantifying the leakage out of blood vessels can help to reveal the severity of injury or therapeutic effects of medications on worsening or improving the brain damage during experimental ischemia (26).

Studies on a variety of medicinal plants, including Cuminum cyminum shows that the more is phenolic content in the plant, the higher is its antioxidant capacity (27,28). Phytochemical reviews show Cuminol, Carvone, Apigenin and Luteolin in Cuminum cyminum compounds (19,20). Apigenin flavonoid inhibits the inflammatory response induced by lipopolysaccharide in macrophages and also prevents the release of inflammatory mediators in HMC-1 cells by blocking NF-Kb paths (29). This compound which is also found in many vegetables such as parsley and celery (30) has a variety of biological activities including antioxidant, anti-inflammatory and anti-tumor and neuron protective properties. Antioxidant and anti-apoptotic properties of Apigenin can be due to affecting the expression of Nrf2, P53 and the downstream target genes transcription (31). When PC12 cells lack glucose, oxygen and blood supply, the mitochondrial membrane potential, cell survival and mRNA levels of antioxidants and also protein expression of Nrf2 are decreased. The pre-treatment of these cells with Apigenin mainly through antioxidant and anti-apoptotic activities decrease the mentioned cases compared to the control group (31).

Bhatt et al. in their study found that aqueous and ethanol extracts of Cuminum cyminum seeds have analgesic and anti-inflammatory effects. They reported that aqueous extracts compared to ethanol extracts reduce more effectively inflammation caused by carrageenan in rats. They stated that anti-inflammatory
activity of the extract is due to inhibitive action of the extract on the release of prostaglandins and that the aqueous extract may be involved in the initial phase of inflammation through inhibiting the release of histamine, serotonin and quinones (32). Monoterpenes, linalol, gamma terpinene, alpha-pinene and beta-pinene Compounds (33) are related to the anti-inflammatory activity of the plant (34).

Coppola et al. also reported that daily administration of Cuminum cyminum extract with three doses of 100, 200 and 300 mg/kg of body weight reduces urinary biochemical changes due to stress induced by forced swimming and induced memory failure with scopolamine and lipid peroxidation in rats (35).

Until now, no scientific study has been reported about the effect of Cuminum cyminum on ischemic stroke. Therefore, the results of this study, conducted for the first time, and other studies about the effects of this plant and its effective compounds, show the useful effects of Cuminum cyminum extract in reducing symptoms of stroke, including blood-brain barrier damage.

Conclusion
Given flavonoids and phenolic compounds in Cuminum cyminum and the results of many studies that emphasize the direct relationship between these compounds and antioxidant and anti-inflammatory activity, Cuminum cyminum can prevent the destruction of the blood-brain barrier caused by ischemic stroke and is recommended as a neurological protection. Obviously, in this regard, complementary studies are recommended.

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Conflict of interests
The Authors declare that there is no conflict of interest regarding the authorship or publication of this paper.

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