Research Paper Pre-nutritional Effects of Hot (Cinnamon) and Cold (Lentil) Temperaments on the Animal Model of Stroke

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ABSTRACT

Background and Aim: Stroke, mainly caused by atherosclerosis, is the second leading cause of death worldwide. Atherosclerosis may be caused by spleen dysfunction, and oxidative stress intensifies the brain damage induced by cerebral ischemia. According to the studies, cinnamon and lentils as hot and cold temperaments, respectively, contain antioxidant compounds and affect spleen function. This study investigated and compared the effect of cinnamon and lentils in preventing stroke.

Materials and Methods: Cinnamon and lentil extracts were injected intraperitoneally daily to adult male Wistar rats for 30 days, and at the end, a rotarod test was carried out. Then, blood samples were taken from their eyes. The rats were submitted to the ischemic stroke, and the activity level of Catalase (CAT), Superoxide Dismutase (SOD), and total antioxidant were measured. The ischemic stroke model was implemented using the MCAO method. Infarct area and ischemic tolerance were measured by the MCAO (Middle Cerebral Artery Occlusion) method, and infarct volume was assessed by 2,3,5-triphenyl tetrazolium chloride.

Results: Chronic use of lentil extract decreased motor function, CAT, SOD, and total antioxidant activity compared with cinnamon extract. The cinnamon extract improved the ischemic tolerance and reduced the infarct size. The group receiving lentil extract could not tolerate ischemia and died during the experiment.

Conclusion: It seems that diet adjustment can effectively reduce the incidence of stroke or its complications. Awareness of food temperament and its relationship with various diseases can reduce disease burden, though further studies should be conducted on this topic.

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1. Introduction

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troke is the primary cause of severe long-term injury in the United States and the second leading cause of death in the world [1, 2]. Stroke patients suffer from motor and sensory disabilities, cognitive deficits, and emotional disorders. Only

25% of these patients return to their pre-stroke level of physical activity and social participation [2]. Most ischemic stroke is caused by atherosclerosis [3-5] and decreased or completely blocked cerebral blood flow owing to thrombi or emboli. This condition causes rapid cell death followed by loss of neurologic functions [6]. In addition, based on Persian Medicine, spleen dysfunction (the spleen is hot in temperament) may cause various diseases such as atherosclerosis because the spleen, as a storage organ, plays an essential role in the absorption and secretion of black bile in the human body (according to the "Al-Qanun fi al-Tibb" [Canon of Medicine" by Avicenna]). Oxidative stress also intensifies the brain damage induced by cerebral ischemia [7]. Therefore, an antioxidant drug and capable of improving and preventing splenic dysfunction should be taken to improve spleen function. Today, due to the widespread acceptance of people and the low side effects of medicinal plants, medicinal plants are more used for prevention and treatment.

The bark of different cinnamon species is one of the most popular spices used worldwide for preparing foods and treating diseases in Persian and modern medicines [3, 4]. Cinnamon plant with a hot and dry temperament [6] can effectively improve and prevent stroke and motor function. It positively affects spleen function and is characterized by antioxidant, antiinflammation, antihyperlipidemic, and antiobesity properties [3, 4].

In contrast, lentils have a cold and dry temperament. Lentil cultivars (Lens culinaris) are leguminous seeds with high natural antioxidants [8-10]. Many researchers have acknowledged the high antioxidant capacity of phenolic compounds, including tannins from tannin components present in plant extracts of lentil [9, 11]. Therefore, the present study aims to compare the effects of lentil and cinnamon extracts on preventing cerebrovascular accidents.

2. Materials and Methods

Animal experimental protocol

Adult male Wistar rats (n=28, body weight=250-300 g, prepared from the animal lab of Zanjan University of Medical Science, Iran) were housed in groups of 4 and maintained under a natural 12/12-hour light/dark cycle, 50%-60% relative humidity, and at a temperature of 23-27°C. The rats were fed with a regular diet and tap water ad libitum. The rats were treated according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. This study was approved by the Ethics Committee of the Zanjan University of Medical Sciences. Twenty-eight rats were randomly grouped into four (each 7 rats): 1) normal control group, 2) vehicle group which received normal saline (150 mg/kg.); 3) cinnamon group which was taken cinnamon extract (260 mg per animal), and 4) lentil group administered by lentil extract (150 mg/kg). Intraperitoneal (IP) injection of cinnamon and lentil extracts (260 mg per animal) was performed daily for 30 days. At the end of the 30th day, the rotarod test was performed. Then, blood samples were taken from the eye, and the rats were submitted to the ischemic stroke. Next, the infarct volume was measured by 2,3,5-Triphenyl Tetrazolium Chloride (TTC), and at the last step, the level of Catalase (CAT), Superoxide Dismutase (SOD), and total antioxidant were evaluated.

Rotarod test

To evaluate motor coordination, rats were trained to stay on a rotarod. All rats underwent a 3-day training program on a 7-cm diameter rotarod (Stoelting, USA). During the training, each rat was placed on the rotarod at a fixed speed (4 rotations per minute). The time for each rat to stay on the rotating bar was documented for three trials (5-min interval). The device automatically records the time in 0.1 s once the rats fall on the rotating shaft.

Superoxide dismutase activity assay

SOD activity was measured based on the method described by Constantine et al. [12]. Inhibition of photochemical reduction of nitroblue tetrazolium by 50% was used as an activity unit (U).

Catalase activity assay

Serum catalase activity was measured as formerly explained by Aebi et al. [13]. For CAT designation, the reaction mixture consisted of 2 mL of sodium phosphate buffer (50 mM, pH 7.0), 0.5 mL of H2O2 (40 mM), and 0.5 mL of sample. The decomposition of H_2O_2 was measured by a decrease in absorbance at 240 nm at 25°C.

Ferric reducing antioxidant power

The method outlined by Benzie et al. was used to determine ferric reducing antioxidant power [14]. The standard curve was prepared by trolox ranging from 0 to 500 µM. The activity was expressed as µmol Trolox Equivalents (TE/g) sample.

Establishment of focal cerebral ischemia by middle cerebral artery occlusion (MCAO) model

The model proposed by Longa et al. with some modifications was used as a basis to develop the ischemic stroke [15]. Rats received chloral hydrate (400 mg/kg) through intraperitoneal injection to be anesthetized. A midline neck incision was done to expose the common carotid artery. It was attentively dissected free from surrounding nerves and fascia from its bifurcation point to the base of the skull, and the occipital artery branches of the left internal carotid artery and external carotid artery were isolated. Then, a 2-cm length of size 3-0 monofilament nylon suture with a round tip was entered to the external carotid artery and pushed through the internal carotid artery to reach the middle cerebral artery. A slight resistance against the suture after passing 20-22 mm of suture length showed it was placed in the correct location. Blood flow was restored

after 60 minutes. Rectal body temperature was measured and maintained at about 37°C during the surgery.

Measurement of infarct size

After sacrificing the rats by chloral hydrate (800 mg/kg), their brain was removed. Seven coronal brain sections were cut at 2 mm in thickness from frontal to temporal lobes, according to the rat brain matrix. Tissue sections were stained in a 1.5% TTC (Sigma, USA, code: 1.38380). Then, a digital camera (Nikon, Japan) was used to take images from sections, which were transferred to a computer for analysis by Image-J Software. Equation 1 was used to calculate infarct size with edema correction:

1. Infarct size=left hemisphere volume - (right hemisphere volume - infarct volume)/left hemisphere volume

Statistical analysis

All data were presented as Mean±SEM. The results of CAT and SOD activity tests and total antioxidant activity were analyzed using 1-way ANOVA (Analysis of Variance) with the Tukey test. The significant effects of hot and cold temperaments on different parameters with P<0.05 were compared. The results of the rotarod test and SOD activity were analyzed using the nonparametric Kruskal-Wallis test.



Figure 1. Effect of cinnamon and lentil extracts on the latency to fall-off

The data are expressed as Mean±SE (n=7). *** P<0.001 compared with the control, vehicle, and cinnamon groups. Co: Control; Ve: Vehicle; Cin: Cinnamon; Len: Lentil.



Figure 2. Effect of cinnamon and lentil extracts on Catalase (CAT) activity in serum

The data are expressed as Mean±SE (n=7); " P<0.01; "" P<0.001 compared with the control and vehicle animals; ⁵⁵⁵ P<0.001 compared with the cinnamon extract. Co: Control; Ve: Vehicle; Cin: Cinnamon; Len: Lentil.

3. Results

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Latency to fall-off assay (Rotarod)

Effects of hot or cold temperament on motor function are illustrated in Figure 1. On the 30^{th} day, the latency to fall-off assay of the group, which received lentil extract significantly (P<0.001) decreased compared with control, vehicle, and cinnamon extract-treated groups.

Catalase activity assay

Figure 2 shows the effects of hot or cold temperament on CAT activity. On the 30th day, the CAT activity of the group receiving cinnamon extract significantly increased compared with the control and vehicle groups (P<0.001). However, it decreased in the group that received lentil extract compared with the control and



Figure 3. Effect of cinnamon and lentil extracts on Superoxide Dismutase (SOD) activity in serum

The data are expressed as mean \pm SE (n=6). " P<0.01 and; " P<0.001 compared with the control and vehicle groups; ⁵⁵⁵ P<0.001 compared with the cinnamon extract. Co: Control; Ve: Vehicle; Cin: Cinnamon; Len: Lentil.



The data are expressed as Mean±SE (n=6). ** P<0. 01 and *** P<0.001 compared with the control and vehicle groups. ^{\$\$\$} P<0.001

vehicle groups (P<0.01) and the group receiving cinnamon extract (P<0.001).

compared with the cinnamon extract. Co: Control; Ve: Vehicle; Cin: Cinnamon; Len: Lentil.

compared with the control and vehicle group (P<0.01) and the group receiving cinnamon extract (P<0.001).

Superoxide dismutase activity assay

Effects of hot or cold temperament on SOD activity are illustrated in Figure 3. On the 30th day, the group receiving cinnamon extract showed a significant increase in SOD activity compared with the control and vehicle group (P<0.001). However, SOD activity significantly decreased in the group treated with the lentil extract

Total antioxidant activity assay

Figure 4 indicates the effects of hot or cold temperament on the total antioxidant activity. On the 30th day, the total antioxidant activity significantly increased in the group treated by cinnamon extract compared with the control and vehicle groups (P<0.001). However, it significantly decreased in the group receiving the lentil



Figure 5. Effect of cinnamon and lentil extracts on ischemic tolerance

The data are expressed as Mean±SE (n=6); *** P<0.001 compared with the vehicle groups; ⁵⁵⁵ P<0.001 compared with the cinnamon extract. Co: Control; Ve: Vehicle; Cin: Cinnamon; Len: Lentil.

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Figure 6. Effect of cinnamon extract on infarct volume

A) Effect of cinnamon on infarct size evaluated by 1.5% TTC staining; B) The cinnamon extract-treated group showing a significant reduction in tissue damage (white area) in comparison with MCAO.

The data are expressed as Mean±SE (n=7). *P<0.05 and **P<0.01 the group receiving cinnamon extract compared with the vehicle groups.Co: Control; Ve: Vehicle; Cin: Cinnamon; Len: Lentil.

extract compared with the control and vehicle group (P<0.01) and the group treated with the cinnamon extract (P<0.001).

Tolerance to induced ischemia

Effects of hot or cold temperament on tolerance to induced ischemia are shown in Figure 5. On the 30th day, tolerance to induced ischemia significantly increased in the group treated with cinnamon extract compared with the vehicle group (P<0.001) and decreased in the group receiving the Lentil extract compared with the vehicle group (P<0.001) and the group receiving cinnamon extract (P<0.001).

Stroke volume

Animals receiving lentil extract could not survive for 24 hours after ischemia induction. Therefore, stroke volume was not measured in this group. Pretreatment with cinnamon extracts significantly reduced total stroke volume (P<0.001) compared with the vehicle group (Figure 6A and 6B).

4. Discussion

Based on the results, motor function decreased by the lentil extract and increased by the cinnamon extract. Furthermore, the antioxidant activity significantly increased in rats receiving cinnamon and decreased in those treated with lentils. The cinnamon extract increased the duration of ischemia tolerance and reduced the infarct size. Animals that received lentils could not tolerate ischemia and died in 24 hours.

The motor function was evaluated in the present study, and the results showed its improvement in the cinnamon-treated group and impairment in the lentils-treated group. Sometimes contradictions can be found between the results of the present study and the previous ones. For example, a study showed that 200 mg/kg of red lentil extract did not significantly decrease the catatonic responses after perphenazine administration compared with the control group, while the groups that received 800 and 1000 mg/kg of red lentil extract demonstrated a significant difference at all the time points [16]. These results could probably be due to the fact that behavioral tests were performed immediately after the extract injection as lentil extract relaxes the muscles for some time after injection, which affects the results.

Ischemic stroke may result from the decrease or complete obstruction of cerebral blood flow owing to thrombi or embolic leading to decreased oxygen and energy supply to the critical tissues of the brain, which results in rapid cell death and, accordingly, loss of neurologic functions [6]. Cerebral ischemia/reperfusion insult damages brain tissues through complex quick pathophysiological mechanisms such as glutamate excitotoxicity, inflammation due to the blood-brain barrier leakage, oxidative damage, loss of ionic homeostasis,

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phospholipase activation, neuronal depolarization, and finally apoptosis. Oxidative stress is the main factor leading to brain damage induced by cerebral ischemia. Brain tissue is susceptible to oxidative damage. This sensitivity is due to the high speed oxidative metabolic activity, severe production of Reactive Oxygen Species (ROS), and high content of polyunsaturated fatty acids [17, 18]. Also, Saif Ahmad indicated in his study that sesamin reduced lipid peroxidation and superoxide anion levels while restoring ischemia-induced evacuation from decreased glutathione and SOD enzyme activity and protein expression in the ischemic brain of MCAO animals [19]. In Persian medicine, any dysfunction of the spleen can lead to some diseases. Spleen dysfunction can cause atherosclerosis because the spleen, as a storage organ, plays an essential role in absorbing and secreting the black bile in the human body [20]. Prolonged use of cold and dry lentils may increase the amount of hydrate and increase the risk of atherosclerosis and stroke. On the contrary, cinnamon with a warm and dry temperament reduces the risk of atherosclerosis and stroke [21].

The present study results showed the desirable effects of cinnamon extract on antioxidative enzyme activities of SOD, CAT, and TAC (total antioxidant activity) compared with the control, vehicle, and lentil extracttreated group. Cinnamon has an oxidative inhibitory mechanism. The chemicals in cinnamon prevent the formation of harmful free radicals [22, 23]. Therefore, due to its phenolic compounds and an antioxidant [24], and anti-inflammatory properties, cinnamon extract can reduce the volume of stroke in ischemic injury [25, 26]. It also improves SOD and CAT activities [27].

5. Conclusion

Our findings suggest that cinnamon may have beneficial brain-protective effects, possibly due to its high antioxidant properties, a neuroprotective factor that inhibits ischemia-reperfusion-mediated damage. It seems that diet adjustment can be effective in reducing the incidence of stroke or stroke complications. Awareness of food temperament and its relationship with various diseases can reduce the burden of disease and may be helpful to conduct further related studies.

Ethical Considerations

Compliance with ethical guidelines

This research was approved by the Deputy of Research and Technology, Zanjan University of Medical Sciences (Ethical code No: IR.ZUMS.REC.1397.342).

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Authors' contributions

Conceptualization and Supervision: Hossein Mostafavi and Mohsen Bahrami; Methodology and Data collection: Hossein Mosafavi , Leila Ghassemifard , Masoumeh Hosseini and Solmaz Najjari; Investigation, Writing-original draft, and Writing-review & editing: All authors; Data analysis: All authors; Funding acquisition and Resources: Hossein Mosafavi and Leila Ghassemifard.

Conflict of interest

The authors declared no conflict of interest.

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