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Original Article

Effect of cinnamon on antioxidant content and ZO-1 gene expression in the brain following middle cerebral artery occlusion in rats receiving high-fat diet

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ABSTRACT

Introduction: Despite all the research, no definitive treatment for stroke has been found yet. Cinnamon is a plant that has been shown to have health benefits effects. In this study, the effect of pretreatment of cinnamon on ischemia tolerance and the expression of Zonula occludens 1(ZO-1) gene in the brains of rats receiving a high-fat diet was investigated.

Methods: In this study, 72 rats were divided into six groups: control, sham, model (stroke), vehicle, lovastatin, and cinnamon. All groups except the control group received a high-fat diet for 8 weeks. Then the last three groups received Carboxymethyl cellulose, lovastatin, and cinnamon 130 mg accordingly for 6 weeks. Stroke was induced by middle cerebral artery occlusion (MCAO). Twelve hours later, the animals were examined for the extent of serum lipids, brain edema, anti-oxidant capacity and gene expression of ZO-1.

Results: Cinnamon was effective in reducing serum cholesterol and triglyceride. Cinnamon treatment significantly diminished brain edema. It also restored anti-oxidant capacity. ZO-1 gene expression was increased in the ischemic brains after cinnamon treatment ($P< 0.05$).

Conclusion: Pretreatment with Cin130 had beneficial effects on the serum lipid profile, edema volume in ischemic brain and anti-oxidant capacity. It increased ZO-1 gene expression and so maintained cellular integrity and prevented the subsequent edema.

Keywords:
Stroke
Cinnamon
MCAO
Brain edema
ZO-1

Introduction

Stroke is a cerebrovascular disorder that occurs due to abnormal blood flow to the part of the brain (Campbell et al., 2019). Stroke is the third leading cause of death after cancer and cardiovascular disease in industrialized countries. Eighty percent of brain strokes are ischemic and only 20% are hemorrhagic. Most studies are aimed at the prevention and treatment of ischemic stroke. One of the events following a stroke is the reperfusion of the ischemic region, which not only cannot restore the condition but also worsen the condition (Farhoudi et al., 2017; Hosseini et al., 2010).

Changes made after ischemia-reperfusion (I/R) include the production of free radicals due to cell mem-

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brane lipid peroxidation, activation of COX-2/NOS-2 enzymes (COX for Cyclooxygenases and NOS for Nitric oxide synthases), and expression of inflammatory factors (Adibhatla and Hatchera, 2008; Sun et al., 2018). Increased generation of reactive oxygen species (ROS) has been contributed as a major mechanism of I/R injury pathogenesis (Sun et al., 2018). Blood-brain barrier (BBB) failure due to infiltration of inflammatory factors into damaged tissue and activation of matrix metalloproteinases is another consequence of I/R (Rempe R. G et al., 2016). Finally, DNA damage and cell death are predictable in I/R (Kalogeris et al., 2016). BBB which is formed by endothelial cells of cerebral blood vessels restricts the blood-to-brain diffusion of particles so conserving neuronal physiologic function. Tight junctions and their related proteins including Zonula occludens play a prominent role in the barrier property (Luissint et al., 2012). Although key mediators of oxidative stress induced-injury in I/R are characterized to date, antioxidant therapeutics of I/R in stroke have not led to an effective outcome (Andreadou et al., 2009; Kalogeris et al., 2014).

Hyperlipidemia and consequent atherosclerosis are among the main preventable risk factors for stroke while approximately two-thirds of deaths from atherosclerosis are due to one or more coronary artery thrombosis (Lewis and Segal 2010; Nelson, 2013). Therefore, factors that improve the abnormal levels of lipids in the plasma can limit atherosclerosis and its consequences.

Considering the side effects of chemical drugs, today herbal compounds with different properties are widely used as alternative therapies (Sedighi et al., 2017). Cinnamon, as a medicinal plant, is one of the well-characterized herbs which has lipid-lowering properties (Rao and Gan, 2014; Vafa et al., 2012). Several studies have shown its beneficial effects on inflammation, neuroprotection, and restoring glucose and lipid profile (Ghosh et al., 2015; Stavinoha and Vattem 2015; Tuzcu et al., 2017; Verspohl et al., 2005).

Since most of the studies regarding the effect of cinnamon on I/R-induced brain injury have been performed in healthy conditions, this study aimed to investigate the effect of the cinnamon hydroalcoholic extract on oxidative stress, ZO-1 expression, and infarct volume of rats undergoing I/R and receiving high-fat diet. Taking into account that previous studies have shown the lipid-lowering and neuroprotective effects of lovastatin (Moradi et al., 2019), in the present study this drug has been used as a positive control to compare the effects of cinnamon.

Materials and Methods

Preparation of a high-fat diet was performed according to the articles with some modifications (Marques et al., 2015; Buettner et al., 2006). Briefly, the usual meal was molded, and for every 800 grams of grilled food, 20 grams of sheep's fat was added. Distilled water was added to the grilled food for dough, and it was mixed completely by kneading. This dough mixture was converted into pellet form and then laid on racks for drying.

Hydroalcoholic extract of cinnamon was prepared from Adonis Gol Darou Company, Tehran, Iran. The lovastatin powder was prepared by Osveh Pharmaceutical Company, Tehran, Iran. According to the recommended dosage in the articles, 10 mg of powder per kg body weight of the animal was used. Carboxymethylcellulose (CMC) 0.5% was used as a solvent for lovastatin powder (Mirhadi, 2011).

In this research, 72 adult male Wistar rats weighing 180-220 g with an age range of approximately 6 to 8 weeks (prepared from the Animal Laboratory of Zanjan University of Medical Sciences) were included. We used 6 animals for each experiment (Gene expression, brain edema, and BBB permeability evaluation). Rats were kept in appropriate bioclimatic conditions, including 12 hours of light, 12 hours of darkness, 22-24° C, and 60% humidity. To adapt to the environment, the animals were transferred to the laboratory for one week before the study. During this time, water and food were freely provided to the animals. All experiments were carried out at 2 pm and according to the Zanjan University of Medical Sciences Laboratory Animal Health Care and Use Guidelines (Ethical No: ZUMS.REC.1395.38).

Animals were randomly divided into six groups; the control group (animals that received the usual diet), the sham group (animals receiving a high-fat diet for eight weeks and undergoing surgical stress in the 14th week), the stroke model group (animals receiving high-fat diet for eight weeks and undergoing MCAO surgery at week 14), the lovastatin group (animals receiving a high-fat diet for eight weeks, then receiving10 mg/kg lovastatin for six weeks by the intraperitoneal (IP) injection and then undergoing MCAO surgery at week 14), the vehicle group (animals receiving high-fat diet for eight weeks and then receiving 0.5% Carboxymethyl cellu-

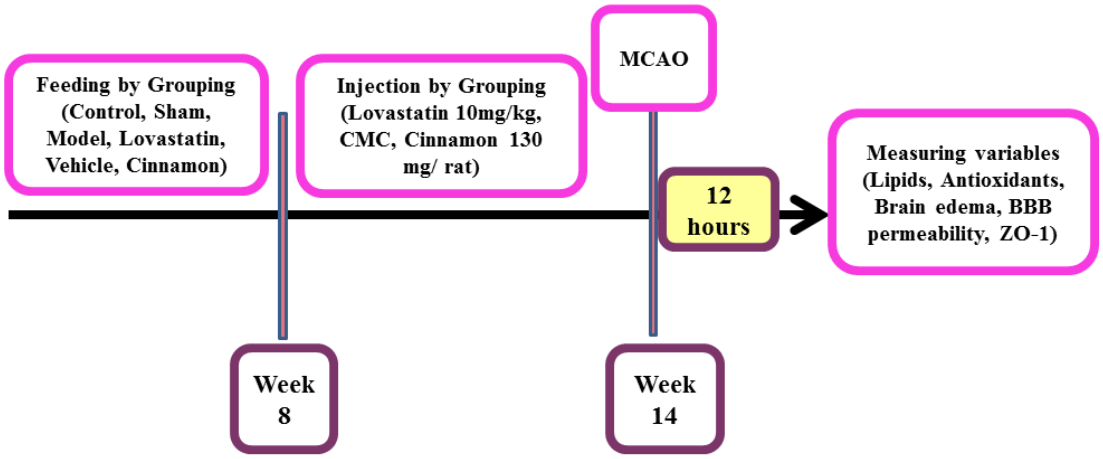


FIGURE 1. Schematic diagram of experimental design.

lose for six weeks by the IP injection and then undergoing MCAO surgery at week 14), The Cin 130 group (animals receiving high-fat diet for eight weeks and then receiving 130 mg hydroalcoholic extract of cinnamon (Ranasinghe et al., 2012) by the IP injection for six weeks and then undergoing MCAO surgery at week 14) (Figure 1).

Induction of stroke with a middle-cerebral artery occlusion model (MCAO)

The induction of middle cerebral artery occlusion (MCAO) was performed according to the intraluminal filament model (Longa et al., 1989). Briefly, rats were anesthetized using 400 mg/kg body weight chloral hydrate (Merck/Germany Cat: 102425) through intraperitoneal injection. The right common carotid artery was isolated, and a silicone-coated nylon was inserted along the right common carotid arteries into the internal carotid artery until it blocked the origin of the middle cerebral artery.

The depth of penetration was approximately 20 mm from the carotid bifurcation to effectively block the middle cerebral artery. Rats were subjected to focal ischemia for 60 min; blood flow was restored by withdrawing the nylon filament. The surgery was performed at 37±0.3°C. After recovery from anesthesia, rats were returned to their cages.

Blood sampling

12 hours after induction of ischemia, the animals were anesthetized with chloral hydrate, and then the blood was taken from the heart. The blood samples were centrifuged at 7000 × g for 10 min at room temperature to obtain plasma. The serum samples were stored at -20 °C until biochemical analysis. Serum levels of cholesterol, triglyceride, and HDL were measured by spectrophotometry (enzymatic colorimetry) and using special measuring kits (Pars Azemon). LDL levels were obtained by the formula LDL (mg /dl) = Total cholesterol - (HDL + TG / 5).

Measurement of blood-brain barrier (BBB) permeability

The BBB integrity was evaluated by measuring the Evans Blue (EB) extravasations. After 60 minutes of ischemia, 4 mL/Kg of 2% Evans blue solution was injected through the animal's tail vein. Twelve hours after reperfusion, the rats were anesthetized, and using transcardial technique, 250 ml of saline was infused through the left ventricle to remove EB from the vessel. Then the brain was removed and hemispheres were separated. The brain tissue was homogenized in 2.5 ml phosphate buffer saline and then 2.5 ml of trichloroacetic acid 60% (Merck, Germany) was added to precipitate protein content. The mixture was then vortexed for 3 min, cooled for 30 min, and centrifuged for 30 min at 1000 ×g. The supernatants were measured at 610 nm for absorbance of EB using a spectrophotometer (UV-visible, USA). The results were expressed as microgram per gram of brain tissue calculated according to a standard curve (G Y Yang and A L Betz 1994).

Evaluating the Brain edema

In order to measure the absolute brain water contents

TABLE 1: Specific primers were used for q-PCR amplification.

Primers	Product Size(bp)	Gene
F: 5′-CCATCTTTGGACCGATTGCTG-3' R: 5'-TAATGCCCGAGCTCCGATG-3'	123	ZO-1
F: 5'-CATGTACGTTGCTATCCAGGC-3' R: 5'-CTCCTTAATGTCACGCACGAT-3'	250	B-actin

(ABWC), the wet/dry weight method was used. Briefly, rats were killed under deep anesthesia and their brain was removed and then placed in the brain matrix to separate the cerebellum and the olfactory bulb. The brain was divided into the right and the left hemispheres through a midline sagittal incision. Each hemisphere was placed in a separate pre-weighed container to measure its wet weight. Then, the container and the tissues were placed in a 110 °C oven for 24 hours to obtain the tissues' dry weight. ABWC of each hemisphere (%) was determined using bellow equation (Panahpour et al., 2014):

ABWC (%) = [(wet weight – dry weight)/ wet weight] × 100

Real-time polymerase chain reaction

Total RNA isolated with Trizol Reagent Invitrogen (Sinacolon RN7713C). The cDNA was synthesized using the Takara Company's PrimeScript™ RT Reagent Kit (Perfect Real Time). Specific primers for rat ZO-1 and β-actin were used for this experiment (Table 1) using Takara SYBR Premix Ex Taq Master (Takara Bio, Inc., Shiga, Japan). Gene expression levels were quantified by Applied Biosystems TM Real-Time PCR Instruments. The relative expression ratio of ZO-1 and β-actin were calculated using the relative expression software tool (REST). The cycling parameters for qPCR were as follows: 10 min at 95 °C for initial denaturation, followed by 40 cycles of 30 s at 95 °C, 30 s at optimum temperature for each gene, and 30 s at 72 °C.

Superoxide Dismutase (SOD) and Catalase (CAT) Activity Assay

Superoxide dismutase (SOD; EC.1.15.1.1) activity was measured with RANDOX kits (Cat.No. SD 125; Randox Labs Ltd., Crumlin, UK) (McCord and Fridovich, 1969). In this method, xanthine and xanthine oxidase is used to generate superoxide radicals, which react with 2-(4-iodophenyl)-3-(4-nitro-phenol)-5-phenyltetrazoliumchloride(I.N.T.) to form a red formazan dye. The degree of inhibition of this reaction was the index for superoxide dismutase activity measurement. The activity was measured at 37 ºC by a UV spectrophotometer, and absorbance was measured at 30 S and 3 min at 505nm. The unit of activity is defined as the amount of enzyme that inhibits the rate of the formazan dye formation by 50%. The activity of SOD was expressed as units/g hemoglobin (U/gHb). Catalase (CAT; EC.1.11.1.6) activity was measured in hemolysates by UV spectrophotometer (Aebi 1984). According to the method, decomposition of the substrate H_2O_2 was checked spectrophotometrically at 240 nm at 0 and 30 S. Catalase activity was expressed as U/gHb.

Statistical analysis

Data were analyzed using SAS9.1 software. All data are presented as the mean± SEM. Analysis of the results of lipid profiles, antioxidant enzyme activity, and BBB permeability were assessed by ANOVA and Duncan post hoc test. Comparison of BBB permeability in two hemispheres was assessed by independent t-test. The relative expression ratios of genes were calculated using the relative expression software tool (REST). A *p*-value of less than 0.05 was considered statistically significant.

Results

Lipid profile

Cinnamon 130 and lovastatin treatment decreased serum cholesterol significantly compared with the animals in model, sham and vehicle groups (Figure 2) ($P<0.05$). Both cinnamon 130 and lovastatin-treated animals showed decreased serum triglyceride compared with the model group. Lovastatin treatment significantly decreased serum triglyceride compared with the Sham group ($P<0.05$). However, there was no serum triglyceride considerable difference in cinnamon-treated and sham animals (Figure 3).

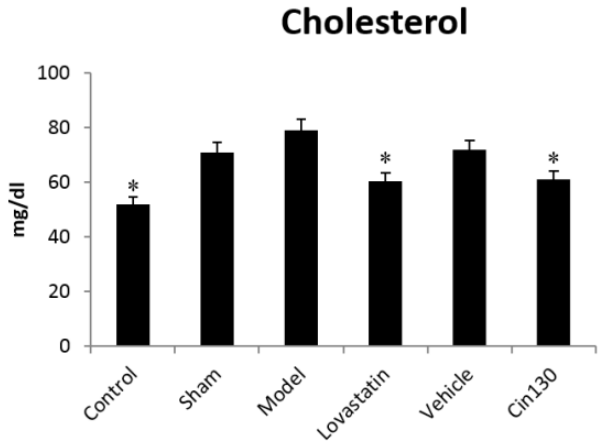


FIGURE 2. Serum Cholesterol plasma levels at the end of the study in the groups; Control, Sham, Model, Lovastatin, Vehicle, and cinnamon 130 (Cin130). $^{*}P< 0.05$ compared with Sham, Model, and Vehicle groups. All the results have been reported as Mean ± SEM. (n=6).

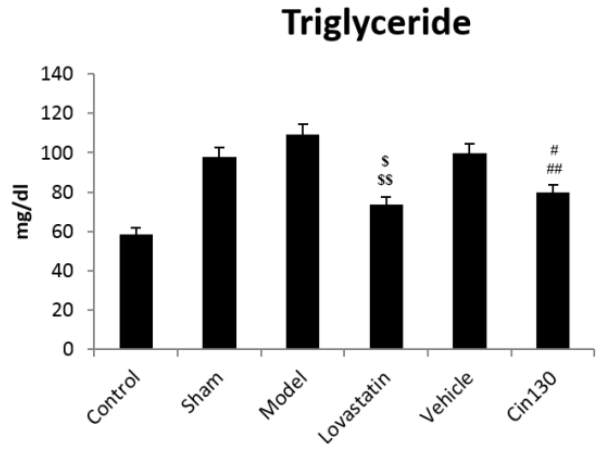


FIGURE 3. Serum Triglyceride plasma levels at the end of the study in the groups; Control, Sham, Model, Lovastatin, Vehicle, and cinnamon 130 (Cin130). $^{\#}P< 0.05$ compared with Model, and Vehicle groups. $^{\#\#}P< 0.01$ compared with control. $^{\$}P< 0.05$ compared with the Sham group. $^{\$\$}P< 0.01$ compared with the Model and Vehicle groups. All the results have been reported as Mean ± SEM. (n=6).

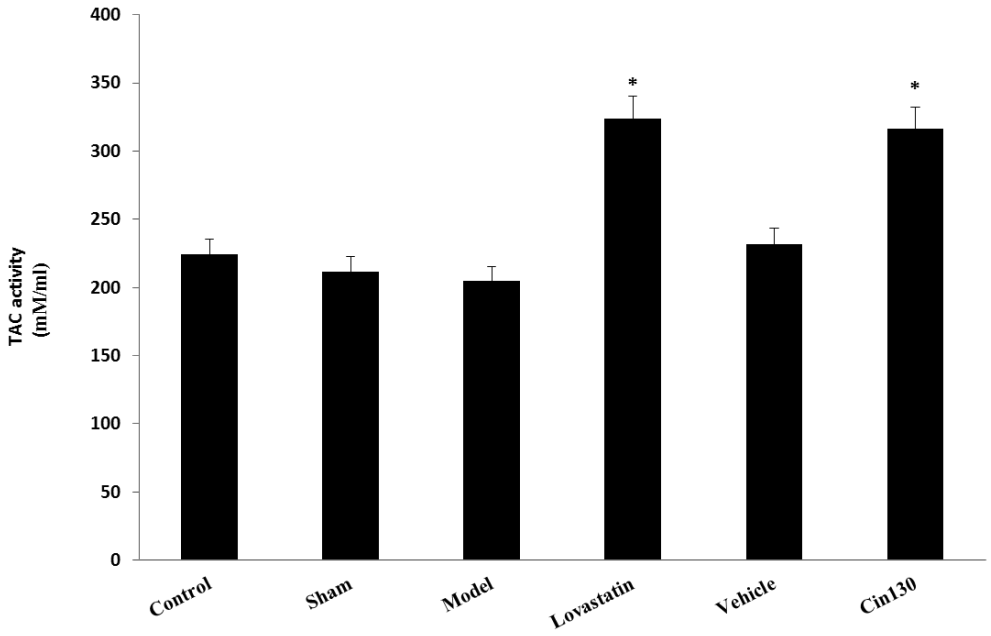


FIGURE 4. Serum total antioxidant capacity at the end of the study in the groups; Control, Sham, Model, Lovastatin, Vehicle, and cinnamon 130 (Cin130). $^{*}P< 0.05$ compared with Control, Sham, Model, and Vehicle groups. All the results have been reported as Mean ±SEM (n=6).

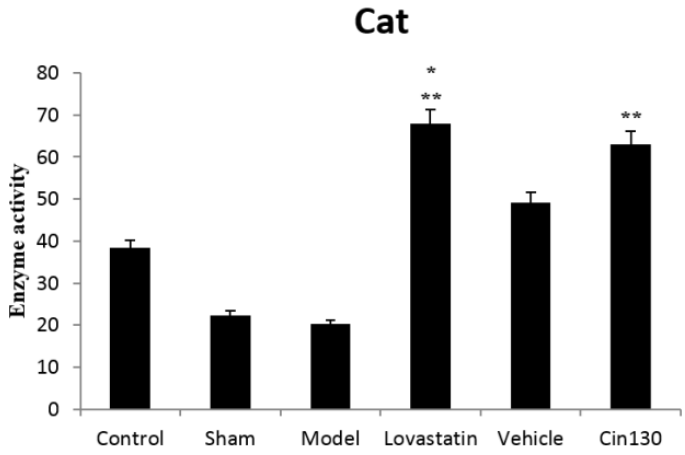


FIGURE 5. Serum catalase levels at the end of the study in the groups; Control, Sham, Model, Lovastatin, Vehicle, and cinnamon 130 (Cin130). $^{*}P<0.05$ compared with control and vehicle groups. $^{**}P<0.01$ compared with sham and model groups. All the results have been reported as Mean ± SEM (n=6).

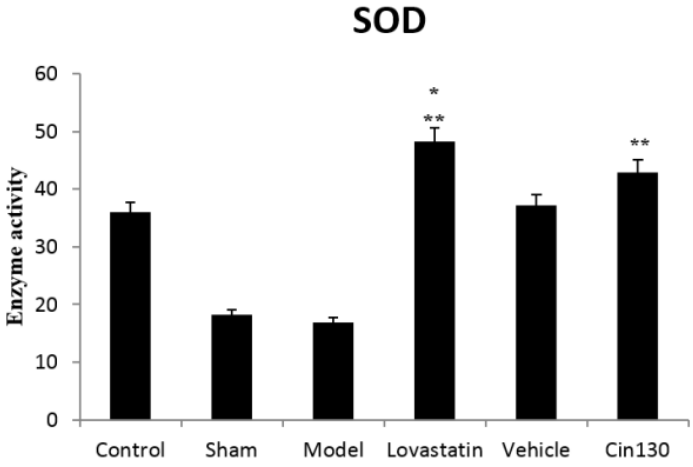


FIGURE 6. Serum Superoxide dismutase levels at the end of the study in the groups; Control, Sham, Model, Lovastatin, Vehicle, and cinnamon 130 (Cin130). $^{*}P<0.05$ compared with control and vehicle groups. $^{**}P<0.01$ compared with sham and model groups. All the results have been reported as Mean ± SEM (n=6).

Anti-oxidants evaluation

The total serum antioxidant capacity (TAC) assay showed that its value in the serum of treated rats was significantly higher than the animals of the other groups (Figure 4) ($P<0.05$). Catalase measurement showed that the enzyme levels in the serum of cinnamon-treated animals were significantly higher than the rats of the other groups. Unexpectedly, the rats of the vehicle group which received lovastatin solvent had much more catalase compared with sham and model animals (Figure 5). As shown in figure 6 the same results were found in Superoxide dismutase changes while cinnamon treatment increased its serum levels significantly.

Effects of the cinnamon extract on BBB permeability

Distribution patterns of Evans blue dye leakage at 12 hours after 1-hour transient focal ischemia-reperfusion are shown in figure 7A. In all groups, BBB permeability to EB was significantly increased in ischemic compared with intact hemispheres except those in control and sham groups ($P<0.05$). The damage severity was more significant in model and vehicle groups ($P<0.01$). On the other hand, BBB permeability to EB in the ischemic hemispheres in lovastatin and Cin 130 groups were significantly less than that of the model and vehicle groups receiving a high-fat diet without any treatment intervention ($P<0.05$) (Figure 7B).

Evaluation of brain edema

As represented in figure 8, brain edema assessment in both hemispheres of each group showed that in all ischemic groups, the volume of edema in the right hemisphere (ischemic) was significantly more than left hemisphere (intact). In the ischemic hemispheres of the

A)

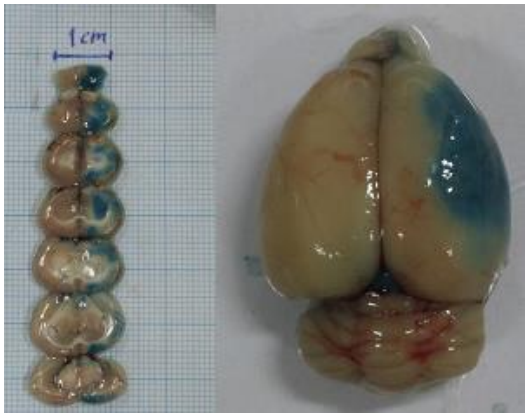
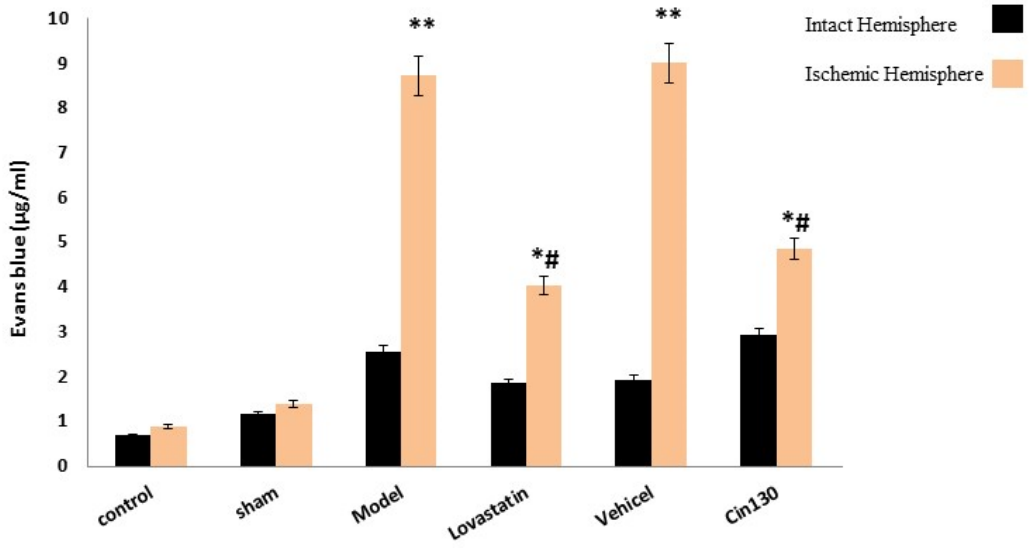


FIGURE 7. Peripheral and central regions of the ischemic cortex undergoing Evans blue dye leakage at 12 hours after 1-hour transient focal ischemia-reperfusion are shown (A). Comparison of the average permeability of the BBB to Evans blue in both hemispheres (within groups) and the damaged hemispheres (between groups) in control, sham, model, lovastatin, vehicle, and cinnamon (Cin130) groups. The data are expressed as the mean ±SEM. ANOVA and LSD post hoc tests were used. $^{*}(P<0.05)$, $^{**}(P<0.01)$ compared with intact hemispheres (within groups). $^{\#}(P<0.05)$ compared with the model and vehicle groups (between groups) (B). (n = 6).

B)



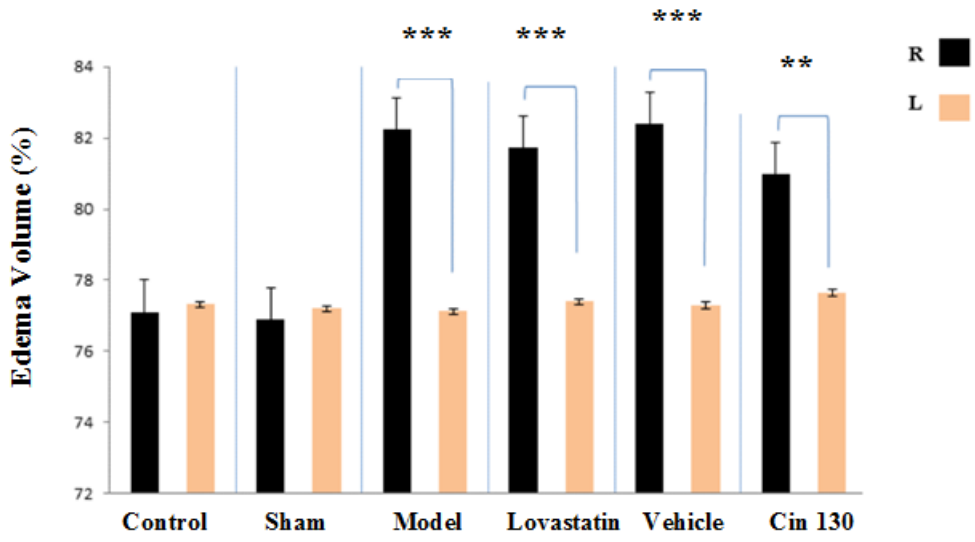


FIGURE 8. The volume of brain edema in ischemic and intact hemispheres at the end of the study in the groups; Control, Sham, Model, Lovastatin, Vehicle, and cinnamon 130 (Cin130). Common letters indicate no statistical difference between groups. $^{**}P< 0.01$ and $^{***}P< 0.001$. All the results have been reported as Mean ±SEM. (n=6).

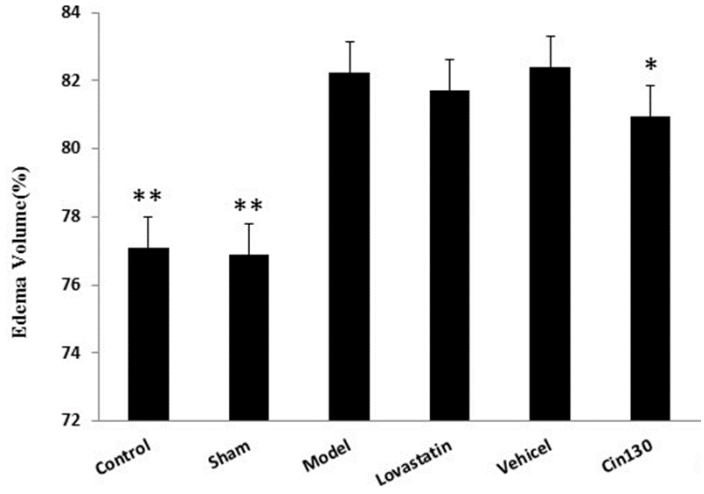


FIGURE 9. The volume of brain edema in ischemic hemispheres at the end of the study in the rats of groups Control, Sham, Model, Lovastatin, Vehicle, and cinnamon 130 (Cin130). $^{*}(P<0.05)$, $^{**}(P<0.01)$ compared with the model, vehicle and lovastatin groups. All the results have been reported as Mean ±SEM (n=6).

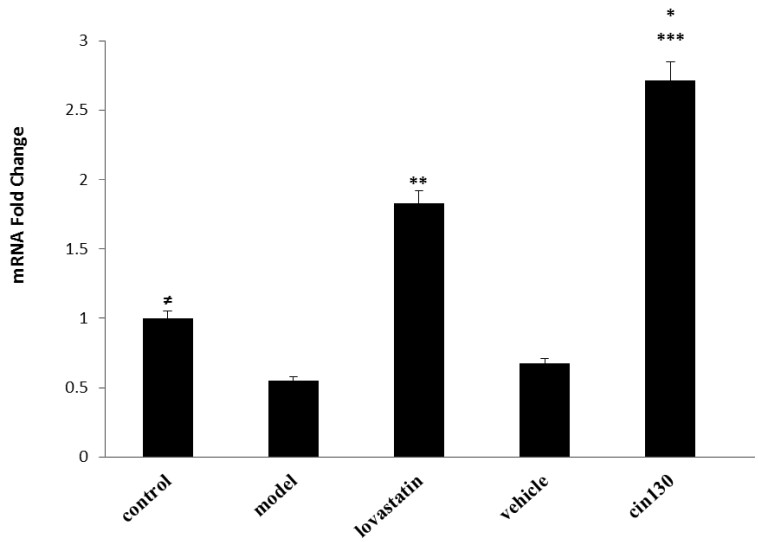


FIGURE 10. ZO-1 gene expression in ischemic hemispheres at the end of the study in the rats of groups Control, Sham, Model, Lovastatin, Vehicle, and cinnamon 130 (Cin130). $^{\#}(P<0.05)$ compared with the model and vehicle groups. $^{*}(P<0.05)$ compared with lovastatin group, $^{**}(P<0.01)$ and $^{***}(P<0.001)$ compared with the model and vehicle groups. All the results have been reported as Mean ±SEM (n=6).

animals that were treated with cinnamon 130, the volume of the edema was significantly lower than untreated ischemic animals (Figure 9).

ZO-1 gene expression

As shown in figure 10, ZO-1 gene expression in the ischemic hemispheres of model and vehicle groups was decreased compared with control animals ($P<0.05$). Treatment with cinnamon 130 and lovastatin significantly elevated the ZO-1 gene expression; however, this elevation was more in the animals under cinnamon 130 treatments ($P<0.001$).

Discussion

Based on the results of this study, cinnamon extract treatment restored lipid profile disturbances in rats that received a high-fat diet. In 2017, Tuzcu et al. have shown that cinnamon's polyphenols diminish body weight, serum glucose, and lipid profile (Tuzcu et al., 2017). Similar findings have been reported previously by other groups consistent with our results (Anderson et al., 2016; Babu et al., 2007). It is clear that hyperlipidemia, as a risk factor, aggravates the survival prognosis in stroke patients (Zeljkovic et al., 2010). So, cinnamon intake in patients with dyslipidemia may reduce their

vulnerability.

After the stroke neurological deficits are inevitable, although the severity depends on the lesion extent and this is important to plan for rehabilitation (Abdelnour and El-Nagi, 2017). According to our result, MCAO induces brain edema and this malfunction was worse in hyperlipidemic rats. However, cinnamon treatment decreased the volume of edema in the suffering animals. As shown by Chen, cinnamaldehyde, an essential ingredient in cinnamon, diminished the infarction area and neurological disorders. It also decreased oxidant agents in I/R-induced injury brain tissue (Chen et al., 2016). A couple of studies have reported some beneficiary effects of cinnamon extract in neurodegenerative situations. As reported by Frydman-Marom et al., oral administration of cinnamon extract diminished β-Amyloid plaques in the brain and improved cognitive loss in an animal model of Alzheimer's disease (Frydman-Marom et al., 2011). Furthermore, in a study by Jana et al., cinnamon up-regulated BDNF, a neurotrophic factor, through PKA signaling pathway and they suggested a therapeutic potential for cinnamon in neurodegenerative disorders (Jana et al., 2013).

During stroke injury, ROS production by several cellular oxidative metabolic processes contributes to pathogenesis. However, when the equilibrium between pro-oxidants and antioxidants is disturbed, consequently excessive production of ROS leads to a phenomenon named oxidative stress (Adibhatla and Hatchera, 2008). Oxidative stress has been correlated progressively more to the onset and/or progression of numerous human diseases (Giustarini et al., 2009). Superoxide dismutase (SOD) and catalase (CAT) are among the first-line antioxidants in the body and the effectiveness of any synthetic or natural antioxidants is based on the activation of these enzymes (Ighodaro and Akinloye, 2018). High-fat diet is a common situation in which the oxidative stress incidence is increased in tissues due to unusual elevated lipid peroxidation (Tan and Norhaizan, 2019). In the present study, we have shown that cinnamon administration increased CAT and SOD activity in brain tissue of animals undergone I/R and received a high-fat diet. Beji et al. have reported the antioxidant property of cinnamon extract in diabetic rats (Beji et al., 2018). This antioxidant effect has been shown in a cold trauma model in mice too. They contributed the neuroprotective activity to the polyphenols present in cinnamon extract (Yulug et al., 2018).

BBB stability essentially is dependent on a family of proteins named Zonula occludens (ZO) (Luissint et al., 2012). Jiao et al. have shown that ZO-1 is one of the proteins in brain microvascular endothelial cells which participates in the control of BBB permeability during reperfusion injury (Jiao et al., 2011). Our results indicated that BBB integrity was disturbed in ischemic high-fat diet animals, a phenomenon leading to edema in their brain. In part, these findings can be related to ZO-1 gene expression decrement in the brain tissue. On the other hand, cinnamon extract treatment elevated ZO-1 gene expression and eliminated brain edema to some extent. Some investigations have revealed the beneficiary effects of polyphenols in brain edema during cerebral ischemic injury (Panickar and Anderson, 2011; Xue et al., 2013), however, we are going to introduce cinnamon and probably its polyphenols acting through BBB tight junction proteins as a mechanism.

Overall, cinnamon treatment led to beneficiary effects on the serum lipid profile, brain edema, and antioxidant capacity in ischemic animals. It also increased ZO-1 gene expression in brain tissue and it seems that through this mechanism lowers the permeability of the BBB and thus prevents subsequent edema due to ischemia-reperfusion.

Acknowledgments

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Conflict of interest

The authors declare that they have no conflict of interest. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

References

Abdelnour L, El-Nagi F. Functional neurological disorder presenting as stroke: a narrative review. J Psychol Abnorm 2017; 6: 2.

Adibhatla RM, Hatchera JF. Phospholipase A2, reactive oxygen species, and lipid peroxidation In CNS pathologies. BMB Rep 2008; 41: 560-7. https://doi.org/10.5483/BMBRep.2008.41.8.560.

Aebi H. Catalase in vitro. Methods Enzymol 1984; 105: 121-6. https://doi.org/10.1016/S0076-6879(84)05016-3.

Anderson RA, Zhan Z, Luo R, Guo X, Guo Q, Zhou J, et al. Cinnamon extract lowers glucose, insulin and cholesterol in people with elevated serum glucose. J Tradit Complement Med 2016; 6: 332-6. https://doi.org/10.1016/j.jtcme.2015.03.005.

Andreadou I, Iliodromitis EK, Farmakis D, Kremastinos DT. To prevent, protect and save the ischemic heart: antioxidants revisited. Expert Opin Ther Targets 2009; 13: 945-56. https://doi.org/10.1517/14728220903039698.

Babu PS, Prabuseenivasan S, Ignacimuthu S. Cinnamaldehyde-a potential antidiabetic agent. Phytomedicine 2007; 14: 15-22. https://doi.org/10.1016/j.phymed.2006.11.005.

Beji RS, Khemir S, Wannes WA, Ayari K, Ksouri R. Antidiabetic, antihyperlipidemic and antioxidant influences of the spice cinnamon (Cinnamomum zeylanicumon) in experimental rats. Braz J Pharm Sci 2018; 54. https://doi.org/10.1590/s2175-97902018000217576.

Campbell BCV, De Silva DA, Macleod MR, Coutts SB, Schwamm LH, Davis SM, et al. Ischaemic stroke. Nat Rev Dis Primers 2019; 5: 70. https://doi.org/10.1038/s41572-019-0118-8.

Chen YF, Wang YW, Huang WS, Lee MM, Wood W G, Leung YM, et al. Trans-cinnamaldehyde, an essential oil in cinnamon powder, ameliorates cerebral ischemia-induced brain injury via inhibition of neuroinflammation through attenuation of iNOS, COX-2 expression and NFκ-B signaling pathway. Neuromolecular Med 2016; 18: 322-33. https://doi.org/10.1007/s12017-016-8395-9.

Farhoudi M, Mehrvar K, Sadeghi-Bazargani H, Hashemilar M, Seyedi-Vafaee M, Sadeghi-Hokmabad E, et al. Stroke subtypes, risk factors and mortality rate in northwest of Iran. Iran J Neurol. 2017; 16: 112-7.

Frydman-Marom A, Levin A, Farfara D, Benromano T, Scherzer-Attali R, Peled S, et al. Orally administrated cinnamon extract reduces β-amyloid oligomerization and corrects cognitive impairment in alzheimer's disease animal models. PLoS One 2011; 6. https://doi.org/10.1371/journal.pone.0016564.

Yang GY, Betz AL. Reperfusion-induced injury to the blood-brain barrier after middle cerebral artery occlusion in rats. Stroke 1994; 25: 1658-64. https://doi.org/10.1161/01.STR.25.8.1658.

Ghosh T, Basu A, Adhikari D, Roy D, Pal AK. Antioxidant activity and structural features of Cinnamomum zeylanicum. Biotech. 2015; 5: 939-47. https://doi.org/10.1007/s13205-015-0296-3.

Giustarini D, Dalle-Donne I, Tsikas D, Rossi R. Oxidative stress and human diseases: Origin, link, measurement, mechanisms, and biomarkers. Crit Rev Clin Lab Sci 2009; 46: 241-81. https://doi.org/10.3109/10408360903142326.

Hosseini AA, Sobhani-Rad D, Ghandehari K, Benamer H. Frequency and clinical patterns of stroke in Iran - Systematic and critical review. BMC Neurol 2010; 10. https://doi.org/10.1186/1471-2377-10-72.

Ighodaro OM, Akinloye OA. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria J Med 2018; 54: 287-93. https://doi.org/10.1016/j.ajme.2017.09.001.

Jana A, Modi KK, Roy A, Anderson JA, van Breemen RB, Pahan K. Up-regulation of neurotrophic factors by cinnamon and its metabolite sodium benzoate: Therapeutic implications for neurodegenerative disorders. J Neuroimmune Pharmacol 2013; 8: 739-55. https://doi.org/10.1007/s11481-013-9447-7.

Jiao H, Wang Z, Liu Y, Wang P, Xue Y. Specific role of tight junction proteins claudin-5, occludin, and ZO-1 of the blood-brain barrier in a focal cerebral ischemic insult. J Mol Neurosci 2011; 44: 130-9. https://doi.org/10.1007/s12031-011-9496-4.

Kalogeris T, Baines CP, Krenz M, Korthuis RJ. Ischemia/Reperfusion. Compr Physiol 2016; 6: 113-70. https://doi.org/10.1002/cphy.c160006.

Kalogeris T, Bao Y, Korthuis RJ. Mitochondrial reactive oxygen species: a double edged sword in ischemia/reperfusion vs preconditioning. Redox Biol 2014; 2: 702-14. https://doi.org/10.1016/j.redox.2014.05.006.

Lewis A, Segal A. Hyperlipidemia and primary prevention of stroke: does risk factor identification and reduction really work? Curr Atheroscler Rep. 2010; 12: 225-9. https://doi.org/10.1007/s11883-010-0117-4.

Longa EZ, Weinstein PR, Carlson S, Cummins R. Reversible middle cerebral artery occlusion without craniectomy in rats. Stroke 1989; 20: 84-91. https://doi.org/10.1161/01.STR.20.1.84.

Luissint AC, Artus C, Glacial F, Ganeshamoorthy K, Couraud PO. Tight junctions at the blood brain barrier: physiological architecture and disease-associated dysregulation. Fluids Barriers CNS 2012; 9: 23. https://doi.org/10.1186/2045-8118-9-23.

Marques C, Meireles M, Norberto S, Leite J, Freitas J, Pestana D, et al. High-fat diet-induced obesity Rat model: a

comparison between Wistar and Sprague-Dawley Rat. Adipocyte 2015; 15: 11-21. https://doi.org/10.1080/21623945.2015.1061723.

McCord JM, Fridovich I. Superoxide dismutase: an enzymic function for erythrocuprein (hemocuprein). J Biol Chem 1969; 244: 6049-55. https://doi.org/10.1016/S0021-9258(18)63504-5.

Mirhadi K. Effect of intraperitoneally injection of different doses of lovastatin on pain and inflammatory response induced by formalin in mice. Am J Anim Vet Sci 2011; 6: 160-5. https://doi.org/10.3844/ajavsp.2011.160.165

Moradi P, Ganjkhani M, Anarkooli IJ, Abdanipour A. Neuroprotective effects of lovastatin in the pilocarpine rat model of epilepsy according to the expression of neurotrophic factors. Metab Brain Dis 2019; 34: 1061-9. https://doi.org/10.1007/s11011-019-00424-1.

Nelson RH. Hyperlipidemia as a risk factor for cardiovascular disease. Prim Care. 2013; Mar; 40: 195-211. https://doi.org/10.1016/j.pop.2012.11.003.

Panahpour H, Nekooeian AA, Dehghani GA. Candesartan attenuates ischemic brain edema and protects the blood-brain barrier integrity from ischemia/reperfusion injury in rats. Iran Biomed J 2014; 18: 232.

Panickar KS, Anderson RA. Effect of polyphenols on oxidative stress and mitochondrial dysfunction in neuronal death and brain edema in cerebral ischemia. Int J Mol Sci 2011; 12: 8181-207. https://doi.org/10.3390/ijms12118181.

Buettner R, Parhofer KG, Woenckhaus M, Wrede CE, Kunz-Schughart LA, Schölmerich J, et al. Defining high-fat-diet rat models: metabolic and molecular effects of different fat types. J Mol Endocrinol 2006; 485-501. https://doi.org/10.1677/jme.1.01909.

Ranasinghe P, Perera S, Gunatilake M, Abeywardene E, Gunapala N, Premakumara S, et al. Effects of Cinnamomum zeylanicum (Ceylon cinnamon) on blood glucose and lipids in a diabetic and healthy rat model. Pharmacognosy Res 2012; 4: 73-9. https://doi.org/10.4103/0974-8490.94719.

Rao PV, Gan SH. Cinnamon: a multifaceted medicinal plant. Evid Based Complementary Altern Med 2014; 2014: 642942. https://doi.org/10.1155/2014/642942.

Rempe RG, Hartz AMS, Bauer B. Matrix metalloproteinases in the brain and blood-brain barrier: Versatile breakers and makers. J Cereb Blood Flow Metab. 2016; 36: 1481-507. https://doi.org/10.1177/0271678X16655551.

Sedighi M, Bahmani M, Asgary S, Beyranvand F, M. R-K. A review of plant based compounds and medicinal plants effective on atherosclerosis. J Res Med Sci 2017; 22. https://doi.org/10.4103/1735-1995.202151.

Stavinoha R. C, Vattem D A. Potential neuroprotective effects of cinnamon. Int J Appl Res Nat Prod 2015; 8: 24-46.

Sun MH, Jin H, Sun X, Huang SH, Zhang FL, Guo ZN, et al. Free radical damage in ischemia-reperfusion injury: an obstacle in acute ischemic stroke after revascularization therapy. Oxid Med Cell Longev 2018. https://doi.org/10.1155/2018/3804979.

Tan BL, Norhaizan ME. Effect of high-fat diets on oxidative stress, cellular inflammatory response and cognitive function. Nutrients 2019; 11: 2579. https://doi.org/10.3390/nu11112579.

Tuzcu Z, Orhan C, Sahin N, Juturu V, Sahin K. Cinnamon polyphenol extract inhibits hyperlipidemia and inflammation by modulation of transcription factors in high-fat diet-fed rats. Oxid Med Cell Longev 2017. https://doi.org/10.1155/2017/1583098.

Vafa M, Mohammadi F, Shidfar F, Sormaghi MS, Heidari I, Golestan B, et al. Effects of cinnamon consumption on glycemic status, lipid profile and body composition in type 2 diabetic patients. Int J Prev Med 2012; 3: 531-6.

Verspohl EJ, Bauer K, Neddermann E. Antidiabetic effect of Cinnamomum cassia and Cinnamomum zeylanicum in vivo and in vitro. Phytother Res. 2005; 19: 203-6. https://doi.org/10.1002/ptr.1643.

Xue R, Lv J, Gao J, Fu R, Li W, Lei X, et al. Protective effect of tea polyphenols on the blood-brain barrier. Translational Neuroscience 2013; 4: 295-301. https://doi.org/10.2478/s13380-013-0133-2.

Yulug B, Kilic E, Altunay S, Ersavas C, Orhan C, Dalay A, et al. Cinnamon polyphenol extract exerts neuroprotective activity in traumatic brain injury in male mice. CNS Neurol Disord Drug Targets 2018; 17: 439-47. https://doi.org/10.2174/1871527317666180501110918

Zeljkovic A, Vekic J, Spasojevic-Kalimanovska V, Jelic-Ivanovic Z, Bogavac-Stanojevic N, Gulan B, et al. LDL and HDL subclasses in acute ischemic stroke: prediction of risk and short-term mortality. Atherosclerosis 2010; 210: 548-54. https://doi.org/10.1016/j.atherosclerosis.2009.11.040