Journal of
Babol University of Medical Sciences
JBUMS
e-ISSN: 2251-7170 p-ISSN: 1561-4107

Neuroprotective Effect of Losartan after Traumatic Brain Injury in Male Rats Based on Marmarou Model

Sh. Shadi (MD)[1], A. Siahposht-Khachaki (PhD)[*1], Gh. Hooshmand (PhD)[2]

1.Immunogenic Research Center, Mazandaran University of Medical Sciences, Sari, I.R.Iran.

2.Department of Pharmacology, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, I.R.Iran.

Article Type

Research Paper

Received:
Sep 4th 2021
Revised:
Nov 13rd 2021
Accepted:
Dec 18th 2021

ABSTRACT

Background and Objective: Traumatic brain injury is the most important cause of death among young people in the world. Considering the neuroprotective effect of losartan on the recovery of brain damage, the present study was conducted to investigate the effects of losartan on neurological severity scores, blood-brain barrier health, cerebral edema, and histological changes after the induction of traumatic brain injury in male rats.

Methods: In this experimental study, 60 male Wistar rats weighing 250 to 280 grams were assigned into 7 groups: 1) Intact, 2) Surgery Sham, 3) Traumatic brain injury, 4) Losartan solvent (saline), 5) Low dose of losartan (5 mg), 6) Medium dose of losartan (10 mg), 7) High dose of losartan (20 mg) and 6-8 rats were used in each group. 30 minutes after brain trauma, different doses of losartan were injected intraperitoneally based on the Marmarou weight drop model. Neurobehavioral testing was performed, and cerebral edema and blood-brain barrier health were evaluated in all groups and the animal's brain was then used for tissue staining.

Findings: Losartan in doses of 5 mg (79.75±4.36) and 10 mg (70.375±5.60) was able to reduce cerebral edema ($p<0.001$) and neurological severity scores (11±1.41, and 12.75±1.90), and improve the animal's coordination on the balance beam task (11.125±3.313) and (6.875±2.94), respectively ($p<0.001$). In the dose of 10 mg, the effects were more significant (6.875±2.94) ($p<0.001$). In terms of histology, losartan decreased perivascular edema, and neuronal necrosis, and decreased astrocytic edema. Losartan had no effect in the dose of 20 mg ($p>0.05$).

Conclusion: The results of the study showed that losartan improves the outcomes of brain injury, which requires more studies in the future to investigate the mechanisms of this effect.

Keywords: *Losartan*, *Traumatic Brain Injury*, *Cerebral Edema*, *Rat*.

Cite this article: Shadi Sh, Siahposht-Khachaki A, Hooshmand Gh. Neuroprotective Effect of Losartan after Traumatic Brain Injury in Male Rats Based on Marmarou Model. *Journal of Babol University of Medical Sciences*. 2022; 24(1): 305-19.

© The Author(S).
Publisher: Babol University of Medical Sciences

[DOR: 20.1001.1.15614107.1401.24.1.38.4]
[DOI: 10.22088/jbums.24.1.305]

[*]Corresponding Author: A. Siahposht-Khachaki (PhD)
Address: Department of Physiology, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, I.R.Iran.
Tel: +98 (11) 33543893. **E-mail:** siahposht@mazums.ac.ir

Introduction

Traumatic Brain Injury (TBI) is the most common cause of brain injury, and the complications caused by traumatic brain injury among the survivors of trauma caused by motor vehicle collision, sports injuries, and war injuries are considerable (1, 2). Trauma causes two types of primary and secondary injuries in brain tissue. In all brain injuries, toxicity occurs due to excessive stimulation of neurons by the neurotransmitter glutamate, which causes successive pathophysiological reactions and causes brain tissue destruction and cell death. Since primary injuries are irreversible, clinical interventions have focused on preventing and controlling neurological damage caused by secondary injuries (3).

Losartan is an anti-hypertensive medicine. It is also effective in reducing kidney failure in people with type II diabetes and nephropathy. The American Diabetes Association has recommended the use of these drugs for reducing nephropathy and proteinuria in patients (4). Sartans that block angiotensin II type 1 receptors are highly neuroprotective, nerve-restorative, and anti-inflammatory (5-7). Losartan (LOS) can improve secondary brain damage caused by CCI (Controlled Cortical Impact). Losartan reduces brain lesion volume, neuronal apoptosis and protein involved in oxidative stress (8). In addition, losartan also improves neurological function (9). Losartan increases the expression of tight junction protein ZO-1 and reduces cerebral edema and blood-brain barrier leakage (10). In addition, losartan inhibits the inflammatory factor TNF-α and improves the anti-inflammatory factor IL-10 (11).

Losartan prevents vasospasm after subarachnoid hemorrhage (SAH). After SAH, losartan positively affects the endothelin-1 receptor pathway in a non-competitive direct and indirect agonistic manner. Losartan reduces endothelin-1-induced maximal contraction. This effect disappears by L-NAME due to inhibition of NO. Considering this spasmolytic effect of losartan, in addition to its known effects (reducing brain inflammation, restoring brain self-regulation and reducing epileptic activity) and improving primary brain injury, losartan seems to have a good therapeutic potential after injury (12). It is known that angiotensin 2 inhibitors such as losartan, which is the subject of this study, have a neuroprotective effect and reduce inflammation and increase the expression of the IL-10 marker (13).

Losartan can improve memory due to increased cholinergic activity (14). In a study, it was shown that the amount of acetylcholinesterase in the hippocampus and cortex is low in rats with renal hypertension compared to healthy rats; so, losartan can be beneficial in improving memory and learning (15). Losartan can reduce stress and tension-related behaviors, but it has been seen to be more helpful in emotional stress than physical stress (16). Losartan has a positive effect in ischemia-reperfusion injury, which is associated with a significant decrease in nitric oxide synthase, and leads to decline in brain infarction and improves neurobehavioral results (17). Therefore, in the present study, according to the available documentation and reasons, there is a debatable part about the neuroprotective effects of losartan (LOS); Does losartan really has neuroprotective effects on traumatic brain injury (TBI) based on the Marmarou weight drop model, which is the method of diffuse axonal injury and involves all brain structures? And secondly, what are the pathological findings associated with these neuroprotective effects? Therefore, in this study, the effects of intraperitoneal (IP) losartan injection on neurological severity scores, cerebral edema and permeability of the blood-brain barrier after induction of traumatic brain injury (TBI) based on the Marmarou model were investigated.

Methods

In this experimental study, after approval by the ethics committee of Mazandaran University of Medical Sciences with the code IR.MAZUMS.REC.1398.1251, 60 adult male Wistar rats weighing 250 to 300 grams

[DOR: 20.1001.1.15614107.1401.24.1.38.4]
[DOI: 10.22088/jbums.24.1.305]

were used under conditions of 12:12-h light-dark cycle and a temperature of 22±2 centigrade and they had no restrictions in terms of access to water and food. In this experimental study, based on previous studies (18, 19), 7 control and experimental groups were used with 6-8 rats in each group, and a total of 60 male rats, and neurological severity scores, cerebral edema, blood-brain barrier health, and coordination and balance scores along with histological findings were evaluated in all groups.

1) Intact group: male rats that were not subject to any intervention (20), 2) Surgery Sham: male rats that underwent surgery but had no traumatic brain injury (20, 21), 3) Traumatic brain injury (TBI) group: rats that have undergone brain trauma after anesthesia (20, 21), 4) Losartan solvent (saline) group (22, 23), 5) Low dose of losartan (5 mg), medium dose of losartan (10 mg) and high dose of losartan (20 mg). Losartan was purchased from Sigma (CAT Number: 99-8-124750) and all injections were done intraperitoneally (IP) (24).

A) The method of inducing traumatic brain injury: Before the operation, animals were anesthetized using ketamine (100 mg/kg) and xylazine (5 mg/kg) and after insertion of cannula in the animal's trachea to control breathing and prevent hypoxia, it was connected to a manual breathing unit (Ambu bag artificial ventilation device). Then, the scalp was opened, traumatic brain injury was induced between the bregma and lambda region of the animal's skull, and immediately after the impact, the metal plate was removed from the animal's head, and after spontaneous breathing, the animal was disconnected from the artificial ventilation device (Ambu bag). At this stage, the animal was transferred to the cage (18, 19).

B) The method of measuring the health of the blood-brain barrier: The permeability of the blood-brain barrier was measured 4-6 hours after the trauma by injecting Evans aqueous solution through the jugular vein. Thus, 20 mg/kg of Evans blue dye 2% (1 ml/kg) was injected through the jugular vein using an insulin syringe. After transcardial perfusion, the brain was quickly and completely removed, cut into pieces, and after chopping, it was poured into 20 ml of solution (14 ml of acetone solution+6 ml of sodium sulfate) and it was kept for 24 hours. The amount of Evans blue solution was placed in the shaker device and measured with a spectrophotometer at the wavelength of 610 nm, and then the amount of Evans blue dye per microgram of brain tissue was calculated based on the following formula (18, 25).

$$\text{Evans blue Dye (μg) in brain tissue (g)} = \frac{13.24 \times 20 \times \text{absorbance}}{\text{tissue weight}}$$

C) The method of measuring brain fluid content: After measuring the wet and dry weight of the brain (at a temperature of 60-70 degrees centigrade for 72 hours), the water content of the brain tissue was calculated as edema index using the following formula (18, 26).

$$\text{Percentage of water content} = 100 \times \frac{\text{Dry tissue weight - Wet tissue weight}}{\text{Wet tissue weight}}$$

D) Evaluation of the outcomes of neurological rating scores (Veterinary Coma Scale): The evaluation of neurological outcomes was done using the neurological score table (Veterinary Coma Scale) which has 15 points (1-15), and the scores of motor activity (1-8), eye activity (1-4) and respiratory activity (1-3) were measured on all days (18, 21).

E) Evaluation of movement and balance performance (beam tasks): Movement-balance performance was evaluated using beam-walk (BW) and beam-balance (BB), scoring criteria for the distance traversed

[DOR: 20.1001.1.15614107.1401.24.1.38.4]
[DOI: 10.22088/jbums.24.1.305]

were based on 0 to 5, while a score of zero indicates the inability to move from the starting point, scores 1 to 4 correspond to the distal sections of 60, 40, 20 or 80 cm from the starting point, respectively, and a score of 5 indicates traversing the full length of the beam (100 cm) and entering the target box. Normal rats performed the beam task without errors (that is, maintaining their balance for 60 seconds in BB and BW in 5 seconds) (19, 27).

F) Hematoxylin and eosin staining method: Animals were anesthetized using thiopental at a dose of 50 mg/kg after 72 hours after brain injury. Brain samples were taken and fixed in 10% formalin buffer solution. Sections were prepared using an automatic microtome (Leica, Germany) and stained with hematoxylin and eosin. The samples were examined by two separate pathologists who were unaware of the animal groups and the drugs used. To evaluate each sample in each group, 5 samples from each slide were examined in 10 fields. The degree of neuronal edema, astrocyte swelling, neuronal degeneration and vascular congestion were graded from 0 (slight), 1 (mild), 2 (moderate) and 3 (severe). This intensity was determined based on 10x magnification under an optical microscope (18).

Data analysis: After the normality of the data was checked with Shapiro-Wilk's test, repeated-measures ANOVA statistical test was used to compare quantitative variables between the studied groups, and Newman-Keuls post hoc test was used in One-Way ANOVA, Tukey's posttest was used in the case of two-way ANOVA, and statistical analyses were performed using GraphPad Prism 8 software, and $p<0.05$ was considered significant.

Results

Repeated-measures ANOVA along with Tukey's post hoc test showed that traumatic brain injury caused a decrease in neurological severity scores in all groups (around 4) compared to the Intact (score 15) and Sham (score 15) groups on D0 ($p<0.001$) (Diagram A1). But in the following days (D1-D3) after the administration of losartan, the neurological severity scores increased and at the end of the third day (D3) in the 5 mg dose group (11±1.41) and in the 10 mg dose group (12.75±1.90), the scores were close to the Intact and Sham groups ($p<0.001$). In the 20 mg dose group, at the end of the third day, there were no changes in the neurological severity scores (7.5±1.77) and this group showed no significant difference compared to the TBI (7.37±2.72) and saline (7.25±2.43) groups. Furthermore, repeated-measure ANOVA statistical analysis and Newman-Keuls post hoc test showed that Intact and Sham groups (Diagram B1) have the highest area under the ROC Curve (AUC) (1400). Intraperitoneal administration of doses of 5 (1350±40) and 10 mg (1000±156) of losartan caused a significant increase in AUC values ($p<0.001$), but in the dose of 20 mg of losartan (520±100), these findings were not significant.

One-way ANOVA and Newman-Keuls post hoc test showed that traumatic brain injury leads to an increase in brain water content (92±5.24) ($p<0.001$). On the other hand, the administration of losartan in the 5 mg dose group (79.75±4.36) and 10 mg dose group (70.375±5.60) was able to reduce this increase in brain water by a large amount ($p<0.001$). However, losartan in a dose of 20 mg (91±3.92) had no effect on brain water content (Diagram 2).

One-way ANOVA and Newman-Keuls post hoc test showed that traumatic brain injury increased the content of Evans blue (46.36±2.088) in the brain tissue ($p<0.001$) (Diagram 3). Losartan in effective doses (5 mg/kg and 10 mg/kg) has reduced the amount of this index (9.43±2.37, 25.925±5.73), i.e., reducing the permeability of the blood-brain barrier ($p<0.001$). However, in the 20 mg losartan dose group (46.625±6.823), no effect on the health of the blood-brain barrier was observed.

[DOR: 20.1001.1.15614107.1401.24.1.38.4]
[DOI: 10.22088/jbums.24.1.305]

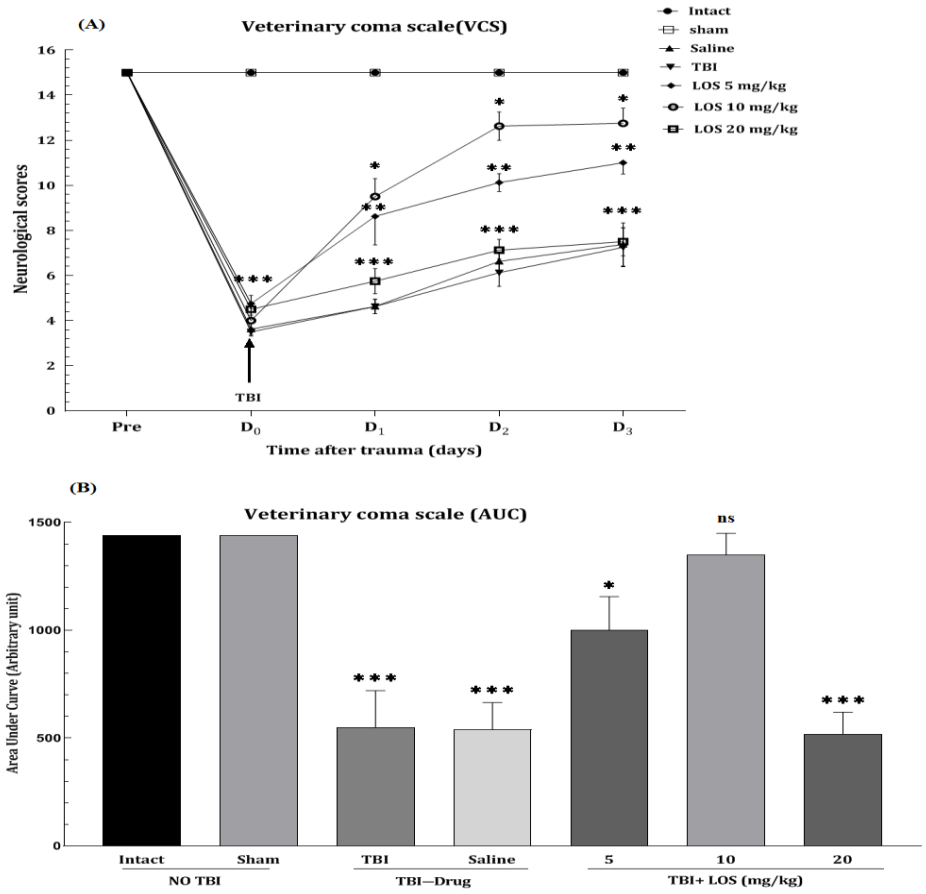


Diagram 1. The effect of losartan administration in doses of 5, 10 and 20 mg on neurological scores (A) and the area under the ROC Curve of neurological severity scores (B) after traumatic brain injury. ***p<0.05, **p<0.01, ***p<0.001 significant difference of all groups compared to Sham and Intact groups. Ns= not significant. Data are displayed as Mean±SD. There are 6 to 8 rats in each group.**

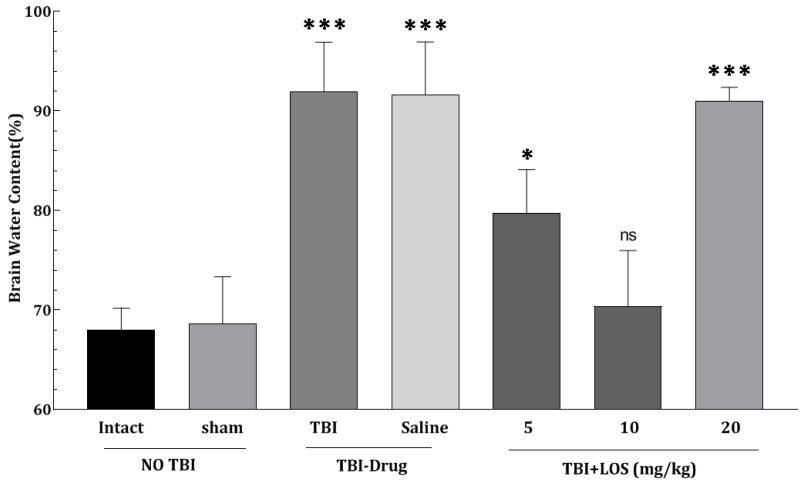


Diagram 2. The effect of losartan administration in doses of 5, 10 and 20 mg/kg on brain water content after traumatic brain injury. ***p<0.05, ***p<0.001 significant difference of all groups compared to Sham and Intact groups. Ns= not significant. Data are displayed as Mean±SD. There are 6 to 8 rats in each group.**

[DOR: 20.1001.1.15614107.1401.24.1.38.4]
[DOI: 10.22088/jbums.24.1.305]

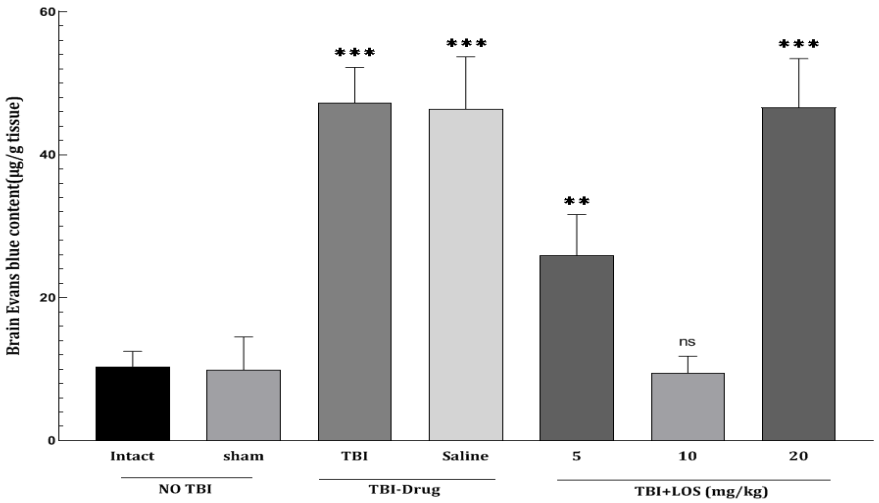


Diagram 3. The effect of losartan administration in doses of 5, 10 and 20 mg/kg on the content of Evans blue dye in the brain tissue after traumatic brain injury. $^{**}p<0.01$, $^{***}p<0.001$ significant difference of all groups compared to Sham and Intact groups. Ns= not significant. Data are displayed as Mean±SD. There are 6 to 8 rats in each group.

Repeated-measures ANOVA along with Tukey's post hoc test showed that brain trauma increased the duration of the animal traversing the beam in all groups (about 16) compared to the Intact (5 seconds) and Sham (5 seconds) groups on D0 ($p<0.001$) (Diagram A4). However, in the following days (D1-D3) and after the administration of losartan, traverse time decreased and at the end of the third day (D3) in the 5 mg dose group (11.125±3.313) and in the 10 mg dose group (6.875±2.94), it was close to the Intact and Sham groups ($p<0.001$). In the 20 mg dose group, at the end of the third day, there were no changes in traverse time (16.25±1.66), and this group showed no significant difference compared to the TBI (16.125±2.03) and Saline (16±1.511) groups. Furthermore, repeated-measure ANOVA statistical analysis and Newman-Keuls post hoc test showed that the Sham and Intact groups (Diagram B4) have the lowest area under the ROC Curve (AUC=480). Traumatic brain injury increases these values (1361±100) ($p<0.001$). However, the administration of doses of 5 mg (901±50) and 10 mg (585±150) of losartan caused the AUC values to be closer to the values in Sham and Intact groups ($p<0.001$), but in the dose of 20 mg of losartan (1332±50), these findings were not significant.

Two-way ANOVA statistical analysis and Tukey's test showed that as soon as traumatic brain injury was induced`, the balance score started to decrease (about 2.5±0.3) and became significant in all groups compared to the sham and intact groups on the day of traumatic brain injury induction (D0) ($p<0.001$) (Diagram A5). However, on days (D1-D3) after losartan injection, this balance score started to increase, and at the end of the third day in the 5 mg (4.125±0.99) and 10 mg (4.625±0.51) losartan groups, balance scores increased to become closer to Intact (score=5) and Sham (score=5) groups ($p<0.001$). With the passage of time, this significant difference between the treatment groups and the Intact and Sham control groups decreased ($p<0.05$). On the other hand, during the first to third days (D1-D3) after traumatic brain injury, as can be seen, the difference between losartan groups at doses of 5 and 10 mg and Saline (2.37±0.91) and TBI (2.25±0.88) control groups showed an increasing trend ($p<0.001$), which indicates the improvement in balance scores, but no effect was observed in the dose of 20 mg (2.23±0.70), and there is no difference between this group and the Saline and TBI control groups. Furthermore, repeated-measure ANOVA statistical analysis and Newman-Keuls post hoc test have shown

[DOR: 20.1001.1.15614107.1401.24.1.38.4]
[DOI: 10.22088/jbums.24.1.305]

that the Sham and Intact groups (Diagram B5) have the highest area under the ROC Curve (AUC=480). Traumatic brain injury causes a decrease (256±33.73) in these values ($p<0.001$). However, the administration of doses of 5 mg (372±43) and 10 mg (450±24.21) of losartan caused AUC values to be closer to its values in Sham and Intact groups ($p<0.001$). On the other hand, in a dose of 20 mg losartan (258±20), these findings were not significant.

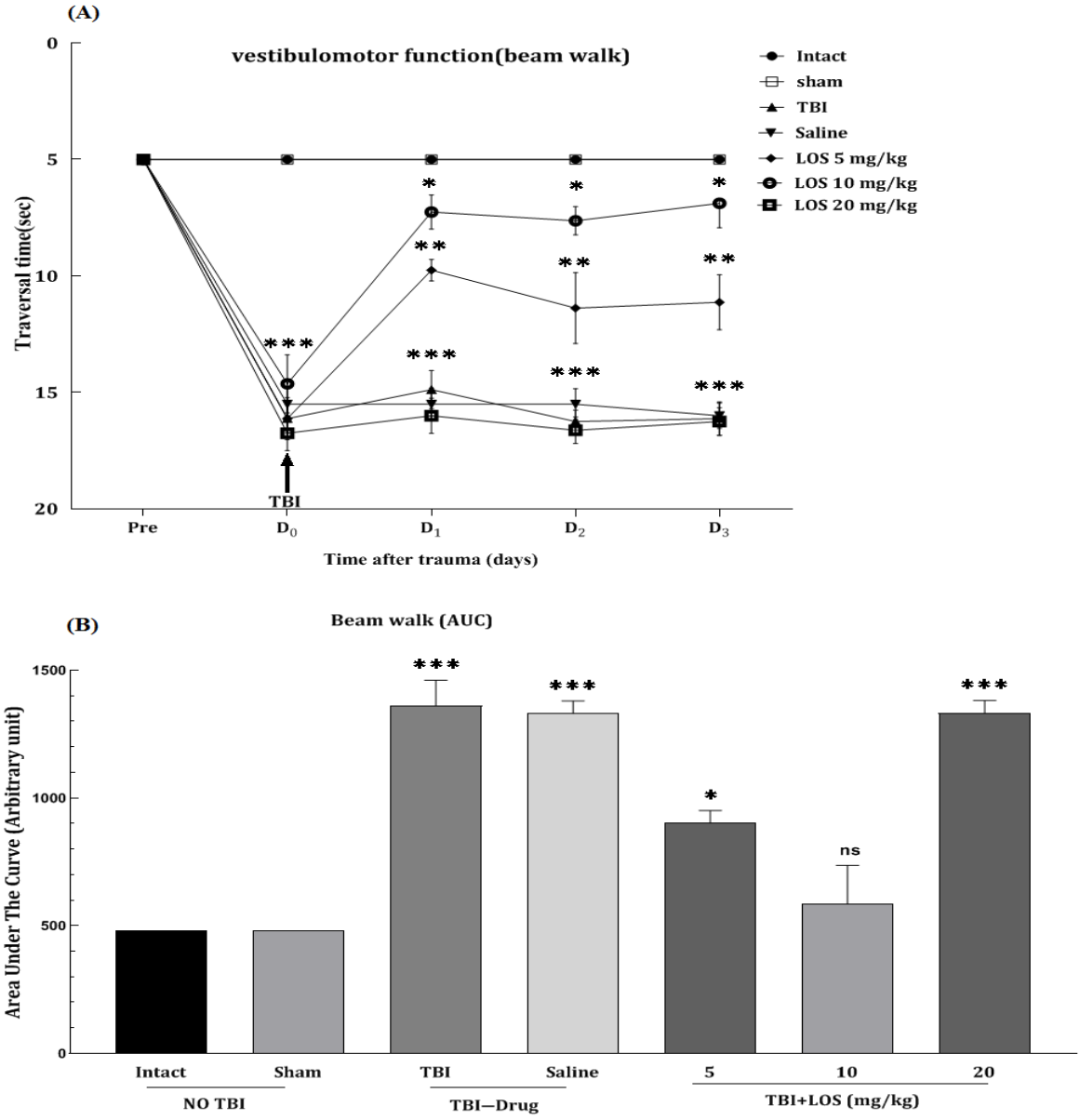


Diagram 4. The effect of losartan administration in doses of 5, 10 and 20 mg/kg on the movement scores of beam walk (A) and area under the ROC Curve of beam walk (B) after traumatic brain injury. **$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ significant difference of all groups compared to Sham and Intact groups. Ns= not significant. Data are displayed as Mean±SD. There are 6 to 8 rats in each group.**

[DOR: 20.1001.1.15614107.1401.24.1.38.4]
[DOI: 10.22088/jbums.24.1.305]

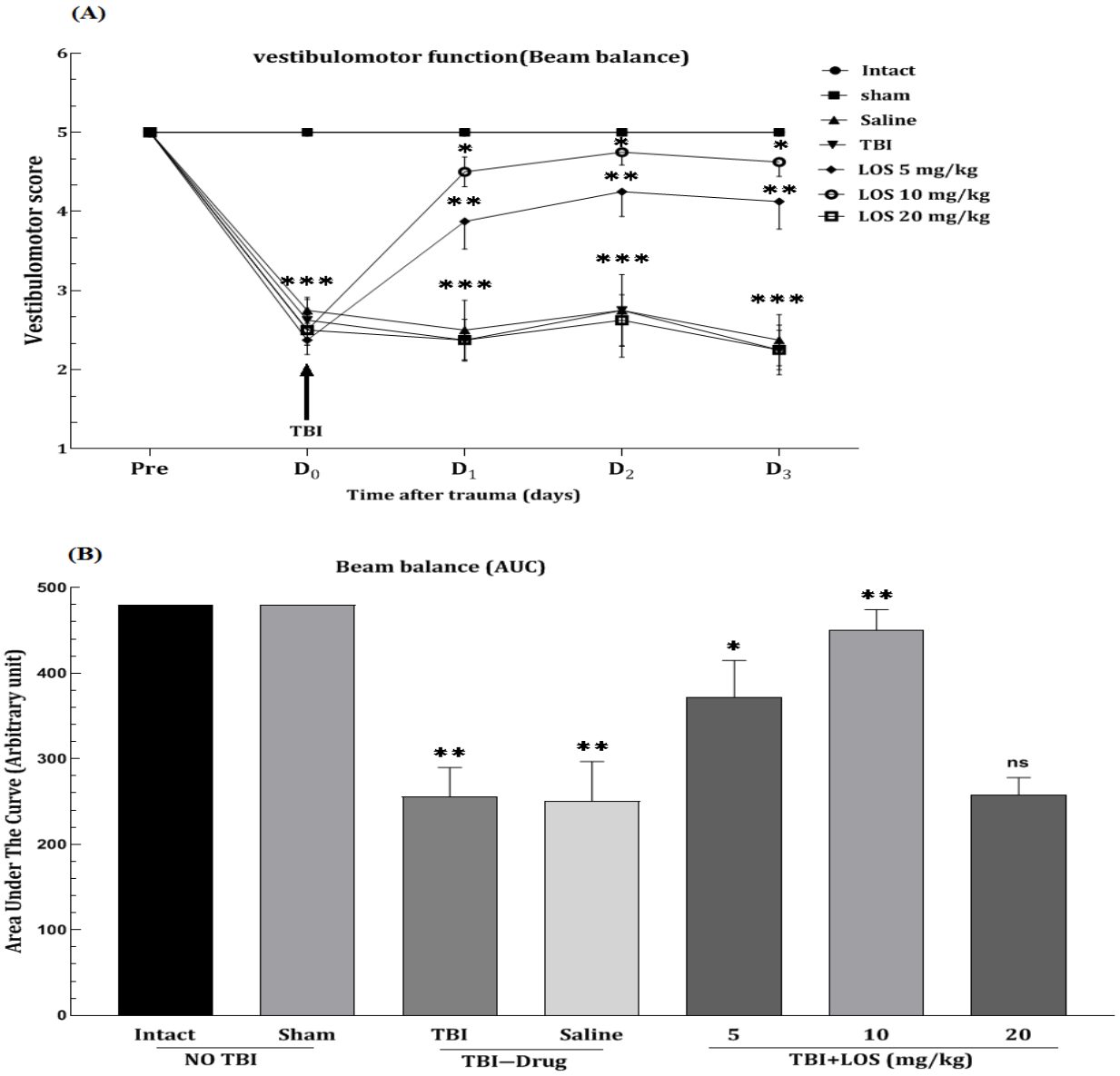


Diagram 5. The effect of losartan administration in doses of 5, 10 and 20 mg/kg on the scores of the balance beam index (A) (on the duration of the animal's stay on the beam) and area under the ROC Curve of balance beam (B) after traumatic brain injury. ***p<0.05, **p<0.01, ***p<0.001 significant difference of all groups compared to Sham and Intact groups. Ns= not significant. Data are displayed as Mean±SD. There are 6 to 8 rats in each group.**

Histological findings: In the histological findings in the intact and sham groups, normal neurons with a large central vesicular nucleus, neuroglia and vessels along with endothelial cells are visible (Figures A 1 and B1, and Table 1). However, after trauma in the TBI and saline (C, D) groups, degenerated neurons with wrinkled and heavily stained nuclei are seen with perineural edema, vascular congestion, and perivascular edema. But in groups treated with (E, F) (Losartan 5 and 10 mg/kg), normal neurons with a large central vesicular nucleus and neuroglia are seen, and in the group treated with (G) (Losartan 20 mg/kg), degenerated neurons with wrinkled and heavily stained nuclei are observed along with perineural edema.

[DOR: 20.1001.1.15614107.1401.24.1.38.4]
[DOI: 10.22088/jbums.24.1.305]

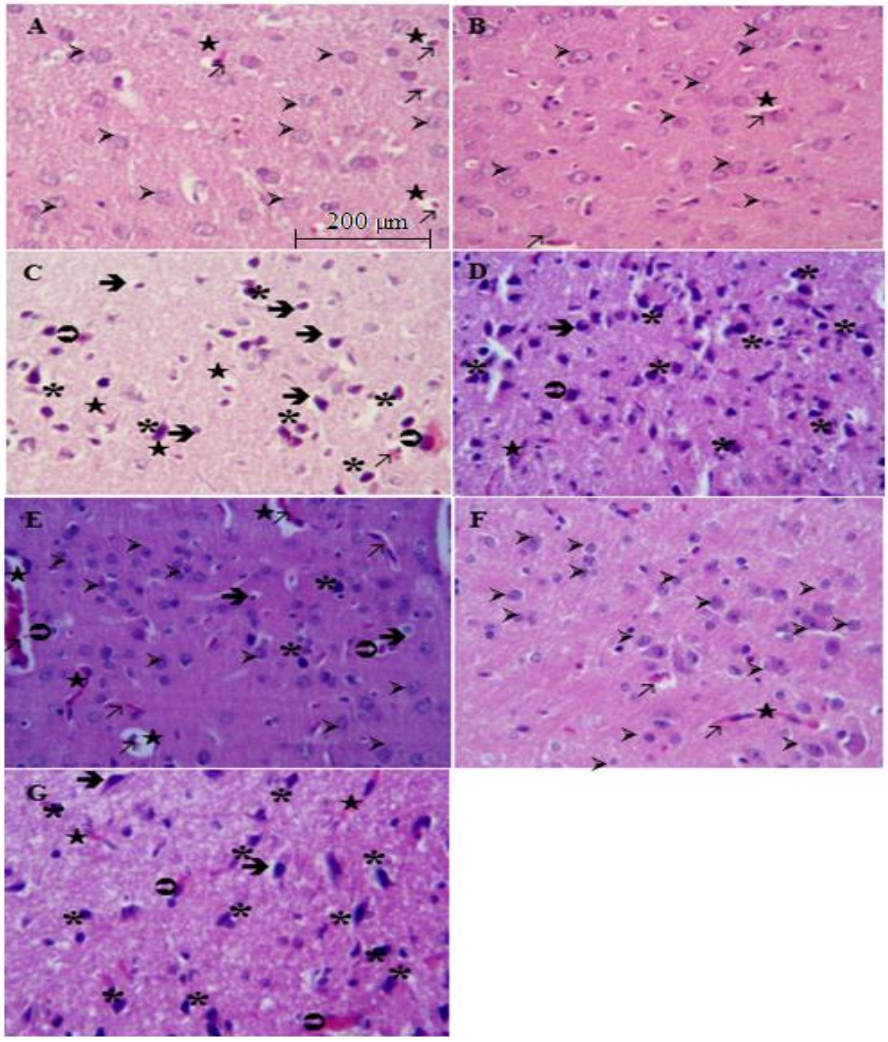


Figure 1. Microscopic image of the cerebral cortex of a male rat. Effects of losartan on histological changes 72 hours after traumatic brain injury (A-G). Magnification of the image is 400× and H&E staining is used. (A) Intact, (B) Sham, (C) TBI, (D) Saline, (E) Losartan 5 mg, (F) Losartan 10 mg, (G) Losartan 20 mg, ➢: normal neuron, *: degenerated neuron, ➔: neuronal edema, ➲: swollen astrocyte, ★: blood vessels, ↗: endothelial cells.

Table 1. Effects of losartan on histopathological changes on the brain cortex sections of rat 72 hours after traumatic brain injury

Groups	Neuronal edema Mean±SD	Astrocyte swelling Mean±SD	Neuronal degeneration Mean±SD	Vascular congestion Mean±SD
Intact	0±0	0±0	0±0	0±0
Sham	0±0	0±0	0±0	0±0
TBI	2.3±0.6***	1.3±0.2**	3.3±0.2***	1.3±0.4**
Saline	2.1±0.2***	1.5±0.3**	3.1±0.3***	1.5±0.2**
Los 5	1.9±0.2**	0.9±0.1*	2.1±0.5**	1.1±0.3**
Los 10	0.3±0.2*	0±0*	0±0*	0.1±0.4*
Los 20	2.5±0.5***	1.6±0.4***	3.2±0.5***	1.6±0.4***

Animals received losartan (5, 10 and 20 mg/kg) or its solvent intraperitoneally. *p<0.05, **p<0.01, ***p<0.001 significant difference of all groups compared to Sham and Intact groups.

[DOR: 20.1001.1.15614107.1401.24.1.38.4]
[DOI: 10.22088/jbums.24.1.305]

Discussion

In this study, the results showed that despite the fact that traumatic brain injury leads to an increase in brain water content and in other words causes cerebral edema, Losartan can reduce the amount of cerebral edema on the third day after the induction of traumatic brain injury. Examining the results of the study, it can be found that the brain water content in the group that received losartan at a dose of 10 mg/kg has no significant difference with the intact and sham groups. This means that losartan at a dose of 10 mg had appropriate effects and was able to minimize its difference with the healthy (control) and sham groups in a dose-dependent manner. Losartan at a dose of 5 mg/kg was also able to reduce brain edema in the group that received this amount of the drug, but clearly, the rate of brain edema reduction was higher in the losartan 10 mg/kg group. Moreover, the content of brain water in the 20 mg/kg group was not significantly different from the TBI and saline groups who did not receive any medication, that is, the 20 mg dosage had no effect on traumatic brain injury and could not cause a change compared to the two trauma groups (TBI and saline). This shows that this dose of the drug had no effect on brain edema. One of the reasons that the dose of 20 mg/kg of losartan could not affect the indices of cerebral edema is that neurotoxic effects may be seen with increasing the dose of the drug, and perhaps the drug in high doses triggers neurodegenerative pathways and applies agonistic effects on angiotensin II receptor type 1, which can lead to seizures, destruction of the blood-brain barrier, and neuroinflammation (28).

Xiong et al. showed that losartan at a dose of 3 mg/kg in rats causes a reduction in brain lesion volume, neuronal apoptosis and stress proteins. Losartan also improves neurological and motor function. Losartan increases the expression of ZO-1 cell binding proteins and reduces brain edema and blood-brain barrier leakage. In addition, losartan inhibits the anti-inflammatory factor TNF-a and improves the anti-inflammatory factor IL-10. Overall, losartan can improve the prognosis of traumatic brain injury and may be a promising therapeutic approach to improve this problem (10). In our study, losartan was able to affect cerebral edema as well as balance and motor performance in the beam task, and probably a part of these effects is due to neuroinflammatory pathways such as TGF-β, even prevention of the breakdown of the blood-brain barrier and the level of matrix metalloproteinases (MMPs), which requires further investigation and analysis (29).

In this study, in order to study the permeability of the blood-brain barrier, it was shown in different groups that traumatic brain injury caused an increase in the content of Evans blue dye in the extravascular tissue of the brain of rats, which indicates an increase in the permeability of the blood-brain barrier, and 5 mg/kg and 10 mg/kg doses of losartan were effective in reducing the level of this index, and the amount of Evans blue dye in these groups was significantly different from the amount of this substance in the TBI and saline groups, and on the other hand, no significant difference was found compared to intact and sham groups. 10 mg/kg losartan is more effective than the dose of 5 mg/kg in terms of improving the permeability of the blood-brain barrier. Furthermore, the dose of 20 mg/kg was not effective in this case and the groups that received this amount of the drug did not show a significant difference in the content of Evans blue dye in their brain tissue compared to the TBI and saline groups.

In their investigations on laboratory animals (mice, rats, and guinea pigs), Friedman et al. concluded that in traumatic brain injury, which is associated with swelling, destruction of neurons, convulsions, and inflammation in general, factors such as TGF-β in the pathway of astrocytes, albumin in the blood and other blood-tissue factors may cause permanent damage to the brain. By intraperitoneally injecting losartan into the mentioned animals, they found that factors such as TGF-β decreased in the CA1 and CA2 layers. Furthermore, factors such as glutamate, which are found in neuronal damage, have been reduced by taking losartan. In addition, the restoration of cerebral vessels has improved cognitive function and reduced the functional disorders of skeletal muscles and eventually improved motor activity. Studies have shown that

[DOR: 20.1001.1.15614107.1401.24.1.38.4]
[DOI: 10.22088/jbums.24.1.305]

losartan activates neuroglia cells and reduces neuronal death and brain inflammation associated with diabetes, and positive results were obtained in mouse behavioral testing. These studies also showed that the use of losartan in rats can reduce blood-brain barrier disorders and the entry of albumin, which leads to the prevention of convulsions in laboratory animals. Therefore, it can be said that according to the experiments conducted by this group, losartan is an important factor for neuroprotective effects (30). Therefore, part of the neuroprotective effects of losartan is due to the effects on neuronal activity (31), reducing the amount of glutamate in the synaptic space and neurotoxicity (32) and reducing neuronal death (22), reducing neuronal inflammation associated with diabetes (33), reducing the destruction of brain tissue and blood-brain barrier in strokes (34).

In another study conducted by Culman et al., it was determined that the intravenous administration of losartan with doses of 30 and 3 mg/kg in rats 90 minutes after stroke induction by middle cerebral artery occlusion caused significant neuroprotective effects through reduction of blood pressure during the improvement of cerebral perfusion and reduction of the volume of liquid leaked into the cerebral ventricles. This study is in line with our research, and it was shown in this study that losartan can have protective effects, and part of these effects are related to cerebral blood flow, which is modulated by losartan (35). In another study, Drews et al. showed that intranasal administration of losartan in a dose of 1 µM reduced beta amyloid plaques in the areas surrounding the cerebral ventricles, and also reduced brain inflammation and increased neurogenesis in the brain, all of which indicate the neuroprotective role of this angiotensin receptor blocker (36).

Royea et al. showed that the administration of 10 mg/kg of body weight losartan in Rat Model of Alzheimer's disease improved cardiovascular defects and reduced beta-amyloid plaques, improved spatial memory in water maze test, and improved neurogenesis, reduced inflammation, and reduced oxidative stress, which expresses the neuroprotective role of losartan and is carried out through the angiotensin IV receptor (AT4R) (37). This study is in line with our study and we also got better results in the dose of 10 mg. However, losartan probably acts by reducing oxidative stress, which was not measured in our work, and needs further evaluations in traumatic brain injury in the future. In this study, in order to investigate the brain function after the traumatic brain injury in the days after the incident, the information related to the parameters of beam task was evaluated in the studied groups. The results of this study showed that the groups of Saline, TBI and 20 mg/kg losartan during the days after the injury, even on the third day after the injury, did not show a significant improvement in the balance-motor score. On the other hand, the groups receiving losartan in doses of 5 mg/kg and 10 mg/kg during the days after the injury were able to quickly approach the Intact and Sham groups and there was no significant difference with these groups on the third day. Therefore, this proves that losartan can possibly improve cognitive, balance and neurological function, because it can affect neuroinflammation as well as the rate of glutamate release and have protective effects in neurodegenerative diseases (38, 39).

In this study, the neurological severity scores of the studied rats were investigated in different groups. Examining the neurological severity scores (VCS) during 3 consecutive days showed that the groups that received losartan in doses of 5 and 10 mg/kg had the highest neurological score at all times, and on the third day, their neurological severity scores were closer to the Intact and Sham groups compared to the other groups, and the increasing trend of VCS in these groups was faster than other groups.

The overall results of this study showed that administration of losartan as an angiotensin II type 1 receptor blocker with doses of 5 mg/kg and 10 mg/kg led to improvement in neurological symptoms, including improvement in motor function, improvement in blood-brain barrier and cerebral edema. There are also good histological changes in the rats that were subjected to brain trauma. As a result, this drug could

[DOR: 20.1001.1.15614107.1401.24.1.38.4]
[DOI: 10.22088/jbums.24.1.305]

possibly have protective effects on neurons and also possibly be effective in clinical cases of traumatic brain injury, which requires further investigations.

Conflict of interest: The authors of the article declare that there is no conflict of interest and the authors are solely responsible for the content and writing of this article.

Acknowledgment

The Deputy of Research and Technology of Mazandaran University of Medical Sciences is hereby thanked for the financial support of this study.

[DOR: 20.1001.1.15614107.1401.24.1.38.4]
[DOI: 10.22088/jbums.24.1.305]

References

1.Peixoto C, Hyland L, Buchanan DM, Langille E, Nahas R. The polytrauma clinical triad in patients with chronic pain after motor vehicle collision. J Pain Res. 2018;11:1927-36.

2.Swan AA, Amuan ME, Morissette SB, Finley EP, Eapen BC, Jaramillo CA, et al. Long-term physical and mental health outcomes associated with traumatic brain injury severity in post-9/11 veterans: A retrospective cohort study. Brain Inj. 2018;32(13-14):1637-50.

3.Bullock MR, Povlishock JT. Guidelines for the management of severe traumatic brain injury. Editor's Commentary. J Neurotrauma. 2007;24 Suppl 1:2 p preceding S1.

4.Kasper D, Fauci A, Longo D, Hauser S, Jameson JL, Loscalzo J. Harrison's principles of internal medicine (cardiovascular disorders) [Translated by Khodaei M, Tayebi Khameneh P], 19th ed. Tehran: Arjmand pub; 2015.p. 5. [In Persian]

5.Benicky J, Sánchez-Lemus E, Honda M, Pang T, Orecna M, Wang J, et al. Angiotensin II AT 1 receptor blockade ameliorates brain inflammation. Neuropsychopharmacology. 2011;36(4):857-70.

6.Torika N, Asraf K, Apte RN, Fleisher-Berkovich S. Candesartan ameliorates brain inflammation associated with Alzheimer's disease. CNS Neurosci Ther. 2018;24(3):231-42.

7.Bhat SA, Goel R, Shukla S, Shukla R, Hanif K. Angiotensin receptor blockade by inhibiting glial activation promotes hippocampal neurogenesis via activation of Wnt/β-catenin signaling in hypertension. Mol Neurobiol. 2018;55(6):5282-98.

8.Li T, Zhang Y, Zhu B, Wu C, Chen Y. Telmisartan regulates the development of cerebral ischemia by alleviating endoplasmic reticulum stress. Pharmazie. 2018;73(10):585-8.

9.Villapol S, Balarezo MG, Affram K, Saavedra JM, Symes AJ. Neurorestoration after traumatic brain injury through angiotensin II receptor blockage. Brain. 2015;138(Pt 11):3299-315.

10.Xiong J, Gao Y, Li X, Li K, Li Q, Shen J, et al. Losartan Treatment Could Improve the Outcome of TBI Mice. Front Neurol. 2020;11:992.

11.Salmani H, Hosseini M, Beheshti F, Baghcheghi Y, Sadeghnia HR, Soukhtanloo M, et al. Angiotensin receptor blocker, losartan ameliorates neuroinflammation and behavioral consequences of lipopolysaccharide injection. Life Sci. 2018;203:161-170.

12.Wanderer S, Andereggen L, Mrosek J, Kashefiolasl S, Marbacher S, Konczalla J. The Role of Losartan as a Potential Neuroregenerative Pharmacological Agent after Aneurysmal Subarachnoid Haemorrhage. Int J Mol Sci. 2020;21(18):6496.

13.Kalynovska N, Diallo M, Sotakova-Kasparova D, Palecek J. Losartan attenuates neuroinflammation and neuropathic pain in paclitaxel-induced peripheral neuropathy. J Cell Mol Med. 2020;24(14):7949-58.

14.Fernandez LA, Caride VJ, Strömberg C, Näveri L, Wicke JD. Angiotensin AT2 receptor stimulation increases survival in gerbils with abrupt unilateral carotid ligation. J Cardiovasc Pharmacol. 1994;24(6):937-40.

15.Srinivasan J, Jayadev S, Kumaran D, Haja Nazeer Ahamed KF, Suresh B, Ramanathan M. Effect of losartan and enalapril on cognitive deficit caused by Goldblatt induced hypertension. Indian J Exp Biol. 2005;43(3):241-6.

16.Aghaei I, Arjmand S, Yousefzadeh Chabok S, Tondar M, Shabani M. Nitric oxide pathway presumably does not contribute to antianxiety and memory retrieval effects of losartan. Behav Pharmacol. 2017;28(6):420-7.

17.Liu H, Liu X, Wei X, Chen L, Xiang Y, Yi F, et al. Losartan, an angiotensin II type 1 receptor blocker, ameliorates cerebral ischemia-reperfusion injury via PI3K/Akt-mediated eNOS phosphorylation. Brain Res Bull. 2012;89(1-2):65-70.

[DOR: 20.1001.1.15614107.1401.24.1.38.4]
[DOI: 10.22088/jbums.24.1.305]

18.Rahimi S, Dadfar B, Tavakolian G, Asadi Rad A, Shabkahi AR, Siahposht-Khachaki A. Morphine attenuates neuroinflammation and blood-brain barrier disruption following traumatic brain injury through the opioidergic system. Brain Res Bull. 2021;176:103-11.
19.Rahimi S, Ferdowsi A, Siahposht-Khachaki A. Neuroprotective effects of metformin on traumatic brain injury in rats is associated with the AMP-activated protein kinase signaling pathway. Metab Brain Dis. 2020;35(7):1135-44.
20.Soltani Z, Shahrokhi N, Karamouzian S, Khaksari M, Mofid B, Nakhaee N, et al. Does progesterone improve outcome in diffuse axonal injury?. Brain Inj. 2017;31(1):16-23.
21.Soltani Z, Khaksari M, Jafari E, Iranpour M, Shahrokhi N. Is genistein neuroprotective in traumatic brain injury?. Physiol Behav. 2015;152(Pt A):26-31.
22.Zhang T-L, Fu J-L, Geng Z, Yang J-J, Sun X-J. The neuroprotective effect of losartan through inhibiting AT1/ASK1/MKK4/JNK3 pathway following cerebral I/R in rat hippocampal CA1 region. CNS Neurosci Ther. 2012;18(12):981-7.
23.Kumar A, Singh B, Mishra J, Sah SP, Pottabathini R. Neuroprotective mechanism of losartan and its interaction with nimesulide against chronic fatigue stress. Inflammopharmacology. 2015;23(6):291-305.
24.Kumar A, Loane DJ. Neuroinflammation after traumatic brain injury: opportunities for therapeutic intervention. Brain Behav Immun. 2012;26(8):1191-201.
25.Siahposht khachaki A, Khaksari haddad M, Shahrokhi Sardo N, Sepehri G. Effects of different phases of estrous cycle on brain edema and neurological outcomes after severe traumatic brain injury in female rats. Koomesh. 2011;13(1):62-72. [In Persian]
26.Khaksari M, Maghool F, Asadikaram G, Hajializadeh Z. Effects of sex steroid hormones on neuromedin S and neuromedin U2 receptor expression following experimental traumatic brain injury. Iran J Basic Med Sci. 2016;19(10):1080-9.
27.Monaco CM, Mattiola VV, Folweiler KA, Tay JK, Yelleswarapu NK, Curatolo LM, et al. Environmental enrichment promotes robust functional and histological benefits in female rats after controlled cortical impact injury. Exp Neurol. 2013;247:410-8.
28.Bar-Klein G, Cacheaux LP, Kamintsky L, Prager O, Weissberg I, Schoknecht K, et al. Losartan prevents acquired epilepsy via TGF-β signaling suppression. Ann Neurol. 2014;75(6):864-75.
29.Min L-J, Mogi M, Shudou M, Jing F, Tsukuda K, Ohshima K, et al. Peroxisome proliferator-activated receptor-γ activation with angiotensin II type 1 receptor blockade is pivotal for the prevention of blood-brain barrier impairment and cognitive decline in type 2 diabetic mice. Hypertension. 2012;59(5):1079-88.
30.Friedman A, Bar-Klein G, Serlin Y, Parmet Y, Heinemann U, Kaufer D. Should losartan be administered following brain injury?. Expert Rev Neurother. 2014;14(12):1365-75.
31.Palkovits M, Šebeková K, Klenovics KS, Kebis A, Fazeli G, Bahner U, et al. Neuronal activation in the central nervous system of rats in the initial stage of chronic kidney disease-modulatory effects of losartan and moxonidine. PLoS One. 2013;8(6):e66543.
32.Wang J, Pang T, Hafko R, Benicky J, Sanchez-Lemus E, Saavedra JM. Telmisartan ameliorates glutamate-induced neurotoxicity: roles of AT(1) receptor blockade and PPARγ activation. Neuropharmacology. 2014;79:249-61.
33.Vargas R, Rincón J, Pedreañez A, Viera N, Hernández-Fonseca JP, Peña C, et al. Role of angiotensin II in the brain inflammatory events during experimental diabetes in rats. Brain Res. 2012;1453:64-76.
34.Smeda JS, Daneshtalab N. The effects of poststroke captopril and losartan treatment on cerebral blood flow autoregulation in SHRsp with hemorrhagic stroke. J Cereb Blood Flow Metab. 2011;31(2):476-85.

[DOR: 20.1001.1.15614107.1401.24.1.38.4]
[DOI: 10.22088/jbums.24.1.305]

35.Culman J, Jacob T, Schuster SO, Brolund-Spaether K, Brolund L, Cascorbi I, et al. Neuroprotective effects of AT1 receptor antagonists after experimental ischemic stroke: what is important?. Naunyn Schmiedebergs Arch Pharmacol. 2017;390(9):949-59.

36.Drews HJ, Yenkoyan K, Lourhmati A, Buadze M, Kabisch D, Verleysdonk S, et al. Intranasal losartan decreases perivascular beta amyloid, inflammation, and the decline of neurogenesis in hypertensive rats. Neurotherapeutics. 2019;16(3):725-40.

37.Royea J, Zhang L, Tong X-K, Hamel E. Angiotensin IV receptors mediate the cognitive and cerebrovascular benefits of losartan in a mouse model of Alzheimer's disease. J Neurosci. 2017;37(22):5562-73.

38.Wanderer S, Grüter BE, Strange F, Sivanrupan S, Di Santo S, Widmer HR, et al. The Role of Sartans in the Treatment of Stroke and Subarachnoid Hemorrhage: A Narrative Review of Preclinical and Clinical Studies. Brain Sci. 2020;10(3):153.

39.Salmani H, Hosseini M, Baghcheghi Y, Moradi-Marjaneh R, Mokhtari-Zaer A. Losartan modulates brain inflammation and improves mood disorders and memory impairment induced by innate immune activation: The role of PPAR-γ activation. Cytokine. 2020;125:154860.

[DOR: 20.1001.1.15614107.1401.24.1.38.4]

[DOI: 10.22088/jbums.24.1.305]