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# Effect of Jujuboside A on Brain Ischemia / Reperfusion Injury Via Expression of Antiapoptotic Gene

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## Abstract

**Background:** Some studies highlighted the role of Jujuboside A as a potent antioxidant on ischemic neurons. However, the definitive effect of this substance on the change in the expression of the neuroprotective genes has not been clearly identified. Therefore, this study aimed to investigate the effects of Jujuboside A on the expression of proliferation-inducing genes and cell count enhancement in the hippocampus after the induction of transient global ischemia/reperfusion. **Materials and Methods:** This experimental study was performed on 24 male Wistar rats in four groups control, ischemia, vehicle, and Jujuboside A. Three days after induction of ischemia, the hippocampus of animals was removed and isolated from brain tissue. In order to investigate the results of the intervention, the expression of *nuclear factor-κB (NF-κB), nerve growth factor (NGF)*, and *Brain-derived neurotrophic factor (BDNF)* genes were determined using real-time polymerase chain reaction. **Results:** The results showed that *NGF* expression was significantly higher in the Jujuboside A group than in the ischemic group ($P<0.05$). Moreover, the expression of *BDNF* in the Jujuboside A group was increased compared to the ischemic group. However, the expression of *NFκB* in the Jujuboside A group was lower than that of the ischemic and control groups, but these changes were not significant ($P>0.05$). **Conclusion:** Jujuboside A can increase the expression of *NGF* by promoting a protective effect on the hippocampus after transient global ischemia/reperfusion.

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**Keywords:** Hippocampus; Ischemia; Jujuboside A; Antiapoptotic; Reperfioen; Brain-derived Neurotrophic Factor

## Introduction

Nowadays, the use of medicinal herbs and traditional medicine in the treatment of diseases has attracted the attention of many researchers [1-3]. *Ziziphi Spinosae Semen* (SZS) the seeds of *Ziziphus jujuba Mill var. Spinosa* belongs to the Rhamnaceae family has been used for more than a thousand years as a sedative drug in the pharmacopeia of China, Japan, Korea, and other Eastern countries [4]. Various studies have





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shown the role of SZS in several activities, including the hypnotic-sedative, antihypertensive, antihyperlipidemic, and anti-inflammatory effects. Moreover, some studies have reported the psychological and anti-anxiety effects of this substance [5, 6]. In addition, the anti-apoptotic effect of this substance has been proven in the study [7].

Stroke is the most common and debilitating neurologic lesion and is the third most common cause of death after heart disease and cancer [8-10]. This disease can be caused by the closure or rupture of one of the blood vessels of the brain. These lesions may lead to permanent disabilities, including impaired motor skills, communication, cognition, memory, and learning. Therefore, stroke decreases the quality of life and burdens the family and society [10]. Usually, there are no warning signs before the stroke and/or only minor symptoms are observed. The risk of developing cerebral embolism, thrombosis, and hemorrhage increases with elevated blood pressure. Typically, 85% of strokes are ischemic, and 15% are hemorrhagic [10, 11].

Since the hippocampus is stocked by the anterior choroidal artery, which is a split of the internal carotid artery, it is always prone to thrombosis due to being long and thin. Therefore, the hippocampus is one of the main zones of the marrow that can be damaged in brain diseases such as stroke and ischemia [12]. One of the most sensitive parts of this region is the CA1 hippocampal pyramidal neurons, which have a high sensitivity to ischemia [13]. Therefore, temporary or permanent reduction of blood flow to the brain causes processes such as cytotoxicity induced by stimulation, acidosis, ion imbalance, oxidative stress, reactive oxygen species (ROS) production, lipid peroxidation, inflammation, and cell apoptosis [14, 15].

The identification of factors that reduce ischemia has always been of interest to researchers, and the use of natural substances and compounds derived from medicinal plants has been precious due to low levels of complications [13-15].

Jujuboside A is a mixture of triterpene glycosides derived from SZS [16]. Previous studies showed Jujuboside A has antioxidant properties and can eliminate cellular ROS [17, 18]. Neurons are highly susceptible to acute oxidative and metabolism stress under ischemic conditions [19]. This may be can induce apoptosis in neuronal cells. Therefore, the use of antioxidants as neuroprotective elements that reduce apoptotic mediators and are the stimuli of neuronal growth may have positive effects on the prevention of ischemic stroke and improving the patients who suffer from ischemic stroke [11, 20]. Many studies highlighted the role of Jujuboside A on ischemic neurons as a potent antioxidant [17, 18]. However, the definitive effect of this substance on the change in the expression of the neuroprotective genes has not been identified. Hence, this study aimed to investigate the effect of Jujuboside A on the proliferation-inducing gene expression and cell enhancement on the hippocampus of male Wistar rats after the induction of transient global ischemia/reperfusion (I/R).

## Materials and Methods

*Study Design*

Twenty-four male Wistar rats (around eight weeks of age and weighing 230±30 g) were used in this study. Animals were kept in plexiglass cages and a room with a temperature of 23±2 °C, relative humidity of 50 to 55%, 12-hour darkness/light cycle with free access to water and food [3]. Two weeks were considered for adaptation to environmental conditions.

Animals were randomly divided into four groups (n=6 per group) as follows:

1. Control group that received no any treatment
2. Ischemia group that underwent transient global I/R for 20 minutes after being anesthetized
3. Treatment group that received 20 µg/kg Jujuboside A (Sigma, Germany) by intraperitoneal (IP) injection 30 minutes before ischemia
4. Vehicle group that received dimethyl sulfoxide 0.3% (DMSO; Sigma, Germany) as the vehicle material through IP injection 30 minutes before ischemia at a volume similar to the treatment group. For anesthesia, ketamine (80 mg/Kg) and xylazine (8 mg/Kg) were administered by IP injection.

All animals were under human caring in accordance with the Principles for the Care and Use of Animals in Research and approved by the ethics committee of Tehran Medical Sciences, Islamic Azad University (code of ethical approval: IR.IAU.PS.REC.1400.182).

*Induction of Transient Global I/R*

Induction of transient global ischemia/reperfusion was as described previously [21]. After exerting the midline incision on the neck, left and right carotid arteries were isolated and separated from the vagus nerve, and then carotid arteries were occluded by microsurgical clamps. Simultaneously hypotension (blood pressure=50 mmHg) was induced by withdrawing blood via the femoral artery. After 20 minutes, the clamps were opened, the blood flow was restored, and the withdrawn blood was re-infused. During the surgical procedure, the temperature of the animal's body was maintained at 37.5 °C by a thermal towel. At the end of the ischemia, the incision was closer by suture thread, and the animals were kept under observation until they were awake and were then kept in separate cages. Three days after ischemia, the animals were anesthetized using ether (inhalation), and immediately after cutting off their head, the hippocampus was separated. Samples were stored at -80 °C.

*RNA Extraction and cDNA Synthesis*

According to the manual's protocol, the TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA) was used for the total RNA extraction from samples. After that, the DNase was applied to remedy all RNA samples. A kit of cDNA synthesis (Yektatajhiz cDNA synthesis kit, Iran) was applied to the synthesis of total cDNA according to the instructions of the manufacture. The proprietary primers were applied to amplify the cDNA for NFκB (nuclear factor kappa-light-chain-enhancer of activated B cells), nerve growth factor (NGF), and Brain-derived neurotrophic factor (BDNF). The glyceraldehyde-3-phosphate dehydrogenase (GHPD) was used for normalization as an internal control. The fast real-time polymerase chain reaction (PCR) System (Biosystems 7500, Thermo Fisher Scientific, USA) was applied in combination with the SYBR Premix Ex Taq II kit (Takara Bio, Iran) to amplify the ensuing cDNA.

*Real-Time PCR*

The sequences of proprietary primer were applied as follows (from 5′ to 3′): NFκB, forward (F): CATACGCTGACCCTAGCCTG, reverse (R): TTTCTTCAATCCGGTGGCGA (135 bp); NGF, F: ATCGCTCTCCTTCACAGAGTT; R: CGCTATGCACCTCAGAGTGG (151 bp); BDNF, F: AATAATGTCTGACCCCAGTGCC; R: TTGTTGTCACGCTCCTGGTC (276 bp) under the following reaction conditions: initial denaturation in 30 seconds at 96 °C, followed by 40 cycles of 20 seconds at 95 °C, 20 seconds at 60 °C, and 30 seconds at 72 °C. The 2-DDCt formula was used for the measurement of the relative quantification of the gene expression levels.

*Statistical Analysis*

The results were analyzed using SPSS software version 16 (SPSS Inc., Chicago IL, USA). Descriptive data were composed of the frequency distribution central indexes, distribution, and percentages. Continuous quantitative data were compared between the different groups using the ANOVA and Tukey's post hoc test, and correlation was used to compare the discrete data between the groups. A P-value lower than 0.05 was considered as statistically significant.

## Results

The results of this study showed that the mean relative expression of *NFκB* gene in the control group was 1.104±0.25. However, the mean relative expression in the ischemic, DMSO, and Jujuboside A groups were 1.141±0.08, 1.025±0.006, and 1.012±0.04, respectively. Also, the expression of *NFκB* gene in the Jujuboside A group was lower than in the other groups. However, there was no significant difference between the groups in terms of relative expression of *NFκB* gene (P>0.05, Figure-1).

Regarding Figure-2, the expression of *BDNF* in control, ischemic, DMSO, and Jujuboside A groups were 0.724±0.01, 0.734±0.011,

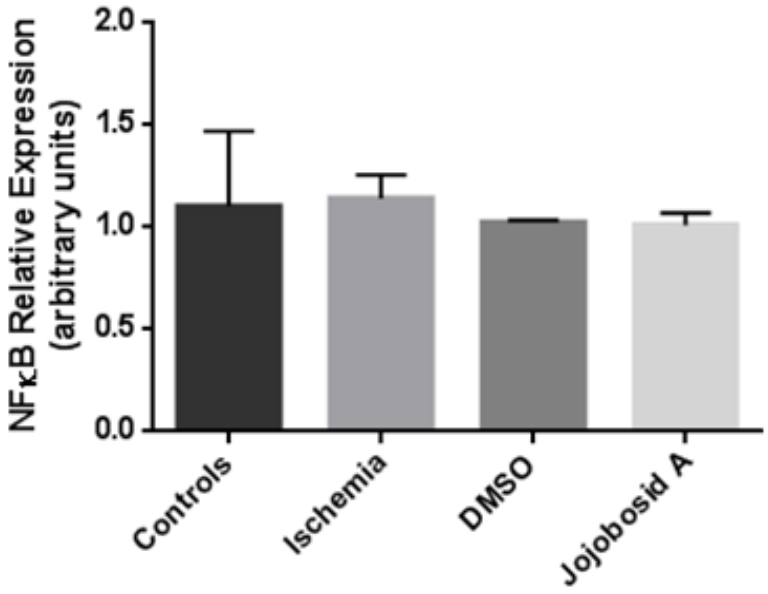


**Figure 1.** The mean relative expression of *NFκB* gene in control, ischemic, vehicle, and Jujuboside A groups.

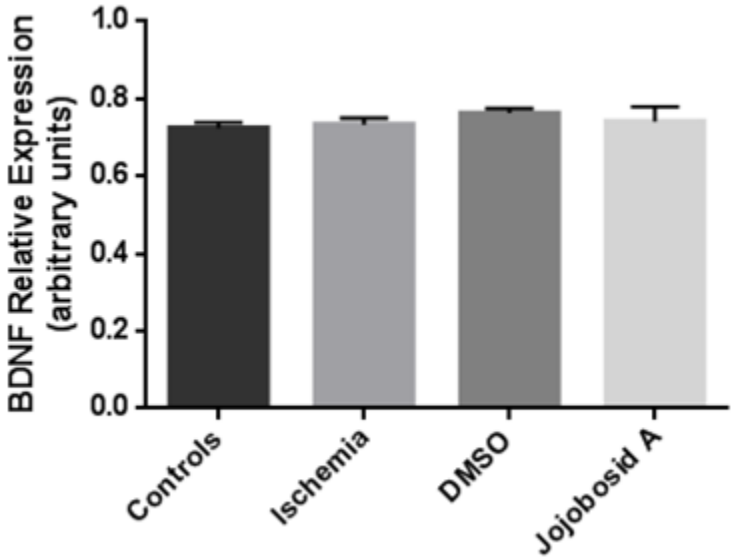


**Figure 2.** The mean relative expression of the *BDNF* gene in control, ischemic, DMSO (vehicle), and Jujuboside A groups.

0.762±0.008, and 0.74±0.02, respectively. The results of statistical analysis showed no significant difference in the expression of the *BDNF* gene between the studied groups (P>0.05, Figure-2).

Also, the expression of *NGF* in control, ischemic, DMSO, and Jujuboside A groups were 0.575±0.035, 0.535±0.003, 0.69±0.036, and 0.699±0.026, respectively (Figure-3). The ANOVA test showed a significant difference between DMSO, Jujuboside A, and ischemic groups in terms of the expression of *NGF* (P<0.05); the gene expression in DMSO and Jujuboside A groups increased significantly compared with ischemic group (Figure-3).

## Discussion

This study showed that Jujuboside A injection before cerebral I/R caused a

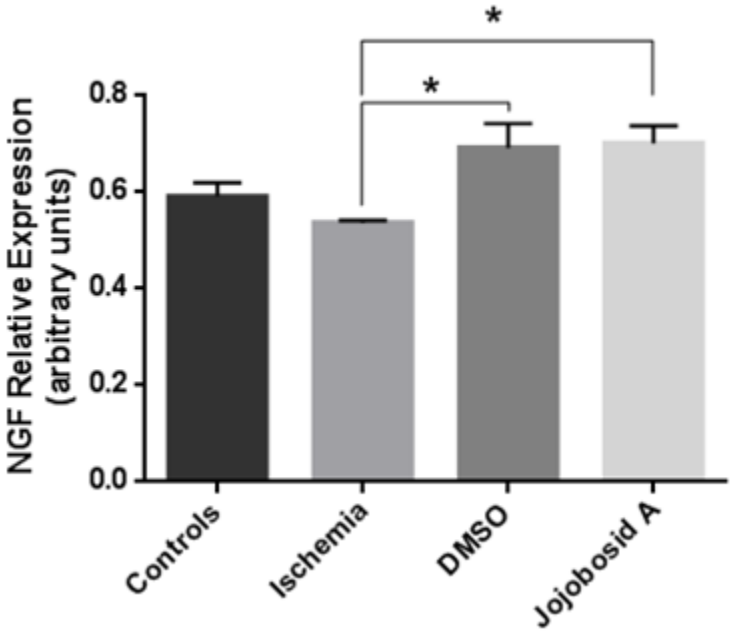


**Figure 3.** The mean relative expression of the *NGF* gene in control, ischemic, DMSO, and Jujuboside A groups. * P<0.05 vs. ischemia group

protective effect on the hippocampus by increasing the expression of some of the neuroprotective genes. Studies have shown that transient global I/R delays the death of nerve cells in sensitive regions of the central nervous system, such as the hippocampal CA1 region [17, 18]. Oxidative stress is a major challenge in ischemia caused by a lack of blood supply to the organs and ultimately leads to the production of free radicals such as ROS and RNA [22, 23]. In recent years, much attention has been paid to the role of antioxidants in preventing the complications of brain damage after stroke [14, 15]. In addition, identifying factors that change the expression of protective genes and cellular growth can be very useful. *NF-κB, NGF*, and *BDNF* are among the genes that are highly involved in the cell proliferation pathway and the growth of nerve cells. These genes effectively control the transcription of DNA, cytokine production, proliferation, and survival of nerve cells (neurons) [24]. In addition, these genes promote the development of the neural network [24, 25]. The role of Jujuboside A as a strong antioxidant has been mentioned in some studies [5, 6, 17, 18].

The current study showed that NGF expression in the hippocampus after transient global I/R was significantly higher in the Jujuboside A group compared with the ischemic group. However, the expression of this gene in the control group was more than that of the ischemic group. In other words, 20 minutes of cerebral I/R reduced the expression of *NGF* from the cells of the hippocampal CA1 region, which was significantly increased by the Jujuboside A injection. This suggests that Jujuboside A injection can decrease degenerated neurons (unpublished data) and increase the expression of the genes to repair the cells. In addition, the level of *BDNF* expression in the treatment group increased compared to the ischemic group, which could be effective in improving the ischemic changes.

In a study by Han *et al*. [18] that investigated the protective effects and potential mechanism of Jujuboside A on isoproterenol-induced cardiomyocytes injury, it was concluded that Jujuboside A pretreatment could increase cell viability and improve injury in H9C2 cells induced by Isoproterenol. Moreover, they showed that Jujuboside A could sharpen the mTOR, Akt, and PI3K phosphorylation [18]. Therefore, they indicated that Jujuboside A could reduce cellular damage by phosphorylation of PI3K, Akt, and

mTOR [18].

Wang *et al.* [26] showed that Jujuboside A has a suppressive effect on the involuntary actions of the mouse through the Rpgrip1 (retinitis pigmentosa transcription factor regulated GTPase regulator interacting protein1) and Mark3 (MAP/microtubule affinity-regulating kinase3) in the hippocampus.

The *NGF* is mostly preoccupied with the growth, preservation, proliferation, and permanence of nervous cells. In fact, the *NGF* is decretory for the permanence and preservation of sympathetic and sensory nerve cells, as they shift to programmed cell death in its lack [27]. However, some evidancs propose that the NGF also regulates the cycle cell of nervous cells by involved pathways [28]. The NGF activity is due to two types of receptors that are related to the neurodegenerative disturbance; the low-affinity NGF receptor (LNGFR/p75NTR) and tropomyosin receptor kinase A (TrkA) [29]. Accordingly, NGF via some pathways could stimulate intracellular factors that increase the survival of NGF-mediated nerve cells. Although in the lack of NGF, the expression of the apoptotic protein is enhanced, stimulation of c-Jun (the transcription factors of apoptosis) is done when all the NGF-mediated pathways are blocked [30-32].

Furthermore, NGF can lead to the expression of some genes by binding to the TrkA receptor, such as bcl-2, which stimulates the survival and proliferation of the neuron cell [30, 31, 33].

High-affinity interactions between pro-NGF, p75NTR, and sortilin may lead to either survival or apoptosis [34]. Also, *NF-κB* controls nuclear gene transcription to reinforce cell survival [24, 25]. Otherwise, apoptosis occurs when both NRIF and TRAF6 are engaged in c-Jun N-terminal kinase activation, which c-Jun phosphorylates. The operated transcription factor c-Jun reconciles nuclear transcription via AP-1 to raise pro-apoptotic gene expression [35, 36].

Therefore, it seems that due to its effect on the signaling pathway resulting from the expression of the *NGF* gene, Jujuboside A has a neuroprotective effect on the hippocampus after transient global I/R and can survive damaged neurons.

## Conclusion

The results of this study show that Jujuboside A can increase the expression of *NGF* by repairing the hippocampus after transient global I/R. In other words, Jujuboside A can prevent negative changes in the hippocampus via increased *BDNF* expression.

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## Conflict of Interest

The author(s) indicated no conflicts of interest.

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