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Research Paper

Effects of *Nigella sativa* Nano-hydroalcoholic Extract on Neuronal Damages in the Hippocampus of Male Rats After Cerebral Ischemia/Reperfusion

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ABSTRACT

Introduction: Cerebral ischemia is one of the leading causes of global mortality and disability in many countries. Damage caused by reperfusion is due to the inflammatory function of the injured tissue. Ischemia-reperfusion causes the formation of oxygen free radicals and other oxidants. The CA1 region of the hippocampus is one of the highly sensitive parts of the brain in ischemia and hypoxia. The *Nigella sativa* plant, with its antioxidant properties, can remove free radicals and cell-damaging compounds to prevent cell death and damage to viable cells. To determine the effects of hydroalcoholic extract and nano-hydroalcoholic extract containing *N. sativa* on the CA1 region of the hippocampus in male Wistar rats following transient global ischemia/reperfusion.

Methods: Four groups of 32 male Wistar rats were randomly formed: Control, ischemia, hydroalcoholic extract of black recipient seed, and nano-extract of hydroalcoholic black seed recipient. Ligation of bilateral common carotid arteries induced an ischemia model. Following the behavioral test, brain removal was completed and prepared for Nissl staining and stereological evaluations, along with the expression levels of *Bax* and *Bcl2*, using the real-time PCR technique.

Results: A considerable rise in the number of viable pyramidal cells was observed in the hydroalcoholic extract and nano-hydroalcoholic extract groups compared to the ischemia group. *Bax* expression was elevated, and the expression of *Bcl-2* declined after transient global I/R in the CA1 region. The effects of transient global ischemia/reperfusion (I/R) on gene expression were reversed by the injection of hydroalcoholic and nano-hydroalcoholic extracts containing *N. Sativa*.

Conclusion: Transient global I/R remarkably promotes cell death and morphological changes. It appears that the hydroalcoholic extract of *N. sativa*, particularly the nano-hydroalcoholic extract, at a dose of 400 mg/kg, may be a suitable solution for treating ischemia-induced brain damage.

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Highlights

- *N. sativa* extracts reduced hippocampal cell death after brain ischemia.
- Nano-hydroalcoholic extract of *N. sativa* showed stronger neuroprotective effects than its hydroalcoholic extract.
- *N. sativa* extracts reversed the decreased *BAX/BCL-2* gene expression caused by ischemia.
- Memory performance in male rats improved with *N. sativa* extracts.

Plain Language Summary

When the brain temporarily loses blood supply, which can happen during cardiac arrest or surgery, it may suffer from a condition called ischemia/reperfusion injury. This condition produces harmful molecules called free radicals, leading to brain cell damage and memory problems. The hippocampus, an important brain area for learning and memory, is vulnerable to ischemia/reperfusion injury. In this study, we tested whether *Nigella sativa* extract, also known as black seed, can protect the brain from brain ischemia. Two forms of extracts were used: Hydroalcoholic and nano-hydroalcoholic. These extracted were given to male Wistar rats after inducing brain ischemia/reperfusion by blocking blood flow to their carotid arteries. The results showed that both types of *N. sativa* extract reduced cell death in the hippocampus and improved memory function compared to untreated rats. The nano-hydroalcoholic extract had a stronger protective effect. At the molecular level, *N. sativa* extracts helped balance the activity of two key genes, *BAX* and *BCL-2*, which control whether brain cells die or survive. These findings suggest that *N. sativa*, particularly its nano-hydroalcoholic extract, may be a promising natural treatment for preventing brain damage caused by brain ischemia/reperfusion. While this research was done in rats, it provides a scientific basis for further studies that could one day help protect human brain health in conditions such as stroke, cardiac arrest, or brain surgery.

1. Introduction

Transient global ischemia in humans occurs due to cardiac arrest or cardiac surgery, which causes selective and delayed nerve death, especially pyramidal neurons in the hippocampus (Tanaka et al., 2000). The key structure of the memory system is the hippocampus, which is used to study phenomena related to ischemia. However, specific hippocampal subsets illustrate different sensitivities to ischemia/hypoxia. The most sensitive area of the hippocampus is CA1. Even if it is exposed to a short-term ischemic attack, the mechanisms of delayed neuronal death leading to a significant reduction of nerve cells occur in this structure (Jiao et al., 2011). It has been reported that CA2-3 segments are relatively resistant to ischemia. Long-term ischemia due to ischemic recurrence causes changes in CA2-3 neurons, but the reduction of neurons, which is evident in this part, is rarely shown (Kirino & Sano, 1984). Nerve death due to global ischemia occurs up to 2-4 days after ischemia in rats, and the fetus is not recognizable. The role of apoptotic death and necrosis in ischemia-induced nerve cell death remains controversial (Yamashima & Oikawa, 2009; Graham & Chen, 2001).

However, the underlying mechanisms of ischemic death are still unclear. The molecular events that determine the fate of neurons could be investigated due to the significant delay between attack and the onset of death. It has been shown that extracts of herbs preserve protection against ischemic damage to various organs such as the kidneys, heart, and brain (Peng et al., 2003). Apoptosis, or programmed cell death, plays a critical role in neurodegenerative conditions and ischemia-induced brain damage. Among the key regulators of apoptosis are the BCL-2 family proteins, which include both pro-apoptotic and anti-apoptotic members. *BAX* (Bcl-2-associated X protein) promotes apoptosis by facilitating mitochondrial outer membrane permeabilization, whereas *BCL-2* (B-cell lymphoma 2) inhibits this process and promotes cell survival. The ratio of *BAX* to *BCL-2* is often used as an indicator of a cell's susceptibility to apoptosis. Changes in the expression of *BAX* and *BCL-2* genes can indicate whether a treatment exerts pro-survival or pro-apoptotic effects on neurons.

Therefore, assessing these markers helps evaluate the neuroprotective potential of experimental treatments (Antonsson & Martinou, 2000). In the past few years, scientists have worked to identify the active compounds in herbal medications and to understand how they work (Meddah et al., 2009). Black seed, or *Nigella sativa*, a member of the Ranunculaceae family, is one of these herbal treatments that is used for a broad range of diseases, including bronchial asthma, headache, allergies, gastrointestinal problems, hypertension, obesity, and various types of cancer. *N. sativa* seeds can also help reduce depression and have immunostimulatory effects on various inflammatory and immunologic diseases, including arthritis, colitis, and experimental allergic encephalomyelitis, as well as in sensitized animals, asthma patients, and chemical warfare victims, due to its documented component (Kaplan & Miller, 2000). Recently, clinical and animal studies on therapeutic drugs of black seed extracts have revealed that these drugs include a variety of effects such as anti inflammation (Hajhashemi et al., 2004) and modulation of the immune system (Tekeoglu., 2007), antioxidant activities (Kanter et al., 2006), and protection from light (Kanter et al., 2006). Recently, considerable attention has been focused on plants containing natural products and agents that can mitigate the damage caused by free radicals. The current research aims to explore the effect of hydroalcoholic and nano-hydroalcoholic extracts containing *N. sativa* on neurons in the CA1 area of the hippocampus in an ischemia model of the Wistar rats.

2. Materials and Methods

Study animals

Test subjects were 32 male Wistar rats, aged 8 weeks and weighing 200-250 g, which were accommodated under standard conditions (23±2 ˚C, 12/12 h light–dark cycle, relative humidity of 50±6%). They were provided with food and water in suitable amounts. All employed experimental procedures, such as caring and handling of the animals, were executed in compliance with the Tehran Medical Sciences Branch, Islamic Azad University Ethics Committee acts.

Study design

The test subjects were haphazardly divided into four groups of 8.

In the control group, the rats were not given any treatment or surgery.

In the ischemia group, after anesthesia, the carotid arteries of the rats were clamped for 20 minutes, followed by reperfusion.

In the experimental group 1, after ischemia and reperfusion for 20 minutes, the intraperitoneal injection of hydroalcoholic extract of black seed at a dose of 400 mg/kg was performed for 14 days straight.

In the experimental group 2, after ischemia and reperfusion for 20 minutes, the intraperitoneal injection of nano-hydroalcoholic extract of black seed at a dose of 400 mg/kg was performed for 14 consecutive days.

Twenty-four hours after the last injection, all animals underwent evaluation using the Y-maze behavioral test. Subsequently, the rat brains were removed for histological studies and gene evaluation.

Surgical process

After anesthesia by injection of pentobarbital sodium (40 mg/kg, IP), a vertical incision was made in the anterior region of the animal's neck, and by pushing the sternocleidomastoid muscle, the common carotid arteries were exposed on both sides. After detachment of the vagus nerve, a microsurgical clamp was applied for 20 minutes to close the arteries. Then the clamps were removed and circulation was restored. During the surgery, the animal's temperature was measured regularly using a thermometer, and a heat lamp stabilized the temperature at 37±0.5 ˚C. The cut was sutured with 04 silicon yarn. All the animals were monitored in separate cages until their conditions were stabilized (Dellu., 1992).

Y-maze behavioral test

A Y-maze apparatus was used to test short-term spatial memory. In simple words, a Y-maze is made up of three arms that cross each other at a 120˚ angle. It is preferred that test respondents examine the fresh arms with a higher frequency than a previously inspected arm. It was thought that revisiting a previously researched arm was a mistake. A lower tendency to explore the most recently visited arm indicated a better memory performance. The occurrence of entries into all three different arms (A, B, and C) in sequence was considered a valid alternation, reflecting genuine memory-based exploration. The total number of entries was recorded (Longa et al., 1989).

Preparation method of the hydroalcoholic extract of *N. sativa*

The alcoholic extraction of *N. sativa* was carried out using the maceration method, where the crushed grain was poured into the decanter's funnel. Each time, 250 g of *Nigella* powder with 60% alcohol was added as the solvent. Then, the solution was placed at room temperature for 8-10 hours. Afterwards, the valve of the decanter was opened and the solution was passed through the filter twice. After drying the extract, it was measured, and 32% w/w extract was obtained (Tavakkoli et al., 2017).

Preparation method of nano-hydroalcoholic extract of *N. sativa*

First, the hydroalcoholic extract was poured into a container. Then, the alcohol was removed, and the extract was dried. After that, 10 g of the dried extract was dissolved in 100 mL of distilled water. The acquired hydroalcoholic extract was put into the ultrasonic probe for 1 h. Then,

The dynamic light scattering (DLS) was used to evaluate the nanoemulsion containing *N. sativa*. DLS is a physical method that is applied to actuate the distribution of particles in solutions and suspensions. Hydrodynamic diameter was used to measure the zeta potential, which serves as a marker to investigate the surface charge of the nanoemulsion. Additionally, transmission electron microscopy (TEM) was employed to examine the shape and size of the nanoemulsion (Tavakkoli et al., 2017).

Real-time PCR

The entire cellular RNA was applied to prepare complementary DNA (cDNA) and used in the measurement of *BCL-2* and *BAX* mRNA expression. Pars Tous Kit (Iran) was utilized to extract total RNA. The method and kit, as instructed by the manufacturer of Yekta Tajhiz Azma Kit (Iran), were used for cDNA preparation. Eventually, the cDNA was maintained at -20 ˚C. The housekeeping gene *GADPH* was used for normalization of target gene expression. The applied primes are shown in Table 1.

First, hippocampus samples were prepared, and then the purification of extracted RNA was performed. The high-quality RNAs were kept at -80 ˚C until they were used for cDNA synthesis. One milligram of RNA was converted to cDNA using the quantitect reverse transcription kit (Qiagen). To carry out real-time PCR, the primers were designed and underwent a large-scale search using the BLAST tool. Real-time PCR was conducted by executing the following cycling conditions: 95 ˚C for 10 min, and 40 cycles at 95 ˚C for 15 s, and 60 ˚C for 1 min. Every single complete amplification stage subsequently had a dissociation stage: At 95 for 15 s, 60 for 30 s, afterwards the temperature was boosted from 60 to 95 (at the rate of 0.03/s). Melting curve analysis was accomplished according to the dissociation stage data and reactions.

Cavalieri's volumetric analysis

Cavalieri's principle was used to assess the volume of the CA1 region. It is essential to ensure that all sections of the object of interest are parallel. The distance between them is known, and the first coronal section indiscriminately hits the object of interest. To estimate the cross-sections, coronal brain sections were stained with hematoxylin and eosin (H&E).

The total volume (V, mm^3) of the CA1 region was calculated by Cavalier's method, where V=ΣP (total number of the volume profiles counted in each rat's hippocampus) × a/p (the area associated with each point) × t (the distance between the sampled sections).

Table 1. The primer sequences

Gene		Prime Sequence (5'-3')
BAX	Forward primer	GCAAACTGGTGCTCAAGG
	Reverse primer	CAGCCACAAAGATGGTCA
BCL2	Forward primer	GAGTGGGATACTGGAGATGA
	Reverse primer	TGGTAGCGACGAGAGAAGTC
GAPDH	Forward primer	AGGTCGGTGTGAACGGATTTG
	Reverse primer	TGTAGACCATGTAGTTGAGGT

Nissl staining

Sections of 10 micron thickness were air-dried after being placed directly onto gelatin-coated glass slides. The slides were dehydrated and cover-slipped with Entellan after being dyed with 1.0% cresyl violet. Each animal had eight photomicrographs taken (between the level of 2.3 and 5 mm posterior to bregma, according to the Paxinos atlas). A blinded investigator randomly picked three of them with a minimum distance of 40 microns and counted them using a light microscope at 400x magnification. Only pyramidal cells with visible nuclei and nucleoli were included in the study. Images were collected with a microscope (Olympus AX-70, Japan) at 400x magnification and analyzed with Image-Pro-Plus software, version 6.0 (LEICA DMLB, Germany).

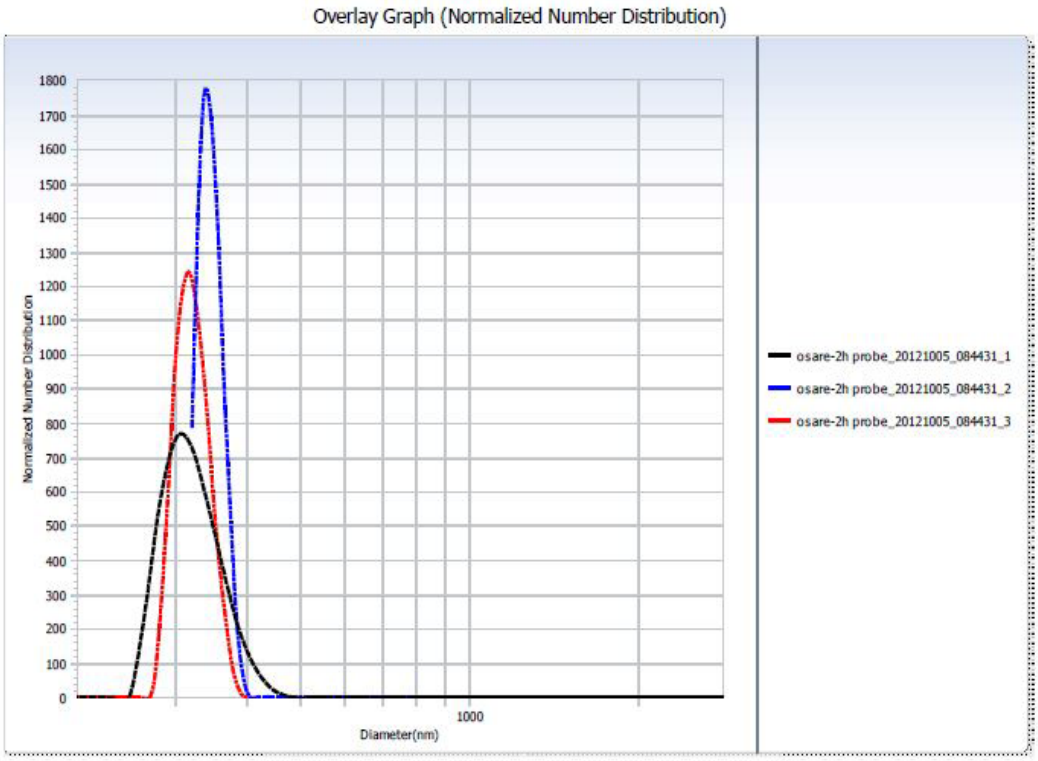
Statistical analysis

Data are reported as Mean±SD. Analysis of variance (ANOVA) and least significant difference (LSD) methods are conducted for the comparison of different groups. In addition, a $P<0.05$ was identified as significant.

3. Results

Investigation of particle size of nanoemulsions

The shape of the graphs is a single peak, and the particle size distribution is narrow, indicating that the particles are small and uniform. The particle size is between 300 and 350 nanometers. Another device was used to ensure the data was accurate. The results of the sample size of black seed hydroalcoholic nano-extract are shown in Figure 1.



No	Data	Repet. No	pH	Ave.Diameter(nm)	PD	Mean.(nm)	D (10%) (nm)	D (50%) (nm)	D (90%) (nm)
1	osare-2h probe_20121005_084431_1	1	NA	382.5	0.171	350.5	266.6	304.9	360.9
2	osare-2h probe_20121005_084431_2	2	NA	1877.4	-94.420	350.5	328.5	339.1	364.0
3	osare-2h probe_20121005_084431_3	3	NA	1188.8	-35.367	329.7	288.2	313.9	346.4
Average :				1149.6	-43.205	343.6	294.4	319.3	357.1

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Figure 1. The DLS of nano-hydroalcoholic extract of the *N. sativa* sample

*Significance ($P<0.05$).

DLS analysis

After the *Nigella* hydroalcoholic sample was prepared, DLS was employed to analyze particle size and distribution, and particle dispersion was examined using diagrams. The dispersion was between 294.4 and 357.1 nm, and a single peak diagram was obtained. The diameter of this diagram increases intensively when the size of particles exceeds 90 nm. If there are smaller particles with lower concentrations in the suspensions, they sometimes fade from the sensors, resulting in no intensity being reported. Following the stability test in 4 different environments, with one test conducted after one month and another after three months, the results remained consistent with the initial findings.

Investigation of particle size by zeta potential

The results of the zeta potential of *N. sativa* nano-hydroalcoholic extract are as follows (Figures 2 and 3).

Zeta potential analysis

Most fluids contain cations and anions, which are atoms with positive and negative charges. When the loaded particles are suspended in a liquid, the opposing load ions are absorbed into the particulate matter. It means that samples with negative loads attract positive ions from the fluid, and conversely, samples with a positive charge attract negative ions from the fluid. The ions near the surface of the particle are highly absorbed while the farther ions will have a loose bond, called the intrusion layer. Zeta potential ranges from -10 to +10, indicating the stability of the sample (Figure 4).

Behavioral test results

The results of the Y-maze test revealed that ischemia remarkably decreased the kinetic ability of test subjects. The aggregate of suitable feedback in the I/R group considerably declined in comparison with the control group. Furthermore, the longer the time of ischemia occurred, the worse the viciousness of neurological function was. Compared with the I/R group, the nano-hydroalcoholic extract of black seed recipient group showed significantly increased memory functions after I/R injury (Figures 4 and 5).

Results of the weight of rats in different groups

As a result of measuring the weight of rats before and after induction of ischemia and drug injection, it was observed that after surgery and during 14 days, when the experimental groups 1 and 2 were receiving the drug, one-way ANOVA test and LSD test showed a significant difference between ischemia group and experimental groups 1 and 2 (Figure 6).

Results of real-time PCR

The relative expression of *BAX* in the hippocampus of the I/R group was considerably higher than that of the control group, whereas the relative expression of *BCL-2* mRNA was substantially lower. (Figures 7 and 8). As shown in Figures 7 and 8, the treatment of rats with hydroalcoholic extract of black seed and nano-extract of hydroalcoholic black seed effectively decreased the relative expression of pro-apoptotic mRNAs *BAX* (Figure 7, $P<0.05$). It increased the relative expression of *Bcl-2* in these groups (Figure 8, $P<0.05$).

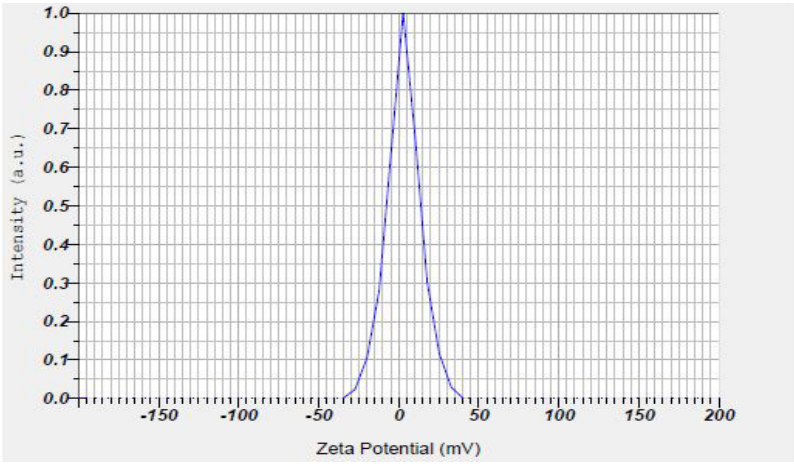

Figure 2. Zeta potential of *N. sativa* nano-hydroalcoholic

*Significance ($P<0.05$).

13990828-Abdolrahimkhan-Sample1-Zeta-Scan3.nzt

Measurement Results

Date	:	Wednesday, November 18, 2020 4:24:31 PM
Measurement Type	:	Zeta Potential
Sample Name	:	13990828-Abdolrahimkhan-Sample1-Zeta
Temperature of the Holder	:	25.1 °C
Dispersion Medium Viscosity	:	0.893 mPa·s
Conductivity	:	0.401 mS/cm
Electrode Voltage	:	3.3 V

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	3.1 mV	0.000024 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean)	:	3.1 mV
Electrophoretic Mobility Mean	:	0.000024 cm^2/Vs

Figure 3. Zeta potential of *N. sativa* nano-hydroalcoholic results

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Nissl staining results

Nissl staining was used to determine viable pyramidal cells in the CA1 area. Cells with round, bright nuclei with an euchromatin appearance and multiple nuclei were considered as viable cells. Cells with dense, multifaceted, and heterochromatin nuclei were considered degenerative cells. The results of ANOVA showed that transient global I/R significantly decreased the number of pyramidal neurons ($P<0.001$). While the injection of hydroalcoholic extract and nano-hydroalcoholic extract containing *N. sativa* reversed the effect of transient global I/R on the number of neurons ($P<0.05$) (Figures 9 and 10).

Most of the pyramidal cells were healthy. Note that a denotes healthy pyramidal cells, and b refers to degenerated pyramidal cells.

Results of stereological evaluation of the volume of the CA1 region of the hippocampus

The results of calculating the volume of the hippocampus CA1 region of 6 histological slides with H&E staining for each rat and a total of 6 rats in each group were observed. The volume in the ischemia group decreased significantly compared to the control group. Furthermore, the volume of the hippocampus CA1 area by injection of hydroalcoholic extract increased significantly compared to the injection of *N. sativa* nano-hydroalcoholic extract (Figure 11). H&E staining were used to measure the volume of the CA1 region (Figure 12).

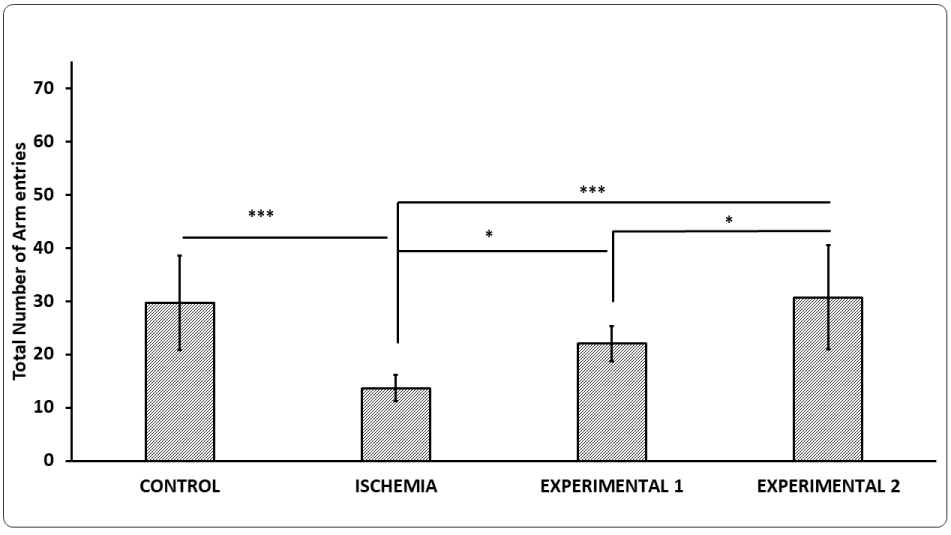

Figure 4. Comparing the total number of arms rats entered into in different groups

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*Significance ($P<0.05$).

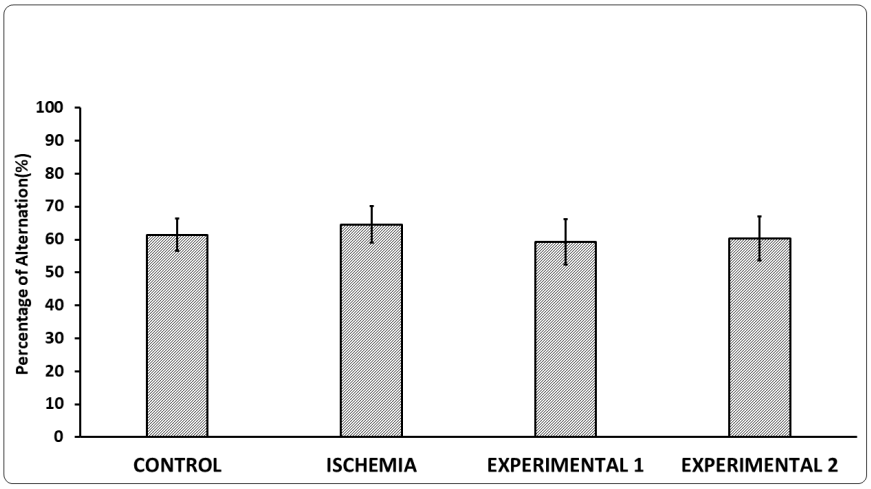

Figure 5. Comparing the spontaneous interval behavior of rats in different groups

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4. Discussion

Our findings showed that cerebral ischemia/reperfusion for 20 minutes caused delayed death of pyramidal cells in the CA1 region.

Ischemia-reperfusion injury is a pathological condition that has both local and systemic effects. The key element during ischemia is cell energy depletion, and the interplay of oxidative and microcirculatory stress, as well as inflammation and apoptosis, takes place during reperfusion (Soares et al., 2019).

Following ischemia, hippocampus may be affected by programmed death of cells. Oxidative stress caused by reactive oxygen species (ROS) production contributes to the programmed death of cells (Itō, 1984). Increased ROS levels inside the cell can lead to DNA and intracellular protein damage through oxidation, resulting in cell death.

In response to global cerebral ischemia, CA1 neurons in the hippocampus are particularly susceptible and experience selective, delayed degeneration. These pyramidal neurons play a crucial role in spatial learning and memory, and their degeneration causes learning and memory problems (Xu et al., 2021; Zuo et al., 2015).

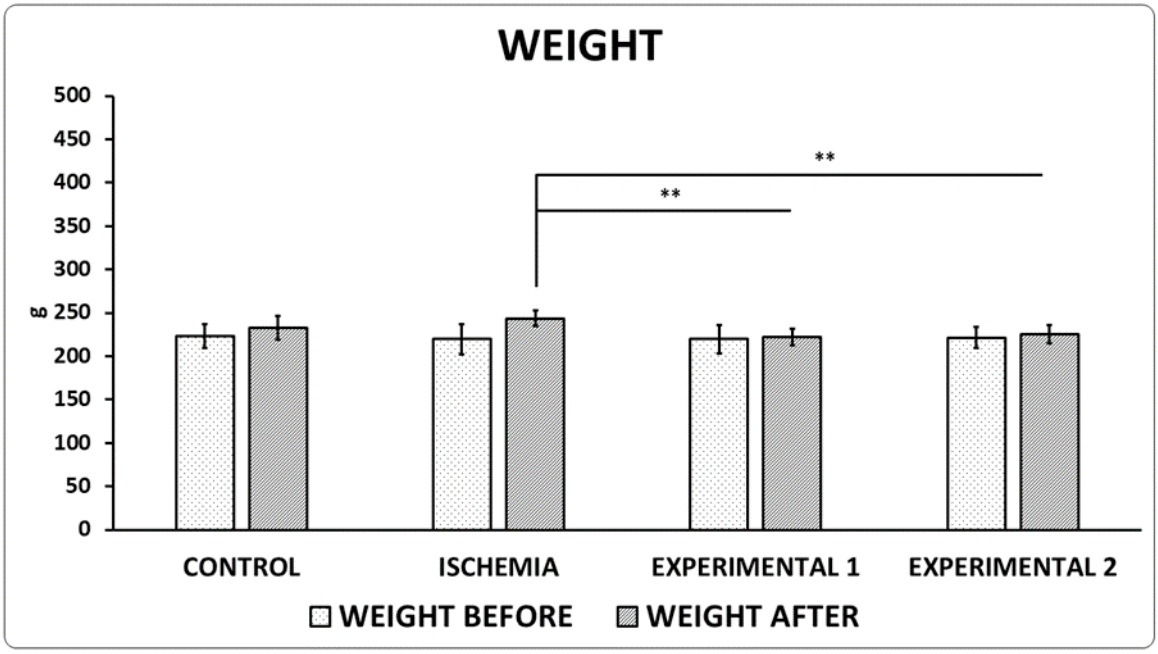

Figure 6. Comparing the weight of rats at the beginning and end of the study in different groups

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*Significance (P<0.05).

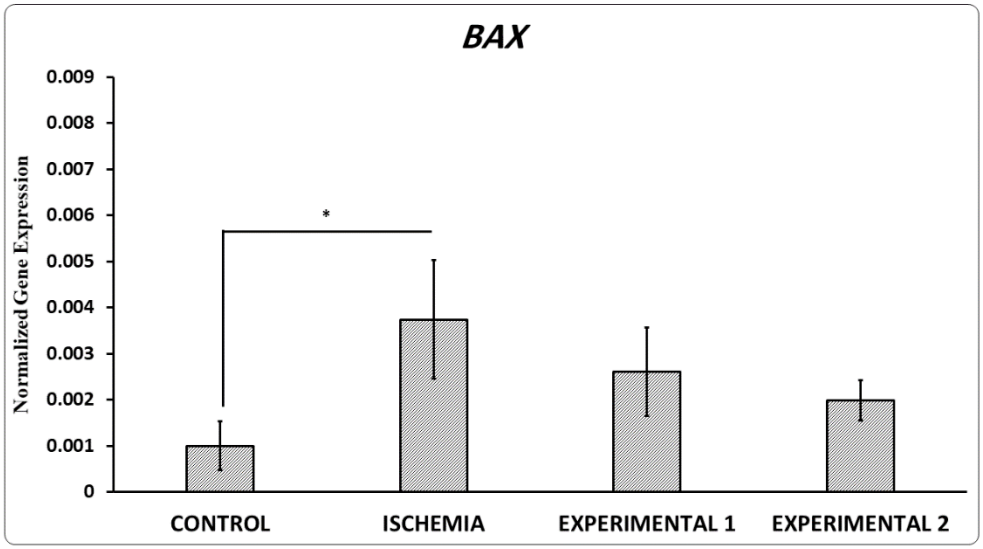

Figure 7. Comparing *BAX* gene expression in different groups

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*Significance (P<0.05).

Antiinflammatory and antioxidant medications should be investigated to provide additional treatment options that can help prevent or reduce these injuries, as current drugs have limited efficacy and offer little to no benefit to patients (Mucha et al., 2021). Antioxidants are compounds that can significantly slow down the oxidative reaction (Molitoris & Wagner, 1996). So far, many natural and synthetic antioxidants have been introduced for the treatment or prevention of patients associated with free radicals (Farahmand & Tajali, 2016). Medicinal plants always have an instrumental role in the management of global health care and are considered the best treatment for diseases (Dehpour et al., 2011). *N. sativa* is a medicinal plant that is highly consumed. In recent years, further attention has been paid to the medicinal and biological properties of *N. sativa*.

One of *N. sativa*'s possible qualities is the ability of one or more of its components to minimize tissue damage following ischemic/reperfusion injury due to antioxidant activity (Bayrak et al., 2008; Erşahin et al., 2011).

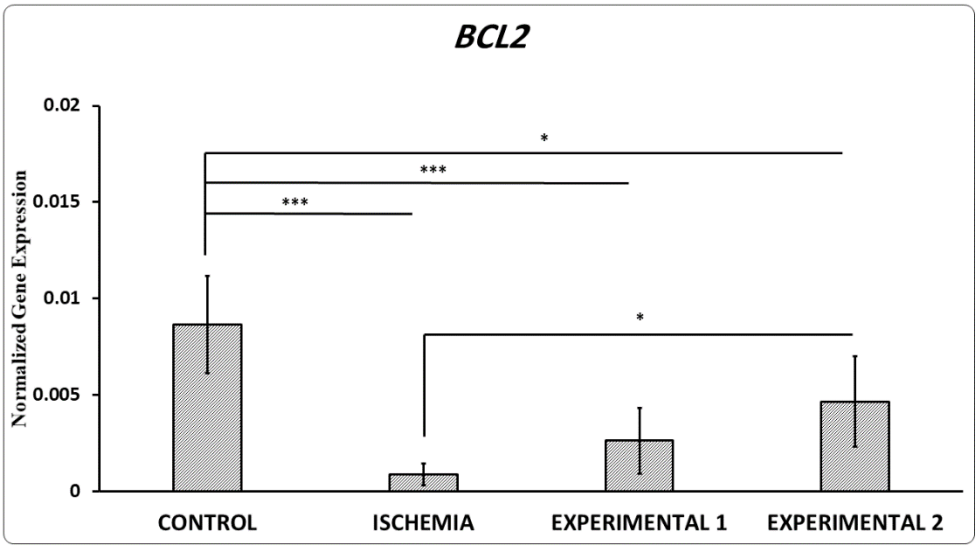

Figure 8. Comparing *BCL2* gene expression in different groups

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*Significance (P<0.05).

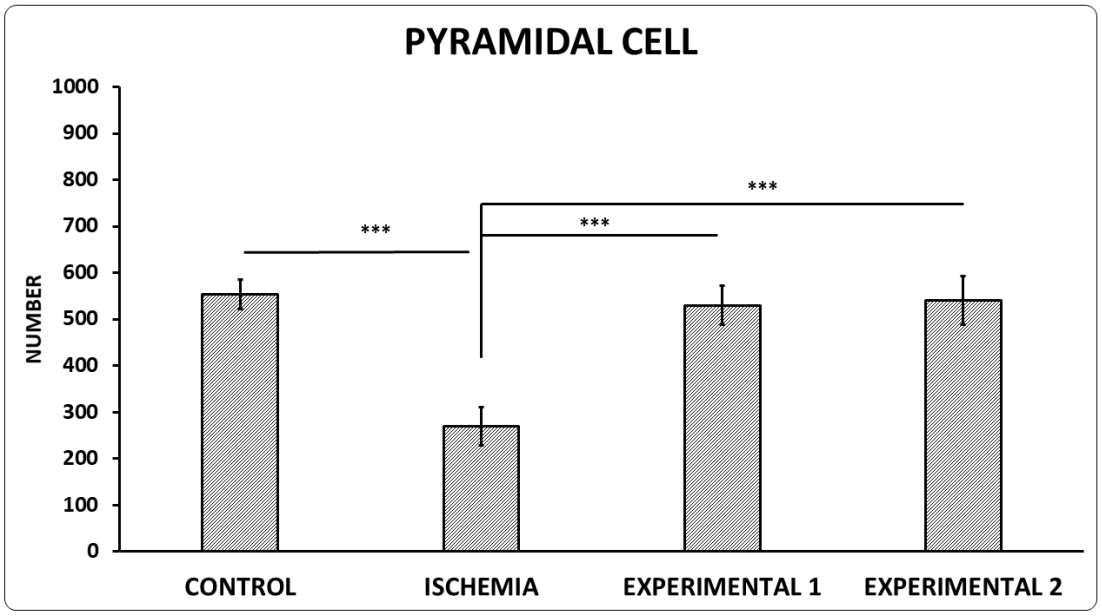

Figure 9. Comparing the average number of pyramidal cells in the CA1 region in different groups

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*Significance (P<0.05).

It has been reported that administration of antioxidant agents before local brain ischemia in laboratory studies protects against ischemic brain lesion (Lipton., 1999). Based on a review of the literature, thymoquinone, which is the most important extract of *N. sativa*, has many pharmacological properties, mostly related to its antioxidant properties. In this regard, Hosseinzadeh et al. (2007) reported that the administration of *Nigella* oils in the global ischemic model of the brain has reduced the amount of lipid peroxidation. Their findings can support the role of antioxidant properties in the protective effects of *N. sativa* in cerebral ischemia. Ziyyat et al. (1997) in-

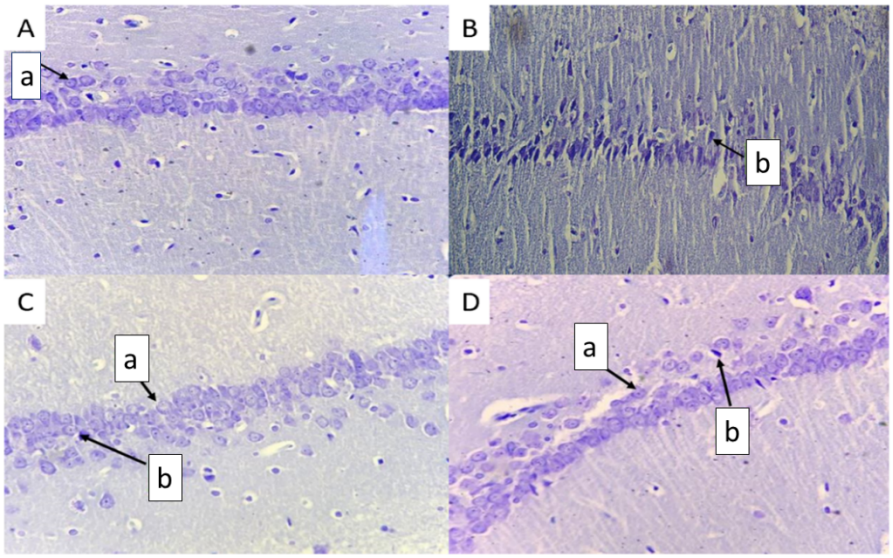

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Figure 10. Photomicrograph Nissel staining of the pyramidal cells of the CA1 region of the hippocampus in different groups (magnification of ×400)

A) Control group, B) Ischemia group, C) Hydroalcoholic extract group with a dose of 400 mg, D) Hydroalcoholic nano-extract group with a dose of 400 mg

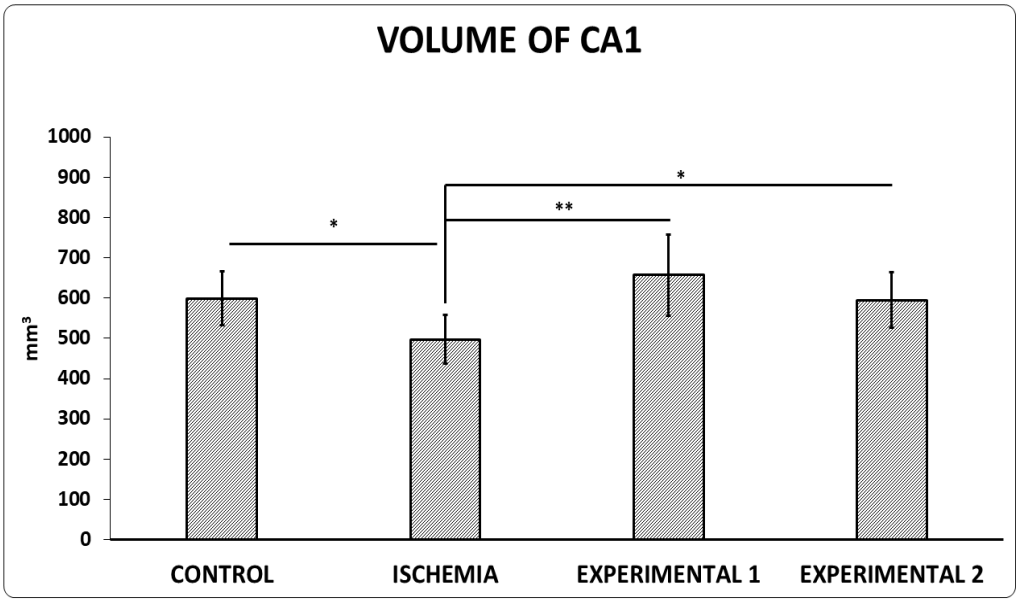

Figure 11. Comparing CA1 area volume in different groups

*Significance (P<0.05).

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vestigated the neuroprotective effects of thymoquinone on transient cerebral ischemia on the hippocampus in rats. Thymoquinone reduces malondialdehyde levels, increases reduced glutathione, catalase, and superoxide dismutase activity until it reaches its normal level. Thymoquinone is a promising factor in healing the damages caused by the destruction of neural cells (neurodegeneration), such as cerebral ischemia. *N. sativa* also enhances the activity of antioxidant enzymes such as superoxide dismutase and glutathione peroxidase, and with its antioxidant properties, reduces lipid peroxidation in biological membranes (Butt & Sultan, 2010). It is known that thymoquinone and its active metabolite thymohydroquinone can prevent lipid peroxidation by sweeping superoxide, radical hydroxyl, and single molecular oxygen (Kanter, 2008). In addition, thymoquinone can inhibit arachidonic acid metabolism and restrict ischemic brain damage caused by inflammation of cyclooxygenase and lipoxygenase pathways (El Mezayen et al., 2006).

Nanoparticle-based drug delivery systems have demonstrated higher potential in treating ischemic stroke and potentially other neurological disorders. Joachim et al. showed that the gelatin of osteopontin nanoparticles in a rat ischemic stroke model augments nerve protection in the nose (Joachim et al., 2014). Nagai et al. showed that intravenous administration of silvestazole nanoparticles improves acute ischemic damage in the brain following

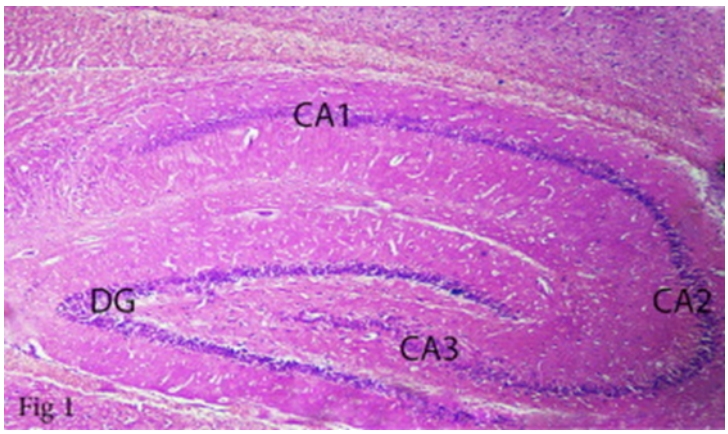

Figure 12. Different areas of the hippocampus of rats (H&E staining)

DG: Dentate gyrus; CA: Cornu ammonis.

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the ischemic/reperfusion (Nagai et al. 2015). According to the previous studies and the results of the current research concerning the function of *N. sativa* plant and its role in preventing the death of neural cells, it can be said that the hydroalcoholic extract and nano-hydroalcoholic extract of *N. sativa* can exert its protective effect on ischemia/reperfusion brain damages, which can be attributed to the properties of oxygen free radicalization of this plant. Our results showed a significant decrease in *BAX* expression and an increase in *BCL-2* expression following treatment with [compound/extract], suggesting a shift toward anti-apoptotic signaling. This *BAX/BCL-2* modulation indicates that the therapy may protect against ischemia-induced neuronal apoptosis by promoting cell survival pathways. Also, the *N. sativa* plant in nano form has more efficacy and higher adsorption than the hydroalcoholic extract. Still, since the role of other compounds in these plants, which has not been studied, and the role of other mechanisms, such as inhibition of inflammatory pathways, and also the possibility of a cumulative effect of these compounds, is not known, this issue needs further research and studies.

5. Conclusion

This research revealed that hydroalcoholic extract and nano-hydroalcoholic extract containing *N. sativa*, by preventing morphological changes and resulting cell death, have an effective role on neurons in the CA1 region of the hippocampus. Therefore, it seems that hydroalcoholic extract and nano-hydroalcoholic extract containing *N. sativa* through antiinflammatory and antioxidant mechanisms can be considered as a treatment candidate for ischemic brain damage.

Ethical Considerations

This study was approved by the Ethics Committee of Tehran Medical Sciences Branch, Islamic Azad University, Tehran, Iran (Code: IR.IAU.TMU.REC.1399.515). The ARRIVE ethical criteria were followed in all of the trials. During the research, the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 86-23) was also followed.

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Authors' contributions

Conceptualization, supervision, data analysis, interpretation, review and editing: Zahra Nadia Sharifi and Atarodalsadat Mostavafinia; Methodology and experimental design: Mahsa Golmohammadi and Atarodalsadat Mostavafinia; Data collection and laboratory work: Mahsa Golmohammadi and Mohammad Mahdi Nazarnejad; Original draft preparation: Mohammad Mahdi Nazarnejad; Final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

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