Title: Effects of Nigella Sativa Nano-Hydroalcoholic Extract on Neuronal Damages of Hippocampus Following Transient Global Ischemia / Reperfusion in Male Wistar Rats

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Abstract

Background: Cerebral ischemia is one of the leading causes of global mortality and the prominent cause of disability in many countries. Damage caused by reperfusion is due to the inflammatory function of the damaged tissue. Ischemia-reperfusion causes the formation of oxygen free radicals and other oxidants. The CA1 region of the hippocampus is one of highly sensitive parts of the brain against ischemia and hypoxia. The Nigella Sativa plant with its antioxidant properties can remove free radicals and Cell damage compounds in order to prevent damage to viable cells and cell death.

Purpose: To determine the effects of Hydroalcoholic Extract and Nano Hydroalcoholic Extract Containing Nigella Sativa on CA1 Region of Hippocampus in Male Wistar Rat 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 groups). Ligation of bilateral common carotid arteries induced Ischemia model. After behavioral test, brains removal was completed and prepared for Nissl staining and soteriological evaluations along with expression level of Bax and Bcl2, by real time PCR technique.

Results: considerable raise in the number of viable pyramidal cells was observed in Hydroalcoholic Extract and Nano Hydroalcoholic Extract groups compared to the ischemia group. Bax expression was elevated and the expression of Bcl-2 was declined subsequent to transient global I/R in the CA1 region. The effects of transient global I/R on genes expression was reversed by the injection of hydroalcoholic and nano-hydroalcoholic extract containing Nigella Sativa.

Conclusion: Transient global I/R remarkably promotes cell death and morphological changes.it seems that Hydroalcoholic Extract of Nigella sativa especially Nano Hydroalcoholic extract of Nigella sativa at a dose of 400 mg / kg, may be a suitable solution for the treatment of ischemia-induced brain damage.

Keywords: Hydroalcoholic extract, Hippocampus, Ischemia-reperfusion, Nigella Sativa, Nano-hydroalcoholic extract.

Introduction

Transient global ischemia among mankind occurs due to cardiac arrest or cardiac surgery, which causes selective and delayed nerve death, especially pyramidal neurons in the hippocampus area (1). The key structure of the memory system is 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's exposed to short-term ischemic attack, the mechanisms of delayed neuronal death leading to significant reduction of nerve cells occurs in this structure (2). 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, are rarely shown (3). Nerve death due to global ischemia 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 still remains controversial (4, 5). 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 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 (6, 7). 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 (8). In the past few years, scientists have worked to identify the active compounds in herbal medications and to understand how they work (9). Black seed, or Nigella sativa, a member of the Ranunculaceae family, is one of these herbal treatments which is used for a broad range of diseases, including bronchial asthma, headaches, allergies, gastrointestinal problems, hypertension, obesity, and various types of cancer. Nigella sativa seeds can also help to reduce depression and applies immunostimulatory effects to a variety of 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 (10). 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-inflammatory (11) and modulation of immune system (12) antioxidant activities (13) and protection from light (14). Lately a lot of attention has been paid to plants with natural products and agents that can limit the harm induced by free radicals. The current research aims to explore the effect of Hydroalcoholic and Nano Hydroalcoholic Extracts Containing Nigella Sativa on neurons in the CA1 area of hippocampus in ischemia model of wistar rat.

Method

Animals

Test subject were 32 male wistar rats with age of 8 weeks and weighed 200-250 g which were accommodated under standard conditions (23 ± 2 °C, 12/12 h light–dark cycle, relative humidity of 50% ± 6%) and were provided with food and water in suitable amounts. All employed experimental procedures, such as caring and handling of the animals, were executed in comply with Tehran Medical sciences, Islamic Azad University Ethical Committee Acts [IR.IAU.TMU.REC.1399.515].

Study Design

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

Control group: The rats were not given any treatment or surgery.
Ischemia group: After anesthesia, the carotid arteries were clamped for 20 minutes followed by reperfusion.
Experimental group 1: After ischemia reperfusion for 20 minutes, intraperitoneal injection of hydroalcoholic extract of black seed at a dose of 400 mg / kg was performed for 14 days straight.
Experimental group 2: After ischemia reperfusion for 20 minutes, intraperitoneal injection of nano hydroalcoholic extract of black seed at a dose of 400 mg / kg was performed for 14 consecutive days.

24 hours after the last injection, all animals were evaluated by Y-Maze behavioral test, then rat brains were removed for histological studies and gene evaluation.

Surgical process

After anesthesia by injection of pentobarbital sodium (40mg/kg, i.p.), a vertical incision was made in the anterior region of the animal's neck, and by pushing the Sternoclidomastoid muscle, the common carotid arteries were exposed on both sides. After detachment of vagus nerve microsurgery clamp was applied for 20 minutes in order to close the arteries. Then the clamps were removed and circulation was restored. During the surgery, the temperature of the animal was measured regularly by thermometer and the temperature was stabilized by heat lamp at 37±0.5°C. The cut was sutured by 04 silicon yarn. All the animals were monitored in separate cages until their conditions were stabilized (15).

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 was indictive of 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 (16).

Preparation method of hydroalcoholic extract of Nigella sativa

The alcoholic extraction of nigella sativa was carried out by maceration method so that the crushed grain was poured into the funnel of decanter and each time 250g of nigella powder with 60 degrees alcohol was added as solvent. Then, they were placed at room temperature for 8-10 hours. Afterwards, the valve of 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 (17).

Preparation method of nano-hydroalcoholic extract of Nigella sativa

First, the hydroalcoholic extract was poured into a container, then alcohol was removed and the extract was dried, after that ,10 grams of the dried extract was dissolved in 100 cc of distilled water. The acquired hydroalcoholic extract was put into the ultrasonic prop for 1 hour. Then,
The DLS (dynamic light scattering) was used to evaluate the nanoemulsion containing Nigella sativa. DLS is a physical method that is applied to actuate the distribution of particles in solutions and suspensions. Hydrodynamic diameter measured the zeta potential as a marker to investigate the surface charge of nanoemulsion and TEM (transmission electron microscopy) was utilised to investigate the shape and size of nanoemulsion (17).

Real time PCR

Entire cellular RNA was applied to prepare complementary DNA (cDNA) in order to be used in measurement of *BCL-2* and *BAX* mRNA expression. Pars tous kit (Iran) was utilized to extract total RNA. Method and kit instructed by manufacturer of Yekta tajhiz azma kit (Iran) was used for cDNA preparation. Eventually the cDNA was maintained at -20℃. Housekeeping gene GADPH was used for normalization of target gene expression. The applied primes are shown in Table 1.

Table 1. The Primers sequences.

Gene		Prime sequence5’ -3’
BAX	Forward Primer	GCAAACTGGTGCTCAAGG
	Reverse Primer	CAGCCACAAAGATGGTCA
BCL2	Forward Primer	GAGTGGGATACTGGAGATGAAG
	Reverse Primer	TGGTAGCGACGAGAGAAGTC
GAPDH	Forward Primer	AGGTCGGTGTGAACGGATTTG
	Reverse Primer	TGTAGACCATGTAGTTGAGGTCA

First hippocampus samples were prepared, then purification of extracted RNA was performed and the high quality RNAs were chosen to be kept at -80℃ until eventually they were used for cDNA synthesis. 1mg RNA converting to cDNA was completed utilizing Quantitect reverse transcription kit (Qiagen). In order to carry out Real-time PCR the primers were designed and underwent a large-scale search implementing BLAST tool. Real-time PCR was conducted by execution of following cycling conditions: 95℃ for 10min, and 40 cycles at 95℃ for 15s, and 60℃ for 1min. Every single complete amplification stage subsequently had a dissociation stage: at 95℃ for 15s, 60℃ for 30s, 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 volumetric analysis.

Cavalieri's principle was used to assess the volume of 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 H&E.

The total volume (V, mm^3) of CA1 region was calculated by Cavalier's method, where $V=\Sigma P$ (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).

Nissl staining

10m-thick sections 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 percent cresyl violet. Each animal had eight photomicrographs taken (between the level of 2.3 and 5 mm posterior to bregma fortune 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 400 magnification. Only pyramidal cells with visible nuclei and nucleoli were included in the study. Images were collected with a microscope (OlympusAX-70, Japan) at 400 magnification and analyzed with Imaging-Pro-Plus (LEICA DMLB, Germany) software.

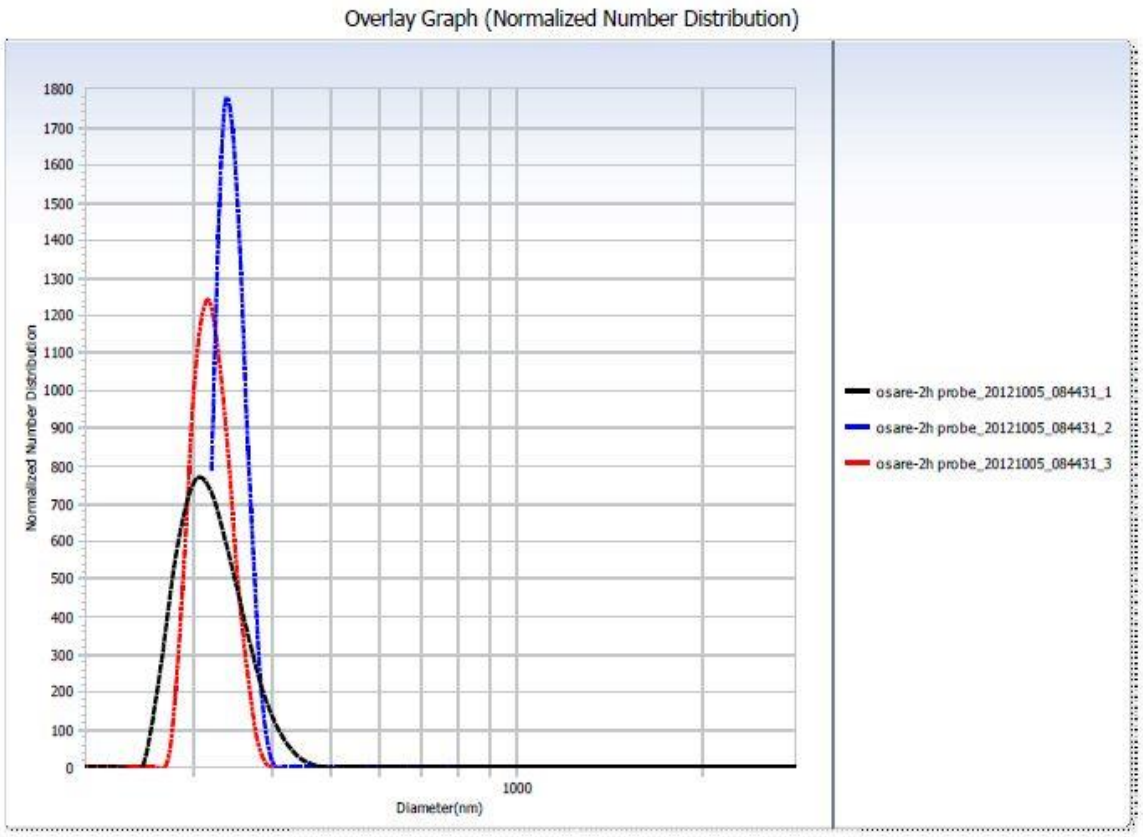
Statistical analysis

Reported data are at mean± standard deviation. Analysis of variance (ANOVA) and least significant difference (LSD) methods are conducted for comparison of different groups. In addition, a p-value of less than 0.05 was identified as significant.

Results

Investigation of particle size of Nanoemulsions (DLS)

The shape of the graphs is single peak and the particle size distribution is narrow, indicating that the particles are small and uniform. The particles size is between 300 and 350 nanometers. Another device was used to ensure the data was accurate. The results of 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

Figure 1. DLS of nano-hydroalcoholic extract of nigella sativa sample

*: is the indicator for level of meaningfulness (P<0.05)

DLS Analysis

After the nigella hydroalcoholic sample was made, DLS was used to investigate particle size and particle size distribution and particle dispersion was analyzed using diagrams. The dispersion was between 294.4 and 357.1 nm and a single peak diagram was obtained. The diameter of this diagram is intensive, when the size of particles is larger than 90 nm, it will rise more intensely. if there are smaller particles with lower concentrations suspensions, sometimes they fade from the sensors and as a result, no intensity is reported. Also, after taking the stability test in four different environments, once after one month and also another one after three months, the results were the same as the initial results.

Investigation of particle size by Zeta potential

The results of zeta potential of nigella sativa nano-hydroalcoholic extract are as follows (Table 2, Graph 1):

Table 2: zeta potential of nigella sativa nano-hydroalcoholic results.

13990828-Abdolrahimkhan-Sample1-Zeta-Scan3.nzt

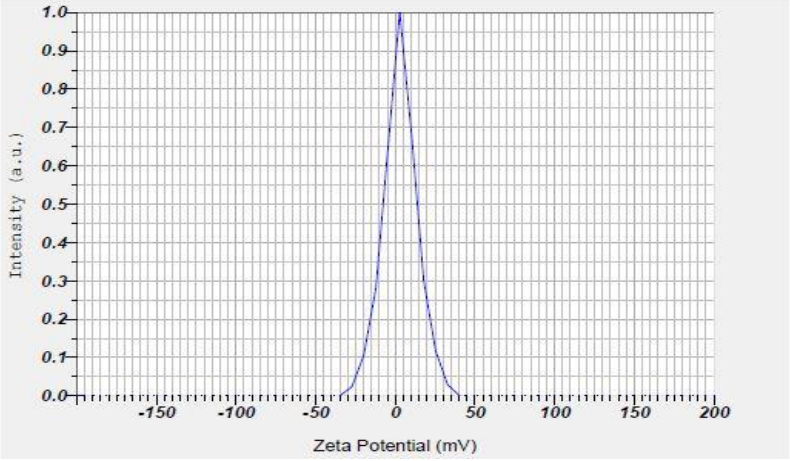
Measurement Results

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



Graph 1: zeta potential of nigella sativa nano-hydroalcoholic

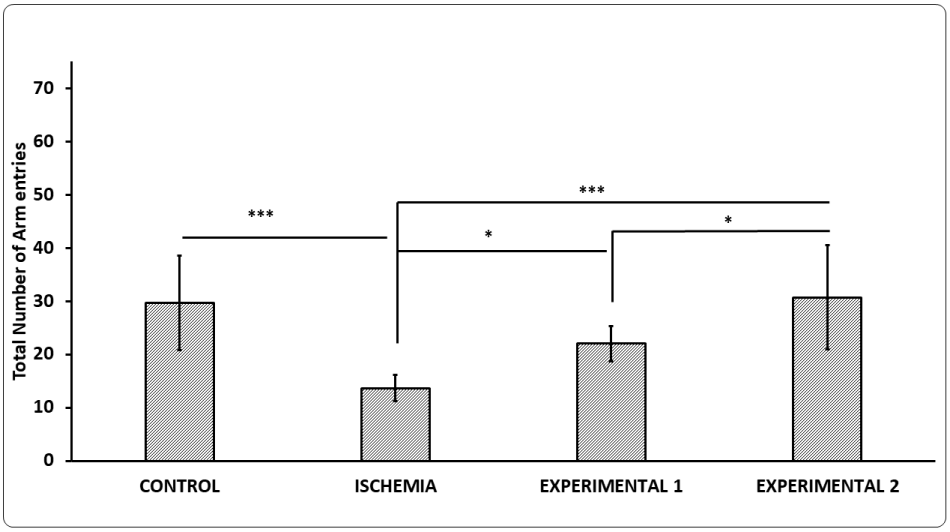
*: is indicator for level of meaningfulness(P<0.05)

Zeta potential analysis

Most fluids contain cations and anions which are the atoms with positive and negative loads. When the loaded particles are suspended in a fluid, the opposing load ions are absorbed into the particulate matter. It means, samples with negative loads attracts positive ions from fluid, and on the contrary, the sample with a positive charge, attracts 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 has a charge from -10 to +10, which shows the stability of sample (Graph 2).

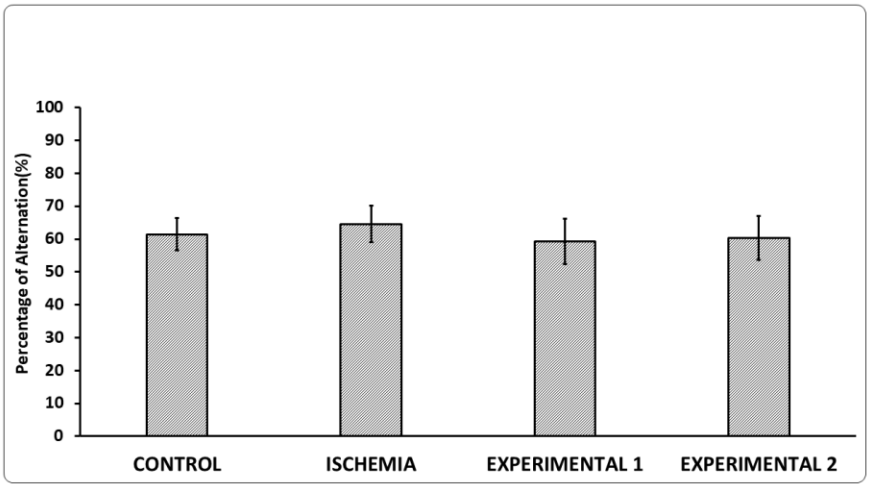
Behavioral Test Results

The results of Y-maze test revealed that ischemia remarkably decreased the kinetic ability of test subjects. The aggregate of suitable feedback in I/R group was considerably declined in comparison with the control group. Furthermore, the longer the time of ischemia occurred, the worse the viciousness of neurological function was. Whilst compared with I/R group, nano hydroalcoholic extract of black seed recipient group memory functions were significantly increased after I/R injury. (Graph 2&3).



Graph 2. Comparison of total number of arms entered into rats in different groups.

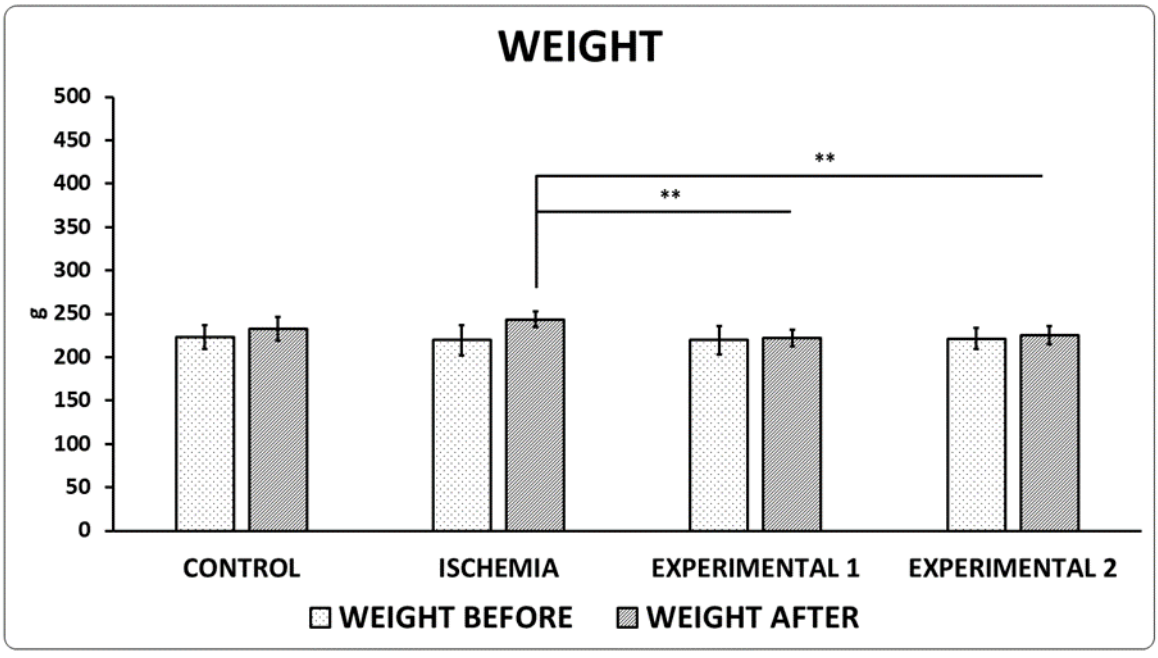
*: is indicator for level of meaningfulness (P<0.05)



Graph 3. Comparison of spontaneous interval behavior of rats in different groups

Results of 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 group 1 and 2 were receiving the drug, one-way ANOVA test and LSD test showed a significant difference between ischemia group and experimental group 1 and 2 (Graph4).

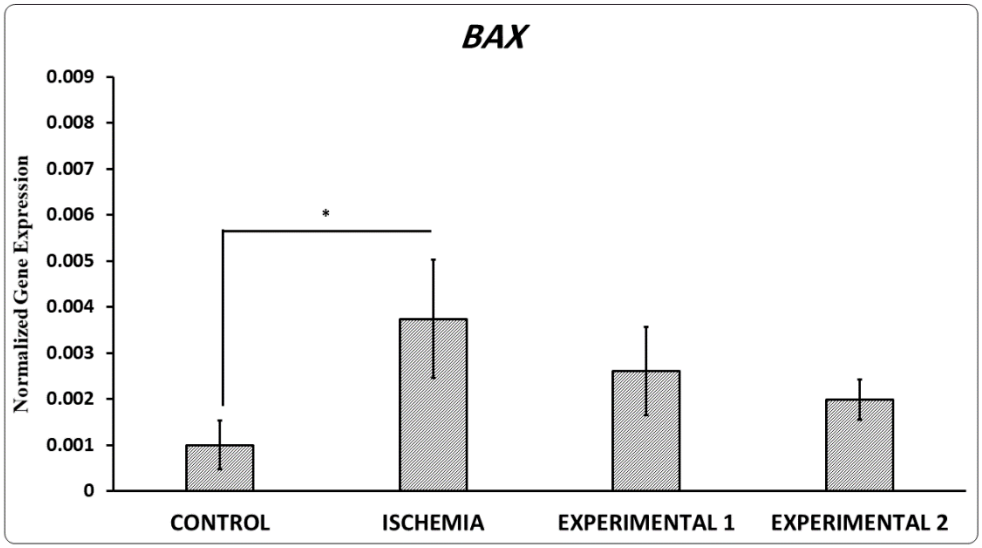


Graph 4. Comparison of weight of rats at the beginning and end of the study in different groups.

*: is indicator for level of meaningfulness(P<0.05)

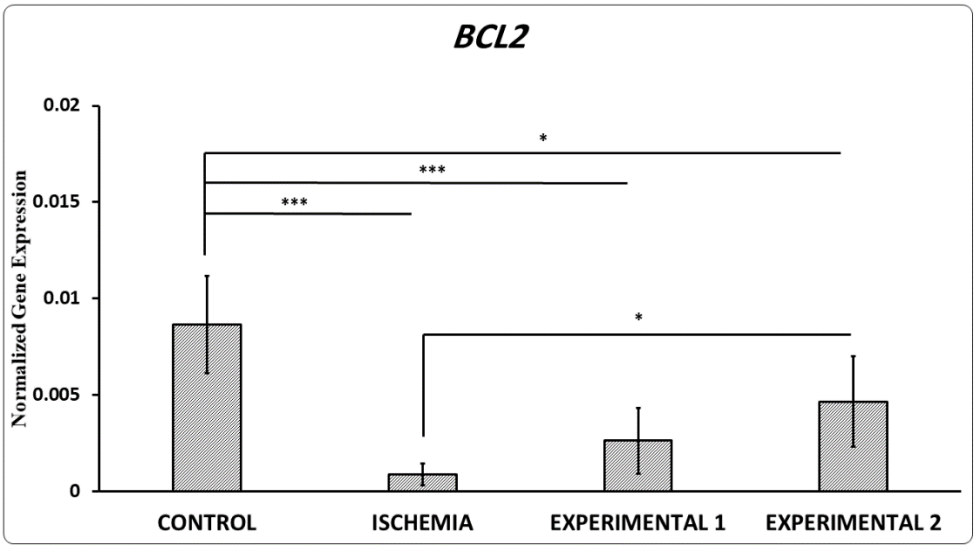
Results of Real Time-PCR

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. (graph 5, 6). As showed in graph 5&6, 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 ,(Graph 5 $P<0.05$), and increased the relative expression Bcl-2 in these groups (Graph 6 $P<0.05$).



Graph 5. Comparison of *BAX* gene expression in different groups

*: is indicator for level of meaningfulness($P<0.05$)

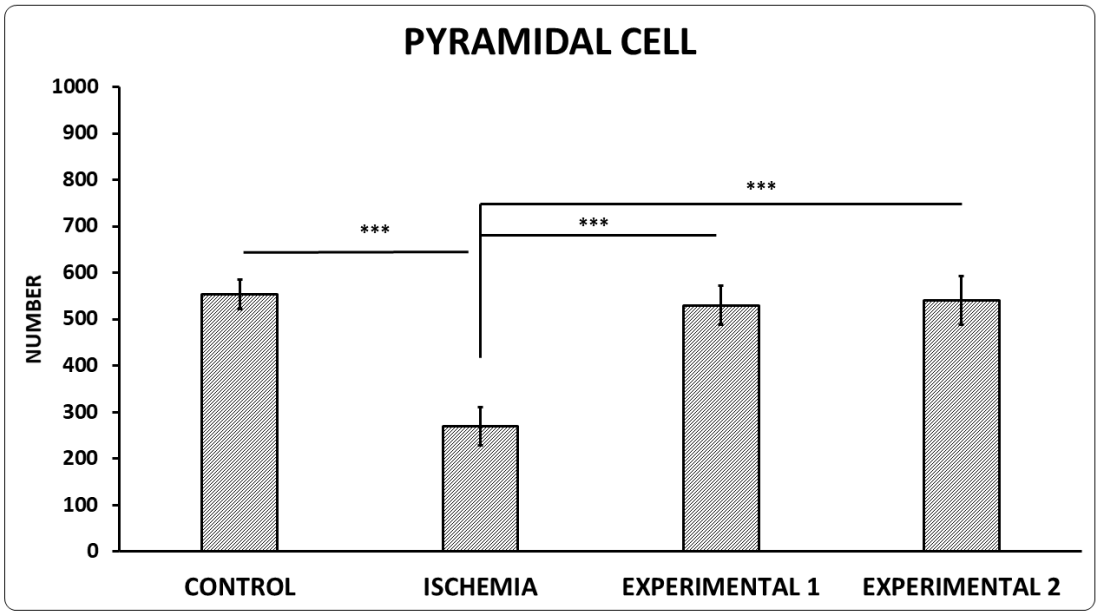


Graph 6. Comparison of *BCL2* gene expression in different groups.

*: is indicator for level of meaningfulness(P<0.05)

Nissl Staining Results

Nissl staining was used to determine viable pyramidal cells in CA1 area. Cells with round, bright nucleus with a euchromatin appearance and multiple nuclei were considered as viable cells. Cells with dense, multifaceted and heterochromatin nucleus were considered as 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 Nigella Sativa reversed the effect of transient global I/R on the number of neurons (P<0.05) (Fig. **2**). (Graph 7)



Graph 7. Comparison between average number of pyramidal cells in CA1 region in different groups.

*: is indicator for level of meaningfulness(P<0.05)

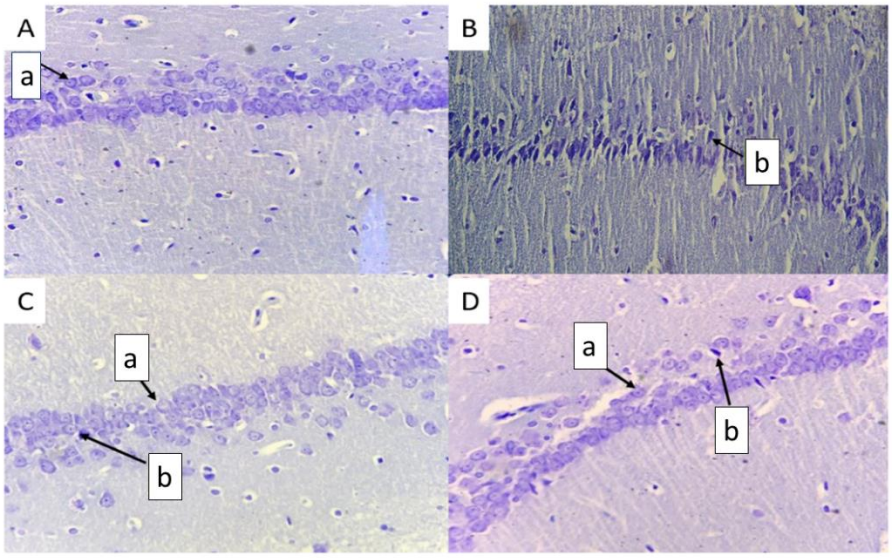
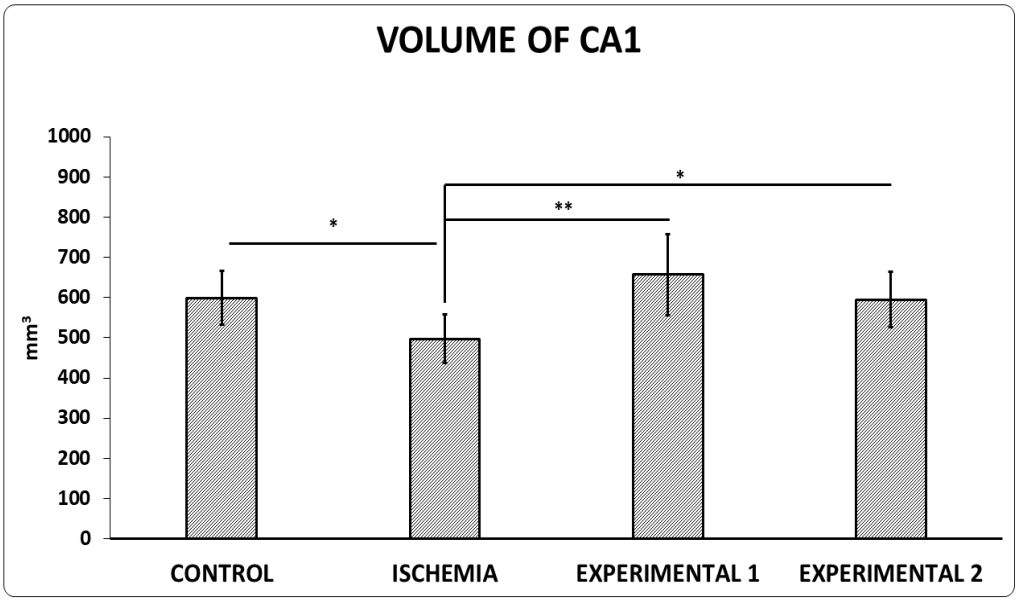


Figure 2. Photomicrograph Nissel staining l of the pyramidal cells of the CA1 region of the hippocampus in A) Control group B) Ischemia group C) Hydroalcoholic extract group with a dose of 400 D) hydroalcoholic nano-extract group with a dose of 400 magnification of 40. Most of pyramidal cells were healthy. a: Healthy pyramidal cells, b: Degenerated pyramidal cells

Results of stereological evaluation of volume of CA1 region of hippocampus

The results of calculating the volume of hippocampus CA1 region of 6 histological slides with hematoxylin and eosin (H&E) staining for each rat and a total of 6 rats in each group were observed. The volume in ischemia group decreased significantly compared to the control group. Furthermore, the increase in the volume of hippocampus CA1 area by injection of hydroalcoholic extract increased significantly compared to the injection of nigella sativa nano-hydroalcoholic extract (Graph 8). Hematoxylin and eosin staining were used to measure the volume of CA1 region (Figure 3).



Graph 8. Comparison of CA1 area volume in different groups.

*: is indicator for level of meaningfulness(P<0.05)

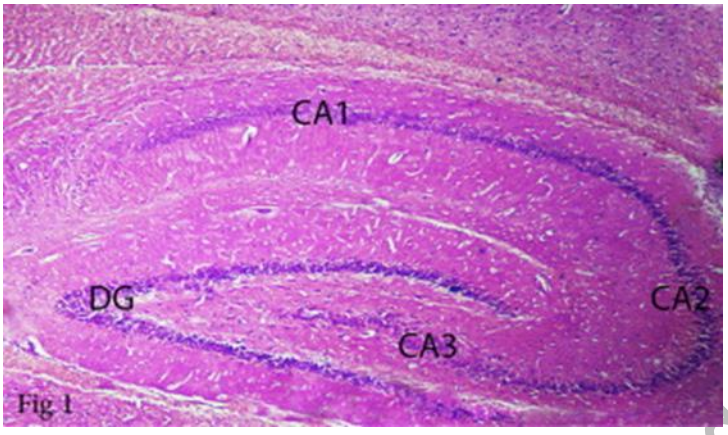


Figure 3. H&E staining sample with display of different areas of hippocampus of rats.

DG: dentate gyrus, CA: Cornu Ammonis

Discussion

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

Ischemia-reperfusion injury (IRI) is a pathological condition that has both local and systemic effects. The key element at play during ischemia is cell energy depletion, and the interplay of oxidative and microcirculatory stress, as well as inflammation and apoptosis, takes place during reperfusion. (18)

Following brain ischemia, the programmed death of neurons in a specific area of the brain that is invaded may be the hippocampus or cerebellum. Oxidative stress caused by ROS production contributes in the programmed death of cells. Although several researches have shown that the production of reactive oxygen species (ROS) contributes in causing cell lesions in the brain ischemia (19). Increased ROS levels inside the cell can lead to DNA and intracellular proteins 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. (20, 21).

Anti-inflammatory and antioxidant medications should be investigated in order to provide other treatment options that can assist prevent or reduce these injuries because current drugs have limited efficacy and provide little or no benefit to patients. (22). Antioxidants are compounds that can significantly slow down the oxidative reaction (23). So far, many natural and synthetic antioxidants have been introduced for the treatment or prevention of patients associated with free radicals (24). Medicinal plants always has an instrumental role in the management of global health care and are

considered the best treatment for diseases (25). Nigella sativa is a medicinal plant which is highly consumed. In recent years, further attention has been paid to the medicinal and biological properties of nigella sativa

One of Nigella 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. (26, 27)

It has been reported that administration of antioxidant agents before local brain ischemia in laboratory studies preserve protection against ischemic brain lesion (28). Based on a review of different literatures and comparisons, thymoquinone, which is the most important extract of nigella sativa, have many pharmacological properties, mostly related to their antioxidant properties in this regard, Hosseinzadeh et al. revealed that the nigella oils administration in the global ischemic model of the brain has reduced the amount of lipid peroxidation. Their findings can enhance the role of antioxidant properties on the protective effects of nigella sativa in cerebral ischemia (29). In 2006, Almajed et al. investigated the neuroprotective effects of thymoquinone on transient cerebral ischemia on hippocampus in rats. Thymoquinone reduces MDA levels (malondialdehyde), increases GSH, catalase and SOD 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 (30). Nigella sativa also enhances the activity of antioxidant enzymes such as SOD و PX-GSH and with its antioxidant properties reduce lipid peroxidation in biological membranes (31). It is known that thymoquinone and its active metabolite thymohydroquinone can prevent lipid peroxidation by sweeping superoxide, radial hydroxyl and single molecular oxygen (32). In addition, thymoquinone can inhibit arachidonic acid metabolism and restrict ischemic brain damage caused by inflammation of cyclooxygenase and lipoxygenase pathways (33). Drug delivery systems of nanoparticles have proven to have higher potential in the treatment of ischemic stroke and probably other neurological disorders. Joachim et al. illustrated that the gelatin of osteopoitin nanoparticles in the ischemic stroke model of rats improves nerve protection in nose (34). Nagai et al. showed that intravenous administration of silvestazole nanoparticles improves acute ischemic damage in the brain following the ischemic/reperfusion (35). According to the previous studies and the results of the current research in relation to the function of Nigella sativa plant and its role in preventing the death of neural cells, it can be said that the hydroalcoholic extract of Nigella satient and nano-hydroalcoholic extract of Nigella sativa can exert its protection 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 treatment may protect against ischemia-induced neuronal apoptosis by promoting cell survival pathways. Also, Nigella sativa plant in Nano majority has more efficacy and higher adsorption than hydroalcoholic extract, but 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 cumulative effect of these compounds is not known, this issue needs further research and studies.

Conclusion.

This research revealed that Hydroalcoholic Extract and Nano Hydroalcoholic Extract Containing Nigella Sativa by preventing morphological changes and resulting cell death has an effective role on neurons in the CA1 region of hippocampus. Therefore, it seems that Hydroalcoholic Extract and Nano Hydroalcoholic Extract Containing Nigella Sativa through anti-inflammatory and Antioxidants mechanisms can be considered as a treatment candidate for ischemic brain damages.

Declarations

- Ethics approval and consent to participate: The Ethics Committee of Tehran Medical Sciences of Islamic Azad University, Iran, authorized all of the study's protocols [approval number: 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.
- Consent for publication: Not applicable
- Availability of data and materials: All data generated or analysed during this study are included in this published article [and its supplementary information files].
- Competing interests: The authors declare that they have no financial or other conflicts of interest.
- Funding: This study was self-funded.
- Authors' contributions: MG made substantial contributions to the conception and design of the work, AM was in charge of the acquisition, analysis and interpretation of data, MMN have drafted the work and substantively revised it, SM also participated in the analysis and interpretation of data, SNS made substantial contributions to the conception and design of the work and also was in charge of the acquisition, analysis and interpretation of data.
- Acknowledgements: For their direction and support, we are grateful to the Herbal Pharmacology Research Center, Tehran Medical Sciences, Islamic Azad University, and all of the personnel.

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List of Abbreviations

- Bax: Bcl-2 Associated X Protein
- Bcl-2: B-cell lymphoma 2
- cDNA: Complementary Deoxyribonucleic Acid
- DLS: Dynamic Light Scattering
- H&E: Hematoxylin and Eosin
- I/R: Ischemia/Reperfusion
- LSD: Least Significant Difference
- mRNA: Messenger Ribonucleic Acid
- PCR: Polymerase Chain Reaction
- ROS: Reactive Oxygen Species
- SOD: Superoxide Dismutase
- TEM: Transmission Electron Microscopy