The effect of nigella sativa extract on learning and spatial memory of adult male rats

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Abstract

Introduction:
Humans have long noted the importance of memory in learning and have always sought the ways to improve memory and prevent its weaknesses. In the current study, the effect of Nigella sativa extract on learning and memory of adult male rats was investigated.

Materials and Methods:
In the current experimental study, 40 adult male rats weighing 180-220 g were divided into five groups of eight rates including experimental groups 1, 2 and 3, which were given daily doses of 100, 200 and 400 mg/kg per body weight extracts of Nigella sativa for 17 days, control group (0.1 ml normal saline gavage) and negative control group (received no substance). The extract was administered 12 days before training and continued throughout the training. On the eighteenth day of the administration of the extract, the memory test was conducted by Barnes maze and latency to reach the target and the number of errors was measured.

Results:
The results showed that learning and spatial memory in groups that receive Nigella sativa extract significantly increased (P<0.05) compared to the control and negative control groups.

Conclusions:
The results suggest that the administration of Nigella sativa extract can enhance spatial memory and learning due to oleic acid, linoleic acid and antioxidant compounds.

Keywords: Nigella Sativa, Memory, Learning, Extract

Introduction
Nigella sativa, also known as black seed, is a plant that grows in vast areas of the world. In Latin, black seed is called panacea meaning healer of all diseases (1). There are about 8 species of this plant (2). Nigella is an annual, short (about 20-30cm) herbaceous plant with 2-3 times pinnate basal leaves, compounded, alternate and finely divided into long linear strands (1, 3). Crushed black seeds have a cumin-like aroma, and have long been used in treatment of many acute and chronic diseases. The seeds from this plant have such properties as enhancing lactation, antimicrobial, diuretic, laxative, blood pressure lowering, and boosting immune system, and in some cases, can cause abortion (4, 5). Over the centuries, black seed oil and plant have been used to enhance health in Asia, Middle-East, and Africa (5). Nigella seeds contain fat, fiber, minerals (ions), and elements such as zinc, copper, sodium, iron, calcium, and various...
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vitamins including ascorbic acid, thiamine, niacin, and folic acid. They are also a rich source of fatty acid esters such as lauric, mirctic, stearic, palmitic, oleic, and linoleic acids (6). Studies conducted on the central nervous system by Abdol-Hakim in 2006 indicate that the back seed oil reduces function of central nervous system and has strong tranquilizing effects (7). Results have shown that black seed oil contains linoleic (55.6%), oleic (23.4%), and palmitic (12.5%) acids, and all these unsaturated fatty acids improve learning. Furthermore, recent studies have shown that antioxidant compounds also improve learning and memory. Since black seed is a known antioxidant and a promising compound in treatment of cerebral ischemia and neurological degenerative diseases, it may also have a role in improving learning and memory (8). In Hoseinzadeh et al. study, effectiveness of Nigella sativa extract in treatment of cerebral ischemia was demonstrated (9).

Results of a study conducted in 2005 on the anticonvulsant healing effect of Nigella in rats showed that thymoquinone can be highly effective in tonic-clonic seizures (10). Study of analgesic effect of oral administration of Nigella sativa seeds in diabetic rats by Roghani et al. in 2006 revealed reduced feeling of pain in rats (11).

In a study by Parvardeh et al. (2003) on the effect of thymoquinone contained in Nigella seeds on motor system activity and coordination in rats, it was found that thymoquinone reduces muscle relaxation and sleeping, improves motor activity, and reduces impaired motor coordination in rats (12). Despite many studies on healing effects of Nigella sativa, no study has yet been conducted on its effect on memory and learning. The present study aims to investigate the effects of Nigella sativa extract on learning process and spatial memory using Barnes Maze.

Materials and Methods

In this in-vitro study, all ethical principles in relation to working on laboratory animals were observed. Forty Wistar adult male rats, weighing 180-220 grams, were procured from the Center for Breeding and Keeping Laboratory Animals of Kazeroun Azad University. All animals in Kazeroun University Animal House were housed in alternate 12 hour light-dark cycle and at constant 25°C. In order for animals to adjust to new conditions, all experiments were conducted after two weeks settlement. Rats received food and water ad lib.

Nigella sativa hydroalcoholic extract preparation:

First, seeds were crushed by an electric mill. The powder was soaked in ethanol 80% for 72 hours, and centrifuged afterwards to ensure absence of suspended particles. The sediment was discarded after sedimentation and the supernatant was concentrated at 40°C (13). Rats were randomly divided into 5 groups of 8, including negative control, positive control, and three experimental groups 1, 2, and 3. Negative and positive control groups received normal food and water. A week before commencement of experiments, 0.1 ml of normal saline was administered to the positive control group rats via gavage. Experimental groups each received 100, 200, and 400 mg per kg body weight of Nigella hydroalcoholic extract via gavage for 17 days. Twelve days following administration of the extract, animals underwent spatial memory training and evaluation for 5 days and 4 times per day using Barnes Maze. On the 6th day, memory assessment was performed.

Memory assessment with Barnes Maze:

In this model, memory assessment was performed using a round-table with 18 running holes around the perimeter. To encourage rats to find the target hole, light source was placed 100cm from table, and to help them navigate, visual signs were installed around the maze during experiment (figure 1).
Following administration of the extract, rats in each group were familiarized with the maze for 1 hour. On this occasion, the target box was absent from the maze, and rats were allowed to roam freely in the maze. Animals were then returned to an open box. After 20 minutes, each rat was returned to the maze and a box was placed on it for 10 seconds. Then, the light above the table was turned on and box was removed, and animal had 90 seconds to find the hole. Once the target hole was found, light was turned off and target hole was covered by a dark screen, and animal was allowed to rest there for 60 seconds. During the first training, researcher guided the rats if they failed to find the target hole after 90 seconds. To avoid detection by olfactory signals, table surface was wiped with alcohol after each training session. In this study, assessment indicators included delay time in finding the hole and number of errors. From the moment of removing the box over animal until touching target hole was recorded as delay time in finding the hole, and going to the wrong hole was considered an error. All training was recorded using video camera, and stored on the computer.

Analysis of data:
Data were analyzed using SPSS-17 software. To determine treatment effect on groups, one-way variance analysis test was used, followed by Tukey test, to assess differences between groups. P<0.05 was considered significant. Data were reported as Mean ± SD.

Results
To investigate learning and memory of animals in Barnes maze, two factors of time to reach the target and frequency of errors in reaching the target were assessed as important variables.

Effect of Hydroalcoholic extract of Nigella sativa on delay time to reach the target during training days:
Delay time to reach the target significantly reduced in experimental groups receiving the extract compared to positive and negative control groups (P<0.05). There was a significant reduction and difference in delay time between experimental group 3 (receiving 400 mg/kg dose of extract) and control groups and groups 1 and 2 (receiving 100 and 200 mg/kg doses respectively) (P<0.05) (table 1 and plot 1).
The effect of nigella sativa extract on frequency of errors during training days:

Comparison of control groups with experimental groups showed a significant difference, as frequency of errors in finding the target hole reduced in experimental groups compared to control groups, and the reduction was significant in groups receiving maximum doses (200, and 400 mg/kg). Moreover, there was a significant difference between group 3 and groups 1 and 2 (P<0.05) (table 2, and plot 2).

Table 2: Mean frequency of errors in negative control, positive control and experimental groups (doses 100, 200, and 400 mg/kg) on training days

<table>
<thead>
<tr>
<th>Groups (n=8)</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>33.25±0.443</td>
<td>31.96±0.227</td>
<td>31.54±0.136</td>
<td>31.64±0.164</td>
<td>31.21±0.306</td>
</tr>
<tr>
<td>Positive control</td>
<td>34.42±0.279</td>
<td>33.17±0.279</td>
<td>31.42±0.154</td>
<td>31.04±0.198</td>
<td>30.5±0.158</td>
</tr>
<tr>
<td>100 mg/kg dose of extract</td>
<td>25.38±0.168</td>
<td>18.22±0.904</td>
<td>5.78±0.437</td>
<td>7.31±0.374</td>
<td>4.21±0.514</td>
</tr>
<tr>
<td>200 mg/kg dose of extract</td>
<td>15.59±0.724</td>
<td>8.53±1.038</td>
<td>8.17±0.848</td>
<td>7.69±0.766</td>
<td>3.82±0.303</td>
</tr>
<tr>
<td>400 mg/kg dose of extract</td>
<td>3.57±0.701</td>
<td>3.57±0.433</td>
<td>3.64±0.534</td>
<td>2.44±0.639</td>
<td>1.52±0.271</td>
</tr>
</tbody>
</table>
The effect of hydroalcoholic Nigella sativa extract on delay time to reach the target on test day:
There was a significant difference in delay time to reach the target between experimental groups receiving Nigella sativa extract compared to positive and negative control groups (P<0.05). The delay time reduction in higher doses depended on the dose, and was significant in 400 mg/kg group compared to all other groups (P<0.05) (table 3, and Plot 3).

Table 3: Mean delay time in negative control, positive control, and experimental groups (doses 100, 200, and 400 mg/kg) on test day (delay time in seconds)

<table>
<thead>
<tr>
<th>Groups (n=8)</th>
<th>Delay time on test day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>300±0</td>
</tr>
<tr>
<td>Positive control</td>
<td>300±0</td>
</tr>
<tr>
<td>100 mg/kg dose</td>
<td>44.33±2.985</td>
</tr>
<tr>
<td>200 mg/kg dose</td>
<td>26.83±1.4</td>
</tr>
<tr>
<td>400 mg/kg dose</td>
<td>17.33±1.909</td>
</tr>
</tbody>
</table>
The effect of hydroalcoholic extract of Nigella sativa on frequency of errors on test day:
Frequency of errors in finding the target hole reduced in experimental groups compared to negative and positive control groups, and the reduction was significant in groups receiving maximum doses. Frequency of errors in reaching the target in negative and positive control groups and all experimental groups was significantly different. There was a significant difference between the 400 mg/kg group and all other groups (in 400 mg/kg group frequency of errors significantly reduced compared to all other groups (P<0.05) (table 4, and plot 4).

Table 4: Mean frequency of errors in negative control, positive control and experimental groups (doses 100, 200, and 400 mg/kg) on the test day

<table>
<thead>
<tr>
<th>Groups (n=8)</th>
<th>Frequency of errors on test day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>30.83±0.307</td>
</tr>
<tr>
<td>Positive control</td>
<td>31±0.365</td>
</tr>
<tr>
<td>100 mg/kg dose</td>
<td>16.5±0.764</td>
</tr>
<tr>
<td>200 mg/kg dose</td>
<td>12.93±0.601</td>
</tr>
<tr>
<td>400 mg/kg dose</td>
<td>6.5±0.885</td>
</tr>
</tbody>
</table>

Plot 4: The effect of hydroalcoholic Nigella sativa extract on frequency of errors on test day
(The effect of hydroalcoholic Nigella sativa extracts on frequency of errors on test day on rats in negative control, positive control, and experimental groups with doses 100, 200, and 400 mg/kg) (n=8) (P<0.05)

The present study results showed dose-dependent reduction in frequency of errors and delay time in reaching the target in experimental groups receiving Nigella sativa extract.

Discussion
The present study results showed that administration of hydroalcoholic extract of Nigella sativa at doses 100, 200, and 400 mg/kg, reduced delay time and frequency of errors in finding target hole in spatial memory in Barnes maze on both training and test days. Thus, it can be asserted that Nigella sativa extract dramatically improves spatial memory. However, this effect is dose-dependent.

Given different habits in using vegetable oils and different conversion mechanisms of fatty acids in the body, researchers conducted various experiments on the effects of fatty acids on memory, and obtained conflicting results. Fats are among the most important sources of
human food, and in a balanced diet, they provide up to 30% of calories needed by the body (14). In addition to providing energy, fats act as thermal insulation in subcutaneous tissues and around some organs. They are also involved in rapid transmission of neural waves along myelinated nerves. In addition, fats are the only source of essential fatty acids and fat-soluble vitamins (15). About one third of fats are provided from vegetable and two thirds from animal sources. Vegetable and animal fats fatty acids and cholesterol are different from one another (16).

The most common unsaturated fatty acids found in combination with glycerol in vegetable oil include oleic and linoleic acids, which have one, two and three double bonds with carbon 18 atom (17). Nigella sativa contains unsaturated fatty acids such as oleic and linoleic acids and antioxidant compounds, which appear to improve learning. This probably occurs through changes in fluidity of membrane, especially in hippocampus area (18).

Furthermore, studies have shown that linoleic acid prevents neuronal cell death by glutamate in cell culture. This means that in cell culture medium, glutamate stimulates neuronal cell death by affecting N and non-N receptors of methylidiaspartate (19). Unsaturated fatty acids increase fluidity of neuronal membrane by reducing membrane cholesterol level. Such fluidity is necessary for emergence of dendrites and axons, and facilitates axonal and dendritic sprouting (20).

Tanaka et al. (2002) reported that unsaturated fatty acids such as arachidonic, oleic, and linoleic acids, increase action of acetylcholine nicotinic receptors in hippocampus through protein C kinase in pre-synaptic neuron. In turn, acetylcholine nicotinic receptors increase release of glutamate neurotransmitters. Thus, increased glutamate facilitates long-term synaptic transmission in hippocampus. Long-term facilitation of a cellular model of learning and memory is similar to long-term strengthening.

Presence of oleic acid is essential in food, since it can affect transmission of signals through activation of protein C kinase and phosphorylation of proteins in hippocampus. Additionally, oleic acid moderates mutual effect of interaction between receptors of benzodiazepines and gamma-aminobuteric acid (a major inhibitory neuromediator in vertebrates’ nervous system), which is also moderator of toxic effects on neuroblatoma, and stimulates proliferation of Schwann cells (21).

Studies have shown that oleic acid is involved in the cascade of secondary messengers. Possible mechanisms of effect of oleic acid on the nervous system have recently been studied. Biochemical studies have demonstrated that action of protein C kinase is due to fatty acids, and subsequent phosphorylation and reconstruction of fractured connections. In vitro studies have shown that oleic acid can accelerate axonal and neuronal clusters growth. Stimulating rapid axonal growth is associated with action and participation of fractured connections via a protein C kinase-dependent mechanism. In vitro studies have shown that oleic acid is involved in creating neuronal phospholipids during rapid growth of neuron clusters (22). Oleic and arachidonic acids activate benta subunit and other subunits of protein kinase, and protein phosphorylation of fractured connections occur afterwards by protein C kinase, and ultimately involve fractured connections in reconstruction process of neuronal cells. Accumulation and action of fractured connections in neuronal growth indicates that oleic acid can act as a neurotrophic factor (23).

Other studies have shown that the number of insulin receptors in cerebral cell membrane increases according to long-chain fatty acids. It is believed that the cerebral insulin receptors are involved in cognitive functions like learning and memory (20). In fact, unsaturated fatty acids control cognitive function through
maintaining level of cerebral insulin receptors (24).

Oxidative stress and lipid peroxidation is an important factor in causing impaired memory, as in Alzheimer's disease, and antioxidants can have a protective effect (25). Unsaturated fatty acids with multiple double bonds are actively collected by the brain before and after birth, and make up the essential lipid membranes. Diet-induced changes in unsaturated fatty acid of cells affect cell neurochemistry and neurophysiology of the cell. Furthermore, cerebral membrane unsaturated fatty acids can change in diet, and synaptosomes fatty acids change accordingly. Today, it has been found that fatty acids in food affect neuronal membrane composition in adult brain through natural destruction and reconstruction of brain tissue and probably also synaptogenesis and dendritic and axonal branches (26). Aging causes major behavioral changes due to changes in synaptosomes. The best explanation is that quality of learning depends on cholesterol-induced changes in membrane fluidity. In aging rodents, cholesterol ratio to phospholipids increases, with subsequent reduction in membrane fluidity, and since cerebral membrane is a data processing site, mental activities, including learning, reduce with aging. On the other hand, in pathological cases, such as Alzheimer's disease, cerebral cholesterol level increases (15).

**Conclusion**

According to the present study, hydroalcoholic extract of Nigella sativa can affect memory, and thus increase learning because it contains oleic and linoleic acids and antioxidant compounds.

**Acknowledgements**

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**References:**

12. Parvardeh S, Hosenzadeh H. Hypotonic and muscle relaxant activity of thymoquinone, the major active constituent of Nigella sativa seed, and its effects on locomotor activity and...