The effect of *Zingiber officinalis* L. on learning and memory in rats

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A B S T R A C T

Background and Objective: According to the importance of learning and memory in the human life and also unavoidable neural degeneration due to aging, finding new compounds (drugs) against this process is valuable. However, there are many recommendations for herbal medicine and constituents which encouraged us to examine a candidate plant *Zingiber officinalis* for the mentioned purpose.

Materials and Methods: Male rats (250-300 g) were divided into control and treatment groups. Treatment groups consist of three subgroups including oral (plant was prescribed to animals mixed in food at a ratio of 6.25%) for 2 weeks, and two groups that received the plant extract at doses of 50 and 100 mg/kg (intraperitoneal, IP). In order to investigate the spatial recognition (alternation) behavior and acquisition-recalling (step through latency, STL), the animals were subjected to Y maze and shuttle box tests, respectively.

Results: In our study, the difference of the initial latency (IL) in oral treatment groups (8.24±1.21 s) and injection (50 and 100 mg/kg) groups versus control group (14.28±1.45 s) were non-significant. However, step through latency (STL) time difference for oral (18.12±0.8 s) group versus control one (13.28±1.33 s) was significant ($p<0.05$). Alternation behavior percentage in injection group (100 mg/kg) and oral one was significant versus control animals ($p<0.05$).

Conclusion: Oral and intraperitoneal administration of the *Zingiber officinalis* could have a significant improving effect on acquisition, retention and recall.

Key Words:
Learning and memory
Y maze
Passive avoidance test
Zingiber officinalis

1. Introduction

Learning and memory are considered as the best functional process of the central nervous system. Learning is a neural phenomenon during which organism changes its behavior by practice; whereas, memory refers to learned information saving process (1). Learning and memory include extensive changes in structure and function of nervous system which is mainly limited to synapses encountered in signal conduction pathways and sensory information in nervous system. Structural changes include change in synapse numbers and change in extension of post-synaptic membrane in contact site and physiologic changes include change in pre- and post-synaptic membrane ion conductance (2). It has been demonstrated that short term memory is linked to cortex and long term memory is linked to limbic system, despite the fact that no specific region of the brain has been considered as memory saving area, because by removing several parts of the brain, memory is

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not lost structural changes mainly occur in synapses at all (3). Researchers are compelling animals to do particular tasks to study memory on them. Recalling a special task after a period of time is considered as the long term memory. It is demonstrated that in long term memory, structural and constant changes occur in the structure of nervous system (4). Physiologic changes, especially in neurotransmitters releasing part have key roles in learning and memory phenomenon of short term memory (5). In long term potentiation (LTP), and receptors making which for the first time were observed in Aplasia snail gill siphon's neurons (6). Following this report, it has been revealed that this phenomenon also occurs in spinal cord grey matter neurons (7), beady neurons of serrated spines in temporal lobe, hippocampus (8) and neocortex.

According to importance of traditional medicine in prophylaxis and treatment of diseases and its low side effects, in this research study, one of the traditional plants of Iran, *Zingiber officinalis* L. was used (9) to evaluate its effect on memory improvement. Main ingredients of this plant could increase acetylcholine levels as an important agent in improving memory (10). Also, induction of LTP by glutamine receptor (11) imitation and inhibition of lipid peroxidation could have an effective role in memory enhancement. According to discussed literature, in this study, the efficacy of *Zingiber officinalis* L. on memory improvement was investigated.

2. Materials and Methods

2.1. Animals

Adult male Wistar rats (Pasteur Institute of Iran, Tehran) with a weight range of 310-350 g at the beginning of experiments were used. They were grouped into 3-4 rat/cage and were kept at 21-23 ºC and at night-day cycle controlled rooms. Animals had free access to food and water and 80-85% of their initial weight was preserved during experiments.

2.2. Method of *Zingiber officinalis* L. alcoholic extract preparation

After buying *Zingiber officinalis* L. from local store and precise recognition by systematic Department of Basic Science College of Shahed University, possible impurities were detached. Then, plant was grinded and powdered, soaked for 24 hours at a ratio of 1 to 4 with 70% methanol, and finally filtered. The obtained extract was placed at 50 °C until its alcohol completely evaporated. Dry extract was made into powder and several concentrations were prepared in normal saline.

2.3. Animals

Rats (n=60) were randomly divided into two control (n=12) and treatment groups (n=48). Treatment group animals were divided into four subgroups (n=12): group1 received *Zingiber officinalis* L. in oral form for 2 weeks and groups 2,3 and 4 received *Zingiber officinalis* L. extract at doses of 25, 50, and 100 mg/kg (IP), respectively.

2.4. Behavioral tests

Evaluation of recognition memory in Y maze test

Y-shape maze is a three arm black box made from Plexiglas. Arms (A, B and C) were alike and each maze arm length, width, and height were 40, 30, and 10, respectively. Arms were arranged with equal angles (120 degree) to each other. In the center, maze arms open to an equilateral triangle (with 15 cm long). Each rat was placed in one of the arms (for example A) and left for 8 minutes to go around freely (for finding food). Number of crossings were manually recorded. If we consider the rat goes to the arms 17 times by this pattern ACB, ABA, CAC, AAC, BAC, AC, the animal has gone 5 triads, of which 2 are non-repetitive. Alternation behavior percentage which demonstrate spatial recognition in animals is the number of non-repetitive triads arms (2×3=6) divided by all triads arms (5×3=15) minus 2 (15-2=13) multiplied one hundred (12).
Alternation behavior percentage= non-repetitive triads arms/ (all triads arms-2)×100
Spatial recognition=6/(15-2)×100=46.51%

Passive avoidance test

The apparatus consisted of an illuminated chamber connected to dark chamber by a guillotine door. Electric shocks were delivered to the grid floor by an isolated stimulator. On the first and second days of testing, each rat was placed on the apparatus and left for 5 min to

habituate to the apparatus. On the third day, an acquisition trial was performed. Rats were individually placed in the illuminated chamber. After a habituation period (5 min), the guillotine door was opened and after the rat entering the dark chamber, the door was closed and an inescapable scrambled electric shock (1 mA, 1 s once) was delivered. In this trial, the initial latency (IL) of entrance into the dark chamber was recorded and rats with ILs greater than 60 s were excluded from the study. Twenty-four hours later, each rat was placed in the illuminated chamber for retention trial. The interval between the placement in the illuminated chamber and the entry into the dark chamber was measured as step-through latency.

2.5. Statistical analysis

All results of passive avoidance experiments were presented as means±S.E.M. Non-parametric Kruskal-Wallis and Mann-Whitney U tests were used for statistical analysis. A $P<0.05$ was considered significant in all calculations.

3. Results

3.1. The effect of *Zingiber officinalis* L. on body weights

According to Table 1, oral and chronic consumption of *Zingiber officinalis* L. caused remarkable and significant decrease of body weight ($p<0.01$) in treatment group. In contrast, its extract injection had no significant effect on mice weight.

3.2. The effect of *Zingiber officinalis* L. on initial latency

According to Figure 1, oral consumption of this plant caused an initial latency of 16.28±1.21 s and its intraperitoneal administration at doses of 50 and100 mg/kg caused an initial latency of 12.28±1.24, 13.51±1.1 s, respectively.

Table 1. The effect of *Zingiber officinalis* L. on weight of experimented animals.

Group		Body weight (g)	
		One week before treatment	Two weeks after treatment
Control		298.3±8.6	311.8±7.3
Oral administration		313.6±9.1	237.3±10.5**
Intraperitoneal administration	50 mg/kg	289.6±9.3	305.2±12.6
	100 mg/kg	320.6±8.1	331.1±7.3

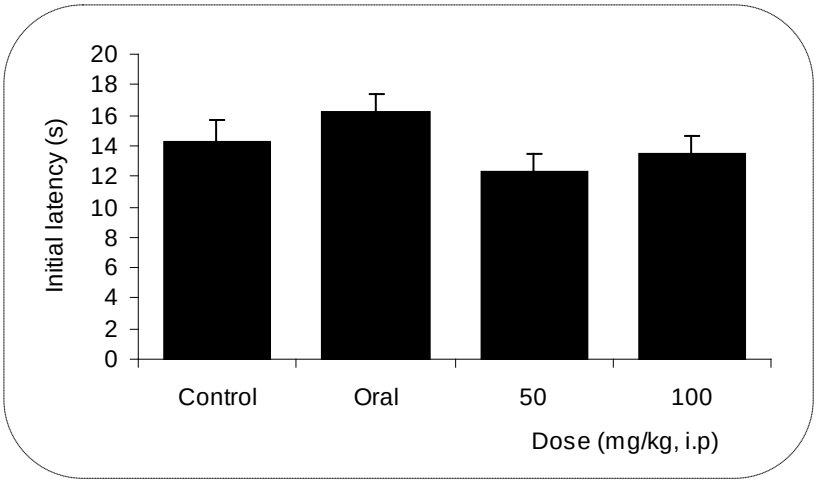

Figure 1. The effect of oral consumption and/or intraperitoneal administration of *Zingiber officinalis* L. on initial latency in male rats. According to the figure, there is no significant difference between the groups. Bars show Mean±S.E.M. Number of samples in each group was 12 animals.

3.3. The effect of *Zingiber officinalis* L. on step through latency

In figure 2, STL is shown in oral and injection groups as compared to control group. In this respect, STL in oral group was 18.12±0.8 s and in injection groups gat doses of 50 and 100 mg/kg, it was 12.12±1.25 and 16.21±1.22 s, respectively. Statistical comparison of these groups with control group (with a STL of 13.28±1.33 s) showed that oral consumption of *Zingiber officinalis* L. significantly increases STL ($p<0.05$) and intraperitoneal injection of its extract has no significant effect on STL in none of the doses, i.e. 50 and 100 mg/kg.

3.4. The effect of *Zingiber officinalis* L. on rat entrance to Y maze arms

According to figure 3, oral consumption of *Zingiber officinalis* L. caused a mean entrance of 27±1.7 times which in comparison with control group (18±1.4 times) was significant ($p<0.05$). Also, it was revealed that mean of entrance to Y maze arms was not significantly affected following its intraperitoneal administration at doses of 50 and 100 mg/kg as compared to control group.

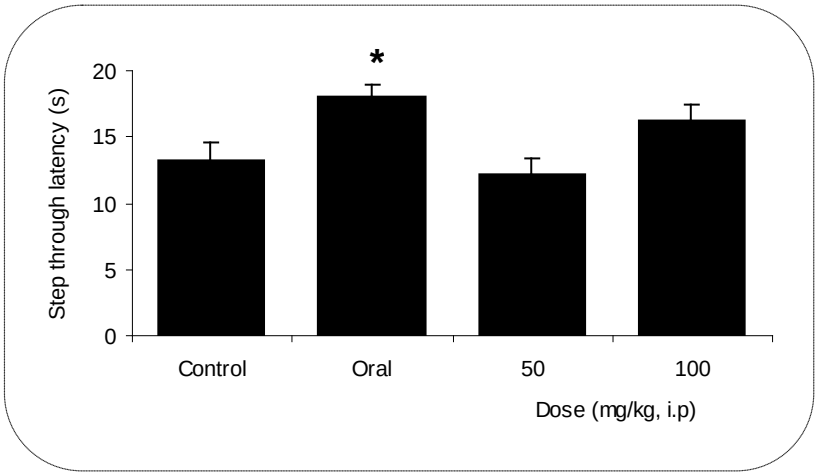

Figure 2. The effect of oral and intraperitoneal administration of *Zingiber officinalis* L. on STL. According to the figure, there is no significant difference between the oral and control groups. Bars show Mean±S.E.M. Number of samples in each group was 12 animals. * shows $p<0.05$ in comparison with control group.

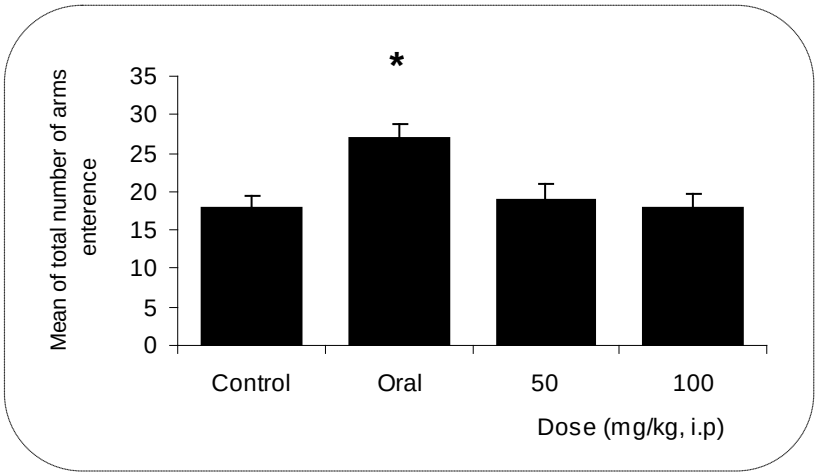

Figure 3. The effect of oral and intraperitoneal administration of *Zingiber officinalis* L. on the mean entrance to Y maze arms. According to the figure, a significant difference was seen between the oral and control groups. Bars show Mean±S.E.M. Number of samples in each group was 12 animals. * shows a significant difference with control group.

3.5. The effect of *Zingiber officinalis* L. on spatial recognition memory

According to figure 4, intraperitoneal administration of *Zingiber officinalis* L. extract at a dose of 100 mg/kg and its oral consumption caused an alternation behavior percentage of 87.3±5.25 and 78.6±4.88, respectively, which was significant ($p<0.05$) as compared to the control group (58.88±6.41)

4. Discussion

Edible and chronic prescription of *Zingiber officinalis* L. at a weight ratio of 1.15 caused a significant change in animals' weight. Intraperitoneal injection of the plant extract had no significant effect on animals' weight. About acute injection, there was not enough time for weight change, but about weight change following its chronic use, there is possibly enough time for weigh change. Bhandan and colleague in 1998 in an experiment working on rabbits with high-fat diet found that *Zingiber officinalis* L. causes a significant amelioration in serum lipid parameters and also causes a decrease in atherosclerosis level in comparison with control group (13). On the other hand, Ahmad and Sharma in 1997 in an experiment on rats with normal diet containing 0.5% of *Zingiber officinalis* L. during 4 weeks found that *Zingiber officinalis* L. causes significant amelioration of serum lipids in comparison with control group (14), which is justifiable according to *Zingiber officinalis* L. adipocyte lipolytic effect.

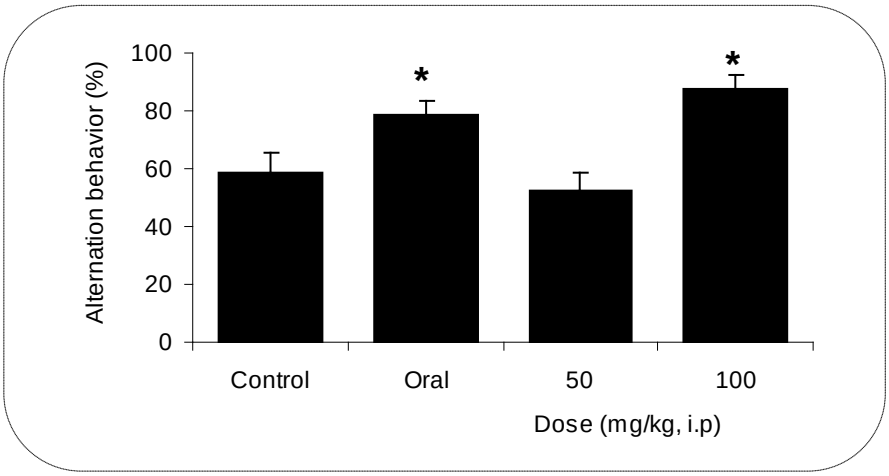

Figure 4. The effect of oral and intraperitoneal administration of *Zingiber officinalis* L. on alternation behavior percentage. According to the figure, there is a significant difference between oral and/or injection group (at a dose of 100 mg/kg) and control group. Bars show Mean±S.E.M. Number of samples in each group was 12 animals. * shows a significant difference with control group.

In our experiments, no significant difference was seen in initial latency of edible and interaperitoneal injection groups in comparison with control group. Regarding STL, it was found out that edible consumption of the plant can increase STL time and as a result improve information recall ability in treated rats. Spatial memory (15) evaluated in Y maze is a valuable test. It was found out that edible and injection use of the plant can cause an increase in alternation percentage. These experimental results are in line with empirical reports of *Zingiber officinalis* L. regarding its enhancer feature (15, 16) and its learning facilitatory effect in another study (15). Based on the latter findings, it has been found out in experimental animals like rats and humans society, impairment in cognitive processes that, memory and learning accompany with dementia and brain atrophy, whether its mechanism has not recognized well, but it is completely clear that brain cortex and hippocampus are two areas of the brain that involve in this process and flowing to improvement and destruction of memory affected vigorously (17). Also, exacerbation of oxidative stress and lipid peroxidation in some regions of brain, especially in hippocampus (18) and besides a decrease in insulin like growth factors and brain-derived neurotrophic factor in

some areas of brain could be effective (via affecting synapses and synaptic conductance) on information acquirement, saving and recall (16, 19). Also, cholinergic system activity is responsible for important processes like memory and information consolidation in hippocampus (12). In terms of electrophysiology, it is found out that cholinergic system activity has an important role in LTP that itself at molecular level affects memory improvement and learning (20). As mentioned above, it can be expressed that the source of all above processes is enhancement of cholinergic system activity, especially at levels of brain cortex and hippocampus. According to chemical compounds in *Zingiber officinalis* L. and intense inhibitory activity of these compounds in inhibition of acetylcholine esterase, level of acetylcholine would be increased (21) that cause memory improvement with the mentioned mechanism. Besides, Masuda and colleagues in an investigation in 1997 on liver microsomes noticed that *Zingiber officinalis* L. has a compound called casumonin-A which is extracted from Corcomoid complex of *Zingiber of casumonin* and has strong antioxidant effect (22). Also, Reddy and lokesh in an experiment in 1992 on rat liver microsomes noticed that zynjron which is a form of *Zingiber officinalis* L. inhibits lipid peroxidation at high doses (23). Additionally, according to important role of peroxidases in memory destruction and booster effect of *Zingiber officinalis* L. as booster of several anti-oxidants (superoxide dismutase, catalase and glutathione peroxidase) and reducer of free radicals, *Zingiber officinalis* L. could have an important role in memory improvement (22,23). Also, there are some reports on anti-inflammatory effects of *Zingiber officinalis* L. by inhibition of cyclooxygenase and 5-lipooxygenase and subsequently reduction in synthesis of prostaglandins and leukotriene (24). Besides, other reports show that 6-gingrol as the main part of *Zingiber officinalis* L. causes a remarkable decrease in 12-o-tetrad canoil-phrbol-13asetate (TPA) which increases in ear inflammation (25). Moreover, according to the importance of inflammatory process in progress of memory destruction and dementia disease (26) and successful use of non-steroidal anti-inflammatory drugs (NSAID) in alleviation of this phenomenon, anti-inflammatory effect of this plant is responsible for its memory booster effect (27). According to this fact that glutaminergic system and NMDA receptors have important roles in memory consolidation by increasing intracellular calcium level and development of LTP phenomenon at synaptic level, especially in hippocampus (28), arguably it could be said that this plant indirectly cause LTP phenomenon in some brain levels by stimulation of NMDA receptors which have important effect on memory by glutaminergic system (29).

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