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# Effect of *Zingiber Officinale* (Ginger Rhizomes) Hydroethanolic Extract on Hyoscine-Induced Memory Impairment in Adult Male Rats

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## ABSTRACT

**Background:** The spice *Zingiber officinale* or ginger possesses antioxidant activity and neuroprotective effects. In this study we hypothesized that treatment with hydroethanolic extract of ginger (50, 100 and 200 mg/kg, p.o) would effect on the hyoscine-induced memory impairment in rats.

**Methods:** In this experimental study 64 male Wistar rats were divided into eight groups (8 rats in each group): normal saline, hyoscine (1 mg/kg), ginger extract (50, 100 and 200 mg/kg), or hyoscine (1 mg/kg) plus ginger extract (50, 100 and 200 mg/kg). Memory impairment was induced by a single injection of hyoscine (1 mg/kg, i.p). Cognitive functions were evaluated using passive avoidance learning (PAL) task. Retention test was carried out 24 h after training, and the latency of entering the dark compartment [step-through latency (STL)] and the total time in the dark compartment (TDC) were recorded. All statistical analysis was carried out at 5% level of significance using SPSS version 21. The data were analyzed by ANOVA followed by Tukey's test.

**Results:** The time latency in hyoscine-treated group was lower than control (133.87±14.60 vs. 242.12±10.58; $p<0.001$, respectively). Treatment of the animals by 100 and 200 mg/kg of ginger extract before the training trial increased the time latency at 24 h after the training trial (277±4.67 and 280.37±7.68; $p<0.01$, respectively). Administration of both 100 and 200 mg/kg doses of the extract in hyoscine received animal groups before retention trials also increased the time latency than the hyoscine-treated groups (247.37±7.62 and 271.87±9.11; $p<0.001$, respectively).

**Conclusion:** The results revealed that the ginger hydroethanolic extract attenuated hyoscine-induced memory impairment.

**Keywords:** Memory; Alzheimer Disease; Ginger; Hyoscine; Rat

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## INTRODUCTION

Dementia is characterized by a progressive decline in cognitive function depending on neurodegeneration, which particularly affects elder population in their daily activities such as memory, speaking, and problem dissolving. The most well-known type of dementia is Alzheimer's disease (AD), which proceeds at stages from mild and moderate to severe and gradually destroys the

brain. Brain aging is known to be related to excessive neuronal loss, decrease in Acetylcholine level, increase in inflammation, and oxidative stress.[1] There are some hypotheses to explain pathogenesis of the disease such as cholinergic hypothesis and amyloid formation hypothesis. Nowadays, the most accepted treatment strategy in AD has been accepted as cholinesterase inhibitors that can inhibit Acetyl cholinesterase (AChE) enzyme in order to increase Acetylcholine level in the brain.[2]

Acetylcholine (ACh) is known to regulate the learning and memory process.[3] It was reported that ACh activity was increased in the training of spatial memory performance and it was decreased in memory deficit.[4] Scopolamine (Hyoscine-N-Butyl bromide), an anti-cholinergic agent, is used as a standard drug for inducing cognitive deficits in healthy humans and animals.[5] It is a muscarinic receptor antagonist with amnestic properties and is a widely used model for characterizing potential cognition enhancing drugs.[6] Blockade of central muscarinic receptors may induce a pattern of cognitive impairment in Alzheimer disease (AD) patients.[7] Therefore, scopolamine challenge is very useful for investigating learning and memory studies in which the cholinergic system is involved.[8]

*Zingiber officinale* Roscoe (Zingiberaceae), or ginger, is one of the frequently used spices in the world. Ginger has been cultivated for thousands of years and used safely in cooking, and medicinally in folk and home remedies. It is used extensively in traditional medicine to treat cold, fever, headache, nausea and digestive problems; and it is also used in Western herbal medical practices for the treatment of arthritis, rheumatic disorders and muscular discomfort.[9] It has been reported that ginger or its extracts possess some pharmacological activities including analgesic, antioxidant, anti-inflammatory, anticonvulsant and neuroprotective effect.[10] In folklore medicine, including Ayurveda and traditional Chinese medicine ginger has been reportedly used for the management/ treatment of Alzheimer's disease either as ginger extract, ginger tea or as inclusion in food formulations and preparation.[11] Numerous experimental studies and clinical observations indicate that ginger influences some central nervous system effects,[12] but accurate mechanisms of its action are not precisely known. Although ginger has been an important ingredient in folk medicine for the treatment of Alzheimer's disease nevertheless, limited information is available on the possible mechanism that ginger renders its anti-Alzheimer properties. Therefore, with regard to the possible effects of ginger on learning and memory and its interactions with cholinergic system, the aim of the present study was to evaluate the effect of hydroethanolic extract of *Z. officinale* on scopolamine-induced memory impairment in rats using the passive avoidance test.

## MATERIAL AND METHODS

2.1. Plant material and preparation of extracts

The fresh rhizomes of ginger (herbarium code no. 1483) was purchased from the Institute of Medicinal Plants Tehran, Iran. The plant material was dried with room temperature (25°C), shade dried and powdered. Approximately 500 g of the dried powder from ginger were extracted with 5 liters of 80% aqueous ethanol using the percolation method at room temperature. The extracts were filtered through filter paper and evaporated to dryness under reduced pressure at a maximum of 40°C using a rotary evaporator. *Z. officinale* yielded 43.25% dried extract. The hydroethanolic extract of ginger was stored in small samples at -20°C until use. The extract was dissolved in saline and was then applied.

### Drugs

The drugs used in the present study was hyoscine-N-butyl bromide (scopolamine) (Osvah, Tehran, Iran). Hyoscine dissolved in physiologic saline solution.

### Animals

In this experimental study 64 locally produced male Wistar rats (250–280 g) from the Iranian Razi Institute, were used in the present experiments. All animals were maintained at a constant temperature (22±2°C) and on a 12 h light/dark cycle. They had free access to laboratory chow and tap water. The animals were maintained under laboratory conditions for an acclimatization period of 7 days before performing the experiment. Each experimental group consisted of eight animals that were chosen randomly from different cages. Each rat was used only once. Animals were handled in accordance with the criteria outlined in the Guide for the Care and Use of Laboratory Animals (National Institutes of Health (NIH) publication 86–23; revised 1985).

### Experimental design

The animals were divided into eight equal groups (n= 8); (1): Control group received normal saline (p.o by gavage), (2): Control group received scopolamine at dose 1 mg/kg via i.p injection 30 min before training, (3-5): groups received ginger extract at doses 50, 100 and 200 mg/kg (p.o by gavage) 30 min before training, (6-8): groups received scopolamine at dose 1 mg/kg and ginger extract at doses 50, 100 and 200 mg/kg (p.o by gavage)

30 min before training. Extract was administered 15 min before scopolamine and 45 min before the training. 30 min after the latest treatment, learning and memory were evaluated. The operator was unaware of the specific treatment groups to which an animal belonged.

**Passive avoidance task**

The apparatus and procedure were basically followed by previous study.[13] The apparatus used for evaluation of the passive avoidance task was two-way shuttle box (Borj Sanaat Co. Iran), which consisted of two adjacent Plexiglas compartments of identical dimensions (27×14.5×14 cm). For the experimental procedure, on the first day (adaptation day) each rat was allowed a 3 min adaptation period and free access to either the light or dark compartment of the box to avoidance training and after being placed in a shuttle box. Following this adaptation period, on the second day (training phase), rats were placed in the illuminated compartment and 30 sec later the sliding door was raised. Upon entering the dark compartment, the door was closed and a 1.5 mA constant-current shock was applied for 3 sec. After 20 sec, the rat was removed from the dark compartment and placed into home cage. In order to test short- and long-term memories, 24 h after receiving foot shock, the rats were placed in illuminated chamber and 30 sec later the sliding door was raised and the latency of entering the dark compartment (step-through latency) and the time spent there during 5 min were recorded again, because the maximum time that was considered in this procedure was 300 sec.

**Statistical analysis**

The data are expressed as mean values with their standard errors. Analysis of data was performed using one-way and two-way ANOVA. Following a significant *P*-value, *post hoc* analysis (Tukey's test) was performed for multiple comparison. Statistical analysis was performed using SPSS (version 21; SPSS Inc., Chicago, IL, USA). A level for $p<0{\cdot}05$ was considered to be significant.

**RESULTS**

The retention test which was conducted 24-h after training. The rat was placed in the lighted chamber as during PAL training. 30 s later, the guillotine door was raised, and the step-through latency during the retention trial (STL) and the time spent in the dark compartment (TDC) were recorded up to 300 s. If the rat did not enter the dark compartment within 300 s, the retention test was terminated and a ceiling score of 300 s was assigned. It revealed a significant difference in the STL among the groups (Figure 1). There was a significant difference in STL between saline treated control groups and ginger treated groups at dose 100 and 200 mg/kg ($p<0.01$ and $p<0.001$, respectively). But there was no significant difference in STL between extract treated group at dose 50 mg/kg and control group. Specifically, the STL of scopolamine-treated group at dose 1 mg/kg were significantly lower than the saline control group ($p<0.001$). Administration of ginger at doses 100 and 200 mg/kg to animals that received scopolamine (1 mg/kg) resulted in longer STL compared to scopolamine-treated animals ($p<0.001$). There was a significant difference in STL between extract treated at dose 200 mg/kg that received scopolamine and saline treated control groups ($p<0.05$). Also there was a statistically significant difference in TDC among the experimental groups (Figure 2).

Consistent with a cognitive impairment, TDC of the scopolamine-treated rats was greater than the saline treated control group ($p<0.01$). Time spent in the dark compartment in the groups that treated extract at doses 100 and 200 mg/kg was significantly less than the respective saline treated control group ($p<0.01$). Also it was a significant difference in TDC between ginger extract treated at doses 100 and 200 mg/kg that received scopolamine and scopolamine-treated groups ($p<0.001$). There was a statistically significant difference in TDC between extract treated at dose 50 mg/kg that received scopolamine than the extract treated groups at doses 100

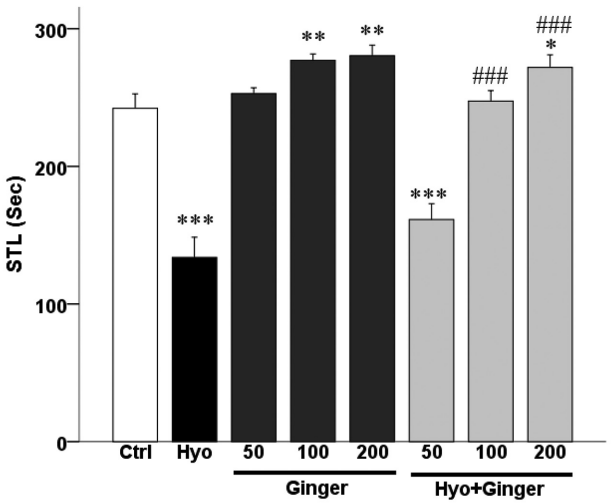


**Figure 1.** Effect of ginger on the step-through latency in the training (STL) of passive avoidance learning (PAL) task in the rats. Columns represent mean ± SEM.
* $p<0.05$, ** $p<0.01$, *** $p<0.001$ as compared with its related Control (Ctrl) group.
### $p<0.001$, as compared with its related hyoscine (Hyo) received group.

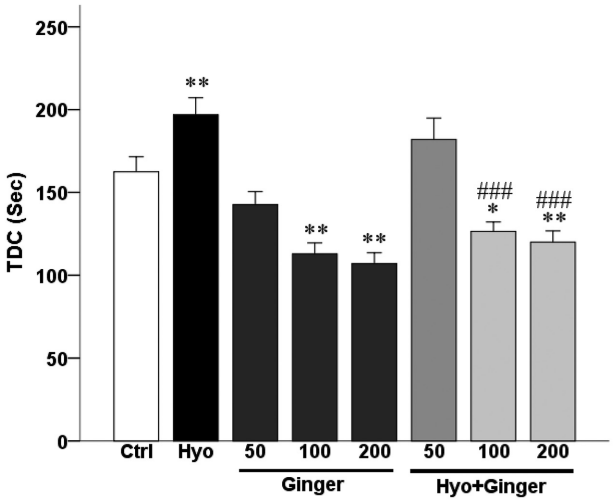


**Figure 2.** Effect of ginger on the time spent in the dark compartment in the training (TDC) which was carried out 24 h after acquisition of passive avoidance learning (PAL) task in the groups. Columns represent mean ± SEM.
* $p<0.05$, ** $p<0.01$ as compared with control (Ctrl) group.
### $p<0.001$ as compared with hyoscine (Hyo) treated group.

and 200 mg/kg that received scopolamine ($p<0.001$). TDC in the group that treated extract at doses 50 mg/kg was significantly less than the respective extract treated group at doses 50 mg/kg that received scopolamine ($p<0.05$).

## DISCUSSION

Cholinergic neurons in the basal forebrain and hippocampus, and acetylcholine as a major neurotransmitter in these neurons, have important roles in learning and memory processes.[14] Age-related dementia and memory deficit observed in AD, are correlated with the loss of cholinergic neurons in the basal forebrain and hippocampus.[15] In addition, pharmacological blockage of cholinergic neurons in these areas causes impairment of memory and learning in experimental animals.[16] Several studies have employed scopolamine; a nonselective muscarinic receptor antagonist, treated animals as a test model of cognitive function,[5] and this model has been widely used to investigate the role of the cholinergic system in learning and memory and test new substances, designed to treat cognitive dysfunction.[3,17] In the present study, preventive effect of ginger on learning and memory deficits was investigated using a scopolamine-induced memory impairment rat model. The results of the present study show that treatment with 50, 100 and 200 mg/kg ginger improved passive avoidance learning (PAL) and memory of control rats and alleviated the negative influence of scopolamine on learning and memory. The decrease in the number of trials to acquisition in the PAL task is evidence of an improvement in memory acquisition. The increase in STL and decrease in TDC during the retention test demonstrates facilitatory effects on memory retention.[18]

It has been shown that the degree of memory impairment is related to the degree of cholinergic loss in early Alzheimer's disease.[19] These findings are further supported by evidence that ACh modulates exploratory behavior in animals,[20] and a host of pharmacological studies showed that manipulating cholinergic transmission affects learning and memory in humans[21] and animals.[22] Further, it is reported that cholinergic transmission is associated with the performance of memory and cue detection.[23] Our current experiments expand on these reports and demonstrate the potential for ginger to protect against memory impairment in scopolamine-induced animals. The results are quite promising. Administration of ginger extract clearly prevented the learning and memory impairments caused by scopolamine administration. Indeed, administration of 50, 100 and 200 mg/kg ginger to scopolamine-induced animals reversed the increased number of trials to acquisition, indicative of a preventive effect of the treatment on acquisition deficits. In the retention trial, decreased STL and increased TDC of diabetic rats were also reversed by ginger treatment.

The determined acetylcholinesterase (AChE) inhibitory activity agreed with some earlier reports where plant phytochemicals from *Citrus medica* inhibited acetylcholinesterase (AChE)[24] and plants extracts of *Ginkgo biloba* and *Salvia lavandulaefolia*, showed a significant improvement in cognitive performance and memory.[25] In the past, different studies have shown that ginger possesses anxiolytic properties,[26] improves inhibitory avoidance learning,[27] facilitates spatial learning along with reducing oxidative stress[28] and inhibits β-amyloid peptide-induced cytokine and chemokine expression in monocytes, thus delaying the onset and progression of neurodegenerative disorders.[29] Moreover, this plant extract and its active component, [6]-gingerol, also inhibited the cholinesterase activity which in turn increased acetylcholine, a neurotransmitter that plays an important role in learning and memory.[30] Therefore, taking all data together, we suggest that the cognitive enhancing effects of ginger might be partly associated with the modulation effect of this plant extract on the alteration of both the monoamine system and the cholinergic system in various brain areas, including the prefrontal cortex and hippocampus. However, the precise underlying mechanism and possible active ingredient responsible for the cognitive enhancing effect of ginger still require further investigation.

## CONCLUSION

In conclusion in the present study we demonstrated that ginger hydroethanolic extract could attenuated scopolamine-induced memory impairment in rats. Ginger extract may provide a new potential alternative for prevention of the neurodegeneration and impaired cognitive functions, and may warrant further clinical studies.

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## CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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