

Morphological Effects of Hydroalcoholic Zingiber Officinalis Extract in the Murine Hippocampus of Male Rat Offspring

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Abstract

Background: The hippocampus is responsible for memory. A diet full of antioxidants improves brain damage and cognitive function. Regard the antioxidant effects of zingiber officinalis (ginger) and its flavonoids components.

Objectives: The aim of this study was to evaluate the effect of the extract of ginger on memory by using hippocampus tissue of the male offspring of rats.

Materials and Methods: In this study, 60 rats, 15 males and 45 females, were used. We separated pregnant female rats from males on the first day of pregnancy (determined by vaginal plug), and during days 16 - 18 of pregnancy, via intraperitoneal injection, three groups received hydroalcoholic extract of ginger, with low (200 mg/kg bw), medium (400 mg/kg bw), and high (800 mg/kg bw) concentration doses. The control group did not receive anything, and the sham group received normal saline during these days. Then at day 50, the males offspring in each group were sacrificed, their brains were removed, and the hippocampus sections were prepared for microscopic studies. Data was analyzed by SPSS 20 and by using one-way ANOVA and then a Tukey post-test ($P < 0.05$ considered as the significance level).

Results: This research showed that the number and thickness of pyramidal and granular layers of the CA1 and dentate gyrus areas of the hippocampus had increased in male offspring according to the increase in the ginger extract dose.

Conclusions: It seems as though ginger extract, which contains compounds such as gingerols, shogaols, and zingerone, can affect memory ability in rats through these compounds' antioxidant properties by affecting embryonic acetylcholine content and place cells.

Keywords: Ginger, Memory, Offspring, Male Rats, Hippocampus

1. Background

The ability to remember is one of the clear features of humans and is a necessity for survival and living normally. An increase in the number of memory disorders during aging has created an incentive to research and discover new medications that boost memory and prevent disorders (1).

Memory involves extensive changes in the structure and functions of the nervous system, mainly exclusive to the synapses involved in the pathways of messages and information in the sensory nervous system. Structural changes occur, including changes in the number of synapses, changes in the extent of the postsynaptic membrane at contact, and physiological changes, including changes in the ionic conductivity of the presynaptic and postsynaptic membranes (2).

Several structures are involved in the brain at different stages of memory. Studies show that during the reading of a text (or the encoding), most of the left hemisphere of

the brain is engaged, while during recall and the remembering of information, most of the right hemisphere of the brain is activated. As well, a part of the brain called the hippocampus has an effect only on long-term memory but not on short-term memory; the majority of researchers in the field of neuroscience believe that the processes associated with short-term memory occur in the frontal area of the brain (3).

A diet full of antioxidants improves brain damage and enhances cognitive function (4), and recent studies have demonstrated that ginger produces both antioxidant activity and neuron-protection activity (5, 6).

Ginger is found in almost half the medicinal combinations of Asian countries (7). The results of studies of plant medicinals with respect to forgetfulness showed that ginger has a mutual efficiency on the cyclooxygenase enzyme, and it is effective with respect to memory loss and Alzheimer's disease (8).

Ginger is effective in treating diseases such as

Alzheimer's disease, irritable bowel syndrome, motion sickness, morning sickness, indigestion, anorexia, joint diseases, and diseases of the upper respiratory tract (9,10). It is also an analgesic, (11) an antiviral, (12) an antimigraine remedy, (13) and a bile secretion regulator, (11) and it has positive inotropic (14) and antitumor effects (15).

It also appears that ginger prevents the synthesis of prostaglandin E2 and thromboxane B2 and affects serotonin receptors (16).

2. Objectives

Some mothers consume ginger during pregnancy in order to relieve nausea and vomiting, but no research has yet been conducted with respect to consumption of ginger during pregnancy and its relation to memory. Thus, the purpose of this study was to investigate the effect of embryonic ginger on memory in mature male rats.

3. Materials and Methods

3.1. Preparing the Extract

First, 1 kg fresh *Zingiber officinalis* L was prepared by an expert botanist. Then the sample was grated and dried at room temperature and converted into dried powder by a mechanical grinder. Of the dried ginger powder, 36 g was macerated in 80% ethanol for 72 hours. The resulting mix was filtered by filter paper, and the filtrate was concentrated using a rotary evaporator at an optimum temperature of 40 - 50°C (17).

3.2. Animals and Experimental Groups

This experimental study used 45 adult female rats and 15 adult male Wistar rats, with an average weight of 220 - 200 g, aged 8 - 9 weeks. They were kept at $20 \pm 2^\circ\text{C}$ in a 12-hour light/12-hour dark cycle and humidity of 50 to 55%, with free access to water and special feed. After two weeks in these conditions, three female rats were placed in a cage for one night with one male rat. A visible vaginal plug the following morning represented the first day of mating and determined the first gestational day (GD0).

Thirty pregnant female rats were randomly divided into five groups. According to the test method, from the 16th day (GD16) until the 18th day (GD18) of pregnancy (the period during which the hippocampus is developed), three of the groups received different intraperitoneal doses of ginger extract at 10 a.m. The concentrations of the doses were low (200 mg/kg bw), medium (400 mg/kg bw), and high (800 mg/kg bw); meanwhile, the control group did not receive any injection, and the sham group received normal distilled water (18, 19). All of the

groups were allowed to pass the course of pregnancy, and the mature infants were born after 21 days. Then at day 50, the male offspring in each group were sacrificed.

3.3. Histological Studies and Preparation of Tissue Dissection

In order to study potential changes in the hippocampus tissue of the rats, the brain tissues were separated and histological studies were conducted by microscopic observations. After removal of the brain from the body, the brains were kept in a 10% formalin solution for 48 hours.

After 24 hours in the formalin solution, the samples were brought out of solution and were cut into small sections. The sections were placed in special baskets of a tissue processor device in which the tissues passed through the following steps. First, the tissues were completely fixed by placement in a 10% formalin solution. The next steps involved placing the sections in 50% alcohol, then 70%, 80%, and 96%, and finally, the samples were placed in absolute alcohol. They were completely dehydrated after passing through these steps. Then the samples were placed in a container of a xylene solution and finally in liquid paraffin. The tissue sections were placed on glass slides and sectioned using a microtome device (4-5 micron thickness). A hematoxylin and eosin staining method was used to stain the samples, and then entellan glue was placed on the tissue.

The sections were imaged to ascertain the number and thickness of the pyramidal and granular layers of areas CA1 and DG.

3.4. Statistical Analysis

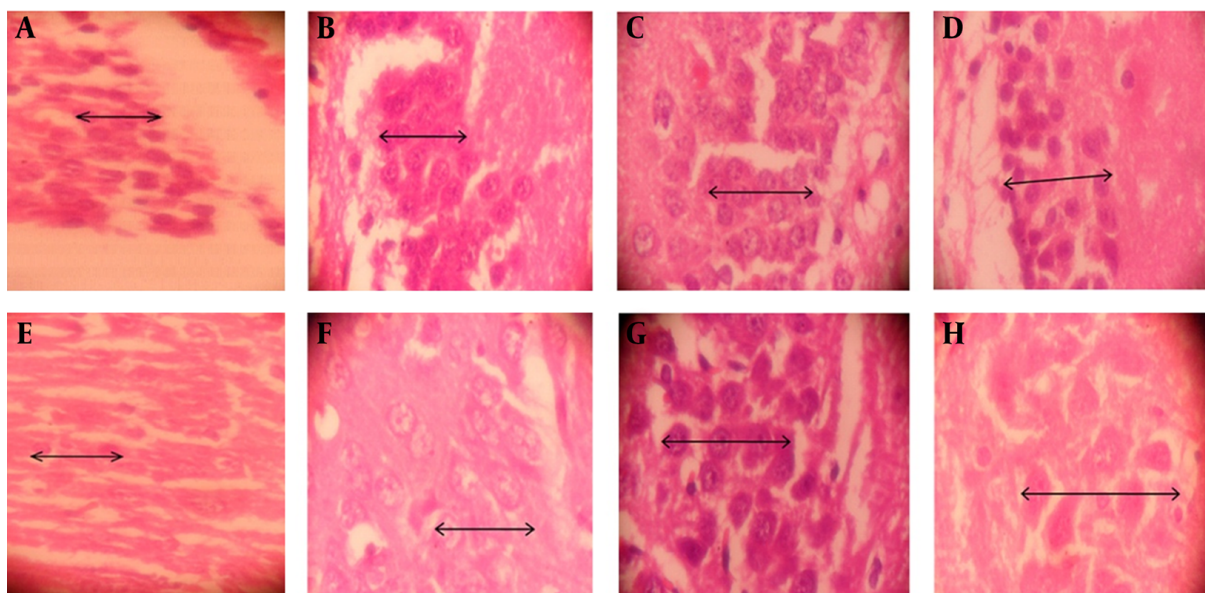
The data was analyzed by SPSS and by using one-way ANOVA and a Tukey post-test. $P < 0.05$ was considered as the significance level.

4. Results

After studying and measuring the tissues taken from the offspring who were recipients of ginger, in comparison with the control and sham groups, it was observed that the number and thickness of the granular layers and pyramidal layer of the CA1 area and the dentate gyrus had increased quite noticeably, with the increment in the number and thickness of this layer having a direct relation to the ginger extract doses (Figure 1, A - D granular layers, E - H pyramidal layer).

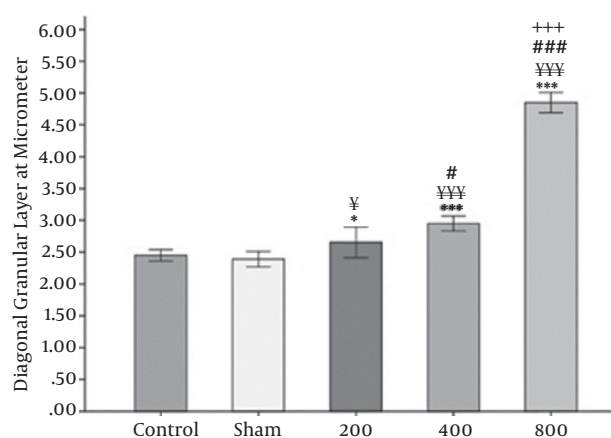
4.1. Granular Layer of the Hippocampus

In the infant male Wistar rats, we investigated the data acquired by measuring the thickness of the granular layer in the control group, in the sham group (that had received

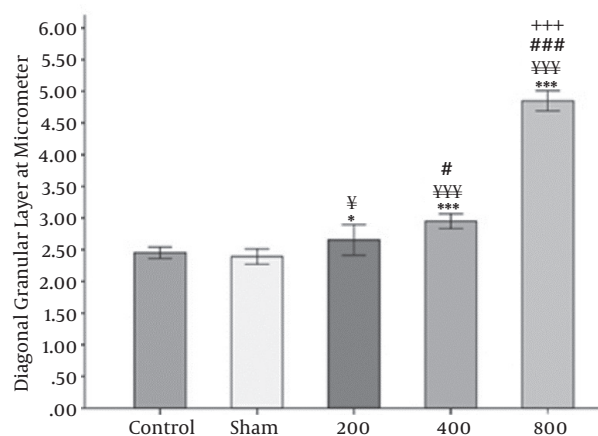
Figure 1. Hippocampus's Granular Layer

Staining (H&E), 100X zoom: A (control group), B (low dose), C (medium dose), D (high dose). Hippocampus's pyramidal layer: E (control group), F (low dose), G (medium dose), H (high dose).

distilled water), and in the groups that received the hydroalcoholic extract of ginger in high, average, and low doses (Figure 2), and we measured the number of granular layers in each group (Figure 3).

Figure 2. Investigating the Data Achieved by Measuring Thickness of the Granular Layer in Control Group, Sham Group, and Groups Receiving Hydroalcoholic Extract of Ginger in High, Average, and Low Doses

* Represents significance compared to the control group, ¥ the sham group (receiving distilled water), # the group that received a low dose of the extract. (***: $P < 0.001$), (¥¥¥: $P < 0.001$), (###: $P < 0.001$).

Figure 3. Investigating Data Acquired by Measuring the Number of Granular Layers in Control Group, Sham Group, and Groups Receiving Hydroalcoholic Extract of Ginger in High, Average, and Low Doses

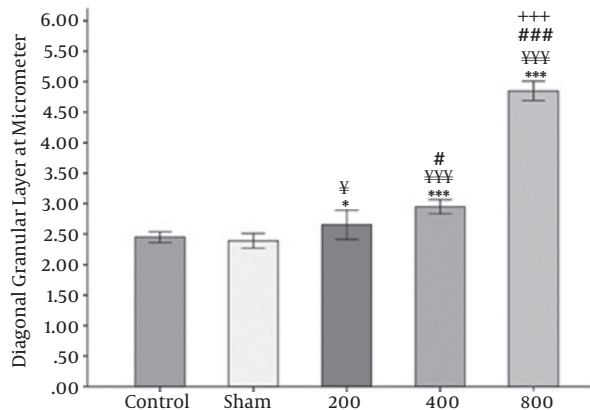
* Represents significance compared to the control group, ¥ the sham group (receiving distilled water), # the group that received a low dose of the extract. (***: $P < 0.001$), (¥¥¥: $P < 0.001$), (###: $P < 0.001$).

4.2. Pyramidal Layer of the Hippocampus

In the infant male Wistar rats, we investigated the data acquired by measuring the thickness of the pyramidal layer in the control group, in the sham group (which had received distilled water), and in the groups that received

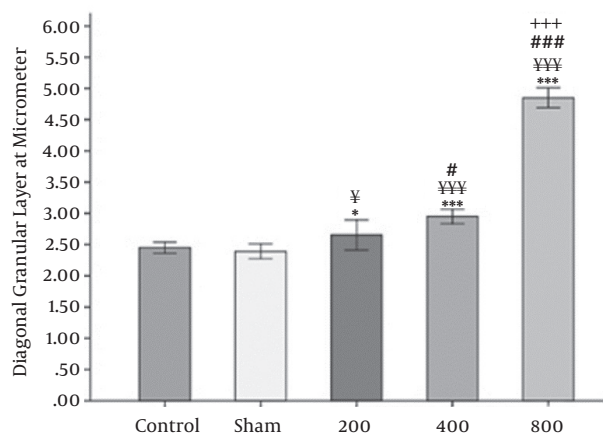
the hydroalcoholic extract of ginger in high, average, and low doses (Figure 4), and we also measured the number of pyramidal layers in each group (Figure 5).

Figure 4. Investigating Data Acquired by Measuring Thickness of the Pyramidal Layer in Control Group, Sham Group, and Groups Receiving Hydroalcoholic Extract of Ginger in High, Average, and Low Doses in Male Wistar Rat Infants



* Represents significance compared to the control group, ¥ the sham group (receiving distilled water), # the group which received a low dose of the extract. (**: $P < 0.001$), (¥¥¥: $P < 0.001$), (###: $P < 0.001$).

Figure 5. Investigating Data Acquired by Measuring Number of Pyramidal Layers in the Control Group, Sham Group, and the Groups Receiving Hydroalcoholic Extract of Ginger in High, Average, and Low Doses



* Represents significance compared to the control group, ¥ the sham group (receiving distilled water), # the group that received a low dose of the extract. (**: $P < 0.001$), (¥¥¥: $P < 0.001$), (###: $P < 0.001$).

5. Discussion

Memory construction in the hippocampus may be affected strongly by hormones, drugs, and other substances. The conditions and environmental characteristics in which a mammal is located can also affect the formation of spatial memory. O'Keefe suggested that some pyramidal cells in hippocampal circuits are only engaged in processing spatial and locational information, calling them "Place cells" (20). Later, several human and animal studies showed that the role of the hippocampus in spatial learning and memory consolidation was more prominent (3).

Due to the increase in the number of hippocampus cells in the rats, it may be concluded that the effects of ginger on different areas of the hippocampus result in an increase in the memory.

Ginger extract contains fragments such as flavin and flavonoids. Flavonoids are capable of acting as antioxidants and may improve catalytic efficiency as allosteric factors; as a result, they reduce the production of free radicals. The protective effects of flavonoids depend on the hydrogenation ability of free radicals (21). It has also been observed that flavonoids strongly protect muscarinic receptors against oxidative factors (22).

Studying rats has shown that ginger significantly decreases peroxidation lipids and increases antioxidant enzymes such as glutathione. Studies have also shown that ginger, as well as ascorbic acid, has antioxidant effects (23) and that using antioxidants can reduce neuronal damage and prevent the oxidation of neuronal proteins, prevent DNA damage, and prevent the peroxidation of membrane lipids. Hence, the use of substances containing antioxidants has great importance in memory enhancement (24).

Ginger has an inhibiting effect on acetylcholinesterase, sodium nitroprusside- (SNP), and quinolinic acid (QA)-induced lipid peroxidation in the rat brain. SNP and QA cause a significant increase in the malondialdehyde (MDA) contents of the brain. However, extract of ginger significantly decreases the SNP and QA and elevates brain MDA contents. Ginger has an inhibitory effect on acetylcholinesterase activities, and some prooxidant-induced lipid peroxidation in a rat's brain may be attributed to the presence of phytochemicals such as flavonoids, tannins, alkaloids, and terpenoids (25).

Cholinergic branches of the brain's basal nucleus (NBM), amygdala, and frontal cortex have roles in spatial learning and memory, and the actions of choline and acetylcholine are very important during the development of the brain and nervous system. In fact, acetylcholine is a regulatory cofactor for many processes associated with the development of the brain and nervous system, and choline containing during an embryo's development adjusts the

amount of learning and memory (26, 27).

Arachidonic acid is a 20-carbon unsaturated fatty acid, a part of cell membrane phospholipids. It is the main precursor of the synthesis of prostanoids, which are in turn precursors of important hormones. The phospholipase enzyme converts cell membrane phospholipids to arachidonic acid. Cyclooxygenase enzyme, then, it converts arachidonic acid to prostaglandins and thromboxane. Lipoxygenase causes the conversion of arachidonic acid to leukotrienes.

Ginger prevents the synthetization of prostaglandin E2 (EGP2) and thromboxane B2 (TBX2). Ginger also prevents thromboxane synthetization, which affects serotonin receptors (10, 16).

Experimental studies have shown that ginger has anti-inflammatory effects and prevents arachidonic acid metabolism by inhibiting cyclooxygenase and lipoxygenase pathways. Thus, it is possible that ginger's anti-inflammatory effects are due to the inhibition of the production of prostaglandins and leukotrienes (28).

Shogaols, other fragments of ginger, have neural protective effects and also cause increasing intestinal blood flow (29). So it may be concluded that ginger facilitates the blood cycle between mother and embryo and that it causes better brain growth of the offspring.

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Footnote

Authors' Contribution: Study concept and design, analysis and interpretation of data, and critical revision of the manuscript for important intellectual content: Fariba Ghodrati, Mino Mahmoodei, Siamak Shahidi, Hamid Reza Ghadimipoure; acquisition of data, statistical analysis: Fariba Ghodrati; administrative, technical, and histological study: Hamid Reza Ghadimipoure, Fariba Ghodrati; study supervision: Mino Mahmoodei. Advisor: Siamak Shahidi.

References

- Barnes C, Joe M, Raymond K. Memory changes during normal aging. New York: Academic Press; 1998.
- Bracciali A, Brunelli M, Cataldo E, Gao DD, Li Q, Fan Y. Hypoglycemic effects and mechanisms of *Portulaca oleracea* L. in alloxan-induced diabetic rats. *J Medicinal Plants, Research*. 2010;4(19):1996–2003.
- Squire LR. Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. *Psychol Rev*. 1992;99(2):195–231. [PubMed: 1594723].
- Bisson JF, Nejdi A, Rozan P, Hidalgo S, Lalonde R, Messaoudi M. Effects of long-term administration of a cocoa polyphenolic extract (Acticoa powder) on cognitive performances in aged rats. *Br J Nutr*. 2008;100(1):94–101. doi: 10.1017/S0007114507886375. [PubMed: 18179729].
- Waggas AM. Neuroprotective evaluation of extract of ginger (*Zingiber officinale*) root in monosodium glutamate-induced toxicity in different brain areas male albino rats. *Pak J Biol Sci*. 2009;12(3):201–12. [PubMed: 19579948].
- Sharif-Abad MH, Esfandiari E, Alaei H, Moattar F. Investigation the effect of water extract of frankincense during lactation on increasing of learning ability and memory of male rat's offspring. *J Basic Med Science Iran*. 003;6(3);6(3):207–11.
- Awang C. Ginger. *Pharm J*. 1992;125:309–11.
- Ali SK, Hamed AR, Soltan MM, Hegazy UM, Elgorashi EE, El-Garf IA, et al. In-vitro evaluation of selected Egyptian traditional herbal medicines for treatment of Alzheimer disease. *BMC Complement Altern Med*. 2013;13:121. doi: 10.1186/1472-6882-13-121. [PubMed: 23721591].
- Nievergelt A, Huonker P, Schoop R, Altmann KH, Gertsch J. Identification of serotonin 5-HT_{1A} receptor partial agonists in ginger. *Bioorg Med Chem*. 2010;18(9):3345–51. doi: 10.1016/j.bmc.2010.02.062. [PubMed: 20363635].
- Agarwal P, Alok S, Fatima A, Singh PP. Herbal Remedies For Neurodegenerative Disorder (Alzheimer's Disease). *Int J Pharm Sci Res*. 2013;3328–40.
- Xiaorui Z. Who manographs on selected medicinal plants. Geneva: World health organization; 1999.
- Mascolo N, Jain R, Jain SC, Capasso F. Ethnopharmacologic investigation of ginger (*Zingiber officinale*). *J Ethnopharmacol*. 1989;27(1-2):129–40. [PubMed: 2615416].
- Mustafa T, Srivastava KC. Ginger (*Zingiber officinale*) in migraine headache. *J Ethnopharmacol*. 1990;29(3):267–73. [PubMed: 2214812].
- Shoji N, Iwasa A, Takemoto T, Ishida Y, Ohizumi Y. Cardiotonic principles of ginger (*Zingiber officinale* Roscoe). *J Pharm Sci*. 1982;71(10):1174–5. [PubMed: 7143220].
- Park KK, Chun KS, Lee JM, Lee SS, Surh YJ. Inhibitory effects of [6]-gingerol, a major pungent principle of ginger, on phorbol ester-induced inflammation, epidermal ornithine decarboxylase activity and skin tumor promotion in ICR mice. *Cancer Lett*. 1998;129(2):139–44. [PubMed: 9719454].
- Abdel-Aziz H, Windeck T, Ploch M, Verspohl EJ. Mode of action of gingerols and shogaols on 5-HT₃ receptors: binding studies, cation uptake by the receptor channel and contraction of isolated guinea-pig ileum. *Eur J Pharmacol*. 2006;530(1-2):136–43. doi: 10.1016/j.ejphar.2005.10.049. [PubMed: 16364290].
- Anosike C, Obidoo O, Ezeanyika L, Nwuba M. Anti-inflammatory and anti-ulcerogenic activity of the ethanol extract of ginger (*Zingiber officinale*). *Afr J Biochem Res*. 2009;3:379–84.
- Kim HG, Lim S, Hong J, Kim AJ, Oh MS. Effects of Myoga on Memory and Synaptic Plasticity by Regulating Nerve Growth Factor-Mediated Signaling. *Phytother Res*. 2016;30(2):208–13. doi: 10.1002/ptr.5511. [PubMed: 26563629].
- Yiming L, Tran VH, Duke CC, Roufogalis BD. Preventive and Protective Properties of *Zingiber officinale* (Ginger) in Diabetes Mellitus, Diabetic Complications, and Associated Lipid and Other Metabolic Disorders: A Brief Review. *Evid Based Complement Alternat Med*. 2012;2012:1–10.
- O'Keefe J. A review of the hippocampal place cells. *Prog Neurobiol*. 1979;13(4):419–39. [PubMed: 396576].
- Vauzour D, Vafeiadou K, Rodriguez-Mateos A, Rendeiro C, Jeremy PES. The neuroprotective potential of flavonoids: a multiplicity of effect. USA: Springer; 2008.

22. Fawcett J, Bordayo E, Jakson K, Liu J, Peterson J. Inactivation of human brain acetyl cholin receptor by oxidative damage is prevented by bioflavonoids and other antioxidants. *Brain*. 2002;**650**(1-2):10–20.
23. Ahmed RS, Seth V, Banerjee BD. Influence of dietary ginger (*Zingiber officinales* Rosc) on antioxidant defense system in rat: comparison with ascorbic acid. *Indian J Exp Biol*. 2000;**38**(6):604–6. [PubMed: [11116533](#)].
24. Di Luca M, Ruts L, Gardoni F, Cattabeni F, Biessels GJ, Gispen WH. NMDA receptor subunits are modified transcriptionally and post-translationally in the brain of streptozotocin-diabetic rats. *Diabetologia*. 1999;**42**(6):693–701. doi: [10.1007/s001250051217](#). [PubMed: [10382589](#)].
25. Oboh G, Ademiluyi AO, Akinyemi AJ. Inhibition of acetylcholinesterase activities and some pro-oxidant induced lipid peroxidation in rat brain by two varieties of ginger (*Zingiber officinale*). *Exp Toxicol Pathol*. 2012;**64**(4):315–9. doi: [10.1016/j.etp.2010.09.004](#). [PubMed: [20952170](#)].
26. Galasko D, Schmitt F, Thomas R, Jin S, Bennett D, Alzheimer's Disease Cooperative S. Detailed assessment of activities of daily living in moderate to severe Alzheimer's disease. *J Int Neuropsychol Soc*. 2005;**11**(4):446–53. [PubMed: [16209425](#)].
27. Carew TJ, Hawkins RD, Kandel ER. Differential classical conditioning of a defensive withdrawal reflex in *Aplysia californica*. *Science*. 1983;**219**(4583):397–400. [PubMed: [6681571](#)].
28. Mustafa T, Srivastava K, Jeusen K. Drug development reports. *Pharmacol Ginger*. 1993;**6**:25–39.
29. Kim DO, Heo HJ, Kim YJ, Yang HS, Lee CY. Sweet and sour cherry phenolics and their protective effects on neuronal cells. *J Agric Food Chem*. 2005;**53**(26):9921–7. doi: [10.1021/jf0518599](#). [PubMed: [16366675](#)].