

**The Assessment of Neuronal Density of CA1 and
CA2 Regions in Hippocampus of Newborn Rats
Born from Mothers Fed by *Cantharellus cibarius*
Hidroalcoholic Extract**

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Abstract

One of the characteristics of human is the ability to change behavior based on previous experience and learning is the gaining the information that makes it possible. Memory is somewhere the preserve and storing this information. The hippocampus is one of centers in the brain responsible for learning and this center is a part of limbic system where new neurons are generated through neurogenesis. The aim of this study was to assess the changes in neuronal density in CA1 and CA2 regions in hippocampus of rats born from mothers fed by *Cantharellus cibarius* extract. In this experimental study, 24 female Wistar rats weighing 200 - 250 g were randomly divided to randomize controlled and 1, 2 and 3 treated groups with *Cantharellus cibarius* hidroalcoholic extract with doses of 100, 250 and 500. Treatment was performed through gavage method 7 days before mating. After delivery, one newborn rat of each mother was accidentally selected and anesthetized with ether, and after removing the skull, tissue processing was per-

formed in order to study the neuronal density in CA1 and CA2 areas by stereology and dissector methods, and all findings were compared by using ANOVA and t-test at significance level, ($p < 0.05$). In control and 1, 2 and 3 experimental groups, the mean of neuronal density of CA1 was 2423 ± 1.59 , 3425 ± 183 , 4003 ± 101 and 4822 ± 19.5 , respectively, in CA2 area it was 2066 ± 390 , 3568 ± 150 , 3993 ± 65.05 and 4629 ± 64.7 . Statistical analysis showed significant increase in neuronal density in (CA1, CA2) areas in experimental group (doses of 100, 250 and 500 mg / kg hydroalcoholic extract) compared to the control group, ($p < 0.05$). It is possible that the *Cantharellus cibarius* extract increases the neurogenesis by excess neuronal density due to minerals such as magnesium, calcium, zinc, vitamin D and vitamin E.

Keywords: *Cantharellus cibarius*, Hippocampus, Neurogenesis

Introduction

Impairment in learning and memory is one of the most important problems in life, and the outcome of damage to these areas (hippocampus) may lead to Alzheimer's disease [1]. Hippocampus is a part of the brain's limbic system responsible for memory, learning process, and regulating the emotions and the encryption of memory is created by the hippocampus and its connections. The hippocampus has numerous connections with the main structures including the limbic system and it is inherently involved in learning and memory processes [2]. Almost any sensory experience activates at least part of the hippocampus and the role of the hippocampus in memory consolidation is well known that if the hippocampus is damaged the stored data fails for the person but the older information which previously have been stabilized and remaining usable [3].

Memory is defined as a kind of activity in mind that makes it possible for us to provide rebuild the states such as feelings and thoughts and even pains. Strengthening the memory is one of the most common issues in today's world; there are solutions for memory reinforcement included healthy diet, fat-free and easy-digestion nutrition. Complete sleeping is the only way enables the brain to recover the information, during the sleep the brain categorizes the data accumulated entire in a day, low intake of caffeine (coffee) strengthens the memory, try to focus on the job during working, this enforces the brain's power and reading the book and newspaper, taking notes, and learning a new language may increase into memory capacity [4].

Cantharellus cibarius from *Cantharillus* species and *Cantharellaceae* family which is endemic of Mazandaran province in northern Iran. This mushroom shows orange color with a particular smell like apricots, this mushroom begins to grow in the autumn and after the first rain, the growing ends after a month, this mushroom is ectomycorrhizal and it is never polluted by the insect and it is rich in protein, with

an antimicrobial effect [5] and antioxidant properties [6] and also organic acids and minerals and elements such as magnesium, zinc, selenium.

Selenium is an antioxidant works with vitamin E and keeps the cells safe against cancer-causing radicals. The pharmacological properties of mushroom: antimicrobial properties, diffusion in agar to explore the antimicrobial potential of the ethanol and methanol extracts, and the sensitivity of some pathogens to both extracts of this mushroom revealed the antimicrobial effect [7].

The assessment of antimicrobial ethanol extract of this mushroom shows its ability to stop the growth of gram positive and negative bacteria. As [8] has reported, this mushroom has antimicrobial and antifungal activity in a wide range. While the ethanol extract just terminates the gram-positive bacteria and this represents a narrow range of antimicrobial activity of this mushroom extract [6].

In this extract, the antioxidant properties were investigated. In this survey, the neutralization of DPPH radicals specifies that both ethanol and methanol extracts of this mushrooms can destroy the neutralized free radicals Extracts taken from *Cantharellus cibarius* suggests that this mushroom could be used in pharmaceutical industry with lower side effects compared with industrial manufactured drugs, flavonoids and phenols can protect the oxidative-induced stress in diseases, and mainly responsible for antioxidant activity [9]. So that the aim of this study was to evaluate the changes in hippocampus neuronal density of newborns rats of mothers fed by the extract of the *Cantharellus cibarius*.

Materials and methods

In this experimental study, which was conducted in 2015 at the Department of Biology, Islamic Azad University of Mashhad, 24 male and 24 female wistar rats with age of 8 weeks and weighing 200-250 g were prepared from Razi Institute Mashhad and were kept at 23 to 25 ° C, 50 of humidity, the light cycle of 12 hours of light, 12 hours of darkness at the animal room of Islamic Azad University of Mashhad, the animals had access to the adequate and standard food and water during the study. Mushrooms were prepared from Mazandaran province and were approved by an expert and herbarium code was dedicated to them at botanical laboratory, the extract was performed by soxhlet method [10]. Female rats were randomly divided into four groups (control and three experimental doses of 100,250,500 mg / kg).

In the experimental group determined doses of extract and in control group only solvent were treated for 7 days by gavage [11]. On 8th day, female rats were kept beside the males for mating. After pregnancy period and birth, of all females one neonate was selected. All neonates were anesthetized and brain was removed from skulls and was kept in 10% formalin salt and after tissue processing, brain was sectioned in 7 microns and stained with toluidine blue. Of all CA1 and CA2 hippocampus regions shooting was done and the neuronal density was assessed by dissector method and was compared with control group [12].

In dissector method, in a reference frame in two consecutive sections, neurons are counted. If a neuron is placed on both contexts, it would not to be counted, but if the neuron is placed in reference frame and not to be in next frame, it would not be counted [13]. Obtained data were analyzed using the Minitab 13 and ANOVA tests to compare the two groups and significance level was considered ($P < 0.05$).

Results

Comparison the average of hippocampal neuronal density in different groups showed that in CA1 region, the neuronal density was 2423 ± 150 in control group. In treated group with 100 mg/kg of hydroalcoholic extract neuronal density was 3425 ± 183 , in treated group with dose of 250 mg/ kg it was 4003 ± 101 , and in extract dose of 500 mg/kg the density was 4822 ± 195 ; there was a significant difference, (diagram 1) ($p = 0$).

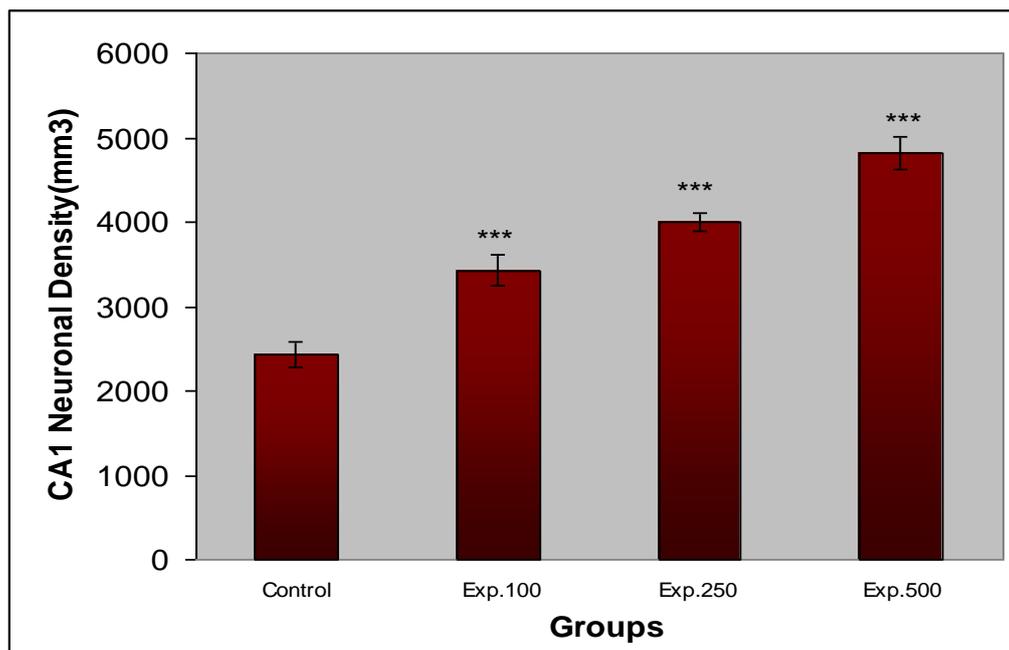


Fig1: Comparison of neuronal density means in CA1 region of newborns in control and three treated groups with the extract of mushrooms
n = 6 per group *** indicates a significance level of $P < 0.001$
The numbers represent the mean \pm SE

The neuronal density mean in CA2 area was 2044 ± 13 in control group, in treated with dose of 100 mg/kg of extract it was 3568 ± 50 ; in group treated with 250 mg/kg of extract it was 3993 ± 65.05 , and it was 4629 ± 64.7 in group treated with the extract dose of 500 mg/kg. In comparison of amount of mean between control group and three treatment groups, there was significant difference (diagram 2) ($p = 0$). Comparison of control group with treated groups in different parts of the hippocampus showed that this extract had additive effect on neuronal density in CA1, CA2 areas.

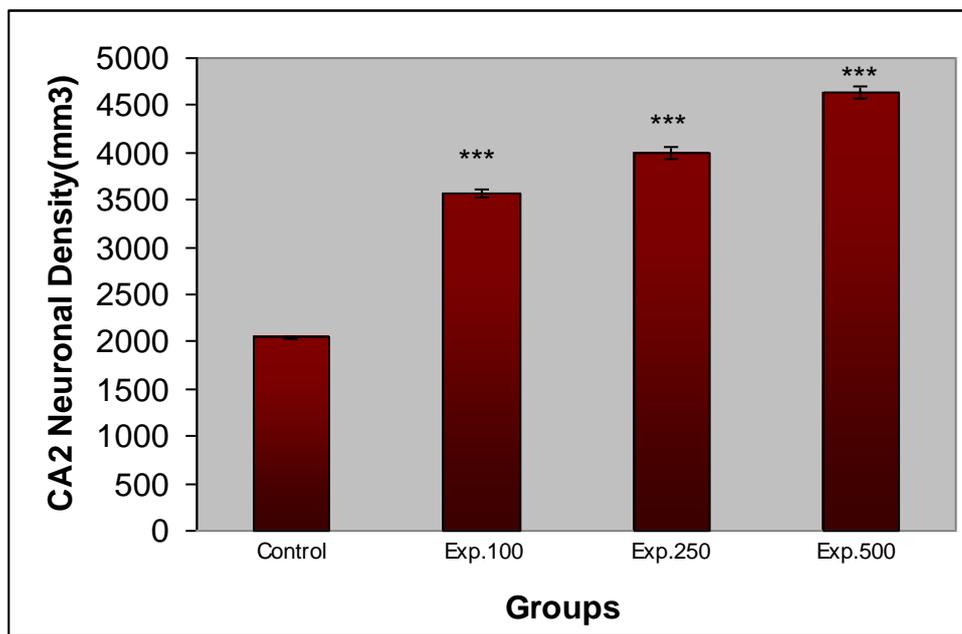


Fig2: Comparison of neuronal density means in CA2 region of newborns in control and three treated groups with the extract of mushrooms
 n = 6 per group *** indicates a significance level of $P < 0.001$
 The numbers represent the mean \pm SE

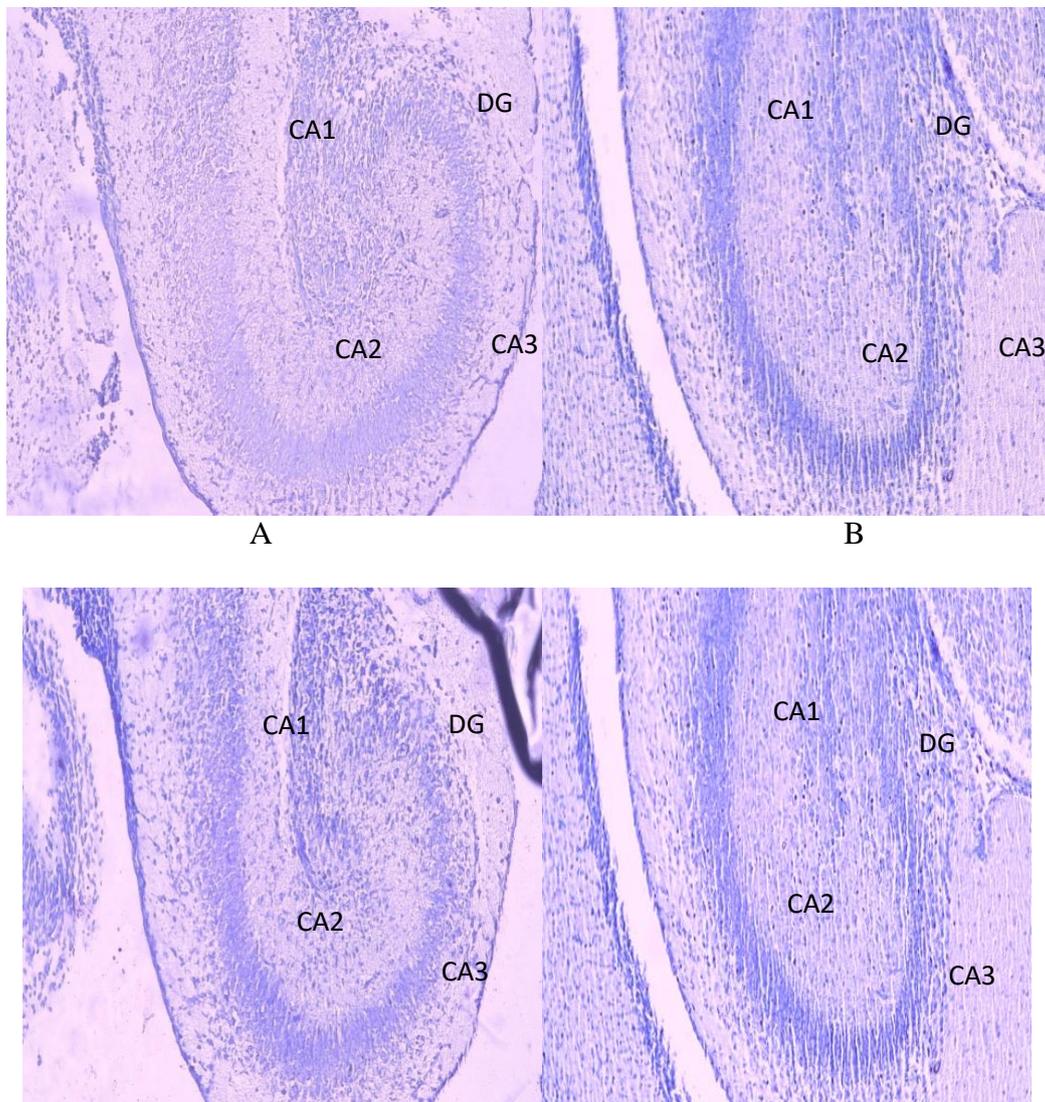


Fig3: various regions of the hippocampus. A (control group), B (experimental dose in 100mg / kg), C (experimental dose in 250mg / kg), D (experimental dose in 500mg / kg) magnification $\times 200$ toluidine blue staining

Discussion and conclusion

This study investigated the effects of *Cantharellus cibarius* extract on hippocampal CA2 and CA1 areas. The results showed that hippocampal neuronal density in the mentioned areas had a significant increase compared with the control group ($p < 0.001$) (Figure 1 and 2). *Cantharellus Cibarius* is a rich source of antioxidants and phytochemicals with health value. It is claimed that the herbal treatment value is within phytochemicals such as alkaloids, tannins, flavonoids and other phenolic compounds that have certain physiological activities on human body, and these

effects has been observed in mushrooms [14]. It has proven that acetylcholine increases the power of learning and memory and plays role in Alzheimer treatment. Currently the only effective treatment for the cognitive decreasing symptoms in Alzheimer's disease is the use of drugs increasing the secretion of acetylcholine and improving the ability of the patient's memory. Acetylcholine is secreted during learning in the brain and plays a key role to prepare and collect the new memories. The role of this matter is the increasing of receptor activity called NMDA. These receptors are proteins controlling the intensity of between neurons connection [15]. Choline is a matter that exists naturally in mushrooms and strengthens the memory. Researchers found that if during pregnancy and lactation, Choline resources of mother is strengthened, the baby's mental capacity would be higher. This matter directly effects on nerve impulses and accelerates the power and production of acetylcholine. These results are in alignment with the findings of our research. The data showed that newborns of mothers fed with mushrooms had higher neuronal density rather than the infants in control group. The increase in neuronal density in the hippocampus suggests a neurogenesis in this area and it is certainly reinforcing the learning and memory and hippocampal function is effective as a center for learning and memory.

Some drugs affecting the acetylcholine receptors may have many benefits in the treatment of cognitive disorders. By using the effects of acetylcholine on specific receptors, it can be strengthened the connections between neurons [16]. Noradrenergic activity is increased during memory consolidation in humans and this increase is associated with increased long-term memory. In fact, we can say that increased norepinephrine activity during memory consolidation can increase memory capacity [17]. Serotonin is a neurotransmitter playing role in nervous mediating systems and regulating the various forms of synaptic connections during development and in the brain, and also for serotonergic system it has been expressed an important role in modulating of memory and learning. Diversity of serotonin receptors leads to serotonin role in cognitive processes; the most important serotonin receptor is located in limbic system a area that is the place for storage memory.

Serotonin induces the inhibition of long-term potentiation in hippocampus by blocking NMDA receptors in postsynaptic neurons [17]. Noradrenergic and cholinergic systems are involved in modulating dopaminergic system in regulation of higher functions. For the synthesis and secretion of neurotransmitters like calcium, zinc, manganese and magnesium are necessary; since these transmitters are required for enforcement of memory, and the synthesis and secretion also are necessary, but the mushrooms could be used because of high doses of elements and could be effective. Therefore, increase in neuronal density in the hippocampus could be result of these elements among newborns from mothers fed with this mushroom. Chronic exposure to nicotine alters these systems; it has been stated that individuals who use the tobacco and its derivatives and may lead to their impaired behavioral and cognitive, administration of nicotine can compensate for this deficiency [18].

The findings showed that opioids have a positive effect on the changes and synaptic plasticity of neuronal network in hippocampus. In fact the opioid receptors located in the hippocampus are abundant and are activated by opioid peptides. Opioids modulate the excitability of neurons in hippocampal pyramidal. Other affective reason is histamine, glucose, vitamin A, and nitric oxide [19]. Antioxidants are fighting with the effects of nicotine. The positive effects of this mushroom extract in neuronal density increase are probably due to its abundant antioxidants content. Use of hydro-alcoholic extract of this mushroom increases the number of neurons in different regions of hippocampus; which is likely provoking some kind of neurogenesis. New cells of human brain in areas such as cerebrum and hippocampus are seen these cells change into neurons and migrate to the smell lobes.

Some researchers have proven the neurogenesis in hippocampus, an important area for memory and learning [20] Survival of neurons consists of distinct neurogenic stages including the phenomenon of induction, differentiation, proliferation, migration, formation of neural pathways and synaptic connections that probably each neuron plays a certain physiological function [21]. Mushrooms with protein content, have positive effects such as sedation, decreased blood pressure, stimulate the immune system, preventing the cancer. *Cantharellus cibarius* is a good source of nutrients and it is considered a low-calorie food. This mushroom is very low in sodium and fat, protein digestibility of mushroom is a very important factor in its nutritional value due to 71 -90 % digestible protein content. Edible mushrooms are rich in thiamin, riboflavin, niacin, vitamin D, folic acid, manganese, magnesium, potassium, zinc and selenium. Selenium is an antioxidant that works with vitamin E and keeps the cells safe to fight with free radicals which are carcinogenesis [22]. Selenium cleanses the harmful reactive oxygen species and other free radicals from brain and shows significantly increase neurogenesis and improved memory [22]. This result is in line with the results of the present study; the increase in neuronal density at different regions of hippocampus of newborns of treated mothers is because of abundant selenium in mushroom extract. The calcium shows a complex relationship with memory, it is clear that calcium deficiency can cause memory loss. Actually drugs blocking the calcium channels in cell membranes can cause memory and neurons loss in the hippocampus. Calcium is necessary for transmission of signals in the brain and releasing neurotransmitters in synapses between neurons [23]. In conclusion we can say a proper diet with adequate calcium can help enhance memory and related-processes. The mushroom extract with high levels of calcium may increase its effects on neuronal density.

Magnesium has antioxidant properties. Antioxidant compounds have tremendous effect on memory and control of related-diseases including Alzheimer's disease, muscles spontaneous contractions, anxiety, disrupted sleep. Magnesium significantly enhances the neurogenesis and improvement in memory, people can achieve these elements from daily food and most of natural foods such as vegetables, legumes, mushrooms and bananas [23]. Thus, the induction of neurogenesis in hippocampus may be due to the abundant presence of magnesium in extract of this mushroom. Hence, according to key role of memory and learning

among individuals and social performance, and taking into account the different diseases that may effect on people's memory and life quality, nowadays the consumption of food, such as plants and mushrooms are recommended particularly because of lack of side effects. So this mushroom would be useful because of its numerous containing elements such as magnesium, zinc, calcium, vitamins D and E and choline, which are effective all on memory and learning and they can effect on the process of memory and learning.

Conclusion

This study shows that the treatment of ethanol extracts of *Cantharellus cibariu* increased the number of neurons in hippocampal CA2, CA1 areas, one week before pregnancy. So the hydroalcoholic extract of this mushroom can induce the neurogenesis in hippocampus and also in neuronal density. This may be due to existence of elements such as magnesium, zinc, calcium, vitamins D and E, and it can be used in the treatment of memory impairment related-diseases.

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