



# Correlation of Aging-Related Anxiety with Dentate Gyrus and Protective Effect of Rosemary Extract

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## Abstract

**Background:** Aging is an unavoidable process that causes destructive changes to the brain. However, using plants with unique properties, such as rosemary, may help prevent complications related to aging. Therefore, we aimed to evaluate the effect of rosemary on anxiety associated with aging.

**Methods:** Male Wistar rats were divided into two groups: young and old, each with two subgroups: old rats receiving solvent (SO), old rats receiving rosemary extract (EO), young rats receiving solvent (SY), and young rats receiving rosemary extract (EY). After 12 weeks, anxiety behavior was tested through the elevated plus maze. Real-time RT-PCR was used to evaluate BDNF expression in the dentate gyrus region. Finally, the thickness of the granular layer and intact neurons in the dentate gyrus were assessed by Nissl staining.

**Results:** The results showed a significant reduction in the percentage of entries and number of entrances into the open arms in aged rats compared to young groups ( $P \geq 0.05$ ). However, the administration of rosemary significantly improved anxiety compared to the SO group ( $P \geq 0.05$ ). Additionally, administration of rosemary (100 mg/kg) proved a significant elevation of BDNF expression in the treated rats compared to the SO group ( $P \geq 0.05$ ). Also, aging meaningly decreased the thickness of the granular layer and the number of intact neurons in the dentate gyrus compared to other groups ( $P \leq 0.05$ ).

**Conclusion:** Normal aging can increase anxiety and tissue changes in the dentate gyrus, while it seems that rosemary extract improves anxiety and can reduce neuronal damage in the dentate gyrus.

**Keywords:** Traditional herb, Rosemary, Dentate gyrus, Anxiety, Aging

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## Introduction

Individuals aged 65 and over are increasing rapidly worldwide, and the global elderly population has been growing at a much faster rate in recent decades (1). The high growth of aging has adverse effects on social and economic development and the health of the elderly (2). Aging causes physiological deterioration in the whole body, especially in the brain, which suffers from a decrease in size, composition, vessels, and plasticity (3). Anxiety and cognitive disorders are common in the elderly, leading to a decrease in performance and quality of life and an increase in mortality (4).

Moreover, aging may affect neurogenesis in different brain regions. The dentate gyrus (DG) is one of the areas of the hippocampus of the brain, and its neurogenesis may gradually decrease with aging (5). The DG is closely related to anxiety-based behaviors (6) and plays a central role in episodic memory, the making of memories, learning, and

creativity (7). Also, it has been proven that different areas of the hippocampus are involved in dementia, Alzheimer's disease, and aging. Therefore, finding a preventive solution for aging complications in the brain is necessary, and it can have a significant effect on reducing treatment costs and issues caused by aging (8).

Although there are various medicines for anxiety treatment, most chemical drugs have numerous side effects. Therefore, effective medicinal plants can be a suitable candidate for prevention and treatment due to their abundance, cheapness, and general acceptance. The rosemary plant, with the scientific name *Rosmarinus officinalis*, is one of the plants native to the Mediterranean region. The extract of this plant is rich in natural antioxidants, tocopherol, and flavonoids (9). This plant is used in traditional medicine as an antispasmodic drug, painkiller, migraine headache treatment, and insomnia treatment caused by emotional distress and anxiety (10).



Various studies have proven the neuropharmacological properties of rosemary, and more recent research has shown that this plant has positive clinical effects on mood, learning, and anxiety (11, 12).

With regard to the effects of rosemary in the treatment of mental disorders, we evaluated the neuroprotective effects of rosemary extract on anxiety, tissue structure, and BDNF gene expression in the dentate gyrus region in a rat model of aging.

## Methods

### *Animal models*

This study was conducted on thirty-two male albino Wistar rats, which were obtained from the Animals Care Center of Qom University of Medical Sciences. During the experiment, the animals were kept under a 12-hour light/dark cycle, and the room temperature was maintained at  $22 \pm 1$  °C. This study was approved by the Ethics Committee of Qom University of Medical Sciences (IR.MUQ.AEC.1401.016).

### *Study design*

This experimental study divided 32 male Wistar rats into young (2–6 months) and old (18–24 months) groups. The weights of the young mice were 200–250 g, while the old mice were 350–450 g. The young group included the healthy young subgroup, which only received the solvent by gavage (SY) ( $n=8$ ), and the young treatment group, which received 100 mg/kg of rosemary extract ( $n=8$ ). The elderly group also includes the healthy elderly group that only received solvent ( $n=8$ ), while the old treatment group received rosemary extract (100 mg/kg) ( $n=8$ ). Animals were treated for 12 weeks.

### *Elevated plus maze test*

The elevated plus maze is a behavioral test used to evaluate anxiety-like responses. The apparatus is constructed from wood and consists of two open arms ( $50 \times 10 \times 1$  cm) and two closed arms ( $50 \times 10 \times 50$  cm), all connected to a central platform measuring  $10 \times 10$  cm. The rats were positioned in the center, and their performance was recorded for 5 minutes using a video camera. The frequency of entries and the duration spent in each arm were measured, with open-arm entries (OAE) and time spent in the open arms (OAT) serving as indicators of anxiety.

### *Real-time reverse transcription polymerase chain reaction (real-time RT-PCR)*

Quantitative real-time RT-PCR was performed to measure the levels of brain-derived neurotrophic factor (BDNF).  $\beta$ -actin was used as a housekeeping gene. RNA was isolated from the dentate gyrus region with a Total RNA Prep Kit (BIOFACT). Reverse transcription was carried out using BioFact™ 5×RT Pre-Mix cDNA Synthesis Kit (BIOFACT). Real-time PCR was conducted

using BioFact™ 2×Real-time PCR Master Mix and the StepOne Plus™ Real-Time PCR Detection System (Applied Biosystems). The sequences of primers were 5' CATCCGAGGACAAGGTGGCTTG 3' forward and 5' GCCGAACCTTCTGGTCCTCATC 3' reverse for the BDNF gene (13).

### *Cresyl-violet staining (Nissl staining)*

The rats were anesthetized with xylazine and ketamine (15 mg/kg and 150 mg/kg, respectively). Then, brain samples were fixed and dehydrated through ascending alcohol concentrations. After clearing with xylene, the tissues were embedded in paraffin. Serial sections (4  $\mu$ m thick) were cut by a rotary microtome (LEICA RM 2235) and stained with 0.1% cresyl violet at 56 °C for 30 minutes. Sections were evaluated under a light microscope (Eclipse E200-LED, Tokyo, Japan). Five images of the dentate gyrus region were selected for counting intact cells. Cell counts in four fields across different groups were performed using ImageJ software (14).

### *Statistical analysis*

All data are expressed as mean  $\pm$  standard deviation. Statistical comparisons were conducted using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test with SPSS version 26 (SPSS Inc., Chicago, IL, USA).  $P < 0.05$  was considered significant.

## Results

### *Elevated plus maze outcomes*

Figure 1A. shows that aging significantly decreased the number of entrances into open arms compared to young groups ( $P \leq 0.001$ ). However, a significant increase in OAE was observed after treatment with rosemary extract in old rats compared to old rats without treatment ( $P \leq 0.001$ ). There was no significant difference in OAE between treated groups with rosemary extract and EY ( $P \geq 0.05$ ).

Also, the percentage of animal entries into the open arms significantly decreased in old rats without the administration of rosemary compared to other groups ( $P \leq 0.001$ ) (Figure 1B).

### *Real-time RT-PCR analysis*

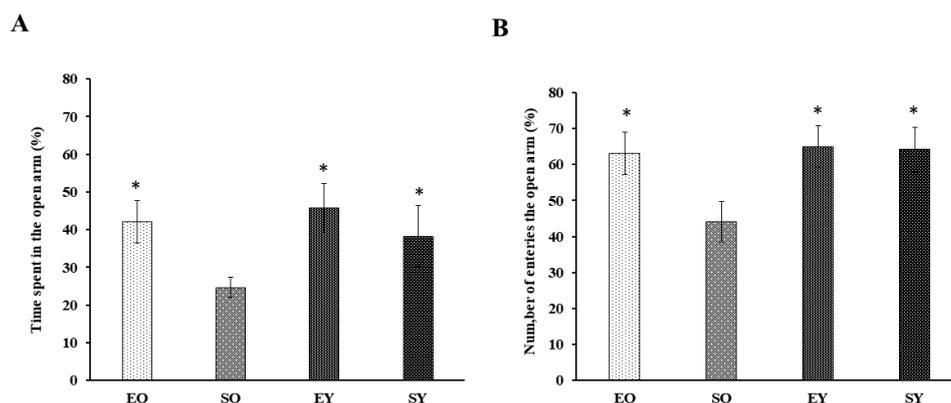
The expression of BDNF was significantly downregulated in the SO group compared to

the other group ( $P \leq 0.001$ ) (Figure 2). However, the administration of rosemary extracts upregulated the level of BDNF expression in old r

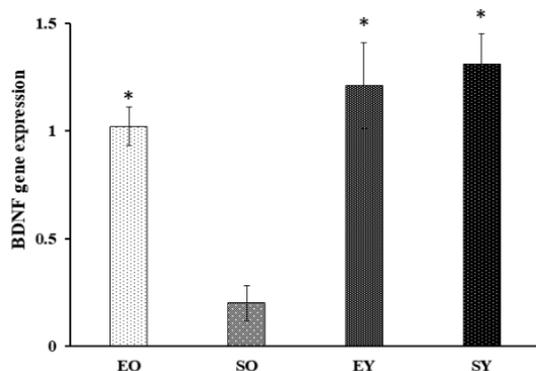
ats, and there was no significant difference between the EO and young groups ( $P \geq 0.05$ ).

### *Histological evaluation*

The Nissl staining results indicated histopathological changes in the dentate gyrus in different groups (Figure 3). The mean intact cell number significantly



**Figure 1.** (A) The percentage of remaining in the open arms of different groups. (B) The percentage of entries into the open arms in different groups. The groups included old rats receiving solvent (SO), old rats receiving rosemary extract (EO), young rats receiving solvent (SY), and young rats receiving rosemary extract (EY) (mean  $\pm$  SEM, a:  $P < 0.05$  \*  $P < 0.001$ , indicating a significant difference between the SO group and the other groups)



**Figure 2.** Comparison of expression of the BDNF marker in the dentate gyrus region by real time RT-PCR in different groups: old rats receiving solvent (SO), old rats receiving rosemary extract (EO), young rats receiving solvent (SY), and young rats receiving rosemary extract (EY) (mean  $\pm$  SEM, \*  $P < 0.001$  compared to the SO groups)

decreased in the dentate gyrus region compared to young rats ( $P < 0.05$ ), while there was no significant difference between the group treated with rosemary and the young group ( $P \geq 0.05$ ) (Figure 4A). Histological evaluation also showed that aging can significantly increase the number of degenerated neurons compared to other groups ( $P < 0.05$ ) (Figure 4B). However, the number of pyknotic neurons significantly decreased in the treated groups compared to the GO group ( $P < 0.05$ ).

In addition, Figure 4C shows that consumption of 100 mg/kg of rosemary extract increases the thickness of the granular layer compared to aged rats ( $P < 0.05$ ).

## Discussion

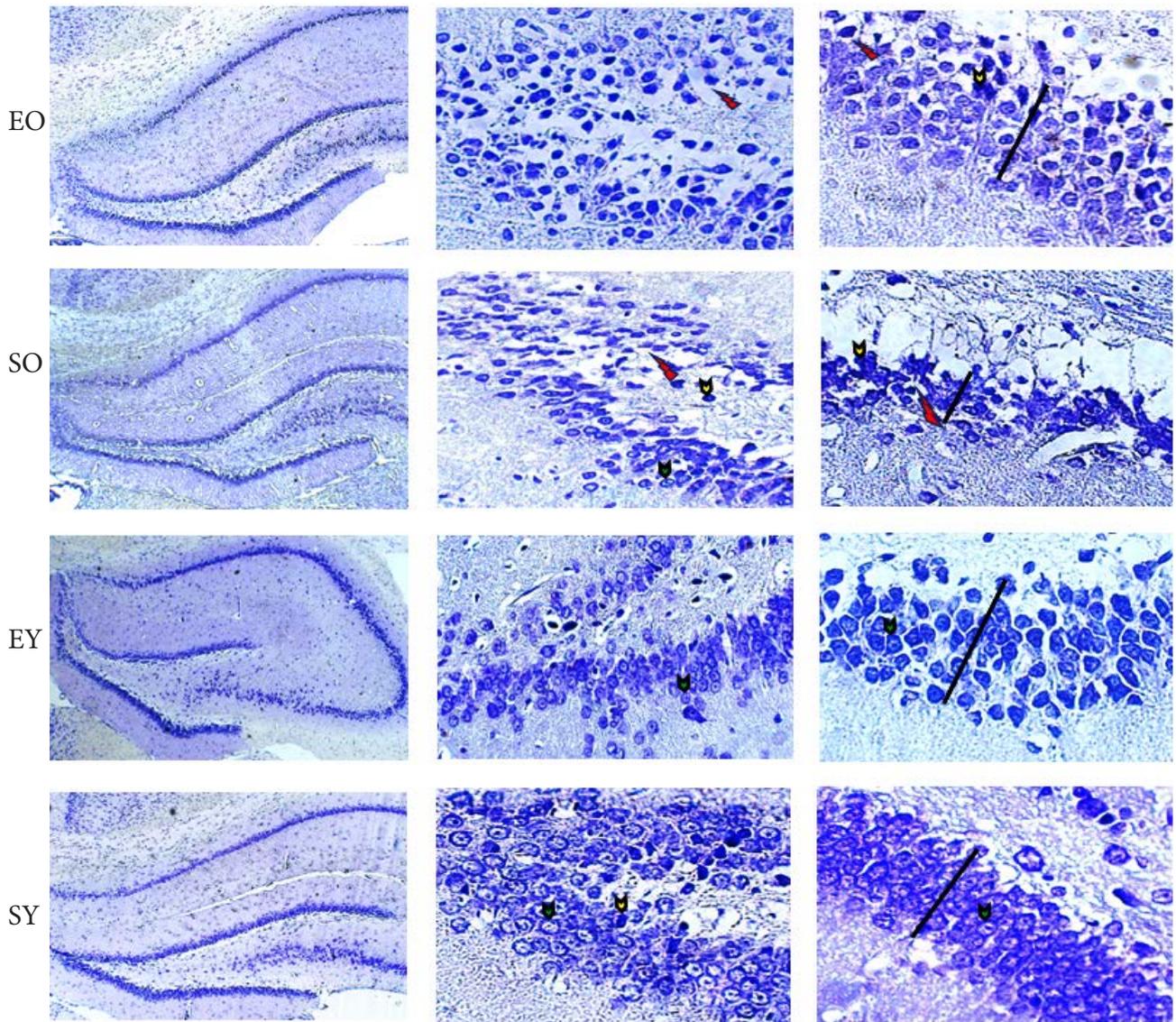
Aging is a series of anatomical and physical changes dependent on time, which causes a decrease in physiological reserves and perceptual, cognitive, and motor functions. Aging is an epidemic phenomenon, but the rate and severity of aging are not the same in different people and groups. The aging process differs depending on genetics and environmental conditions. Aging is known as a slow and chronic inflammation, and the destruction of

inflammatory regulatory mechanisms may contribute to the susceptibility of the elderly to infection and different chronic diseases (15). Also, as neurodegenerative changes occur following aging, brain health is one of the key components of the health of the elderly, requiring special attention to prevent neurodegenerative disorders (16).

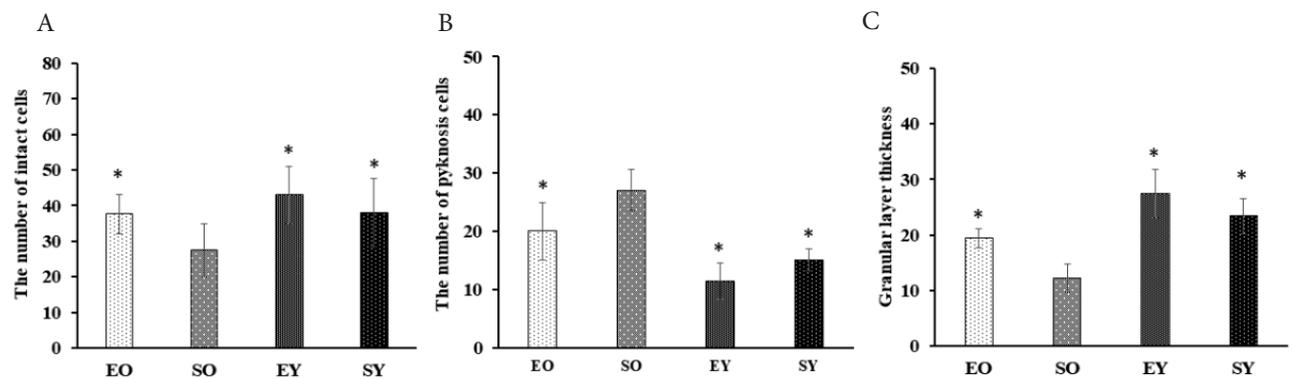
The rosemary plant has positive clinical effects on mood, learning, anxiety, and cognitive performance (17). Also, administration of rosemary extract can increase anti-inflammatory cytokines and antioxidant factors in the cerebellum tissue (18). Carnosic acid is an important component of rosemary extract that improves the survival of pyramidal cells in the CA1 area of rats and protects neurons from ischemic damage by removing ROS. In fact, the effects of rosemary extract may be due to the orientation of neurogenesis in the hippocampus (19). In addition, this traditional herb plays an antioxidant role in male fertility following aging (20).

The results showed that OAT is higher in the young group compared to the old group, which can indicate the effect of anxiety caused by aging. However, the use of 100 mg/kg of rosemary extract results in improvement in anxiety. Also, a study showed that rosemary extract, especially at a concentration of 100 mg, can improve the spatial memory score in middle-aged rats (21). Another study demonstrated that consumption of 100 mg/kg rosemary extract during 3 months may improve working memory and increase the level of antioxidant factors in the hippocampal area in old rats (22). Moreover, the use of rosemary extract could improve attention deficit hyperactivity disorder (ADHD) in mouse models through its anti-inflammatory and neuroprotective properties (23).

BDNF is a neurotrophic factor that affects the survival and growth of nerves (24). Moreover, the level of BDNF expression contributes to the differentiation, development, and survival of neurons in the nervous system, and BDNF expression decreases in neurodegenerative diseases such as Alzheimer's, MS, and Huntington's (25). In the present study, BDNF gene expression significantly decreased



**Figure 3.** Histological images of the dentate gyrus area following Nissl staining under light microscopy in different groups: old rats receiving solvent (SO), old rats receiving rosemary extract (EO), young rats receiving solvent (SY), and young rats receiving rosemary extract (EY). ( $\times 40$ ,  $\times 100$ , and  $\times 400$  magnification). The empty spaces created by the degenerated neurons in the dentate gyrus area (red crooked arrow), pyknotic neurons (yellow arrow), and intact neurons (green arrow). The granular layer thickness has been shown by a black line



**Figure 4.** (A) The comparison of the number of intact neurons in different groups. (B) The comparison of the number of pyknosis neurons in different groups. (C) The comparison of the average thickness of the granular layer in different groups. Groups include: old rats receiving solvent (SO), old rats receiving rosemary extract (EO), young rats receiving solvent (SY), and young rats receiving rosemary extract (EY). (mean  $\pm$  SEM, \*  $P < 0.05$  compared to the SO groups)

in the old group without treatment, which may be related to brain injury following aging, while receiving rosemary extract in the old group resulted in a significant

upregulation in BDNF expression in the dentate gyrus region. A study evaluated the effect of rosemary tea on anxiety and depression biomarkers in healthy individuals.

Similar to the present investigation, the results showed that the use of 5 g of rosemary in 100 mL of boiled water can significantly increase the plasma concentration of BDNF (26).

The histopathological findings showed an increase in degenerated neurons in the OS group. These neurons have a space around their small pyknotic nucleus; the empty spaces are created due to the neuronal destruction in the granular layer. Also, a reduction of cell density was observed in the granular layer in old rats without treatment. A study induced a model of memory retention deficit in neonates following sleep deprivation in pregnant rats. The findings showed that consumption of rosemary extract at 100 mg/kg concentration in mothers can increase the number of neuronal cells in the dentate gyrus in the neonate, while the use of 50 and 200 mg/kg concentrations during pregnancy does not have a positive neuroprotective effect on the neonate's brain (27). Also, it has been proven that receiving 50 mg/kg/day rosemary extract orally can increase the number of pyramidal cells and improve the histopathology of the hippocampal region in a neurotoxicity model rat (28).

## Conclusion

This study demonstrated that aging can cause tissue changes in the dentate gyrus region, and administration of 100 mg/kg rosemary improved anxiety. Also, three months of consumption of rosemary could induce neuroprotective effects on the dentate gyrus following a normal aging process.

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## Authors' Contribution

**Conceptualization:** Mohsen Eslami Farsani.

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**Investigation:** Reihaneh Seyedebrahimi, Shima Ababzadeh.

**Methodology:** Mohsen Eslami Farsani.

**Project administration:** Shima Ababzadeh.

**Writing—original draft:** Ronak Zarei.

## Competing Interests

The authors declare that they do not have any conflict of interest.

## Ethical Approval

This study was approved by the Ethics Committee of Qom University of Medical Sciences (IR.MUQ.AEC.1401.016).

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