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Boswellia serrata gum resin aqueous extract upregulates *BDNF* but not *CREB* expression in adult male rat hippocampus

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Background/aim: Boswellia from the family *Burseraceae* has been proposed for prevention of amnesia; however, the molecular mechanism by which it affects memory is not clear. To reveal the potential molecular mechanism, the effects of boswellia on the expression of two memory related genes, *CREB* and *BDNF*, were investigated.

Materials and methods: Twenty-one male rats were randomly divided into 3 groups (n = 7): the control group received distilled water and the treatment groups received two doses of aqueous extract of *Boswellia serrata* gum resin (boswellia) (50 and 100 mg/kg) every day for 4 weeks. The animals were tested by the Morris water maze (MWM) and their hippocampus was isolated. Expression of *CREB* and *BDNF* genes was analyzed by Q-RT-PCR.

Results: The MWM test showed improvement in spatial learning and memory in both treatment groups. Gene expression analysis revealed a significant increase in *BDNF* but not *CREB* expression in rats treated with both 50 and 100 mg/kg doses in comparison with the control group.

Conclusion: Although boswellia exerts its effects on memory formation at least partly by affecting the expression of *BDNF*, the results imply that boswellia probably affects memory via another BDNF-related pathway than the BDNF–CREB–BDNF cycle.

Key words: Boswellia, memory enhancing, BDNF, CREB, traditional medicine

1. Introduction

Medicinal plants are widely used for therapy of various diseases throughout the world (1). Among plants with medicinal value, the family *Burseraceae* is very important, because its members have important pharmacological properties (2). Gum resins of *Boswellia* (known as boswellia, olibanum, or frankincense) from the family *Burseraceae* are natives of Africa, India, and the Arabian Peninsula (3). In traditional medicine, boswellia is recommended for improving memory (4) and has been used for centuries for the prevention of amnesia (5) as well as other pathologic conditions such as inflammation (6). Evidence available indicates that this gum resin could increase memory power (5,7,8). Despite its historical and medical importance, gaps still exist between our knowledge of the traditional uses of boswellia and the scientific data available (9). In particular, the molecular mechanism by which it affects memory is not clear.

Memory is divided in two distinct forms based on duration of data retention (10). Short-term memory (STM) is independent of gene transcription and protein synthesis whereas long-term memory (LTM) typically relies on these procedures, since inhibition of protein synthesis could lead to impairment of LTM formation (10). Protein synthesis can be regulated by transcription factors capable of tuning transcription rates such as cAMP-response element binding protein (CREB) (11).

It was reported that CREB (12) and brain-derived neurotrophic factor (BDNF) (13) are critical factors involved in memory formation. CREB, a nuclear protein, is implicated in many important neuronal processes such as development, survival, and neuronal plasticity, which are important for memory formation (12). Moreover, CREB is critical for converting short-term memory to long-term memory (14). It is a master transcription factor that acts at a convergent point of three downstream target

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signal transduction pathways of BDNF. BDNF plays a vital role not only in neuronal survival, but also in various aspects of neural plasticity, such as neurogenesis, long-term potentiation (LTP), learning, and memory (15). Effects of BDNF levels/expression on memory have been investigated by several studies (13,15–17). For example, upregulation of BDNF by incensole acetate, a fraction of petroleum ether extract of boswellia, has been reported (18). However, there is no report on considering the effects of aqueous extract of boswellia on expression profiles of genes involved in memory formation.

This study addresses the effects of aqueous extract of *Boswellia serrata* gum resin on the expression of *BDNF* and *CREB* genes with the aim of providing some insight into the molecular mechanism by which boswellia affects memory formation. To do this, male rats treated with aqueous extract of boswellia were tested by Morris water maze (MWM) to analyze spatial learning and memory. Following the MWM test, the expression levels of *CREB* and *BDNF* genes in the hippocampi of the rats were studied by quantitative real-time RT-PCR.

2. Material and methods

2.1. Animals

Twenty-one male Wistar rats (8 weeks old and weighing 250 ± 50 g) were obtained from Tehran University of Medical Sciences and housed in groups of 7 per polycarbonate cage. The animals were given standard laboratory chow and water ad libitum. The animal care and protocols met the NIH/USDA guidelines, under the approval of the Institutional Animal Care and Use Committee (19). Temperature in the animal room was maintained between 20 and 22 °C, and there was a 12-h light/dark cycle (light from 0800 to 2000).

2.2. Preparing aqueous extract of boswellia and treatments

The *Boswellia serrata* gum resin (boswellia) was purchased from MOTHER HERBS PVT LTD (New Delhi, India). Crushed granules or lumps of the gum exudates were ground. One hundred grams of the powder was dissolved in 1000 mL of distilled water and allowed to stand at room temperature for 24 h. The supernatant was transferred to 50-mL Falcon tubes and centrifuged at 1000 rpm for 10 min. Subsequently, the supernatants were filtered using Whatman No. 1 filter paper and stored at 4 °C.

The rats were randomly divided into three groups (n = 7) and treated for 28 days. Group 1 (control) received distilled water; group 2 received 50 mg/kg body weight from boswellia aqueous extract orally with an oral feeding needle, while group 3 received 100 mg/kg body weight. The volumes were adjusted daily for each animal according to weight, to reach the intended dosage, and were administered once a day at a constant time.

2.3. Morris water maze test

After 28 days of treatment, the animals were tested by the MWM test to study spatial learning and memory. The MWM is a circular, black-painted tank with 136-cm diameter and 60-cm height containing water (20~24 °C). This tank was divided into four quadrants (N, E, W, and S) and filled with water to 40-cm depth. An invisible round disk platform (made of Plexiglas) 10 cm in diameter was used and located 1 cm beneath the surface of the water (20). For spatial orientation different geometric shapes were presented in the surroundings of the pool. The training process was performed in six consecutive days as four trials per day manner. Each trial was initiated by placing the animals randomly in one of the four quadrants of the pool. The rats must locate the immersed platform as their only means of escape from the water. Each rat was allowed 60 s to find the platform in each trial. If the rat was unable to find the platform, it was gently guided and led to the platform, where it remained for 10 s before being removed from the pool. The intertrial interval time was 10 s in each block. Behavior in the maze was recorded by a video-tracking computer system. More specifically, the time to reach the hidden platform (escape latency), the length of the swim path (distance traveled), and swimming speed for each rat were recorded and used to assess the performance of the animal in this memory test.

2.4. Gene expression analysis

After the MWM test, the rats were sacrificed and their hippocampi were dissected following decapitation under RNase-free conditions. Tissues were frozen in liquid nitrogen immediately and were stored at –70 °C. Total RNA was extracted with RNx-Plus RNA Isolation Reagent according to the manufacturer's instructions (Cinnagene Inc., Iran), and quantified by Nanodrop ND1000. The integrity of the extracted total RNA was checked by 1.2% agarose gel electrophoresis. RNA was reverse transcribed, using Thermo Scientific First Strand cDNA Synthesis kit (Fermentas, Thermo Fisher Scientific, USA) with random primers, according to the manufacturer's instructions. Then mRNA levels were analyzed by quantitative real-time RT-PCR (qRT-PCR). qRT-PCR was carried out by iQ5 Real-Time PCR detection system (Bio-Rad Laboratories, Inc., Hercules, CA, USA) apparatus using Syber green I. Primers were as follows: CREB (Forward: 5'-CACATAGCCCAGGTATCC-3' and Reverse: 5'-TGAACTGTTTGGACTTGTGG-3'); BDNF (Forward: 5'-GTGACAGTATTAGCGAGTGGG- 3' and Reverse: 5'-ATTGCGAGTTCCAGTGCC-3') GAPDH (Forward: 5'-AACGACCCCTTCATTGACC-3' and Reverse: 5'-TCCACGACATACTCAGCACC-3'). The GAPDH expression level was used as an endogenous normalization factor. The primers were designed using Gene Runner software and synthesized by Bioneer Inc. (Korea). All PCR reactions were run in duplicate.

2.5. Data analysis

The relative expression levels of *CREB* and *BDNF* genes in the hippocampus of rats treated with boswellia to that of control animals were calculated by $2^{-\Delta CT}$ method. ΔC_T was considered as the difference in threshold cycle (C_T) values corresponding to target gene and GAPDH (C_T of target gene – C_T of GAPDH). qRT-PCR data were analyzed using GraphPad Prism version 5.02. One-way ANOVA followed by Tukey's multiple comparison test with SPSS 10 analytic software (SPSS Inc., Chicago, MI, USA) was used to evaluate differences in behavioral scores in the MWM test. The unpaired Student's t-test was used to analyze the difference between two groups of animals. A P value of 0.05 was set as the level of significance.

3. Results

3.1. Effect of boswellia on the spatial memory parameters in the MWM test

To examine whether boswellia aqueous extract affected the memory power of animals, the spatial memory parameters were investigated by MWM test. The swimming speed was not changed during the trials in any of the periods of boswellia aqueous extract administration, indicating no motor disturbances occurred in the animals. Therefore, the escape latency and distance traveled parameters could be compared within groups.

3.2. Boswellia aqueous extract significantly decreased escape latency in rats

All groups of rats including control and treated animals learned to find the hidden platform in the MWM test. A significant difference ($P < 0.001$) between the first and sixth days of training was observed in escape latency of all three groups (Table). This means that training of animals appropriately occurred and all animals are normal regarding mental health. Comparing the escape latencies of the three groups on the sixth day revealed that both groups treated with 50 mg/kg and 100 mg/kg of boswellia extract had lower scores than the control group (Figure 1). This difference was statistically significant ($P < 0.05$), which shows the positive effects of boswellia aqueous extract on memory enhancement. Comparison of the escape latencies of groups treated with 50 mg/kg and 100 mg/kg revealed no significant differences.

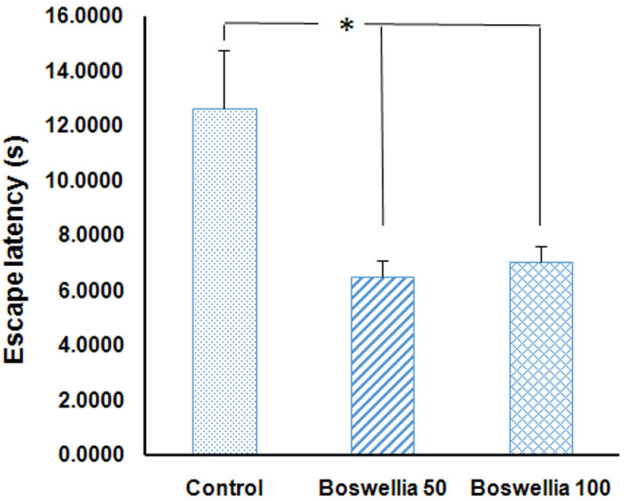

Figure 1. Comparison of effects of the boswellia aqueous extract on the escape latencies of three rat groups on day 6 of the MWM test. Both 50 and 100 mg/kg boswellia extracts led to significant reductions in escape latencies on day 6 in comparison to the control group (*$P < 0.05$). There was no significant difference between the two treated groups.

3.3. Boswellia aqueous extract significantly decrease distance traveled of rats

The time of swimming to reach the platform was compared in all three groups. The comparison revealed significant differences ($P < 0.001$) in distance traveled between the first and sixth days of training among all three groups (Table), which implies learning appropriately occurred in all three groups. Comparison of distance traveled scores of the sixth day of the three groups revealed that both groups treated with boswellia (50 and 100 mg/kg) had lower scores than the control group and the difference was statistically significant ($P < 0.05$) (Figure 2). There was no

Table. Effects of boswellia aqueous extract on spatial memory parameters in the Morris water maze test.

Groups	Escape latency (s)		Distance traveled (cm)	
	Day 1	Day 6	Day 1	Day 6
Control	33.37 ± 1.7	12.63 ± 2.1**	402.3 ± 23.8	154.8 ± 26.6***
Bos 50 mg/kg	30.94 ± 2.9	6.49 ± 6.0**	451.8 ± 42.4	76.4 ± 13.9***
Bos 100 mg/kg	30.87 ± 1.7	7.68 ± 6.3**	383.5 ± 19.6	88.5 ± 13.3***

Values are mean ± SEM for 7 animals per group. A significant difference between day 1 and day 6 of training was observed in escape latency (** $P < 0.001$) and distance traveled (*** $P < 0.001$) of all three groups.

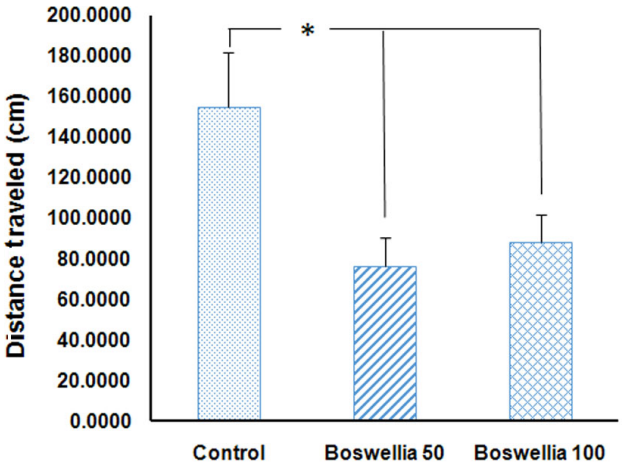

Figure 2. Comparison of effects of the boswellia aqueous extract on the distance traveled scores of three rat groups on day 6 of the MWM test. Both 50 and 100 mg/kg boswellia extracts led to significant reductions in distance traveled scores on day 6 in comparison to the control group (*P < 0.05). There was no significant difference between the two treated groups.

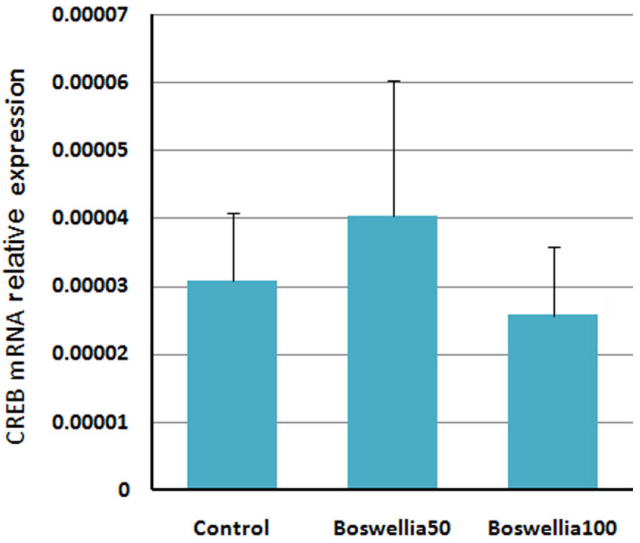

Figure 3. The expression levels of *CREB* transcripts were quantified by quantitative real-time PCR in the hippocampus of rats treated with 50 and 100 mg of boswellia aqueous extract per kg of body weight as well as the control. The expression levels were normalized to expression level of *GAPDH* internal control. The results are mean values of 7 samples per group and error bars indicate the SD.

significant difference between the distances traveled scores of the two treated groups.

3.4. Boswellia aqueous extract did not alter hippocampal expression of *CREB* transcripts

To understand the potential molecular mechanism by which the boswellia affects learning and memory we analyzed expression levels of *CREB* transcripts in the hippocampus of rats treated with boswellia aqueous extracts in comparison to the control group. Following MWM tests, the animals were anesthetized and their hippocampus was isolated. Total RNA was extracted and reverse transcribed to cDNA. The expression levels of *CREB* transcripts were quantified by quantitative real-time PCR normalizing to *GAPDH*. Analysis of the results showed no significant differences in expression levels of *CREB* in the treated groups in comparison to the control group (Figure 3).

3.5. Boswellia aqueous extract increases the hippocampal expression of *BDNF* transcripts

To evaluate the effects of Boswellia aqueous extract on *BDNF* expression, the transcripts of *BDNF* in the hippocampus of the rats were analyzed by quantitative real-time PCR. The results obtained showed that the boswellia aqueous extract upregulates expression of *BDNF* transcripts. Rats treated with both doses of boswellia (50 mg/kg and 100 mg/kg body weight) exhibited significantly higher (P < 0.05) *BDNF* expression levels in comparison to the control group (Figure 4). Moreover, the *BDNF* expression in the rats treated with the dose of 100 mg/kg was significantly higher than that of the 50 mg/kg (P

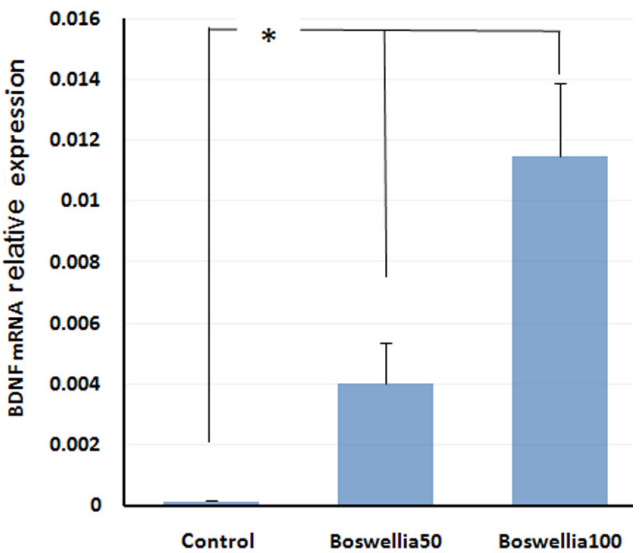

Figure 4. The expression levels of *BDNF* transcripts were quantified by quantitative real-time PCR in the hippocampus of rats treated with 50 and 100 mg of boswellia aqueous extract per kg of body weight as well as the control. The expression levels were normalized to expression level of *GAPDH* internal control. The results are mean values of 7 samples per group and error bars indicate the SD. Data points marked with an asterisk are significant at P < 0.05.

< 0.05), which implies dose-dependent upregulation of BDNF with boswellia.

4. Discussion

In the present study, the effect of boswellia aqueous extract on spatial memory retention was investigated in adult Wistar rats by MWM test followed by quantitative analysis of *CREB* and *BDNF* expression levels in their hippocampi tissues. The results demonstrated that administration of the boswellia aqueous extract led to significant reductions in escape latency and distance traveled values of rats compared to the distilled water-treated control group, which implies the boswellia aqueous extract may positively affect spatial memory. This finding is in line with reports by others on the effect of boswellia resin for memory enhancement (5,7,8,21,22) as well as a study on prevention of memory impairments caused by methimazole-induced hypothyroidism in male rats (23). It was reported that treatment of rats with boswellia might induce morphological changes in hippocampus subregions of the rat brain. These changes include increases in the volume of the cellular layer and individual neurons of dentate gyrus and cornu ammon 3 (CA3) regions (21), neurite outgrowth, branching, and tubulin polymerization dynamics (5) as well as number of neurons and neuronal dendritic spines (7). These morphological changes are governed by several gene transcription and protein synthesis procedures, which are mediated by transcription factors. A master transcription factor that plays a critical role in memory formation is CREB (11,12). This protein acts in a signaling pathway in which BDNF functions both upstream and downstream of it. To understand the molecular mechanism by which boswellia aqueous extract affects memory, we analyzed expression of *CREB* and *BDNF* genes using quantitative real-time PCR. The results showed no significant change in the expression of *CREB* transcripts in the hippocampus of the rats treated with boswellia in comparison to the controls. However, a significant increase in the expression of *BDNF* transcripts was observed.

It was reported that BDNF plays a crucial role in cognition, learning, and memory formation by modulating synaptic plasticity (24). It exists in pre- and postsynaptic compartments and can undergo both retrograde and anterograde transport. When BDNF releases into the synaptic cleft, it may interact with and activate both pre- and postsynaptically tropomyosin related kinase (TrkB) receptors (25). TrkB activation triggers activation of three intracellular pathways: extracellular signal-regulated kinase (ERK), phosphotidylinositol-3 kinase (PI-3K), and phospholipase C-ɣ (PLC-ɣ) cascades (25,26), which, in turn, may activate transcription factors such as CREB. Finally, CREB activates transcription of other genes involved in memory formation and synaptic plasticity including BDNF (27). Therefore, release of intracellular BDNF reservoir from neuronal spines can control production of BDNF itself (BDNF–CREB–BDNF cycle). According to the results of the present study, BDNF was upregulated while CREB was not affected by the aqueous extract of boswellia. This means that it may affect memory via another pathway than the BDNF–CREB–BDNF cycle. One potential pathway might be the mammalian target of rapamycin (mTOR) signaling, since it was also reported that BDNF affects the memory process by activating the mTOR pathway, which is independent of CREB transcription factor. BDNF activates the mTOR kinase to regulate GluR1 expression, a glutamate receptor subunit that is required for memory formation (28). Increased expression levels of GluR1 results in increased proportion of GluR1-containing AMPA receptors. These receptors are trafficked to the synapse in an activity-dependent manner, which may promote LTP. This pathway is inactive under basal conditions, but it can be triggered upon stimulation (29).

In conclusion, our results showed some enhancements in the spatial memory of the rats treated with aqueous extract of boswellia. This improvement might be at least partly due to BDNF, which is upregulated by aqueous extract of boswellia. However, the expression of CREB was not affected. These results imply that boswellia probably affects memory via another BDNF-related pathway than the BDNF–CREB–BDNF cycle.

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