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ORIGINAL ARTICLE

Effect of *Boswellia serrata* gum resin on the morphology of hippocampal CA1 pyramidal cells in aged rat

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Abstract Experimental evidence indicates that administration of *Boswellia* resin, known as olibanum or Frankincense, increases memory power. It is reported that beta boswellic acid, the major component of *Boswellia serrata* gum resin, could enhance neurite outgrowth and branching in hippocampal neurons. We therefore studied whether *Boswellia* treatment produces morphological changes in the superior region of cornu ammonis (CA1) in aged rats. Sixteen male Wistar rats, 24 months of age, were randomly divided in experimental and control groups. The experimental group was orally administered *Boswellia serrata* gum resin (100 mg/kg per day for 8 weeks) and the control group received a similar volume of water. The Cavalieri principle was employed to estimate the volumes of CA1 hippocampal field, and a quantitative Golgi study was used to analysis of dendritic arborizations of CA1 pyramidal cells. Comparisons revealed that *Boswellia*-treated aged rats had greater volumes than control animals in stratum pyramidale and stratum radiatum lacunosum-moleculare. The neurons of CA1 in experimental rats had more dendritic segments (40.25 ± 4.20) than controls (30.9 ± 4.55), $P = 0.001$. The total dendritic length of CA1 neurons was approximately 20 % larger in the experimental group compared to control. Results also indicated that the aged rats treated with *Boswellia* resin had more numerical branching density in the apical dendrites of CA1 pyramidal neurons. The results of the present study show that long-term administration of *Boswellia* resin can attenuate age-related dendritic regression in CA1 pyramidal cells in rat hippocampus.

Keywords Aging · *Boswellia* · Dendrite · Hippocampus · Rat

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Introduction

As the aging population is growing, it is important to understand age-related alterations in brain function and structure. Aging is usually linked to a deficit in learning and memory (Rosenzweig and Barnes 2003). Numerous investigations examining neurobiological substrates of age-associated memory decline have been focused on the hippocampus, because it is a brain structure that is vital for normal memory and also is sensitive to the process of aging (Driscoll and Sutherland 2005). Experimental studies on age-related changes in dendritic morphology are of particular interest, because dendrites bear the majority of synapses and contribute to functional changes (Horner 1993).

Markham et al. (2005) proposed that dendritic regression in cornu ammonis region 1 (CA1) in the hippocampus is a morphological correlate of the memory impairment in aging. A severe decrease in microtubule-associated proteins was also observed in the dendrites and axons of hippocampal CA1 neurons in aged mice (Himeda et al. 2005). It is reported that axonal degeneration is accompanied by disruption of microtubule protein and leads to neurodegenerative diseases and memory loss (Nakayama and Sawada 2002).

One approach to age-related memory has focused on finding drugs to improve declining memory. *Boswellia* resin (known as olibanum or Frankincense) from the trees of the *Boswellia* species native to Arabia, Eastern Africa and India (Archier and Vieillescazes 2000) has been used for many centuries in traditional medicine in Europe, Asia, and Africa as a major anti-inflammatory agent (Moussaieff and Mechoulam 2009; Ernst 2008; Ammon 2006). Growing evidence now indicates that this gum resin could increase memory power (Mahmoudi et al. 2011; Farshchi et al. 2010; Hosseini et al. 2010). It has also been shown that incensole acetate, isolated from *Boswellia* resin, attenuates hippocampal neurodegeneration and improves cognitive ability (Moussaieff et al. 2008a). In vitro studies have indicated that beta boswellic acid, the major component of *Boswellia serrata* (Bs) gum resin, could enhance neurite outgrowth and branching in hippocampal neurons (Karima et al. 2010). It also increased microtubule protein length distribution and the polymerization rate of tubulin (Karima et al. 2012).

Based on this background, this study aimed to evaluate the effects of Bs gum resin on the volumes of the CA1 hippocampal field, i.e. stratum oriens, stratum pyramidal and stratum radiatum lacunosum-moleculare (Fig. 1), and the dendritic morphology of hippocampal CA1 neurons in aged rats. The Cavalieri principle (Gundersen et al. 1988), an unbiased stereological method, was employed to estimate the volumes, and a quantitative Golgi study was used to analyze dendritic arborizations.

Materials and methods

Animals and treatment

Male Wistar rats, 24 months old and weighing 537–568 g, obtained from the animal house of Isfahan Medical Faculty, Iran, were maintained under standard laboratory conditions with food (Khorak Daam Pars, Iran) and water ad libitum under a 12:12 light–dark cycle (lights on at 7:00 a.m.). Aged rats were randomly divided into two groups ($n = 8$ in each group). The first group, the Bs group, was orally administered *Boswellia serrata* gum resin (100 mg/kg per day for 8 weeks). The second group, the control group, was administered a similar volume of water. All animal experiments and housing was carried out in accordance with rules approved by the Ethics Committee at the Shahid Sadoughi Medical University of Iran. The *Boswellia* gum resin with certified botanical origin, *Boswellia serrata*, was received as a gift from Gol darou phytolaboratory (Isfahan, Iran). This powdered extract was dissolved in and then diluted with water to the desired concentration, and administered orally with oral feeding needle in a volume of 5 ml/kg body weight.

Histological procedure

Rats were deeply anesthetized with urethan (Merk, Germany) and transcardially perfused with a phosphate-buffered solution (pH 7.2, 0.12 mol/L) of 4 % formaldehyde and 1 % glutaraldehyde. The brains were divided into hemispheres. One hemisphere was selected at random for estimating the volumes of CA1 field layers, and the other for morphometeric analysis of CA1 neuronal dendrites. They were postfixed in the perfusate, overnight. A posterior portion of each hemisphere that contained hippocampus was taken. Brains were blind coded before sectioning. Coronal sections of 100 μm thickness were cut serially with a vibratome (Diapath, Italy), and were collected along the entire extent of the hippocampus.

Volume estimation

Using systematic uniformly random sampling, every fifth section (with an interval of 500 μm) was taken. The sections were mounted on gelatin-coated object glasses immediately after the sectioning and fixed on these by air-drying at room temperature. The dry sections were stained using hematoxylin: dip in distilled water, 4 min in hematoxylin, washed in running tap water for 10 min, rinsed with distilled water, dehydrated in 70 % (10 min), 96 % (2′ 5 min) and 99 % ethanol (2′ 8 min), cleared 15 min in xylene; and coverglasses were mounted.

Discrimination between the different subdivisions of the hippocampal formation (Fig. 1) was made according to cell morphology (West et al. 1991). Dentate gyrus contains densely packed granule cells, with the smallest neuronal cell bodies in the hippocampus. Pyramidal cell layer (cornu ammonis) of the hippocampus is divided into CA3, CA2 and CA1. The CA3 pyramidal cell layer is composed of large perikari, located near dentate gyrus and the CA1 pyramidal cell layer, lying closer to the subiculum and contained smaller pyramidal cells. The CA2 region consists of a narrow zone of cells located between the CA3 and CA1 region. It contains large pyramidal neurons similar to those seen in CA3, but more loosely packed. The subiculum contains pyramidal cells with neuronal cell bodies similar to those seen in the CA1. Transition from CA1 pyramidal cell layer to subiculum is defined as the point at which the deeper cells of the layer become progressively more loosely packed and cease to be contiguous. The borders of each sector of the cornu ammonis (CA1–CA3) and the subiculum were delineated using the atlas of the rat brain (Paxinos and Watson 2007).

The Cavalieri principle was used to estimate the reference volume of the constituent layers of the hippocampal CA1 field. A grid with a tessellation of points was randomly positioned on each section, and the points hitting each layer of CA1 were counted. The number of points, ΣP, was multiplied with the area associated with each point, $a(P)$, to obtain an unbiased estimate of sectional area of each profile. The sum of sectional areas of the layers was used to estimate reference volume, $V(\text{ref})$, from the following relationship, where t represents the distance between sections:

$$V(\text{ref}) = t.\Sigma P.a(p) = t.\Sigma A$$

No areal shrinkage correction was used in the study, because of the insignificant magnitude of the shrinkage and because no difference in shrinkage was found between groups (mean areal shrinkage of 4 % was detected).

Dendritic arborization analysis

Sections were processed according to a modified version of the single-section Golgi impregnation procedure (Gabbott and Somogyi 1994). Brain sections were incubated in 3 % potassium dichromate in distilled water overnight. The sections were then rinsed in distilled water, mounted on plain slides and a coverslip was glued over the sections at four corners. These slide assemblies were incubated in 1.5 % silver nitrate in distilled water overnight in darkness. On the following day, the slide assemblies were dismantled, tissue sections rinsed in distilled water and then dehydrated first in 95 % ethanol, followed by absolute ethanol. The sections were then cleared in xylene, mounted onto gelatinized slides and coverslipped under Permount (Fisher Scientific, Pittsburgh, PA, USA).

For morphological quantification, ten pyramids of the CA1 region were selected and pooled per animal in a single group. The morphological criteria used for selecting the neurons to be measured were as follows (De Ruiter and Uylings 1987): (1) dark and consistent impregnation throughout the extent of dendrites; (2) cell bodies located in the middle part of the section thickness in order to minimize branch segments cut off at the plan of the section; and (3) relative isolation from other neighboring impregnated cells, blood vessels and silver deposits. Because these criteria were fulfilled solely by apical dendrites, the basal dendritic trees of pyramidal cells were not included in the estimations.

The presence of cut terminal segments on a neuron was not considered as a criterion for exclusion from the estimations, because the elimination of these neurons would have biased the sample towards smaller neurons (Uylings and Van Pelt 2002; Uylings et al. 1986). The likelihood that these cut branches could have interfered with the final results is negligible, because we found that in the Golgi sections of experimental and control rats, there was a similar percentage of cut branches.

Following these criteria, the dendritic trees of CA1 pyramidal cells were traced by hand with the aid of a camera lucida (Leitz Orthoplan, Wetzlar, Germany), at a final magnification of 640×. The centrifugal ordering of dendritic trees was used to estimate the number of dendritic segments per cell (Uemura et al. 1995). Accordingly, order 1 was assigned to the dendrites arising from the soma, and the successive orders were sequentially attributed to each branching point up to the terminals. The total number of segments per cell was calculated by summing the number of dendritic segments of all orders. For metric analysis, the dendritic length was measured using a Zeiss interactive digitizing analysis system (Zeiss, Germany). The branching density of dendritic trees was evaluated by applying the Sholl ring method. A grid of concentric rings (successively 25 μm apart) was placed over the camera lucida drawing of the dendritic field, centered on the soma, and the number of dendritic intersections crossing each concentric ring was counted (Uylings et al. 1986). Whenever the dendrites extended beyond 375 (circle 15), they were included in circle 15.

Statistical analysis

Independent Samples T test was performed on data from the experimental and control rats. Differences were considered to be significant for $P < 0.05$.

Results

The results showing an effect of Bs on the volumes of the different layers of the CA1 region are represented in Table 1. Comparisons between the two groups revealed that Bs-treated aged rats had greater volumes than control animals in stratum pyramidale and stratum radiatum lacunosum-moleculare ($P < 0.05$). The mean volume of stratum oriens, however, did not differ between Bs and control groups (Fig. 1).

Our results also showed that a significant increase of the dendritic branches of CA1 pyramidal cells was present in sections from the Bs group as compared to the controls (Fig. 2). Comparisons between the two groups revealed that the neurons of CA1 in experimental rats had more dendritic segments (40.25 ± 4.20) than in controls (30.9 ± 4.55), $P = 0.001$. A significant effect of Bs treatment was also observed for total dendritic length, where an approximate 20 % increase was observed in the

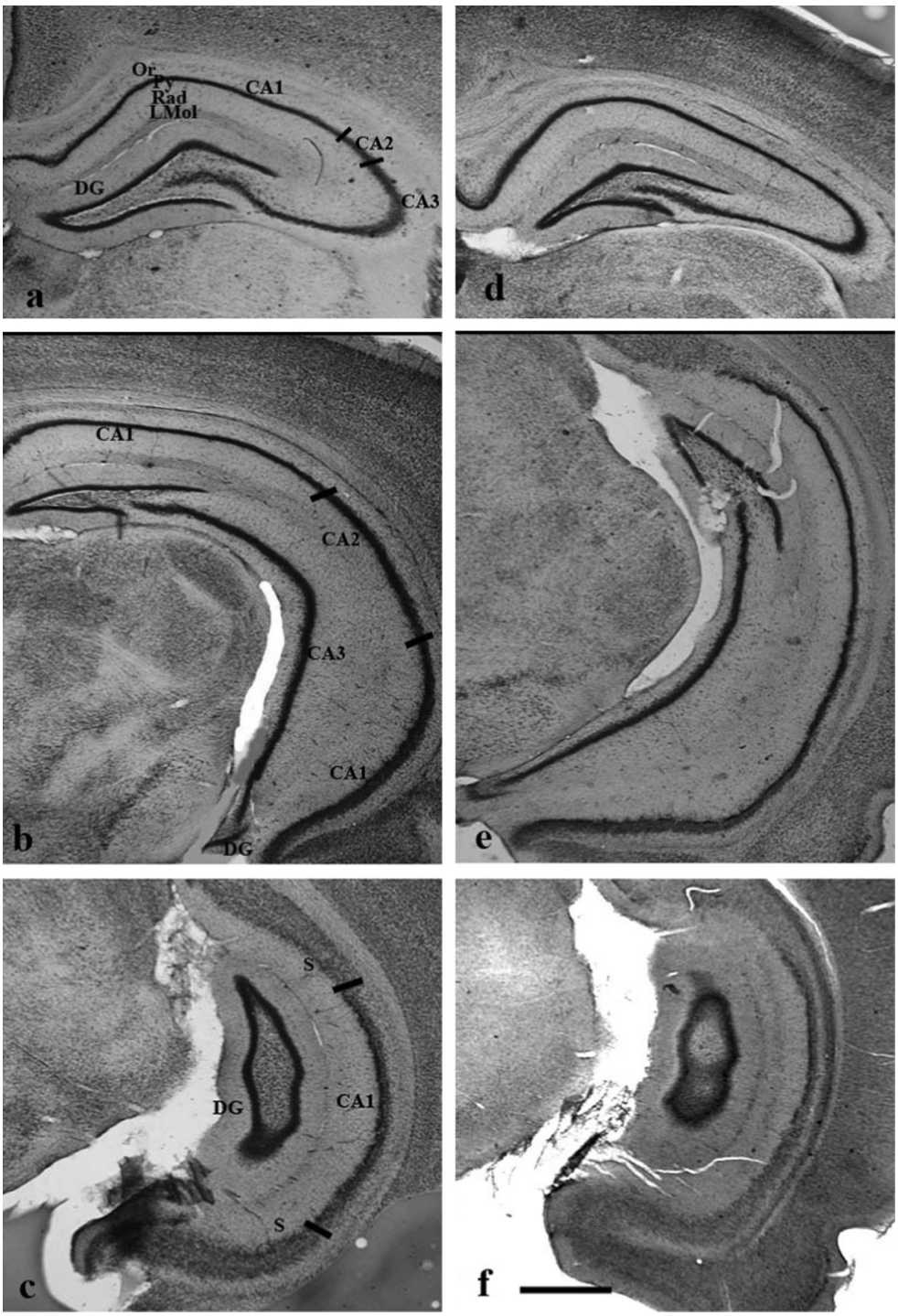

Fig. 1 Coronal sections at various levels along the rat hippocampus of both controls (**a**, **b** and **c**) and *Boswellia* treated groups (**d**, **e**, and **f**). *DG* dentate gyrus, *CA* cornu ammonis, *S* subiculum, *Or* stratum oriens, *Py* stratum pyramidale, *Rad* stratum radiatum, *LMol* stratum lacunosum-moleculare. The *dark thick lines* show the borders between CA1 and CA2, CA2 and CA3, and the subiculum and CA1. *Boswellia*-treated aged rats display greater size of stratum pyramidale, and stratum radiatum lacunosum-molecular in the CA1 hippocampal field than controls. *Scale bar* = 800 μm and applies to all frames

experimental group. The mean values were 1,830 ± 171 μm for Bs-treated aged rats and 1,536 ± 135 μm for control rats.

Study of the dendritic intersections showed that the effect of *Boswellia* treatment was significant for circles 10, 11, 12 and 13 (Fig. 3). The number of intersections in these

Table 1 Volumes of the layers of the hippocampal CA1 field (mm^3) in experimental (*Boswellia* administrated) and control rats

Hippocampal CA1 field	Control ($n = 8$) Mean (SD)	Experimental ($n = 8$) Mean (SD)	*P*
Stratum oriens	3.28 (0.38)	3.06 (0.48)	0.273
Stratum pyramidale	1.86 (0.24)	2.14 (0.31)	0.036
Stratum radiatum + lacunosum moleculare	6.18 (0.86)	7.08 (0.89)	0.033
Total	11.21 (1.21)	12.46 (1.32)	0.040

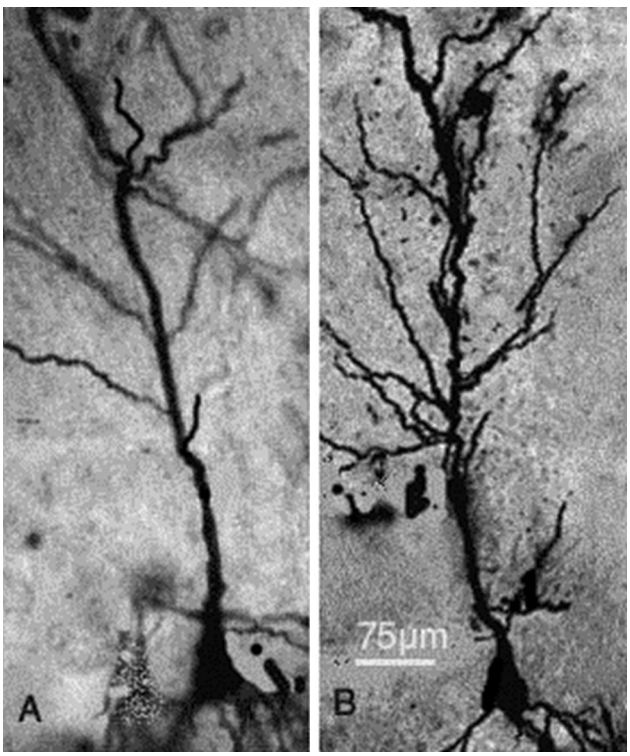

Fig. 2 Photomontages of Golgi-impregnated CA1 neuron from control (**a**) and *Boswellia*-administrated aged rats (**b**). CA1 pyramidal cells from *Boswellia*-treated rats display an obvious enhancement of their intermediate and terminal dendritic segments, when compared to similar neurons from control rats

circles in the Bs group was higher than in the control group ($P < 0.05$).

Discussion

The purpose of the present study was to determine whether long-term administration of *Boswellia* gum resin influences the morphology of CA1 hippocampal field in male aged rats. Our study shows that the administration of Bs for 8 weeks (once per day) induced increases in the volumes of

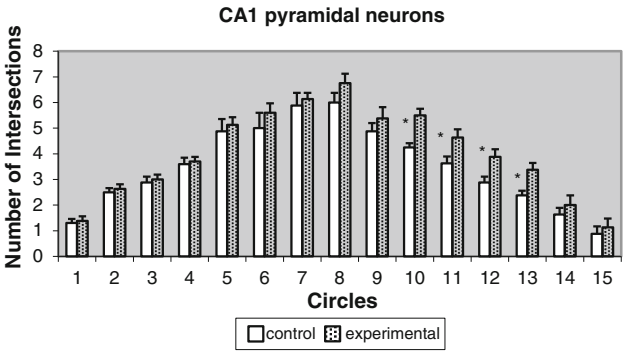

Fig. 3 Graphic representation of the dendritic branching density of CA1 hippocampal neurons of experimental (*Boswellia*) administrated aged rats and control. *Vertical bars* represent SEM. *Circles* 10, 11, 12 and 13, $P < 0.05$

pyramidal layer and the radiatum lacunosum-moleculare layer of the CA1 field, where cell bodies and apical dendrites of CA1 pyramidal cells are situated in aged rat hippocampus. Quantitative morphological analysis of dendritic architecture of Golgi-impregnated CA1 hippocampal neurons also indicated that the aged rats treated by *Boswellia* gum resin showed more branches in the apical dendrites of CA1 pyramidal neurons.

Although we didn't investigate the mechanisms underlying the increase in the dendritic material in this study, the observed increase in the number of dendritic segments and the branching density might be the result of (1) an outgrowth of new dendritic branches, or (2) reducing the speed of dendrite regressive changes, which has been observed during normal aging (Markham et al. 2005; Uylings and De Brabander 2002; De Brabander et al. 1998). It is reported that beta boswellic acid, the major active component of *Boswellia serrata* gum resin, could enhance neurite outgrowth and branching in hippocampal neurons (Karima et al. 2010). It also prevents the disruption of microtubule protein integrity (Karima et al. 2012) as a cause for neurodegeneration during aging (Himeda et al. 2005). It should be considered that *Boswellia* resin contains several active ingredients in addition to boswellic acids. Incensole acetate, another main component of *Boswellia* resin, and its derivatives also attenuate hippocampal neurodegeneration via anti-inflammatory effects on the brain (Moussaieff et al. 2008a). Furthermore, the phytochemical analysis of *Boswellia* gum resin demonstrated the presence of bioactive components, including flavonoids, alkaloids and saponins (Farshchi et al. 2010), that mediate antioxidant properties. It might be suggested that *Boswellia* treatment possibly protects the hippocampus against oxidative stress in aged rats. However, further studies need to be designed to define the precise biological mechanism behind the *Boswellia*-induced neuritic enhancement. To understand the

cellular mechanisms of these alterations, we are planning a detailed analysis of the effects of *Boswellia* and its components (incensole acetate and boswellic acid) on indices of oxidative stress in brain tissue after chronic treatment.

In preliminary experiments, our data showed that the *Boswellia*-treated aged rats had better spatial memory performance in a water maze test than that observed for the boswellic acid-treated group. Several studies also indicated that the activity of *Boswellia* resin can be superior to that of purified boswellic acid (Poeckel and Werz 2002). Therefore, in this study, we examined the effect of *Boswellia* resin administration on the hippocampal morphology, instead of employing *Boswellia* ingredients for the experiment.

While anti-inflammatory (Duwiejua et al. 1993; Moussaieff et al. 2007), anti-arthritic (Sharma et al. 1989), antimicrobial (Camarda et al. 2007), anticarcinogenic (Huan et al. 2000) and hypolipidemic (Dixit et al. 1980) activities of *Boswellia* and its components have been well documented (Ernst 2008; reviewed by Moussaieff and Mechoulam 2009), only a few studies have examined the effect of *Boswellia* resin and its constituents on the central nervous system. Menon and Kar (1971) reported that an ether extract of Boswellia serrata resin produces analgesic and sedative effects in rats. Moussaieff et al. (2008b) found that incensole acetate, a *Boswellia* resin component, causes anxiolytic and antidepressive effects in behavioral mice models. Moussaieff et al. (2008a) reported a neuroprotective effect of incensole acetate in an experimental model of traumatic brain injury, and demonstrated that it reduced the amount of degenerating neurons in the hippocampus. In this view, in agreement with earlier studies, results of our study showed that Bs had neuroprotective effect on CA1 hippocampal neurons.

Since ancient times, many compounds from herbal medicines have been reported to improve cognitive function (Hsieh et al. 2010). In the past few years, in Iran, extensive basic research studies have been focused to investigate the effect of *Boswellia* gum resin on learning and memory. For example, Hosseini et al. (2010) reported that long-term administration of 100 and 500 mg/kg olibanum prevented learning and memory impairment in hypothyroid male rats. Moreover, improvement of memory performance in male Wistar rats and male NMRI mice that were administrated *Boswellia papyrifera*, 100 and 150 mg/kg, 30 min before radial arm maze and Morris water maze tests, was observed (Farshchi et al. 2010). The enhancement effect of *Boswellia papyrifera* and β-boswellic acid on spatial memory retention in male Wistar rats has also been shown (Mahmoudi et al. 2011).

Results of this study provide a neuroanatomical basis that may be relevant to the early reported improvement of learning and memory abilities in BS-treated rats. The precise mechanisms responsible for the efficacy of *Boswellia serrata* are still a matter of debate and remain to be clarified.

In conclusion, our results show that long-term administration of *Boswellia* resin in the aged Wistar rat has neuroprotective activity and enhances dendritic arbors in CA1 pyramidal cells. The findings of the present study suggest the therapeutic potential of this herbal drug in neurodegenerative diseases.

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Conflict of interest None.

References

Ammon HP (2006) Boswellic acids in chronic inflammatory diseases. Planta Med 12:1100–1116

Archier P, Vieillescazes C (2000) Characterization of various geographical origin incense based on chemical criteria. Analysis 28:233–237

Camarda L, Dayton T, Di Stefano V, Pitonzo R, Schillaci D (2007) Chemical composition and antimicrobial activity of some oleogum resin essential oils from *Boswellia* spp. (Burseraceae). Ann Chim 97:837–844

De Brabander JM, Kramers RJ, Uylings HB (1998) Layer-specific dendritic regression of pyramidal cells with ageing in the human prefrontal cortex. Eur J Neurosci 10:1261–1269

De Ruiter JP, Uylings HBM (1987) Morphometric and dendritic analysis of fascia dentata granule cells in human aging and senile dementia. Brain Res 402:217–229

Dixit VP, Joshi S, Sinha R, Bharva VS, Varma M (1980) Hypolipidemic activity of guggal resin and garlic in dogs and monkeys. Biochem Exp Biol 16:421–424

Driscoll I, Sutherland RJ (2005) The aging hippocampus: navigating between rat and human experiments. Rev Neurosci 16:87–121

Duwiejua M, Zeitlin IJ, Waterman PG, Chapman J (1993) Anti-inflammatory activity of resins from some species of the plant family Burseracea. Planta Med 59:6–12

Ernst E (2008) Frankincense: systematic review. BMJ 17(337):a2813

Farshchi A, Ghiasi G, Farshchi S, Malek Khatabi P (2010) Effects of *Boswellia Papyrifera* gum extract on learning and memory in mice and rats. Iran J Basic Med Sci 13:9–15

Gabbott PL, Somogyi J (1994) The single section Golgi impregnation procedure: methodological description. J Neurosci Methods 11:221–230

Gundersen HJG, Bendtsen TF, Korbo L et al (1988) Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. APMIS 96:379–394

Himeda T, Mizuno K, Kato H, Araki T (2005) Effects of age on immunohistochemical changes in the mouse hippocampus. Mech Ageing Dev 126:673–677

Horner CH (1993) Plasticity of the dendritic spine. Prog Neurobiol 41:281–321

Hosseini M, Hadjzad M, Derekhshan M et al (2010) The beneficial effects of olibanum on memory deficit induced by hypothyroidism in adult rats tested in Morris water maze. Arch Pharm Res 33:463–468

Hsieh MT, Peng WH, Wu CR, Ng KY, Cheng CL, Xu HX (2010) Review on experimental research of herbal medicines with anti-amnesic activity. Planta Med 76:203–217

Karima O, Riazi G, Yousefi R, Moosavi Movahedi AA (2010) The enhancement effect of beta-boswellic acid on hippocampal neurites outgrowth and branching (an in vitro study). Neurol Sci 31:315–320

Karima O, Riazi G, Khodadadi S et al (2012) An in vitro study of the role of β-boswellic acid in the microtubule assembly dynamics. FEBS Lett 586:4132–4138

Mahmoudi A, Hosseini-Sharifabad A, Monsef-Esfahani HR et al (2011) Evaluation of systemic administration of *Boswellia papyrifera* extracts on spatial memory retention in male rats. J Nat Med 65:519–525

Markham JA, McKian KP, Stroup TS, Juraska JM (2005) Sexually dimorphic aging of dendritic morphology in CA1 of hippocampus. Hippocampus 15:97–103

Menon MK, Kar A (1971) Analgesic and psychopharmacological effects of the gum resin of *Boswellia serrata*. Planta Med 19:333–341

Moussaieff A, Mechoulam R (2009) *Boswellia* resin: from religious ceremonies to medical uses; a review of in vitro, in vivo and clinical trials. J Pharm Pharmacol 61:1281–1293

Moussaieff A, Shohami E, Kashman Y et al (2007) Incensole acetate, a novel anti-inflammatory compound isolated from *Boswellia* resin, inhibits nuclear factor-kappaB activation. Mol Pharmacol 72:1657–1664

Moussaieff A, Shein NA, Tsenter J et al (2008a) Incensole acetate-a novel neuroprotective agent isolated from *Boswellia carterii*. J Cereb Blood Flow Metab 28:1341–1352

Moussaieff A, Rimmerman N, Bregman T et al (2008b) Incensole acetate, an incense component, elicits psychoactivity by activating TRPV3 channels in the brain. FASEB J 22:3024–3034

Mt Huan, Badmaev V, Ding Y, Liu Y, Xie JG, Ho CT (2000) Anti-tumor and anti carcinogenic activities of triterpenoid, beta boswellic acid. BioFactors 13:225–230

Nakayama T, Sawada T (2002) Involvement of microtubule integrity in memory impairment caused by colchicines. Pharmacol Biochem Behav 71:119–138

Paxinos G, Watson C (2007) The rat brain in stereotaxic coordinates, 6th edn. Academic Press, New York

Poeckel D, Werz O (2002) Boswellic acids: biological actions and molecular targets. Curr Med Chem 13:3359–3369

Rosenzweig ES, Barnes CA (2003) Impact of aging on hippocampal function: plasticity, network dynamics, and cognition. Prog Neurobiol 69:143–179

Sharma ML, Bani S, Singh GB (1989) Anti-arthritic activity of boswellic acids in bovine serum albumin (BSA)-induced arthritis. Int J Immunopharmacol 11:647–652

Uemura E, Carriquiry A, Kliemann W, Goodwin J (1995) Mathematical modelling of dendritic growth in vitro. Brain Res 671:187–194

Uylings HB, De Brabander JM (2002) Neuronal changes in normal human aging and Alzheimer's disease. Brain Cogn 49:268–276

Uylings HB, Van Pelt J (2002) Measures for quantifying dendritic arborizations. Network 13:397–414

Uylings HB, Ruiz-Marcos A, Van Pelt J (1986) The metric analysis of three-dimensional dendritic patterns: a methodological review. J Neurosci Methods 18:127–151

West MJ, Slomianka L, Gundersen HJG (1991) Unbiased stereological estimation of the total number of neurons in the subdivisions of the rat hippocampus using the optical fractionator. Anat Rec 231:482–497