

IMPROVEMENT OF AMYLOID- β -INDUCED MEMORY DEFICITS BY *JUNIPERUS COMMUNIS* L. VOLATILE OIL IN A RAT MODEL OF ALZHEIMER'S DISEASE

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Abstract

Juniper volatile oil is extracted from *Juniperus communis* L., of the *Cupressaceae* family, also known as common juniper. The therapeutic properties of juniper oil are antiseptic, antirheumatic, antispasmodic, astringent, carminative, depurative, diuretic, rubefacient, stimulating, stomachic, sudorific and tonic. In traditional medicine, juniper oil was used to relieve anxiety, nervous tension and mental exhaustion.

In the present study, the effects of inhaled juniper volatile oil (1% and 3%, daily, for 21 days) extracted from *J. communis* L. on spatial memory performance were assessed in an A β (1-42) rat model of Alzheimer's disease. The A β (1-42)-treated rats exhibited the following: decrease of spontaneous alternations percentage within Y-maze task and increase of working memory and reference memory errors within radial arm maze task. Exposure to juniper volatile oil significantly improved these parameters, suggesting positive effects on spatial memory formation. Therefore, juniper volatile oil could be a potential candidate for further preclinical study aimed at the treatment of cognitive deficits in Alzheimer's disease.

Rezumat

Juniperus communis L. din familia *Cupressaceae*, denumit popular ienupăr sau ienibahar, reprezintă o sursă bogată de ulei volatil. Proprietățile terapeutice ale acestuia din urmă sunt: antiseptice, antireumatice, antispastice, astringente, carminative, diuretice, revulsive, stimulatoare, stomahice, sudorifice și tonice. Totodată, în medicina tradițională uleiul volatil de ienupăr se folosea în: anxietate, tensiune nervoasă, epuizare mentală.

În cadrul acestui studiu, au fost evaluate efectele inhalării de ulei volatil izolat din *J. communis* L. (administrare zilnică de ulei 1% sau 3%, timp de 21 zile) asupra memoriei spațiale pe model animal de Alzheimer indus prin administrare de amiloid- β -(1-42). Șobolani tratați cu A β (1-42) au manifestat modificări comportamentale precum: diminuarea alternării spontane în labirintul tip Y, precum și creșterea erorilor de lucru și de referință în testul labirintului cu brațe radiale. Expunerea la uleiul volatil de ienupăr a îmbunătățit semnificativ acești parametri, sugerând astfel un efect pozitiv asupra memoriei spațiale. De aceea, uleiul volatil de ienupăr ar putea constitui o alternativă care merită

explorată în studii preclinice și clinice viitoare care au în vedere ameliorarea și tratarea deficitelor de cogniție din demența Alzheimer.

Keywords: juniper oil, β -amyloid peptide 1-42, memory, Alzheimer.

Introduction

The major pathological hallmark of Alzheimer's disease (AD) is the accumulation of β -amyloid ($A\beta$) peptides in the brain. There is no animal model available that can mimic all the cognitive, behavioral, biochemical, and histopathological abnormalities observed in patients with AD. However, partial reproduction of AD neuropathology and cognitive deficits has been achieved by pharmacological and genetic approaches. Most injection models were performed using synthetic peptide $A\beta$ (1-40) or $A\beta$ (1-42) analogous to peptides found in neuritic plaques in AD patients [11]. Previous studies have demonstrated that intracerebral infusion of $A\beta$ causes brain dysfunctions as evidenced by neurodegeneration and an impairment of learning and memory [3, 5].

The needles and berries of *J. communis* L. have a long traditional history of use. It is used medicinally for urinary infections such as cystitis and urethritis; for respiratory problems such as bronchitis, colic and coughs; as well as gastro-intestinal infections and worms [2, 4]. It helps expel the build-up of uric acid in the joints, and is recommended in gout, rheumatism, arthritis and it is currently included in the British Herbal Pharmacopoeia as treatment for rheumatic pain and cystitis. Also, in aromatherapy the juniper volatile oil is used against anxiety, nervous tension and stress-related conditions [8].

In the present study, we investigated whether chronic inhalation of the juniper volatile oil prevents memory impairment in $A\beta$ (1-42)-induced a rat model of AD.

Materials and Methods

Essential oil and chemical analysis

Plant samples were bought from the Romanian pharmaceutical market in 2012, identified and the voucher specimen for ready reference was preserved at the Department of Pharmacognosy, Faculty of Pharmacy, University of Medicine and Pharmacy "Gr T. Popa", Iasi, Romania. Juniper berries were subjected to hydro-distillation for 2 hours using a Clevenger-type apparatus to obtain the volatile oil.

GC-MS/FID analysis of the juniper volatile oil was performed using Agilent 6890 GC-MS system, equipped with a split/splitless injector (200°C)

and the used parameters were as follows: temperature 280°C, carrier gas (1 mL/min) helium, capillary column DB 5MS (30 m x 0.25 mm; film thickness 0.25 µm; Agilent, Palo Alto, CA, USA), injected volume 0.30 µL, scan time 32 min. The thermal program was 40°C-280°C at a rate of 10°C/min; split ratio 100:1. Two replicates of juniper oil were processed in the same way. Acquisition mass range 40-400 amu. The identification of the compounds was based on comparison of their retention indices (RI), their retention times (RT) and mass spectra with those obtained from authentic Wiley libraries (available through Hewlett Packard) and the literature [1, 13].

The main compounds identified in the essential oil of juniper were: α -thujene (3.78%), α -pinene (41.13%), sabinene (11.73%), myrcene (10.16%), limonene (8.63%), γ -terpinene (1.57%), terpinen-4-ol (3.42%), followed by lower quantites of β -elemene (1.1%), β -caryophyllene (1.03%), germacrene D (1.51%), eremophyllene (1.17%). Most of the compounds are within the limits imposed by the European Pharmacopeia [14].

Animals

40 male Wistar rats (3 months old) weighing 250 ± 50 g at the start of the experiment were used. The animals were housed in a temperature and light-controlled room (22°C, a 12-h cycle starting at 08:00 h) and were fed and allowed to drink water *ad libitum*. The rats were divided into 4 groups (10 animals per group): (1) Control group (sham-operated) received saline treatment (0.9% NaCl); (2) A β (1-42) alone-treated group; (3) A β (1-42)-treated group received by inhalation juniper volatile oil 1% (JO1%+ A β (1-42)); and (4) A β (1-42)-treated group received by inhalation juniper volatile oil 3% (JO3%+ A β (1-42)). Control and A β (1-42) alone-treated groups were caged in the same conditions but in the absence of the tested oil. Rats were treated in accordance with the guidelines of animal bioethics from the Act on Animal Experimentation and Animal Health and Welfare from Romania and all procedures were in compliance with Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. This study was approved by the local Ethic Committee and also, efforts were made to minimize animal suffering and to reduce the number of animal used.

Neurosurgery

All surgical procedures were conducted under aseptic conditions, under sodium pentobarbital (50 mg/kg b.w., i.p., Sigma-Aldrich, Germany) anesthesia. Rats were mounted in the stereotaxic apparatus with the nose oriented 11° below horizontal zero plane. Animal model of AD was established by intracerebroventricular (i.c.v.) injection of 400 pmol A β (1-42) (beta-amyloid peptide 1-42, Rat, Sigma-Aldrich, Germany), 20 days

prior to inhalation of juniper volatile oil (JO1% and JO3%) according to the procedure established by Laursen and Belknap [7]. A β (1-42) was administered right-unilaterally through Hamilton syringe over 4 min, and the syringe was left in place for 5 min after injection before being slowly removed. The injection volume (4 μ L) was delivered gradually (1 μ L/min) using the following coordinates: 1.5 mm lateral to the midline; 7.4 mm ventral to the surface of the cortex [10]. The sham-operated rats were injected with saline.

Y-maze task

Short-term memory was assessed by spontaneous alternation behavior in the Y-maze task. The Y-maze used in the present study consisted of three arms (35 cm long, 25 cm high and 10 cm wide) and an equilateral triangular central area. 60 min after the inhalation of juniper volatile oil (JO1% and JO3%), rats were placed at the end of one arm and allowed to move freely through the maze for 8 min. An arm entry was counted when the hind paws of the rat were completely within the arm. Spontaneous alternation behavior was defined as entry into all three arms on consecutive choices. The number of maximum spontaneous alternation behaviors was then the total number of arms entered minus 2 and percent spontaneous alternation was calculated as (actual alternations/maximum alternations) X 100 [7]. The maze was cleaned with a 10% ethanol solution and dried with a cloth before the next animal was tested. Spontaneous alternation behavior is considered to reflect spatial working memory, which is a form of short-term memory.

Radial arm-maze task

The radial 8 arm-maze used in the present study consisted of 8 arms, numbered from 1 to 8 (48 x 12 cm), extending radially from a central area (32 cm in diameter). The apparatus was placed 50 cm above the floor, and surrounded by various extra maze visual cues placed at the same position during the study. At the end of each arm there was a food cup that had a single 50 mg food pellet. Prior to the performance of the maze task, the animals were kept on restricted diet and body weight was maintained at 85% of their free-feeding weight over a week period, with water being available *ad libitum*. Before the actual training began, three or four rats were simultaneously placed in the radial maze and allowed to explore for 5 minutes and take the food freely. The food was initially available throughout the maze, but was gradually restricted to the food cup. The animals were trained for 4 days to run to the end of the arms and consume the bait. To evaluate the basal activity of rats in radial 8 arm-maze, the rats were given 5 consecutive training trials per day to run to the end of the arms and consume the bait. The training trial continued until all 5 baits have been consumed or until the 5 min have elapsed which have been set as the performance

criteria. After adaptation, all rats were trained with 1 trial per day. Briefly, 60 min after the inhalation of juniper volatile oil (JO1% and JO3%), each animal was placed individually in the center of the maze and subjected to working and reference memory tasks, in which same 5 arms (nos. 1, 2, 4, 5 and 7), were baited for each daily training trial. The other 3 arms (nos. 3, 6 and 8) were never baited. The selection of the baited arms is based on the fact that animals prefer to solve the maze using an adjacent arm selection strategy. In this case, we altered adjacent arm patterning behavior by only baiting 5 arms (nos. 1, 2, 4, 5, and 7) subjecting animals to change their strategy and avoid the unbaited arms. An arm entry was counted when all four limbs of the rat were within an arm. Measures were made of the number of working memory errors (entering an arm containing food, but previously entered) and reference memory errors (entering an arm that was not baited). The maze was cleaned with a 10% ethanol solution and dried with a cloth before the next animal was tested [7].

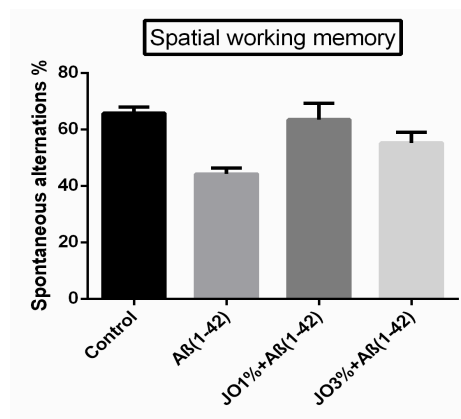
Statistical analysis

The animal's behavioral activities in Y-maze and radial arm-maze tasks were statistically analyzed with analysis of variance (ANOVA). All results are expressed as mean $\pm$ S.E.M. F-values (analysis factor: between groups variance/within groups variance) for which $p < 0.05$ were regarded as statistically significant.

Results and Discussion

Effect of juniper volatile oil on spatial memory in Y-maze task

Analysis of the spontaneous alternation percentage within Y-maze task showed significant differences between $A\beta(1-42)$, JO1%+ $A\beta(1-42)$ and JO3%+ $A\beta(1-42)$ groups ($F(3,36)=6.8$, $p < 0.001$) (Figure 1), indicating that juniper volatile oil significantly improved spatial working memory. At the moment, there is no similar research on juniper essential oil, but there are a few studies that confirm that other types of essential oils (coriander, lavender) with a similar chemical composition could improve anxiety and depression in rat AD model [3, 6, 12]. Also, *in vitro* juniper oil inhibited the heat shock-induced apoptosis in human astrocyte CCF-STTG1 cells [9].

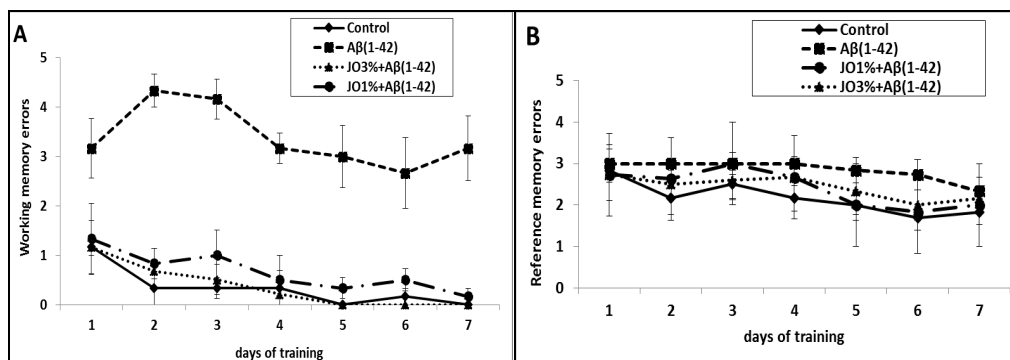
**Figure 1**

Effects of the juniper volatile oil (JO1% and JO3%) on spontaneous alternations % in the Aβ(1-42)-treated rats. Values are means $\pm$ S.E.M. (n = 10 animals per group)

Effect of juniper volatile oil on spatial memory in radial arm-maze task

In radial arm maze-task, groups exposed to JO1% and JO3% showed significant decrease of the number of working memory errors ($F(3,36)=5.6$, $p<0.001$) (Figure 2A) compared to Aβ(1-42) group, during 7 days training, suggesting positive effects on working memory.

Regarding long-time memory, explored by the number of reference memory errors (Figure 2B), was unimpaired ($F(3,36)=7$, $p<0.001$) during 7 days training of rats exposed to JO1% and JO3%, suggesting positive effects of long-term memory, too.

**Figure 2**

Effects of the juniper volatile oil (JO1% and JO3%) on the working memory errors (A) and the reference memory errors (B) during 7 days training in radial arm-maze task. Values are means $\pm$ S.E.M. (n = 10 animals per group)

Conclusions

In summary, the present study indicated that multiple exposures to juniper volatile oil could effectively restore memory impairment by administration of A β (1-42). Therefore, juniper volatile oil could be a potential candidate for further preclinical study aimed at the treatment of cognitive deficits in AD.

Acknowledgements

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