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Neuroprotective effects of inhaled lavender oil on scopolamine-induced dementia *via* anti-oxidative activities in rats

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ABSTRACT

Lavender is used in traditional medicines in Asia, Europe, ancient Greece and Rome, and was mentioned in the Bible and in ancient Jewish texts. Also, lavender is reported to be an effective medical plant in treating inflammation, depression, stress and headache. The present study was undertaken in order to investigate the antioxidant and antiapoptotic activities of the lavender essential oils from *Lavandula angustifolia* ssp. *angustifolia* Mill. and *Lavandula hybrida* Rev. using superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) specific activities, total content of reduced glutathione (GSH), malondialdehyde (MDA) level (lipid peroxidation) and DNA fragmentation assays in male Wistar rats subjected to scopolamine-induced dementia rat model. In scopolamine-treated rats, lavender essential oils showed potent antioxidant and antiapoptotic activities. Subacute exposures (daily, for 7 continuous days) to lavender oils significantly increased antioxidant enzyme activities (SOD, GPX and CAT), total content of reduced GSH and reduced lipid peroxidation (MDA level) in rat temporal lobe homogenates, suggesting antioxidant potential. Also, DNA cleavage patterns were absent in the lavender groups, suggesting antiapoptotic activity. Taken together, our results suggest that antioxidant and antiapoptotic activities of the lavender essential oils are the major mechanisms for their potent neuroprotective effects against scopolamine-induced oxidative stress in the rat brain.

Introduction

Alzheimer's disease (AD) has been estimated to account for 50–60% of dementia cases in persons over 65 years of age worldwide. Characteristic pathological features of the central nervous system (CNS) in AD are senile plaque, neurofibrillary tangle formation, aberrant oxidative and inflammatory processes and neurotransmitter disturbances. Cholinergic deficits are neuropathological occurrences that are consistently associated with memory loss and are correlated with the severity of AD (Kwon et al. 2010).

Despite continued efforts, the development of an effective treatment for AD remains elusive. Current therapeutic strategies are limited to those that attenuate AD symptomology without deterring the progress of the disease itself, and thus only postpone the inevitable deterioration of the affected individual. As the population of AD cases is growing faster than ever (Bonda et al. 2010; Jellinger 2006) the demand for an adequate method of treatment is also on the rise. Moreover, most of the synthetic drugs have severe side effects that limit the dosage and the use by the patients. Notably, as oxidative stress is perhaps the earliest feature of an AD brain (Bonda et al. 2010; Zhu et al. 2007) the successful neuronal protection from oxidative damage will potentially prevent the disease altogether, if appropriately administered.

The damaging effect of the oxidative stress is most notable in AD. That is, oxidative damage marked by lipid peroxidation, nitration, reactive carbonyls, and nucleic acid oxidation is increased in vulnerable neurons in AD, relative to unaffected patients, whether or not they contain any other corresponding pathology (*i.e.*, neurofibrillary tangles (NFTs), *etc.*) (Castellani et al. 2001; Nunomura et al. 2001). Furthermore, reduced metabolic activity, deemed the result of oxidative damage to vital mitochondrial components, has been demonstrated in AD (Hirai et al. 2001). Specifically, cytochrome oxidase, the pyruvate dehydrogenase complex, and the α-ketoglutarate dehydrogenase complex showed reduced activity as a result of oxidative damage (Aliev et al. 2003; Castegna et al. 2002). Thus, alternative and complementary therapies are needed to develop novel anti-dementia agents (Ren et al. 2004).

Scopolamine, a muscarinic antagonist, interferes with memory in animals and humans, particularly the processes of learning acquisition and short-term memory (Hefco et al. 2003). Scopolamine has been used to induce experimental models of AD (Beatty et al. 1986; Collerton 1986; Kopelman and Corn 1988). Scopolamine significantly increases acetylcholinesterase (AChE) activity and malondialdehyde (MDA) level in the cortex and hippocampus

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(Ben-Barak and Dudai 1980; Fan et al. 2005; Jeong et al. 2008; Sakurai et al. 1998) and has been used to screen antiamnesic drugs for age-related CNS dysfunction. The elevation of brain oxidative status after administration of amnesic doses of scopolamine further substantiates the value of scopolamine-induced amnesia as an animal model to test for drugs with potential therapeutic benefits in dementia (El-Sherbiny et al. 2003).

Lavender essential oil is popular as a complementary medicine in its own right and as an additive to many over the counter complementary medicine and cosmetic products (Muyima et al. 2002). The essential oil is traditionally believed to have sedative (Buchbauer et al. 1991), carminative (Catherine and Kathi 2001), anti-depressive (Delaveau et al. 1989) and anti-inflammatory properties (Valiollah et al. 2003) in addition to its recognized antimicrobial effect (Moon et al. 2004). The lavender oil is commonly used in aromatherapy and massage therapy (Welsh 1995). Its major clinical benefits are on the central nervous system (Delaveau et al. 1989).

Silexan[1] is an essential oil produced from fresh *Lavandula angustifolia* flowers by steam distillation that has been licensed in Germany as herbal medicinal product for the treatment of states of restlessness during anxious mood (Uehleke et al. 2012). Silexan acts *via* the GABA receptors (Aoshima and Hamamoto 1999), and pre-clinical data have suggested that it may have anxiolytic and antidepressant potential (Kasper et al. 2010; Woelk and Schläfke 2010).

Lavender extracts display antioxidant (Atsumi and Tonosaki 2007) and AChE inhibitory activities (Adsersen et al. 2006). Inhibitory effects of lavender on glutamate-induced neurotoxicity have also been reported (Adsersen et al. 2006). Based on these findings, it is assumed that lavender may alleviate dementia in some neurodegenerative disorders such as AD. Furthermore, recently, we demonstrated that the lavender essential oils possess a wide spectrum of biological activities, including anxiolytic and antidepressant actions, as well as positive effects on spatial memory formation (Hritcu et al. 2012). Moreover, we suggested that the effects of the lavender essential oils could be attributed to the presence of various constituents, such as linalool and linalyl acetate.

Therefore, the aim of the present study was to investigate the relationship between the antioxidant and antiapoptotic action of the lavender essential oils and their neuroprotective proprieties in scopolamine-induced a dementia rat model.

Materials and methods

Essential oil and chemical analysis

Lavandula angustifolia ssp. *angustifolia* Mill. and *Lavandula hybrida* Rev. were harvested from the Botanical Garden Galati (South-East of Romania) in July 2010 and identified. Vouchers specimens are preserved at the Department of Pharmacognosy, Faculty of Pharmacy (University of Medicine and Pharmacy "Gr. T. Popa", Iasi, Romania), for ready reference. Organic volatile fractions of *Lavandula angustifolia* (LO1) and *Lavandula hybrida* (LO2) were obtained by hydro-distillation of dried flower heads.

The chemical composition was determined by GC–MS, injection volume 1 μl (Column: HP, 5MS bonded phase 5% phenylmethylsiloxane; 0.25 mm i.d.; 30 m length; 0.25 μm film thickness; splitteron, ratio 1:100. Carrier gas: helium. Injector 250 °C, detector 280 °C. Column 50 °C, 2 min; 10 °C/min to 250 °C for 10 min. GC/MS: Agilent Technologies 6890N/5975 insert XL Mass Selective detector).

The identification of the volatile compounds was based on comparison of their retention indices (RIs), and mass spectra with those obtained from authentic samples and/or NIST/NBS, Wiley libraries and literature. The main components in both analyzed samples, LO1 and LO2, were linalool (28.0% and 21.5%, respectively) and linalyl acetate (17% and 22.5%, respectively) followed by terpinen-4-ol (3.3% and 16.7%), lavandulyl acetate (8.3% and 8.4%, respectively). Interesting is that the presence of camphor and borneol was in trace amounts, which is inconsistent with literature data (Lis-Balchin 2002).

Animals

50 male Wistar rats 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 5 groups (10 animals per group): (1) control group received saline treatment (0.9% NaCl); (2) scopolamine (Sco)-treated group received silexan, as positive control. Although silexan is currently the only pharmaceutical quality lavender oil preparation for oral use (Kasper et al. 2010), we administered silexan by inhalation as a reference compound for our lavender oils activities; (3) scopolamine (Sco) alone-treated group; (4) scopolamine-treated group received *Lavandula angustifolia* essential oil (LO1 + Sco); and (5) scopolamine-treated group received *Lavandula hybrida* essential oil (LO2 + Sco). Control and scopolamine alone-treated groups were caged in the same conditions but in the absence of the tested oils. 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 the European Council Directive of 24 November 1986 (86/609/EEC). This study was approved by the local Ethic Committee (registration number 2032) and also, efforts were made to minimize animal suffering and to reduce the number of animal used.

Drugs administration

The inhalation apparatus consisted of a Plexiglas chamber (50 cm × 40 cm × 28 cm). Two chambers were used, one to the control and scopolamine alone-treated animals, which were not exposed to any substance, and the other one to the experimental animals, which were exposed to silexan and lavender oils. Silexan and lavender oils were diluted with 1% Tween 80 (v/v). Silexan and lavender exposure (200 μl) was *via* an electronic vaporizer placed at the bottom of chamber, but out of reach of the animals. Rats in the silexan and lavender groups were exposed to oil vapors for controlled 60 min period, daily, for 7 continuous days. 60 min is a suitable inhalation period for the expected effects (Linck et al. 2010). Chambers were always cleaned up (10% ethanol solution).

Scopolamine hydrobromide (Sigma, Germany) was dissolved in an isotonic solution (0.9% NaCl) and 0.7 mg/kg scopolamine was injected intraperitoneally (i.p.), daily, for 7 continuous days, 30 min after silexan and lavender exposition procedure.

Biochemical parameter assay

One week after the silexan and lavender exposure, all rats were anesthetized rapidly decapitated and whole brains were removed. The temporal lobes were collected. Each of brain tissue samples were weight and homogenized (1:10) with Potter Homogenizer coupled with Cole-Parmer Servodyne Mixer in ice-cold 0.1 M potassium phosphate buffer (pH 7.4), 1.15% KCl. The homogenate was centrifuged (15 min at 3000 rpm) and the supernatant was used

[1] Silexan is the active substance of LASEA® (W. Spitzner Arzneimittelfabrik GmbH, Ettlingen, Germany).