Research Report

Effect of lavender oil (Lavandula angustifolia) on cerebral edema and its possible mechanisms in an experimental model of stroke

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ABSTRACT
Lavender belongs to the family Labiatae and has a variety of cosmetic uses as well as therapeutic purposes in herbal medicine. The present study was conducted to evaluate the protective effect of lavender oil against brain edema and its possible mechanisms in an experimental model of stroke. Under Laser-Doppler Flowmetry, focal cerebral ischemia was induced by the transient occlusion of the middle cerebral artery for 1 h in rats. Lavender oil (100, 200, and 400 mg/kg ip (and/or vehicle was injected at the onset of ischemia. Infarct size, cerebral edema, functional outcome, and oxidative stress biomarkers were evaluated using standard methods. Western blotting was used to determine the protein expression of VEGF, Bax, and Bcl-2. Treatment with lavender oil at doses of 200 and 400 mg/kg significantly diminished infarct size, brain edema, and improved functional outcome after cerebral ischemia (P<0.001). Lavender oil (200 mg/kg) also reduced the content of malondialdehyde and increased the activities of superoxide dismutase, glutathione peroxidase, and total antioxidant capacity (P<0.001). Although lavender oil enhanced VEGF expression (P=0.026), it could not decrease the Bax-to-Bcl-2 ratio (pro- to anti-apoptotic proteins) in the rat brain (P>0.05). The results indicated that lavender oil has neuroprotective activity against cerebral ischemia and alleviated neurological function in rats, and the mechanism may be related to augmentation in endogenous antioxidant defense, inhibiting oxidative stress, and increasing VEGF expression in the rat brain. However, lavender oil could not suppress the apoptosis pathway.

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1. Introduction
Cerebral edema is a life-threatening complication in patients with stroke, for which no effective treatment has yet been found (Rosand and Schwamm, 2001). At present, various approaches are used to alleviate brain edema, such as osmosis, diuretics, corticosteroid therapy, and hyperventilation (Rosand and Schwamm, 2001). Despite considerable efforts, a specific therapy

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for brain edema has not yet been identified. However, new therapeutic strategies are needed to more effectively treat brain edema.

Lavender belongs to the family Labiatae (Lamiaceae) and has a variety of therapeutic and cosmetic uses. Traditionally, lavender was used as a sedative, carminative, antidepressant, anticonvulsant, anti-inflammatory, antispasmodic, analgesic, and treats cerebrovascular disease in many nations (Cavanagh and Wilkinson 2002; Chu and Kemper 2001; Koulivand et al., 2013). Lavender is comprised of over 100 constituents, among which the primary components are linalool (51%) and linalyl acetate (35%), α-pinene, limonene, 1,8-cineole, cis- and trans-ocimene, 3-octanone, camphor, caryophyllene, terpinen-4-ol and lavandulyl acetate, cineole, and flavonoids (Cavanagh and Wilkinson, 2002), which are responsible for its pharmacological activity (Lis-Balchin and Hart, 1999).

Lavender oil is rapidly absorbed by the skin, and the constituent's linalool and linalyl acetate are detectable in the blood 5 min after topical application, peak at 19 min, and largely disappear from the blood within 90 min (Jager et al., 1992). Very recent evidence suggests that lavender has a protective effect against ethanol-induced damage in HepG2 cells (Farshori et al., in press), scopolamine-induced oxidative stress in the rat brain (Hancianu et al., 2013), and improved spatial performance in a rat model of Alzheimer disease (Kashani et al., 2011). There is growing evidence that lavender extract has a protective effect against cerebral ischemia in vivo (Wang et al., 2012) as well as in vitro (Büyükokuroglu et al., 2003). However, studies on lavender and cerebral ischemia are limited. In addition, the effect of lavender on cerebral edema and its possible mechanisms have not yet been studied in an experimental stroke model. Therefore, the present study aimed to evaluate the effects of lavender oil (Lavandula angustifolia) on ischemic lesions, brain edema, neurologic outcome, oxidative stress biomarkers, apoptosis (Bcl-2 and Bax expression), and vascular endothelial growth factor (VEGF) following an ischemic stroke in rats.

2. Results

2.1. Local cerebral blood flow

Measurements of local cerebral blood flow (CBF) by Laser-Doppler Flowmetry (LDF) showed that occlusion of the middle cerebral artery caused a reduction in the local CBF to less than 20% of baseline during 60 min of middle cerebral artery occlusion (MCAO) in all animal groups. Local CBF returned to near baseline after the suture was removed, but CBF recovered slowly after reperfusion in 400 mg/kg lavender oil treated group. There was no significant difference in CBF between groups of MCAO (Fig. 1, P > 0.05). Statistical analysis showed there was a significant difference in CBF after reperfusion in 400 and 200 mg/kg lavender oil treated groups compared with the vehicle group (Fig. 1, P = 0.021).

2.2. Effect of different lavender oil doses on infarct size and brain edema

Treatment with lavender oil at doses of 100 and 200 mg/kg significantly reduced the infarct volume compared with the vehicle group (Fig. 2A and B; P ≤ 0.001). Lavender oil administration at a dose of 50 mg/kg had no significant effect on infarct volume (Fig. 2, P > 0.05).

Induction of focal cerebral ischemia in the vehicle group significantly increased brain water content percent (BWC %) of ischemic hemisphere compared with that in the sham group (P < 0.001, Fig. 3). Treatment with lavender oil at doses of 200 and 400 mg/kg significantly reduced the post-ischemic increase of the BWC% compared with the vehicle group (P < 0.001, Fig. 3). No significant difference in the BWC% was observed in the non-ischemic hemisphere between the groups (Fig. 3; P > 0.05).

2.3. Effect of different doses of lavender oil on neurological outcome

The scores of neurological deficit in lavender oil-treated groups at doses of 200 and 400 mg/kg were significantly improved compared with the vehicle group (P < 0.05, Fig. 4). Lavender oil at a dose of 100 mg/kg did not change the neurological score (P > 0.05, Fig. 4).

2.4. Effect of lavender oil on activities of SOD, GSH-Px enzymes, total antioxidant capacity, and MDA content in the rat brain

As shown in Table 1, after 1 h MCAO that followed by 23 h of reperfusion, the activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) enzymes, and total antioxidant capacity (TAC) were significantly reduced in the vehicle group compared with that in the sham group (P < 0.001). Treatment with lavender oil (200 mg/kg) showed a significant increase in the activities of SOD, GSH-Px, and total antioxidant capacity compared with the vehicle group (P ≤ 0.001).

The malondialdehyde (MDA) was used as a biomarker of lipid per-oxidation and oxidative stress. Ischemia caused a significant increase in the MDA content in the ischemic cortex.
of the vehicle group ($P \leq 0.001$). Treatment with lavender oil (200 mg/kg) at the onset of ischemia caused a significant reduction in MDA content ($P \leq 0.001$; Table 1).

Ischemia causes a significant decrease in Ferric-reducing antioxidant power (FRAP, as a TAC marker) in the brain tissue compared with the sham group. Treatment with lavender oil (200 mg/kg) at the start of ischemia significantly increased TAC compared with the vehicle group ($P = 0.001$; Table 1).

2.5. **Effect of lavender oil on the proteins Bcl-2 and Bax and VEGF expression in the rat brain**

Induction of cerebral ischemia significantly increases the Bax-to-Bcl-2 ratio (pro- to anti-apoptotic proteins) as a marker of apoptosis in the brain tissue of vehicle group compared with the sham group ($P = 0.03$, Fig. 5). Treatment with lavender oil (200 mg/kg) at the onset of ischemia could not lead to a significant reduction in the Bax-to-Bcl-2 ratio compared with the vehicle group ($P > 0.05$, Fig. 5).

Western blotting showed that treatment with lavender oil (200 mg/kg) resulted in a significant increase in vascular endothelial growth factor (VEGF) expression (18%) compared with the vehicle group ($P = 0.026$, Fig. 6).

3. **Discussion**

Infarction and cerebral edema are the two major pathophysiological changes observed following the acute phase of stroke. Our study showed that lavender oil, at doses of 100 and 200 mg/kg, reduces infarct size by 32% and 47%, respectively. Consistent with these results, a recent study reported the neuroprotective activity of lavender oil in transient focal cerebral ischemia in mice (Wang et al., 2012). Moreover, for
the first time, another finding of this research exhibited that lavender oil attenuated cerebral edema induced by MCAO in rats. It is important to know that cerebral edema is a major life-threatening complication in acute phase stroke that causes neurological deterioration and or death (Rosand and Schwamm, 2001). These results provided novel evidence of the neuroprotective potential of lavender oil in a rat model of focal cerebral ischemia.

Some evidence indicates that lavender oil significantly attenuated tumor necrosis factor (TNF-α) in isolated rat mast cells (Kim and Cho, 1999). Recent studies from our group and by others have shown that TNF-α, a pro-inflammatory factor, plays an important role in the pathophysiology of cerebral edema and brain injuries after stroke (Vakili et al., 2011; Han, 2013). However, based on available evidence (Kim and Cho, 1999), a part of the neuroprotective and anti-edematosis effect observed in this study may be related to the effects of lavender oil in the suppression of TNF-α. Although the TNF-α expression levels were not measured in this study, further studies are needed to clarify and confirm this assumption in the future. Likewise, it has been reported that the aqueous extract of lavender reduces glutamate-induced neurotoxicity in the cerebellar granular cell culture of rat pups (Büyükokuroğlu et al., 2003). We assume that the suppression of glutamatergic neurotoxicity may also be responsible for the neuroprotective effect of lavender oil that was observed in this study.

In most animal studies, the effectiveness of a drug is determined by measuring the infarct size (Bae et al., 2013; Schaar et al., 2010). Nevertheless, in clinical trials, neuroprotective efficacy is measured by neurological function (Schaar et al., 2010), and our present study showed that the neurological outcome was improved by treatment with lavender oil. Thus, these observations further verify the neuroprotective activity of lavender oil in rats.

Because the brain has high concentrations of peroxidisable lipids, low levels of antioxidative enzymes (SOD, GSH-Px), high oxygen consumption are very susceptible to ROS-induced damage following ischemia-reperfusion, which causes oxidative damage to brain lipids, proteins, and DNA, finally leading to

### Table 1 – Effects of lavender oil (200 mg/kg) on the antioxidant enzyme activities (SOD, GSH-Px), FRAP (as a marker of total antioxidant capacity) and MDA content in the rat brain.

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (IU/mg Pr)</th>
<th>GSH-Px (U/mg Pr)</th>
<th>FRAP (μmol/mg Pr)</th>
<th>MDA (nmol/mg Pr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated</td>
<td>9.08 ± 0.06</td>
<td>0.27 ± 0.02</td>
<td>0.81 ± 0.004</td>
<td>4.13 ± 0.11</td>
</tr>
<tr>
<td>Vehicle (MCAO+maize oil)</td>
<td>7.78 ± 0.32</td>
<td>0.22 ± 0.01</td>
<td>0.75 ± 0.01</td>
<td>5.32 ± 0.27</td>
</tr>
<tr>
<td>Lavender oil (200 mg/kg)</td>
<td>12.34 ± 0.22</td>
<td>0.3 ± 0.006</td>
<td>0.83 ± 0.003</td>
<td>3.5 ± 0.11</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.
* P<0.05 vs. vehicle.
# P<0.05 vs. sham-operated.

![Fig. 5 – Bax-to-Bcl-2 ratio in sham-operated, vehicle (MCAO+maize oil) and lavender oil treated group at dose 200 mg/kg (Lav-200) in the rat brain.](image)

![Fig. 6 – VEGF expression in sham-operated, vehicle (MCAO+maize oil) and lavender oil treated group at dose 200 mg/kg (Lav-200) in the rat brain.](image)
brain dysfunction and cell death (Murakami et al., 1997; Allen and Bayraktutan, 2009). Since reactive oxygen species (ROS) has a short half-life, its direct measurement in the brain is fairly difficult (Allen and Bayraktutan, 2009). MDA level is commonly used as a biomarker of oxidative stress in in vivo studies (Allen and Bayraktutan, 2009). MDA is a peroxidation product of polyunsaturated fatty acids in cells that are primarily induced by ROS (Allen and Bayraktutan, 2009). Our study showed that lavender oil reduced the post-ischemic-enhanced MDA content as a biomarker of oxidative stress, and increased the activities of SOD, GSH-Px as major antioxidant enzymes. This result corroborates the result of another study (Wang et al., 2012; Hancianu et al., 2013). Our findings suggested that lavender oil attenuated the cerebral ischemia–reperfusion injury in rats by over expression and augmentation of antioxidant enzymes and by suppressing the oxidative stress pathway.

Apoptosis is one of the most important mechanisms that lead to neuronal death after cerebral ischemia (Chelluboina et al., in press; Ferrer, 2006). The Bcl-2-associated X protein (BAX) a protein of the Bcl-2 gene family that promotes apoptosis by competing with Bcl-2 as an anti-apoptotic protein (Raghupathi et al., 2003; Chan, 2004). Our results showed occlusion of MCAO significantly increased apoptosis (Bax-to-Bcl-2 ratio) in the ischemic brain tissue compared with the sham-operated group, but treatment with lavender oil could not reduce the apoptosis after cerebral ischemia in the rat brain. However, our results are in contrast with a recent study indicating that inhaled lavender oil promotes anti-apoptotic activity in a scopolamine-induced dementia rat model (Hancianu et al., 2013). In this study (Hancianu et al., 2013), lavender oil inhaled for 7 days and apoptosis was measured with DNA fragmentation assays in a scopolamine-induced dementia rat model, while in our present study, effects of lavender oil (200 mg/kg ip) were assessed in a transient model of focal cerebral ischemia and apoptosis, evaluated using western blotting. These results indicated that the apoptosis pathways may not be involved in neuroprotection by lavender oil that was observed in the present study. This finding suggests that other mechanisms may also contribute.

Another finding of our study indicated that lavender oil induced VEGF expression in the rat brain during the acute phase of stroke. It appears that at least a part of the neuroprotective effect of lavender oil that was observed in this study maybe related to increased VEGF expression in the ischemic area. The mechanisms underlying VEGF-induced reduction of ischemic injury are not clear in the present study. VEGF is induced following focal cerebral ischemia and promotes the formation of new cerebral blood vessels in the ischemic area (Rosenstein et al., 1998; Sun et al., 2003). Revascularization in the brain infarct usually occurred at 2–5 days after stroke and continued for several months (Hayashi et al., 1997; Sun et al., 2003). Since the current study ischemic injury was determined 24 h after stroke, the neuroprotective effect of VEGF may be independent of the angiogenesis function and may be related to other mechanisms. VEGF protects capillary endothelial cells from apoptotic cell death (Alon et al., 1995) and also relaxes vascular smooth muscle cells (Ku et al., 1993). Thus, part of the direct neuroprotective activity of VEGF may be associated with these effects.

In conclusion, the present study showed that lavender oil reduced the brain infarct size, edema, and improved the neurological function in a rat model of focal ischemic stroke.

The protective effects of lavender oil were mediated through the augmentation of endogenous antioxidant defenses, inhibition of the oxidative stress pathway, and increased VEGF expression in the rat brain. Moreover, lavender oil did not exhibit anti-apoptotic effects in this study. These findings suggest that lavender oil may have immense potential and prove to be a beneficial agent for the treatment of stroke patients.

4. Experimental procedures

4.1. Animals

Adult male Wistar rats (300 ± 20 g) were obtained from the breeding colony of Semnan University of Medical Sciences (SUMS), Semnan, Iran. Animals were housed in polycrylic cages, with not more than four rats per cage under standard laboratory conditions, under natural light and dark cycles (approximately 12 h light/12 h dark), at a temperature of 24 ± 2 °C. They were fed a pellet diet and tap water, ad libitum. All procedures were performed in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

4.2. Surgical procedure and measurement of CBF

Transient focal cerebral ischemia was induced under chloral hydrate (400 mg/kg, Merck, Germany) anesthesia in rats (Vakili et al., 2011; Longa et al., 1989). A 3-0 nylon suture was introduced into the internal carotid artery and gently advanced until LDF (Moor Instruments DRT4, UK) showed a sharp decrease in the ipsilateral CBF to less than 20% of baseline, which confirmed an occlusion of the middle cerebral artery. After 60 min of middle cerebral artery occlusion (MCAO), reperfusion was started by withdrawing the nylon thread for 23 h. To measure CBF, an LDF probe was positioned in direct contact with the right temporal bone after a limited dissection of the temporals muscle at an equal distance between the eye and ear (Lecrux et al., 2007). To fix and prevent the displacement of the LDF probe, a burr hole (2-mm diameter) was drilled 5 mm lateral and 1 mm posterior to the bregma without injury to the dura mater. CBF was continuously monitored 15 min before, during MCAO, and up to 15 min after the reperfusion.

4.3. Neurobehavioral test

The neurobehavioral (sensory and motor) test was performed 24 h after MCAO as described previously (Reglodi et al., 2003). The sum of partial scores yielded the total neurological score, with a maximum of 42 points and a minimum of 0 in normal rats (Reglodi et al., 2003). The neurological score of rats was evaluated blindly by an investigator who was unaware of the animal groups.

4.4. Measurement of infarct size

After neurological evaluation, rats were sacrificed with an anesthesia overdose and the brains were rapidly excised. The
brain was coronally sectioned into seven 2-mm thick slices using a brain matrix. Coronal sections were stained in 2% 2,3,5-triphenyltetrazolium chloride (TTC; Sigma, Germany) at 37 °C in a water bath for 15 min, and then fixed in 4% paraformaldehyde for 24 h. The brain slices were then photographed separately using a digital camera (Cannon-Japan). Infarct areas of all sections were measured using an image analysis software (NIH Image Analyzer). The infarct volume of each slice was calculated by multiplying the infarct area by the thickness of the brain slice by its thickness. The injury volume was calculated from the onset of MCAO. To determine the effect of lavender oil on brain edema, we used five different groups: sham operated (n = 6), vehicle (MCAO+maize oil, 1 ml/kg, n=6), and lavender oil at doses 100 mg/kg (n=7), 200 mg/kg (n=7), 400 mg/kg (n=7), administered at onset of MCAO.

4.6. Oxidative stress marker assay

Following cerebral ischemia, the whole ischemic hemisphere was homogenized (1:10 w/v) in cold 1.15% KCl and centrifugation at 20,000g at 4 °C for 10 min. The supernatants were used for biochemical analyses. The total protein level in the supernatants was determined by the Bradford method using bovine serum albumin as standard (Bradford, 1976). The MDA content or thiobarbituric acid reactive substances (TBARS) were measured using the thiobarbituric acid method as described previously by Mihara and Uchiyama (1978). SOD and GSH-Px activities were measured using a commercial kit (Randox Laboratories Ltd., UK) and according to manufacturer’s protocol. FRAP assay is a colorimetric method for measuring the TAC (Benzie and Strain, 1999).

4.7. Protein measurement and western blotting

Brain samples were homogenized and prepared in lysis buffer (137 mM NaCl, 20 mM Tris–HCl pH 8.0, 1% NP-40, 10% glycerol, 1 mM phenylmethyl sulfonyl fluoride, 10 μg/ml aprotinin, 1 μg/ml leupeptin, and 0.5 mM sodium vanadate). Tissue extracts were centrifuged to remove insoluble material (12500g for 20 min at 4 °C) and total protein concentration was determined according to the Micro BCA procedure (Pierce, Rockford, IL, USA). Equal amounts (25 μg) of protein from each sample were loaded on 15% polyacrylamide gels and separated by a standard SDS-PAGE. Protein bands were transferred to PVDF membranes and then blocked using 5% skim milk and 0.1% Tween-20 in Tris-buffered saline. Membranes were incubated with primary antibodies overnight at 4 °C [anti-Bax, 1:500 (sc-493); anti Bcl-2, 1:200 (sc-492) anti-actin, 1:300 (sc-130065); anti VEGF, 1:1000 (sc-152)] and then with a secondary antibody [goat anti-rabbit IgG horseradish peroxidase conjugated antibody, 1:2000 (sc-2004)] for 1 h at room temperature. Immunocomplexes were visualized by chemiluminescence using the ECL kit (Pierce, Rockford, IL, USA) according to the manufacturer’s instructions. The results for target proteins, Bax, Bcl-2, VEGF, and actin were quantified by densitometric analysis (using Gel-Pro analyzer imaging software). The results for the Bax-to-Bcl-2 ratio were calculated and the results for VEGF were normalized for beta-actin levels.

4.8. Experimental design and treatment plan

To determine the effect of lavender oil (Sigma-Aldrich, Germany) on infarct size and neurological outcome, 28 animals were randomly divided into four experimental groups, including vehicle (MCAO+maize oil, 1 ml/kg, n=7) and treatment groups in which lavender oil was administered intraperitoneally at doses of 50 mg/kg (n=7), 100 mg/kg (n=7), and 200 mg/kg (n=7), at the onset of MCAO.

To evaluate the therapeutic effect of lavender oil on brain edema, we used five different groups: sham operated (n=6), vehicle (MCAO+maize oil, 1 ml/kg, n=6), and lavender oil (200 mg/kg, n=6)-treated, administered at the onset of MCAO.

To determine the effect of lavender oil on the expression of Bcl-2 (anti-apoptotic protein) Bax (pro-apoptotic protein), and VEGF protein in the rat brain, 18 animals were randomly divided into three experimental groups, including sham operated (n=6), vehicle (MCAO+maize oil, 1 ml/kg, n=6), and lavender oil (200 mg/kg, n=6)-treated, administered at the onset of MCAO.

4.9. Statistical analysis

All results were presented as mean ± SEM. Statistical analysis was conducted using Kruskal–Wallis one-way analysis of variance (ANOVA) followed by the Dunn’s test and ANOVA followed by the Holm–Sidak method as post hoc analysis. Differences were considered significant at P<0.05 (SigmaStat 2.0; Jandel Scientific, Erkrath, Germany).

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References

newly formed retinal vessels and has implications for retinopathy of prematurity. Nat. Med. 1, 1024–1028.


