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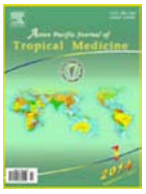

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# Neuroprotective effect of pretreatment with *Lavandula officinalis* ethanolic extract on blood-brain barrier permeability in a rat stroke model

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ABSTRACT

**Objective:** To evaluate the protective effect of *Lavandula officinalis* (*L. officinalis*) extract against blood-brain barrier (BBB) permeability and its possible mechanisms in an experimental model of stroke.

**Methods:** Focal cerebral ischemia was induced by the transient occlusion of the middle cerebral artery for 1 h in rats. Lavender extract (100, 200 mg/kg *i.p.*) was injected for 20 consecutive days. BBB permeability and oxidative stress biomarkers were evaluated using standard methods.

**Results:** The results of this study showed that *L. officinalis* ethanolic extract significantly reduced the BBB permeability in experimental groups when compared with ischemia group. The lavender extract significantly reduced malondialdehyde levels of plasma and brain tissue in intact group when compared with control group.

**Conclusions:** *L. officinalis* extract reduced blood brain barrier permeability and alleviated neurological function in rats, and the mechanism may be related to augmentation in endogenous antioxidant defense and inhibition of oxidative stress in the rat brain.

## 1. Introduction

Stroke is the third leading cause of death in industrialized countries and the most frequent cause of permanent disability in adults worldwide. Three million Americans are currently permanently disabled[1].

Three months following a stroke, 15%-30% of stroke survivors are permanently disabled and 20% require institutional care. Deficits can include partial paralysis, difficulties with memory, thinking, language, and movements[1].

Fifteen percent of ischemic strokes caused by hemorrhage and 85% occured following by ischemia. Ischemia caused by factors such as thrombosis, embolism and reducing in systemic perfusion[2].

Experimental models of cerebral ischemia have been developed to improve our understanding of the deleterious mechanisms involved in brain ischemic damage, and to study the potential efficiency of therapeutic strategies[3].

Many phenomena observed during brain ischemia and reperfusion can be accounted for by damage to membrane lipids, specifically by lipolysis during ischemia and by radical-mediated peroxidation of polyunsaturated fatty acids during reperfusion[4].

Blood-brain barrier (BBB) integrity protects the neuronal microenvironment. When this barrier integrity is lost, inflammatory cells penetrate the brain, causing cell death[5].

The brain consumes a large quantity of oxygen, making it particularly susceptible to oxidative stress[6].

During brain ischemia, the brain increases its reliance on anaerobic glycolysis, thus, cerebral glucose utilization increases substantially and brain glucose levels rapidly fall to barely detectable levels, despite near normal plasma levels[7].

During brain ischemia and reperfusion, a complex cascade of metabolic events initiated, several of which

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involve the generation of nitrogen and oxygen free radicals. These free radicals and related reactive chemical species mediate much of damage that occurs after transient brain ischemia[6].

During brain ischemia, the reduction in cerebral blood flow and oxygen delivery initiates a cascade of deleterious biochemical events. Decrease of oxygen precludes oxidative phosphorylation and results in a switch to anaerobic metabolism[8].

Mitochondrial respiratory chain is the major source for the production of reactive oxygen species (ROS) and following the destruction of the mitochondrial necrotic cell death occurs. Death of endothelial cells causes damage to BBB and resulting in cerebral edema[9].

Damage to energy-dependent ion pumps depolarizes cells and causes the release of glutamate into the extracellular space, N-methyl-D-aspartate receptors are activated resulting in increased intracellular calcium that responsible for the production of ROS and nitric oxide. When nitric oxide combines with superoxide, the proxy nitrites produce that can cause lipid peroxidation[10].

Brain tissue is not properly equipped with antioxidant defense agents. Thus, ROS, other free radicals and oxidants that are produced by inflamed cells, threaten the life of the tissue surrounding the ischemic region[11].

Impermeability of BBB is maintained by microvascular endothelial cells through their tight junctions and basal lamina. During brain ischemia damage to endothelial basal layer starts about 2 h after the onset of ischemia[12].

ROS have a critical role in destruction of the BBB in brain ischemic stroke. Mice lacking of superoxide dismutase are highly susceptible to focal cerebral ischemia[13]. ROS not only has roles in the destruction of the BBB, but also in many incurable diseases like diabetes[14,15], atherosclerosis[16,17], cardiovascular disease[17,18], neurological[19,20], and cancer[21,22]. ROS also create complications[23,24], and toxins[25].

Today, herbs are an important part of traditional medicine in many countries and various studies have shown that plants with high antioxidant effects could be effective in treatment or prevent of these diseases[26-28].

*Lavandula officinalis* is well known among people as a powerful aromatic and medicinal herb. The plant is used in traditional and folk medicines of different parts of the world for the treatment of several gastrointestinal, nervous and rheumatic disorders[29].

Lavender is comprised of over 100 constituents, among which the primary components are linalool and linalyl acetate, α-pinene, limonene,1,8-cineole,cis-andtrans-ocimene, 3-octanone, camphor, caryophyllene, terpinen-4-ol, and flavonoids[30].

Lavender extract has positive effects on spatial learning and memory, motor coordination, avoidance learning and analgesic effect[29].

The major source of free radicals during ischemia is mitochondrial damage and inflammatory response. The brain is protected against free radicals by collectors of exogenous free radicals such as ascorbate, techopherol, or by endogenous antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and catalase. Although the expression of these enzymes is increased during ischemia, but their capacity is limited and will result in increased concentration of free radicals[11].

In this study, the amount of polyphenols and antioxidant compound of lavender plant were assayed and the effect of lavender on the degree of permeability of BBB in a rat model of stroke was examined.

## 2. Materials and methods

### 2.1. Extract preparation

The lavender flowers were purchased from a local provider in Shahrekord. A voucher specimen was deposited in Herbarium unit of this university (No. 421). For preparation of ethanolic extract, air-dried and powdered flowering branches of the plant were macerated with ethanol for 48 h. The macerated powder was then shaked, filtered and evaporated in a rotating evaporator under reduced pressure until dryness. Stoke of extract provided in distilled water then used.

### 2.2. Animals

All experimental animal procedures were conducted with the approval of the Ethics Committee of Shahrekord University of Medical Science of Iran.

A total of 49 male Wistar rats weighing 250 g to 300 g were housed in a temperature and light-controlled room (22 °C, a 12-h cycle starting at 08:00 a.m.) and were fed and allowed to drink water *ad libitum*. The rats were divided into 7 groups with 7 rats per group.

Group I was ischemia rats received distilled water and underwent middle cerebral artery occlusion (MCAO) surgery. Group II was Sham-operated rats which were managed according to the ischemia group, but underwent surgery without MCAO. Group III was ischemia rats received lavender extract with dose 100 mg/kg and underwent MCAO surgery. Group IV was ischemia rats received lavender extract with dose 200 mg/kg and underwent MCAO surgery. Group V was ischemia rats as the control group received distilled water without any surgery, serum and brain tissue were collected

for biochemical analysis. Group VI received lavender extract with dose of 100 mg/kg without any surgery, at the end of experiment serum and brain tissue of intact groups were collected for biochemical analysis. Group VII received lavender extract with dose of 200 mg/kg without any surgery, at the end of experiment serum and brain tissue of intact groups were collected for biochemical analysis.

Two hours after the last dose, the groups of ischemia underwent of MCAO surgery.

### 2.3. MCAO

The rats were weighed and intraperitoneally anesthetized with ketamin 50 mg/kg and xylazine 5 mg/kg. MCAO was performed as described by Longa *et al*[31]. Briefly, using microscopic surgery, a 3-0 silicone coated nylon suture was introduced through the external carotid artery stump. The occlusion was advanced into the internal carotid artery 20-22 mm beyond the carotid bifurcation until mild resistance indicated that the tip was lodged in the anterior cerebral artery and blocked the blood flow to the middle cerebral artery. Reperfusion was started by withdrawing the suture after 60 min of ischemia. Rectal temperature was monitored (Citizen-513w) and maintained at 37.0 °C by surface heating and cooling during surgery.

### 2.4. Assessment of BBB integrity

Focal cerebral ischemia confirmation was also evaluated by using Evans blue (Sigma Chemicals, USA) dye extravasations. Briefly, the rats received 4 mL/kg of 2% Evans blue solution in saline by tail vein injection 30 min after MCAO. Then, 24 h after reperfusion, the thoracic cavity was opened under anesthesia. The rats were perfused with 250 mL saline transcardially to wash out intravascular Evans blue until colorless perfusion fluid was obtained from the atrium. After decapitation, the brains were removed and the hemispheres separated and weighed. The right and left hemispheres were separately homogenized in 2.5 mL phosphate-buffered saline to extract the Evans blue and to precipitate protein, 2.5 mL of 60% trichloroacetic acid was added and mixed by vortex for 3 min. The samples were then placed at 4 °C for 30 min and centrifuged for 30 min at 2991 r/min. The amount of Evans blue in the supernatants was measured at 610 nm using a spectrophotometer. Evans blue levels were expressed as μg/g of brain tissue against a standard curve.

### 2.5. Measurement of plasma malondialdehyde (MDA) levels

The plasma MDA level was measured as lipid peroxidation by the thiobarbituric acid (TBA) reactive substances method. Briefly, to 100 μL plasma or standard, 100 μL sodium dodecyl sulfate (8.1%) and 2.5 mL TBA/buffer (prepared by dissolving of 0.53% TBA in 20% acetic acid as adjusted to pH 3.5 with NaOH) were added. The tubes were covered with caps and incubated at 95 °C for 60 min. The reaction was stopped by placing tubes on ice followed by centrifugation at 4000 r/min for 10 min to separate two phases. The supernatant (20 μL) was injected into the high performance liquid chromatography system. Chromatographic determinations were performed on a high performance liquid chromatograph equipped with an 1100 series pump and a UV absorbance detector. An HP 3395 integrator was employed to record retention times, chromatograms, and evaluate peak heights. A technopak 10 IU C18 reversed-phase column (emission 553 and excitation 515) was used[32].

### 2.6. MDA levels of brain tissue

Following the behavioral tests, rats were killed. The brains were quickly removed and were washed twice with cold saline solution, different parts of brains such as hippocampus, cortex and subcortex separated, placed into glass bottles, labeled, and stored in a deep freeze (-80 °C) until processing. Tissues were homogenized in ice-cold Tris-HCl buffer (50 mmol/L, pH 7.4) for 2 min at 5000 r/min. The homogenized solution was then centrifuged for 60 min at 6688 r/min. MDA level was then measured. Lipid peroxidation was evaluated by measuring the content of TBA reactive substances according to TBA test method with slight modification. The MDA level was determined by a method based on the reaction with TBA at 90-100 °C. The reaction was performed at pH 2-3 at 90 °C for 15 min. The sample was mixed with 2 volumes of cold 10% (w/v) trichloroacetic acid to precipitate protein. The precipitate was pelleted by centrifugation and an aliquot of the supernatant was reacted with an equal volume of 0.67% TBA (w/v) in a boiling water bath for 10 min. After cooling, the absorbance was read at 532 nm using spectrophotometer[33].

### 2.7. Analysis of total phenolic compounds in the extract

The amount of phenolic compounds was measured. Absorbance of the samples was compared with a standard curve and total phenolic content of each extract was calculated milligrams per gram of dry extract[29].

### 2.8. Statistical analysis

Evans blue extravasation, plasma MDA level, brain MDA level were compared using One-way ANOVA (SPSS 11.0; *post*

*hoc* Tukey). Data were expressed as mean±SD. $P<0.05$ was considered significant.

## 3. Results

The amount of phenolic compounds of lavender extract was assessed. The total phenol of extract was 80.8 mg/g of dry extract.

### 3.1. Effect of lavender extract on the permeability of BBB

This study showed that pretreatment of rats with dose 200 mg of lavender extract caused a significant decrease in the permeability of the BBB compared with the ischemia group ($P=0.004$). A significant difference was observed in BBB permeability between sham group and ischemia group ($P=0.000$).

On the other hand, a significant difference was observed between BBB permeability in the right hemisphere (under ischemic) and left hemisphere (normal) in ischemia group ($P=0.034$), Figure 1.

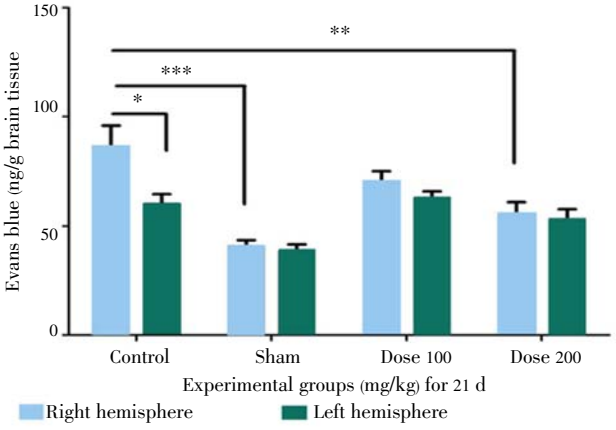


**Figure 1.** Outflow of Evans blue extravasation from BBB in the different experimental groups in the right hemisphere (under ischemic) and left hemisphere (normal)

*: $P<0.05$; **: $P<0.01$; ***: $P<0.001$; $n=7$.

### 3.2. Effect of lavender extract on lipid peroxidation in the brain and serum

In control-intact group the amount of lipid peroxidation (MDA levels) in penumbra and core significantly higher when compared with intact group that received extract with dose 200 mg ($P=0.044$, $P=0.021$ respectively) (Figure 2). On the other hand, in intact group that received extract with dose 200 mg, serum MDA levels significantly lower than control group ($P=0.003$), while lavender extract with dose 100 mg had no remarkable effect (Figure 3).

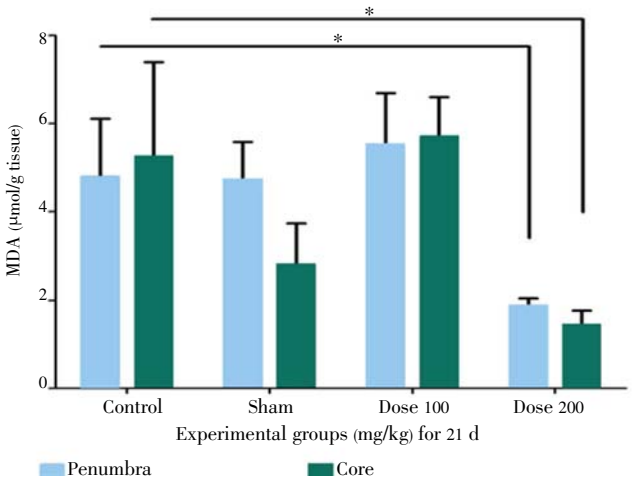


**Figure 2.** Effect of lavender extract on MDA levels in penumbra (cortex) and core (subcortex) of brain tissue (*$P<0.05$) in intact groups.

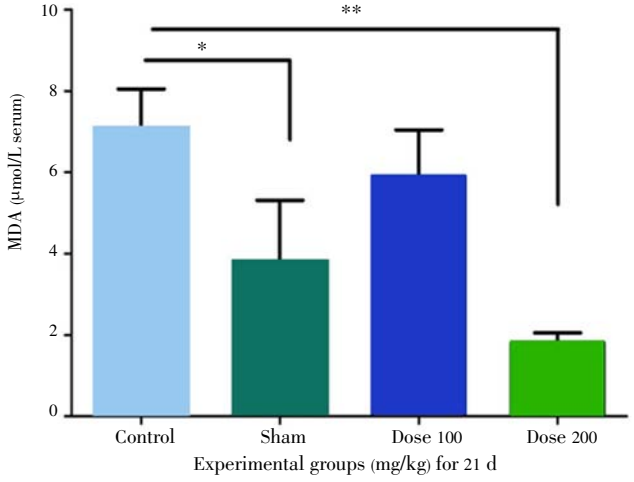


**Figure 3.** Effect of lavender extract on serum MDA levels (*$P<0.05$; **$P<0.01$; $n=7$) in intact groups.

## 4. Discussion

The results of this study indicated that treatment with ethanolic extract of lavender significantly decrease the permeability of BBB in a rat model of stroke. Lavender extract reduced serum and brain MDA levels in intact groups that proved lavender extract may be increased antioxidant capacity in brain and serum.

Matrix metalloproteinases (MMPs) are proteolytic enzymes cleaved to their fully active form in the interstitial space. Among the MMPs, MMP-2 and MMP-9 are able to digest the endothelial basal lamina leading to the opening of BBB[12].

During cerebral ischemia, endothelial basal lamina dissolution starts as soon as 2 h after the onset of ischemia, continues during reperfusion[34], and is rapidly followed by an increase in BBB permeability. The MMPs play an active role in secondary brain injury after focal ischemia[12].

Oxygen radical is one of the byproducts of the production of energetic compounds in body. Abundant presence of oxygen in aerobic tissues such as the brain, can converted these critical

molecule to ROS, that these highly fatal. In the brain, increased ROS cause oxidative damage to all cellular components by modified nucleic acid bases, breaking the DNA structure, break the glycosylphosphatidyl bands between the ribose and bases. Cerebral ischemia was produced by a cascade of metabolic events and by production of oxygen free radicals and nitrogen worsen the cell damage[35].

Many phenomena observed during brain ischemia and reperfusion can be accounted for by damage to membrane lipids, specifically by lipolysis during ischemia and by radical-mediated peroxidation of polyunsaturated fatty acids during reperfusion[36].

Oxidative stress can cause brain damage, directly by damage to membrane phospholipids and nucleotides and indirectly through the mediation of the cellular cascades. Therefore, studies for the development of neuroprotective agents for the treatment of stroke have focused on antioxidants. Antioxidants in many experiments *in vitro* and *in vivo* environments have been evaluated in clinical and preclinical studies for the treatments of various diseases[31,35,37-39].

Inflammatory changes can eventually lead to destruction of BBB and edema formation and the result is cell death. Some medicinal plants have also shown anti-inflammatory activities[40,41]. Thus, neuronal inflammation pathways can be targets for further drug therapy of ischemic[2].

Lavender belongs to the family Labiatae and has a variety of therapeutic and cosmetic uses. Lavender was used as a sedative, carminative, anti-depressant, anti-inflammatory, antispasmodic, analgesic, cerebrovascular disease in many nations[42].

Hydroalcoholic extract of lavender could not exert a significant decrease of abdominal twitches, in acid acetic test while the polyphenolic fraction at doses of 400 and 800 mg/kg and the essential oil at doses of 100 and 200 mg/kg significantly reduced writhes[30] lavender essential oil at a dose of 200 mg/kg has good effects on reducing inflammation[30].

MDA levels used as a key biomarker of oxidative stress to *in vivo* studies[43]. Our study shows that treatment with lavender extract reduces MDA levels in serum and brain tissue. The results of this study suggest that lavender extract reduces damage caused by ischemia-reperfusion through inhibition of oxidative stress.

The results of this study indicate that this plant have useful neuroprotective effects, but it should be noted that high doses of this plant has toxic effects on kidneys[44], which should be taken into consideration when consumption. Herbs are generally less toxic than synthetic drugs but being a natural of this material should not be considered as proof of their safety and caution should be taken when using these materials[45].

## Conflict of interest statement

We declare that we have no conflict of interest.

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