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Protective Effect of *Lavandula officinalis* on Pentylenetetrazol-Induced Epilepsy in Mice: Expression of NOS Genes and Caspase-3

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Abstract

Objective Given the incidence of epilepsy and the adverse effects of unconventional antiepileptic medicines, there is a need for a novel medical treatment strategy for epileptic patients.

Materials and Methods The current study involved the selection of 80 male mice, which were then separated into 10 experimental groups: pentylenetetrazol (PTZ), negative control which received normal saline, treatment which received *Lavandula officinalis* in two doses of 200 and 400 mg/kg, *L. officinalis* 200 mg/kg and 1400 w, *L. officinalis* 200 mg/kg and 7-NI, *L. officinalis* 200 mg/kg and diphenylene iodonium chloride (DPI), *L. officinalis* 400 mg/kg and 1400 w, *L. officinalis* 400 mg/kg and 7-nitroindazole (7-NI) and group which received *L. officinalis* 400 mg/kg and DPI. Each group was stimulated with an 11-day injection cycle (every 48 hours) of PTZ at a dosage of 35 mg/kg. All groups underwent PTZ challenge dosage (75 mg/kg) testing during the 12th injection. Ultimately, the brains of all mice were extracted, and the activity of genes related to neuronal nitric oxide, inducible nitric oxide, and endothelial nitric oxide was assessed. The enzyme-linked immunosorbent assay (ELISA) method was used to assess the quantity of caspase-3 in the groups.

Results *Lavandula officinalis* decreased the severity of seizures. The findings of our study demonstrated that the extract had a suppressive effect on the expression of inducible nitric oxide synthase (*iNOS*) and neuronal *NOS* (*nNOS*; $p < 0.05$), while it had a stimulatory effect on endothelial *NOS* (*eNOS*; $p < 0.05$). In addition, *L. officinalis* reduced caspase-3 levels in the groups who were administered the extract.

Keywords
- *Lavandula officinalis*
- seizure
- pentylenetetrazol
- mice
- apoptosis

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Conclusion The hydroalcoholic extract of *L. officinalis* has been found to be effective. *Lavandula officinalis* enhanced the expression of endothelial nitric oxide and reduced the levels of neuronal and inducible nitric oxide to a greater extent in brain tissue affected by epilepsy. The groups receiving extract derived from *L. officinalis* exhibited a reduction in the level of caspase-3.

Introduction

Epilepsy affects almost 65 million individuals globally, with around 80% of them living in underdeveloped nations.[1] Over the past two decades, more than 15 antiepileptic medications have been developed, several of which possess distinct modes of action. Nevertheless, medication fails to improve epilepsy in over 30% of people.[2] Medicinal herbs have long been utilized in the management of epilepsy in diverse cultural settings. In recent years, there has been a growing interest in using medicinal plants as a supplementary or alternative form of treatment. This approach has had a substantial impact on the management of seizures and exhibits fewer adverse effects in comparison to conventional antiepileptic medications.[3]

Lavandula officinalis, a member of the Lamiaceae family, is a well-known plant in Iranian traditional medicine for its therapeutic effects on nerve illnesses like epilepsy and dementia. *Lavandula officinalis* is commonly recognized for its sedative, antidepressant, diuretic, anticonvulsant, and general tonic properties.[4] In a prior study, we demonstrated that the extract of *L. officinalis* has the ability to decrease the advancement of seizure phases. Furthermore, biochemical tests have revealed that this extract possesses antioxidant properties and can mitigate oxidative stress and damage caused by the elimination of free radicals.[5] Nitric oxide[2] (NO) is generated by the oxidation of L-arginine by three distinct isoforms of nitric oxide synthase (NOS): neuronal nitric oxide synthase (nNOS), endothelial nitric oxide synthase (eNOS), and inducible nitric oxide synthase (iNOS).[6] NOS is extensively distributed throughout different parts of the brain and nervous system.[7]

Pentylenetetrazol (PTZ) kindling is a long-term model of epilepsy that triggers epileptic seizures. PTZ activates the N-methyl-D-aspartate (NMDA) receptor, which in turn triggers the activation of nNOS and the generation of superoxide anion.[8] The substantial quantity of superoxide anion generated in the hippocampus of kindling mice treated with PTZ promptly combines with NO from nNOS, resulting in the formation of peroxynitrite. These effects have a role in causing damage to neurons and are part of the underlying mechanisms of epilepsy.[9] Activated microglia and astrocytes generate proinflammatory cytokines and iNOS synthases. Chronically activated microglia can lead to apoptosis, which, in conjunction with excessive stimulation, leads to neurodegeneration, establishing a feedback loop of heightened excitability and the development of epilepsy.[10]

There is a scarcity of knowledge addressing alterations in the activity or manifestation of NOS in various brain areas. The inquiry at hand is the impact of the PTZ kindling method on the expression of *NOS* types, as well as the potential effect of the aqueous alcoholic extract of lavender on the expression of *NOS* genes.

Caspase-3 is an endoprotease that belongs to the family of enzymes responsible for controlling inflammation and apoptosis signaling networks. It is referred to as an executioner caspase in apoptosis due to its function in orchestrating the destruction of cellular structures, such as deoxyribonucleic acid (DNA) fragmentation or degradation of cytoskeletal proteins.[11] The discovery of the involvement of cell death regulating genes in the neuropathology of epilepsy has been made.[12] Caspase-3 antibodies are effective indicators for monitoring apoptosis induction by measuring the amounts of pro-caspase-3 and its active form. Therefore, the level of caspase-3 was investigated as the most reliable determinant.[13]

The molecular processes behind the effects of numerous therapeutic plants remain unknown. Proper integration of herbal medicines into society, with measures to prevent the uncontrolled use of chemical medications and their associated adverse consequences, can exert a substantial influence on the health and economy of people and society as a whole.

A previous study[5] discovered that lavender had a substantial impact in reducing NO levels and mortality. This study aims to investigate the effects of lavender on molecular mechanisms, specifically its influence on the expression of *NO* isoforms. In addition, our objective is to examine the plant's capacity to decrease mortality and comprehend its impact on apoptosis.

Materials and Methods

Animals

Male mice weighing 25 to 35 g, obtained from Hamadan University, were first placed in cages with a maximum capacity of five mice per cage for the duration of the study. The mice were subjected to a 12-hour light/dark cycle, with the lights being turned on at 6 a.m. They were provided with unlimited access to regular pellet food and tap water. The experimental protocol received approval from the Ethics Committee of Hamadan University.

Drugs

The desiccated aerial components of *L. officinalis* were acquired from the pharmaceutical research center of Bu Ali Sina University and were authenticated by Professor Dastan, an Associate Professor of Pharmacognosy at Hamadan

University of Medical Sciences. The *L. officinalis* powder was pulverized using a grinder and subsequently extracted through maceration using a shaker apparatus. A total of 600 g of plant powder was introduced into a 2,000 mL Erlenmeyer flask, and then 2 L of solvent, consisting of a mixture of 70% water and 30% ethanol, was added. To mitigate any possible chemical reactions, the extraction procedure was conducted in a location shielded from direct sunshine, and the container was securely shut to avoid the evaporation of the solvent. The plant underwent a 72-hour immersion on a shaker machine, and the extraction procedure was iterated three times. Subsequently, the solution was passed through Whatman filter paper and microbiological filters. The extract was condensed using a rotary evaporator under vacuum at a temperature of 45°C, and thereafter stored at a temperature of 4°C in a light-free environment until the time of the study. The extraction process resulted in a yield of roughly 12%. The sample was stored at a temperature of 4°C until the day of testing. During the experiment, the extract was dissolved and diluted in normal saline. PTZ (Sigma) was dissolved in sterile isotonic saline and injected intra peritoneal (ip). The 1400w, which is an inhibitor of iNOS, was diluted by combining it with sterile isotonic saline solution. The compound diphenylene iodonium chloride (DPI), which is known to inhibit eNOS, was solubilized in the solvent dimethyl sulfoxide (DMSO). The compound 7-nitroindazole (7-NI), which inhibits nNOS, was mixed with an isotonic solution and a little amount of Tween-80 was added to enhance its solubility. While we did not examine the chemical components of the above-ground parts of *L. officinalis* in this research, a previous study has already investigated the chemical components of the essential oil extracted from the flowers of *L. officinalis* grown in Isfahan. This investigation utilized the thin-layer chromatography (TLC) and gas chromatography-mass spectrometry (GC-MS) techniques. The investigation found that the primary components of the substance were linalool (34%), 1,8-cineole (18.5%), borneol (14.5%), camphor (10.2%), terpinen-4-ol (4.5%), linalyl acetate (3.7%), α-bisabolol (3%), α-terpineol (2.2%), and (z)-β-farnesene (2.2%).

Induction of Kindled Seizures and Seizure Observation Procedures

The treatments involved administering a subconvulsant dosage of PTZ at 35 mg/kg i.p. on alternate days, specifically days 1, 3, 5, and so on. The animals were categorized into ten distinct groups, with each group comprising eight mice. Group 1 was given 0.3 mL of normal saline. Group 2 received 35 mg/kg of PTZ. Groups 3 and 4 were administered different doses of the extract (200 and 400 mg i.p.). The remaining six groups were given 1400w (iNOS inhibitor, 1 mg/kg), DPI (eNOS inhibitor, 1 mg/kg), and 7-NI (nNOS inhibitor, 10 mg/kg) after the injection of the extract every other day, 30 minutes before PTZ injection. The mice were watched for a duration of 30 minutes immediately after the last drug administration. Following an extra 30-minute period, the mice were observed for mortality prior to being placed back into their original cage. For this investigation, a dosage of 75 mg/kg of PTZ was chosen as the challenging dose for kindled mice on day 24. This administration of PTZ resulted in convulsions characterized by clonic and tonic movements, as well as death. Seizures elicited by the 75 mg/kg PTZ challenge dose were assessed in all groups of kindled mice.

Sample Preparation

The mice that were ignited were euthanized by decapitation at the conclusion of the observation time on the day of testing. The brains were expeditiously extracted, cleansed twice with chilled saline solution, transferred into a cryotube, identified with a label, and preserved in a nitrogen tank at a temperature of −70°C until further handling.

Quantitative Real-Time RT-PCR Analysis of mRNA Expression of *eNOS*, *iNOS*, and *nNOS*

RNA extraction: Initially, a quantity of around 20 mg of brain tissue was utilized. The subsequent RNX solution (Sinacolon, Iran) was employed for the purpose of homogenization and disruption of the cell membrane. In addition, chloroform was used to achieve phase separation. The topmost stratum, comprising RNA, was isolated and an equivalent amount of isopropanol was introduced. The RNA that was obtained was thereafter preserved in a freezer at a temperature of −70°C for subsequent analysis. The RNA concentration and contamination levels in the extracted solution (protein, phenol, and DNA) were determined using a NanoDrop device (BioTek). The ratio of wavelengths at 260/280 nm is indicative of the purity of RNA and the presence of any contaminants during the extraction process. A ratio between 1.8 and 2 indicates a good level of RNA purity, whereas values below 1.8 indicate either contamination or a low concentration of RNA. The quality of RNA was evaluated using TBE buffer (Tris, Boric acid, and EDTA) and electrophoresis methods.

cDNA synthesis: This was achieved by utilizing the Moloney Murine Leukemia Virus (M-ML V) enzyme in a reaction mixture, following the guidelines provided by the manufacturer (Yekta Tajhiz Azma, Iran).

The experiment involved the use of quantitative real-time polymerase chain reaction (qRT-PCR) with SYBR Green fluorescence detection. This was performed utilizing a Sequence Detection system from ROCHE, Germany, along with appropriate primers. The primer pairs were sequenced as follows:

- *eNOS*: F: 5′- TAGTCCTCGCCTCGCCTCCTCG-3′, R: 5′-ACCACTTCCATTCTTCGTAGC-3′.
- *iNOS*: F: 5′- TTGTGCGAAGTGTCAGTGG-3′, R: 5′-TCCTTTGAGCCCGTGC-3′.
- *nNOS*: F: 5′-CGGAGATTGGCGTTCGTG-3′, R: 5′-CACCTTGTCACTCTGGAAGC-3′.
- GAPDH: F: 5′- CAAATTCAACGGCACAGTCAAGG-3′, R: 5′-GACTCCACGACATACTCAGCAC-3′.

The mRNA levels of different genes were determined following normalization using the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The data were normalized using the GAPDH mRNA levels and expressed as fold increases compared with the control.

Measurement of Caspase-3 Plasma Levels

Enzyme-linked immunosorbent assay (ELISA) is a quick diagnostic test utilized to identify and quantify antibodies, antigens, viruses, bacteria, or other compounds in plasma. The ELISA technique utilizes a solid phase composed of a 96-well plate constructed from polystyrene or alternative materials. The primary purpose of the solid phase is to immobilize the antigen or antibody in the sample through the process of binding. The sandwich ELISA method involves the presence of either known or unknown antigens in the samples, with the addition of a buffer to reduce the binding to the solid phase. Subsequently, enzymes that have been marked with labels are introduced to antibodies for the purpose of detection. This technique involves sandwiching the antigen between two particular antibodies. In this investigation, we employed the ZellBio kit to quantify the levels of caspase-3.

Statistical Analysis

The data are displayed as the average value plus or minus the standard error of the mean (SEM). The statistical analyses were performed using SPSS software version 25 (SPSS, United States) and GraphPad Prism version 8 (GraphPad, United States). The data among groups were compared using a one-way analysis of variance (ANOVA), and the means of each group were compared using least significant difference (LSD) multiple group comparison tests. Significance was attributed to *p*-values below 0.05.

Results

In our previous investigation, we found that repeated administrations of *L. officinalis* at doses of 200 and 400 mg/kg showed strong anticonvulsant effects against the development of PTZ kindling. *Lavandula officinalis* showed a superior efficacy as an anticonvulsant compared with Val, a pharmaceutical utilized for the management of epilepsy in clinical practice. This is due to the fact that *L. officinalis*, unlike Val, totally suppressed the fifth stage of seizures. *Lavandula officinalis* showed a superior efficacy compared with Val in mitigating the effects of the test dose of PTZ (75 mg/kg) in mice. Furthermore, our findings indicated that *L. officinalis* exhibited a greater inhibitory impact on NO compared with Val. Our research also showed that administering the extract from the aerial part of *L. officinalis* (at doses of 200, 400, and 800 mg/kg) effectively prevents seizures triggered by PTZ, with the level of inhibition increasing in proportion to the dosage. Nevertheless, the administration of the extract at a dosage of 800 mg/kg resulted in a less potent impact on the suppression of PTZ-induced convulsions. This could be attributed to the stimulatory or poisonous effects of the extract at higher concentrations[5] (►**Fig. 1**).

PTZ-induced kindling led to a notable elevation in brain tissue malondialdehyde (MDA) level, which serves as a marker for lipid peroxidation, in comparison to the control group. Administration of 400 mg/kg of *L. officinalis* prior to kindling significantly decreased the MDA content in the brain tissue compared with the PTZ group.

PTZ stimulation led to a statistically insignificant elevation in brain tissue NO levels as compared with the control

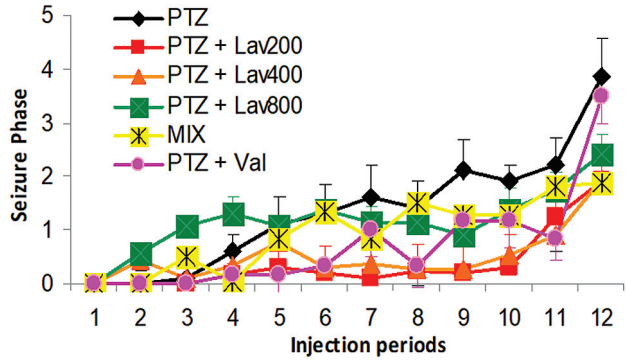

Fig. 1 Effect of aqueous-alcoholic extract of lavender with doses of 200, 400, 800 mg/kg and valproic acid with a dose of 150 mg/kg and a combination group on the progress of convulsive stages in different phases of kindling. Lav, *Lavandula officinalis*, lavender; Val, valproic acid 150 mg/kg; Mix: *L. officinalis* 200 mg/kg and valproic acid 100 mg/kg; PTZ, pentylenetetrazol.

group. Nevertheless, administering Val and three doses of *L. officinalis* prior to treatment notably inhibited the levels of NO in brain tissue, in comparison to both the control and PTZ groups. In addition, pretreatment with a dosage of 200 mg/kg of *L. officinalis* resulted in a decrease in NO levels when compared with the Val group. However, PTZ kindling resulted in a statistically insignificant drop in superoxide dismutase (SOD) levels. Conversely, pretreatment with Val and *L. officinalis* had a statistically insignificant enhancing effect on SOD levels.

Effects of *eNOS* and *eNOS* Inhibitor Expression in Different Groups

The expression of the *eNOS* gene in the group treated with 400 mg of *L. officinalis* extract exhibited a notable increase compared with the group treated with PTZ (1.7 ± 0.8, $p < 0.05$). The group that was administered a dosage of 200 mg of *L. officinalis* extract in combination with the eNOS inhibitor experienced a notable reduction in the expression level of the target gene (3.8 ± 0.8; $p < 0.05$). In contrast, the group that received the iNOS inhibitor together with the 200-mg extract showed a substantial increase in the expression of *eNOS* (3.8 ± 0.8; $p < 0.05$; see ►**Fig. 2**, ►**Fig. 3**).

Effects of *iNOS* and iNOS Inhibitor Expression in Various Groups

The group that was administered 400 mg of *L. officinalis* extract saw a substantial drop in the expression level of the *iNOS* gene compared with the PTZ group (5.1 ± 0.78, $p < 0.05$). In addition, the 200-mg extract also reduced *iNOS* gene expression in comparison to the PTZ group (5 ± 0.88, $p < 0.05$). In the group that received both 400 mg of *L. officinalis* extract and an iNOS inhibitor, there was a statistically significant increase in gene expression (5.3 ± 0.78, $p < 0.05$; see ►**Fig. 4**, ►**Fig. 5**).

Effects of *nNOS* and nNOS Inhibitor Expression in Different Groups

The gene expression level dropped in the group receiving a 400-mg extract. Conversely, the group treated with nNOS

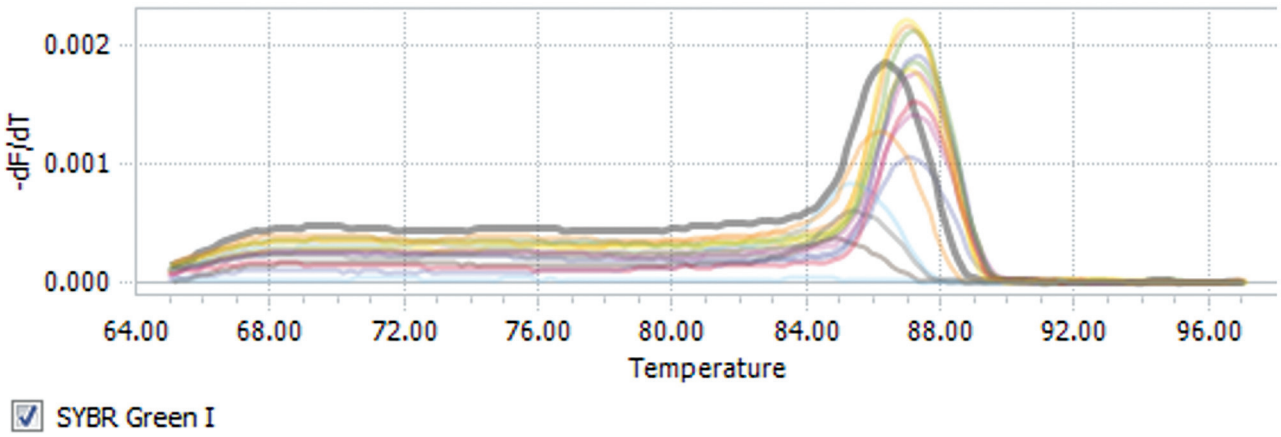

Fig. 2 Melting curve of endothelial nitric oxide synthase (*eNOS*) gene in different groups.

inhibitors had a substantial increase in gene expression level (1.83 ± 0.78). In addition, the group that received 200 mg of extract plus nNOS inhibitor demonstrated a substantial rise in gene expression (2 ± 0.78). In contrast, the group that received 200-mg extract along with eNOS and iNOS inhibitors showed a notable reduction in gene expression, with values of 1.63 ± 0.78 and 3.9 ± 0.78, respectively (►**Fig. 6**, ►**Fig. 7**).

Investigation of Caspase-3 Levels in Different Groups Using the ELISA Method

The study examined the impact of the extract on apoptosis by quantifying the quantity of caspase-3 with a sandwich ELISA technique and kit. The groups that received 200 and 400 mg of the extract showed significant decreases compared with the PTZ group, with values of 0.13 ± 0.013 and 0.15 ± 0.013, respectively.

In addition, there was a notable decrease in caspase-3 levels in the group that was administered a combination of 200 mg of *L. officinalis* extract and an eNOS inhibitor, with a value of 0.12 ± 0.013 compared with PTZ. In addition, the group that was administered lavender extract in combination with iNOS and eNOS inhibitors exhibited a noteworthy reduction in caspase-3 levels, with values of 0.11 ± 0.013 and 0.15 ± 0.013, respectively. In addition, in the group that was administered 400 mg of the extract coupled with inhibitors of eNOS, iNOS, and nNOS, the levels of caspase-3 reduced dramatically to 0.14 ± 0.013, 0.11 ± 0.013, and 0.13 ± 0.013 (►**Fig. 8**).

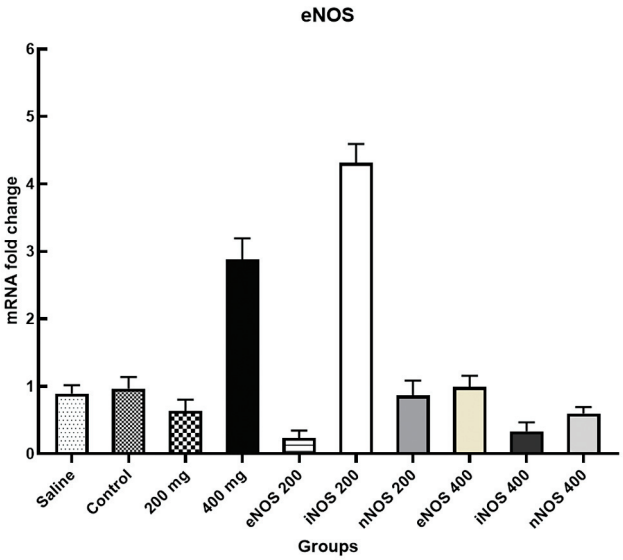

Fig. 3 Endothelial nitric oxide synthase (*eNOS*) gene expression in different groups. All data were normalized against the glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) messenger RNA (mRNA) levels and expressed as fold increases relative to control ± standard error of mean (SEM). The expression level of this *gene* increased significantly in the 400-mg *Lavandula officinalis* extract group compared with the pentylenetetrazol (PTZ) group (1.7 ± 0.8, $p < 0.05$). In the group of 200 mg of *L. officinalis* extract that received the eNOS inhibitor, the expression level of the target gene decreased significantly (3.8 ± 0.8; $p < 0.05$). iNOS, inducible nitric oxide synthase; nNOS, neuronal nitric oxide synthase.

Discussion

In our previous investigation, it was demonstrated that doses of 200 and 400 mg/kg of the aqueous-alcoholic extract of *L. officinalis* effectively decreased seizure phases produced by PTZ. In addition, varying concentrations of the botanical extract exhibited antioxidant properties in the cerebral tissue of mice during epileptic seizures, hence reducing oxidative stress and mitigating harm caused by free radicals. The administration of a 200-mg dose of the extract resulted in a decrease in NO levels, whereas a 400-mg dose led to a reduction in MDA levels and the inhibition of lipid peroxidation. To gain a deeper understanding of how different doses of *L. officinalis* work, we examined the levels of expression of *eNOS*, *iNOS*, and *nNOS* genes. Additionally, we assessed the antiapoptotic activity by measuring the level of caspase-3. NO has been acknowledged to stimulate receptors associated with guanylyl cyclase in the central nervous system, where it functions in the regulation of cerebral blood flow and neuronal plasticity. NO is produced in mammals through the synthesis of L-arginine by three specific enzymes known as NOS. The enzymes are classified as eNOS, nNOS, and iNOS. Each of these enzymes is regulated by a distinct gene.[14] eNOS serves as the main origin of NO within blood

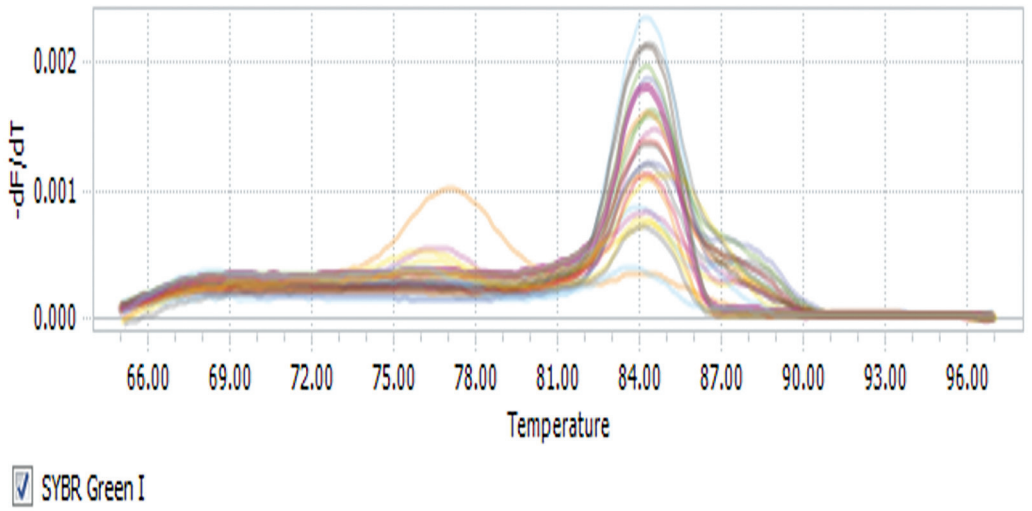

Fig. 4 Melting curve of the inducible nitric oxide synthase (*iNOS*) gene in different groups.

arteries. Studies have shown that a lack of eNOS in the brain might result in a higher occurrence of ischemia infarction and hindered arteriogenesis following a stroke, highlighting the crucial role of eNOS in brain function.[15] A 2022 study conducted by Bu et al found that rats lacking eNOS showed higher blood pressure, larger infarction sizes, and reduced blood flow compared with normal rats following a stroke. Furthermore, in constricted blood arteries, the interruption in the process of rebuilding blood channels after a lack of blood supply has been associated with a reduction in the flow of blood to the brain.[16] Zhang et al's 2016 work revealed that upregulated genetic expression of *eNOS* had a role in safeguarding hepatocytes from ischemia-reperfusion injury.[17] The results of this investigation, combined with the completed research, suggest that the group receiving 400 mg/kg extract saw an increase in the expression of *eNOS*.

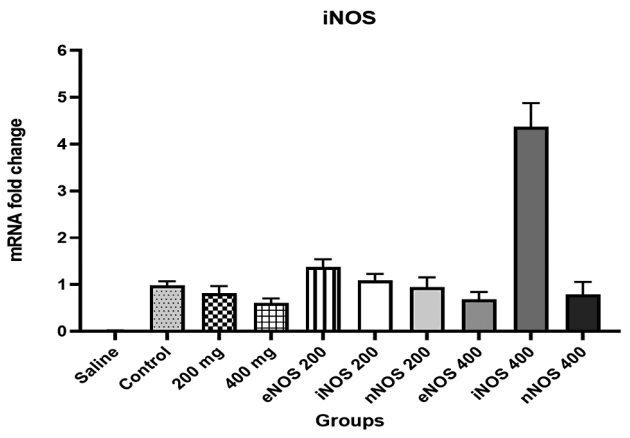

Fig. 5 Inducible nitric oxide synthase (*iNOS*) gene expression in different groups. All data were normalized against the glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) messenger RNA (mRNA) levels and expressed as fold increases relative to control ± standard error of mean (SEM). The 200- and 400-mg extracts decreased *iNOS* gene expression compared with the pentylenetetrazol (PTZ) group (5 ± 0.88; $p < 0.05$). enNOS, endothelial nitric oxide synthase; nNOS, neuronal nitric oxide synthase.

Given that the utilization of this extract was linked to a postponed initiation time of seizures and a reduced length of seizures, it can be deduced that one of the potential mechanisms could be the enhanced expression of this gene, which enhanced blood flow to the brain and hindered epileptic episodes. There is a correlation between the use of the inhibitor and an escalation in the duration of seizures. The study found that the groups that were administered 200 and 400 mg/kg of lavender extract showed a decrease in *iNOS* expression. However, in the group that was administered the iNOS inhibitor, there was an observed elevation in the expression level of this gene. The findings of our study are corroborated by research suggesting that iNOS is not excessively produced in the healthy brain. However, after experiencing trauma, inflammatory damage, stimulation, or hypoxia (such as stroke or ischemic events), the expression of *iNOS* is stimulated in both glial cells and neurons. Within some cells, the accumulation of iNOS occurs in proximity to the areas of brain damage, suggesting a correlation between inflammation and generation of NO from glial cells, which in turn leads to neuronal harm.[18] While iNOS is necessary for normal physiological processes, excessive levels of NO produced as a result of *iNOS* overexpression or dysregulation have been linked to many illnesses. There is evidence indicating that NO plays a function in infections. In animal models, upregulation of this gene induces vasoconstriction, leading to a decrease in blood pressure and cardiovascular dysfunction in response to stress induced by toxins and cytokines.[19] The synthesis of NO by iNOS[6] in cardiac myocytes led to cardiac complications in mice, including ventricular hypertrophy and disruptions in systolic pressure. This was linked to abrupt mortality.[20] The administration of miconazole, which is comparable to the extract used in our investigation, has demonstrated an elevation in the threshold for seizures. Furthermore, it suppresses the nuclear factor-kappa B (NF-κB) pathway and the generation of iNOS. Miconazole also induces a temporary suppression of the melastatin receptor (TRPMZ), a protein that functions as

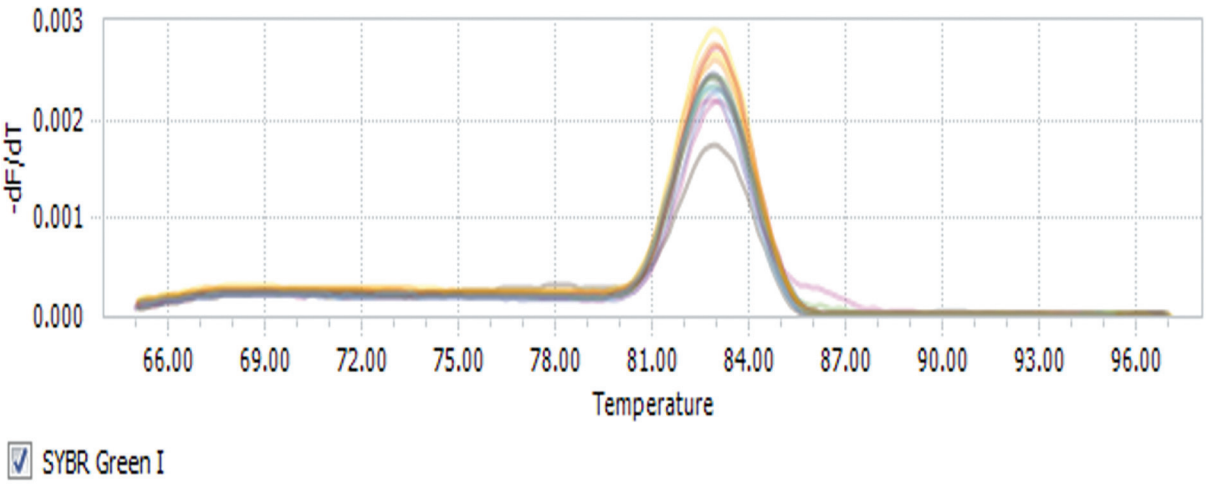

Fig. 6 Melting curve of the neuronal nitric oxide synthase (*nNOS*) gene in different groups.

a nonspecific cation channel allowing the passage of calcium ions. This can result in heightened neuroinflammation and worsening of several forms of central nervous system disorders, such as epilepsy.[21] However, it remains unclear if the expression of *iNOS* and its activity directly cause nerve damage or are only associated with specific stages of neurodegenerative disorders. Additionally, it is uncertain whether iNOS has protective effects in certain circumstances, highlighting its dual and ambiguous nature. In the group administered with 400 mg of the extract, there was a reduction in gene expression, whereas in the group given the nNOS inhibitor, there was a significant rise in gene expression. In addition, the group that was administered 200 mg of lavender extract along with the nNOS inhibitor exhibited a noteworthy augmentation in *nNOS* gene expression. Several conducted studies corroborate our findings. Zhu et al's 2017 study discovered a strong association between epilepsy and a high occurrence of cognitive problems and depressive-like behaviors. However, the specific reasons behind this connection remain unknown. Norepinephrine (NE), a neurotransmitter involved in maintaining the equilibrium of other neurotransmitters, plays a significant role in the development of cognitive problems.[22] Our investigation found that the group receiving 400 mg of extract showed a decrease in *nNOS* gene expression compared with the control group. Hwang et al demonstrated that acupuncture effectively suppressed the elevation of blood pressure in different anatomical locations of hypertensive rats by modulating the production of nNOS.[23] *nNOS* is predominantly expressed in the hippocampus, cortex, hypothalamus, amygdala, and other regions associated with stress and illnesses inside the brain. Alterations in the expression of *nNOS* have been detected in several conditions including major depression, bipolar disorder, stroke, Parkinson's disease, and Alzheimer's disease.[24] In contrast, Zhu et al demonstrated that altering the *nNOS* gene not only conferred protection against stroke but also enhanced neurogenesis. Additionally, it plays a role in ischemia resulting from cerebral injury in the central

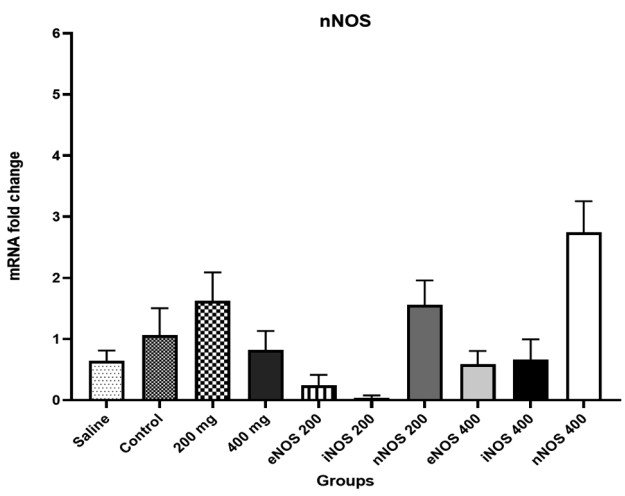

Fig. 7 Neuronal nitric oxide synthase (*nNOS*) gene expression in different groups. All data were normalized against the glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) messenger RNA (mRNA) levels and expressed as fold increases relative to control ± standard error of mean (SEM). The group receiving 200-mg extract and nNOS inhibitor had a significant increase in gene expression (2 ± 0.78). eNOS, endothelial nitric oxide synthase; iNOS, inducible nitric oxide synthase.

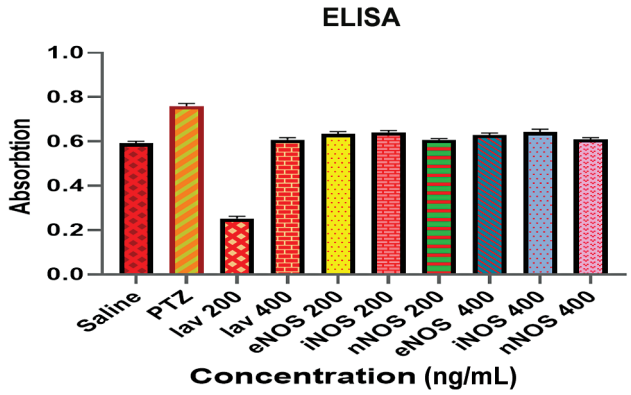

Fig. 8 Concentration of caspase-3 in different groups in the group of 400-mg extract and inhibitors of endothelial nitric oxide synthase (eNOS), inducible NOS (iNOS), and neuronal NOS (nNOS) caspase-3 decreased significantly (0.14 ± 0.013, 0.11 ± 0.013, 0.13 ± 0.013, respectively). PTZ, pentylenetetrazol.

nervous system.[25] However, different investigations have been undertaken that suggest an additional role beyond what is indicated in our study. Percival et al demonstrated that nNOS isoforms play a role in controlling fatigue, oxidative capacity, and oxygen transport, all of which are crucial for achieving the highest possible exercise capacity in skeletal muscles. Furthermore, mice lacking nNOS exhibit the presence of type 2 fibers, along with vascular control and resilience to fatigue.[26] Despite the progress made in medicinal and surgical interventions for epilepsy, our understanding of the underlying processes and mechanisms that cause epilepsy remains incomplete. Apoptosis, neuroinflammation, and oxidative stress are key components involved in the development of epilepsy. Epilepsy involves an intricate series of cellular and molecular processes that lead to excessive stimulation, oxidative stress, and inflammation. These processes result in the release of cytokines that cause cell damage and trigger cell death.[27] Apoptosis is an essential biological process that has a significant impact on the development and injury of the nervous system. Gaining insight into the molecular mechanisms that initiate apoptosis is a crucial milestone in the development of successful treatment approaches for neurological disorders. Caspases are a collection of molecules that are controlled by the presence of Bax. Caspases are a group of cysteine proteases that trigger apoptosis and function as proenzymes that undergo division and cleavage in response to apoptotic signals. Recent results indicate that caspases have a substantial impact on neuronal cell death during development, as well as after neuronal injury.[28] The 2010 study conducted by Yamada et al showed that the pathophysiology of Parkinson's disease involves the action of caspases and the death of dopaminergic neurons. Caspase-3 initiates a series of caspase activations in the process of programmed cell death known as apoptosis. Caspase-3 has been detected in the substantia nigra of individuals diagnosed with Parkinson's disease.[6] Our investigation revealed a notable reduction in caspase-3 levels in the group administered with 200 and 400 mg/kg of the extract, as compared with the control group. The decrease in seizure occurrence can be ascribed to the extract's ability to raise the threshold for seizures and reduce their duration. Cregan et al observed an elevation in caspase-3 levels in the brains of mice affected by Parkinson's disease in their investigation.[28]

Conclusion

This study demonstrated that the aqueous-alcoholic extract of lavender, when administered at doses of 200 and 400 mg/kg, can impact the expression of *eNOS*, *iNOS*, and *nNOS* genes. Specifically, it was found that the expression of *eNOS* increased, while that of *iNOS* and *nNOS* decreased. Additionally, the use of this extract led to a reduction in the levels of caspase-3.

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Conflict of interest
None declared.

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