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Anti-epileptogenic and antioxidant effect of *Lavandula officinalis* aerial part extract against pentylenetetrazol-induced kindling in male mice

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ABSTRACT

Ethnopharmacological relevance: Repeated application of *Lavandula officinalis* (*L. officinalis*) has been recommended for a long time in Iranian traditional medicine for some of nervous disorders like epilepsy and dementia. However, there is no available report for the effect of chronic administration of *Lavandula* extract in development (acquisition) of epilepsy. Therefore, this study was designed to investigate the anti-epileptogenic and antioxidant activity of repeated administration of *Lavandula officinalis* extract on pentylenetetrazol (PTZ) kindling seizures in mice model.

Materials and methods: *Lavandula officinalis* was tested for its ability (i) to suppress the seizure intensity and lethal effects of PTZ in kindled mice (anti-epileptogenic effect), (ii) to attenuate the PTZ-induced oxidative injury in the brain tissue (antioxidant effect) when given as a pretreatment prior to each PTZ injection during kindling development. Valproate (Val), a major antiepileptic drug, was also tested for comparison.

Results: Val and *Lavandula officinalis* extract showed anti-epileptogenic properties as they reduced seizure score of kindled mice and PTZ-induced mortality. In this regard, *Lavandula officinalis* was more effective than Val. Both *Lavandula officinalis* and Val suppressed brain nitric oxide (NO) level of kindled mice in comparison with the control and PTZ group. Meanwhile, *Lavandula officinalis* suppressed NO level more than Val and *Lavandula officinalis* also decreased brain MDA level relative to PTZ group.

Conclusion: This is the first report to demonstrate NO suppressing and anti-epileptogenic effect of chronic administration of *Lavandula officinalis* extract on acquisition of epilepsy in PTZ kindling mice model. In this regard, *Lavandula officinalis* extract was more effective than Val, possibly and in part via brain NO suppression.

1. Introduction

Epilepsy is a complex neurological disorder affecting 50 million of world's total population (Prilipko et al., 2006). Various anticonvulsant agents are available to grapple with this neurological disorder. Despite treatment with available antiepileptic drugs (AED), epilepsy remains refractory in one third of patients. Further, adverse effects associated with AED and recurrent seizures limit their use. Recent data show the efficacy of some substances with antioxidant properties in the therapy of convulsive disorders and provide support for the hypothesis on the crucial role of free radicals in the pathogenesis of neurotoxic brain damage, i.e., epilepsy (Kabuto et al., 1998). Therefore one of the main trends of current investigations is the search for novel antiepileptic drugs with neuroprotective properties. The use of plants and plant extracts to treat diseases is a therapeutic modality. According to the world health organization (WHO), about three-quarters of the world population relies upon traditional remedies (mainly herbs) for the health care of its people (Gilani and Rahman, 2005).

Lavandula officinalis (Lamiaceae), commonly known as Ustu khuddoos or Lavandula has been used for a long time in Iranian traditional medicine for some of nervous disorders like epilepsy and dementia (Sharafkandi, 1991). *Lavandula officinalis* generally has been considered as an sedative, antidepressive, antispasmodic, antiflatulent, antiemetic, diuretic, anticonvulsant, antibacterial, and a general tonic (LaGow et al., 2004). In recent studies, *Lavandula* oil has been found useful as nocturnal sedative (Hardy, 1991; Hudson, 1996), and has also beneficial effects in stress (Tisserand, 1992). There are also some reports for anticonvulsive activities of the *Lavandula officinalis* extract (Salah and Jager, 2005; Shahriyari, 2004Merabani et al., 2006; Gilani et al., 2000). However, there is no report available for chronic administrations of Lavandula extract in acquisition of epilepsy. Also, the

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mechanism of action of *Lavandula officinalis* on epilepsy has not been clearly determined.

PTZ-kindling is an animal model which simulates clinical epilepsy. In this model, repeated injection of sub-convulsive dose of PTZ causes gradual development of seizure, culminating to generalized-tonic–clonic seizures. Further reports indicate that the free radical generation due to the increased activity of glutamatergic transmitter plays a crucial role in neuronal cell death of the PTZ kindling in rodent (Schroder et al., 1993; Rocha et al., 1996; Sechi et al., 1997; Sejima et al., 1997; Becker et al., 1997; Rauca et al., 1999). However, chemical kindling is also a suitable model for testing efficacy of repeating administrations of *Lavandula officinalis* for prevention and treatment of epilepsy development.

Therefore, one of the aims of this study was to provide a scientific basis for the traditional chronic use of *Lavandula officinalis* extract in epilepsy in addition to better understand the mechanisms of action of *Lavandula officinalis* extract.

2. Materials and methods

2.1. Animals

Male NMRI albino mice weighing 25–30 g (Razi Institue, Karaj) at the beginning of the study were housed in cages with a maximum of 5/cage. The mice were housed under a 12-h light/dark cycle with lights on at 6 a.m. The mice had standard pellet food and tap water available ad libitum. The experimental protocol was approved by Ethics Committee of Shahed University.

2.2. Drugs

PTZ (Sigma) was dissolved in sterile isotonic saline and administered i.p. Val (Sigma, USA) was diluted by addition of sterile isotonic saline solution and it was also administered i.p. every second day before PTZ administration. Dried aerial parts of *Lavandula officinalis* (500 g) were purchased from the local market and was identified by professor Amin, the head of Herbarium of Faculty of Pharmacy, Tehran Medical University, where a Voucher specimen number was deposited under the reference number PMP-314. *Lavandula officinalis* powder was prepared using a grinder. Then, plant powder was soaked in double distilled water and ethanol 70% (1:4) for three days at room temperature (25 °C), and filtered. Filtration was repeated three times. All filtrates evaporated to dryness in water bath at 70 °C. The yield of the extract was about 12%. It was stored at −20 °C until test day. Extract was dissolved and diluted in normal saline on the day of experiment. In this study, although we did not analyze chemical constituents of aerial part of *Lavandula officinalis*, but chemical constituents of the essential oil of flowers of *Lavandula officinalis* growing in Isfahan (Iran) were previously studied by TLC and gas chromatography–mass specrtometery (GC–MS) methods. On this basis, its main ingredients were linalool (34.1%), 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%).

2.3. Induction of kindled seizures and seizure observation procedures

Kindling was induced by a total of 11 treatments with a subconvulsant dose of PTZ 35 mg/kg i.p. on every second day (i.e. day 1, day 3, day 5, etc.). Animals were divided into five groups of ten mice each. Group 1 was given 35 mg/kg of PTZ, group 2 received 150 mg/kg of Val. i.p, and the other three groups were given different doses of the extract (200, 400, and 800 mg/kg; i.p.) every second day 30 min before each PTZ injection. Mice were observed for 30 min after the last drug administration; after an additional 30 min, the mice were observed for lethality before returning to home cage. Seizure intensity was evaluated using the following modified scale (Erakovic et al., 2001): 0 no response; 1 ear and facial twitching; 2 convulsive waves axially through the body; 3 myoclonic; 4 generalized clonic convulsions turn over into side position; 5 generalized convulsions with tonic extension episode and status epilepticus; and 6 mortality. The animal was considered to be kindled after having received 11 PTZ injections. In the present study, 75 mg/kg of PTZ was selected as a challenge dose in kindled mice on day 24 (test day). This dose of PTZ produced convulsions (clonic and tonic) and lethality. All groups were tested for PTZ challenge dose (75 mg/kg)-induced seizures in kindled mice. Latent period and duration of phases 2 and 5 were determined. Chimney test was also performed for studying probable motor activity disturbances induced by drugs (Luszczki et al., 2009).

2.4. Sample preparation and biochemical evaluation

Kindled mice were sacrificed by decapitation at the end of the observation period on the test day. The brains were quickly removed and were washed twice with cold saline solution, placed into glass bottles, labeled, and stored in a deep freeze (−30 °C) until processing (maximum 10 h). Tissues were homogenized in four volumes of ice-cold Tris–HCl buffer (50 mM, pH 7.4) for 2 min at 5000 rpm, after cutting up the brains into small pieces with scissors. Malondialdehyde (MDA), nitric oxide (NO), and protein levels were then measured. The homogenized solution was then centrifuged for 60 min at 5000 × g to remove debris. The supernatant solution was then extracted with a mixture of ethanol/chloroform (a volume with ratio of 5:3). After centrifugation at 5000 × g for 30 min, the clear upper layer (the ethanol phase) was taken and used for evaluation of the superoxide dismutase (SOD) activity and protein assay. All experiments were carried out at +4 °C.

2.4.1. Malondialdehyde (MDA) determination

The MDA level was determined by a method based on the reaction with thiobarbituric acid (TBA) at 90–100 °C (Esterbauer and Cheeseman, 1990). In the TBA test reaction, MDA or MDA-like substances and TBA react together for production of a pink pigment having a maximum absorption at 532 nm. 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% (w/v) TBA in a boiling water bath for 10 min. After cooling, the absorbance was read at 532 nm.

2.4.2. Nitric oxide (NO) determination

As NO measurement is very difficult in biological specimens, tissue level of nitrite and nitrate was considered as an index of NO production. The method for brain nitrite and nitrate levels was based on the Griess reaction (Cortas and Wakid, 1990). Principle of the assay is the conversion of nitrate into nitrite by cadmium and followed by color development with Griess reagent (sulfanilamide and N-naphthyl ethylenediamine) in acidic medium (Cortas and Wakid, 1990).The total nitrite was measured by Griess reaction. The absorbance was determined at 540 nm with a spectrophotometer (Ihan et al., 2005).

2.4.3. Superoxide dismutase (SOD) activity determination

Total SOD activity was determined according to the method of Sun et al. (1988). The principle of the method is based on

the inhibition of nitroblue tetrazolium (NBT) reduction by the xanthine–xanthine oxidase system as a superoxide generator. Activity was assessed in the ethanol phase of the brain homogenate after 1.0 ml ethanol/chloroform mixture (5/3, v/v) was added to the same volume of sample and centrifuged. One unit of SOD was defined as the enzyme amount causing 50% inhibition in the NBT reduction rate.

2.5. Statistical analysis

Data were expressed as means ± S.E.M. Statistical analysis was carried out using repeated measure ANOVA followed by post-hoc Tukey *post-hoc* test for parametric data and Kruskal–Wallis one-way analysis of variance by ranks followed by *post-hoc* Mann–Whitney test for non-parametric data. *P* values less than 0.05 were considered as significant.

3. Results

3.1. Effect of Val and different doses of Lavandula officinalis on seizure intensity induced by PTZ

All mice in each group survived without any complications at the end of the kindling period. Only on the test day (PTZ, 75 mg/kg, i.p.), mortality in PTZ group was 10% (Table 2). In the PTZ group, repeated administration of a subconvulsant dose of PTZ (35 mg/kg, i.p.) on every second day (for 21 days, 11 injections) resulted in increasing convulsive activity leading to generalized clonic–tonic seizure. Fig. 1 shows that pre-administration of Val at a dose of 150 mg/kg inhibits seizure scores ($p < 0.05$). On the test dose, pretreatment with Val protected against mortality but did not prevent the development of seizure. Table 1 shows that in kindle period, although pre-administration of Val could not suppress seizure score of 5, but could significantly reduce its duration ($p < 0.05$). Fig. 1 also shows that pretreatment with 200 and 400 mg/kg of *Lavandula officinalis* extract in kindling period and at PTZ test dose could significantly reduce the seizure scores ($p < 0.05$), so that none of the animals could achieve a score of 5 with 12 injections of PTZ (Tables 1 and 2). Effectiveness of *Lavandula officinalis* at a dose of 200 mg/kg was greater than the other drugs and it significantly decreased mean seizure scores in periods 7, 8, 9, 10,11 and 12 relative to the PTZ group ($P < 0.05$) although pre-administration of 800 mg/kg of *Lavandula officinalis* failed to reduce the seizure score in PTZ treated mice (Fig. 1), but this dose like other doses of the extract completely suppressed 5th phase of seizures (Tables 1 and 2). Table 1 also shows that Val and the extract at all doses could significantly reduce ($p < 0.05$) duration of 2th phase of seizures. Meanwhile, Table 2 also shows that in chimney test, pretreatment with Val (150 mg/kg) and *Lavandula officinalis* (200, 400 and 800 mg/kg) does not induce any motor disturbance.

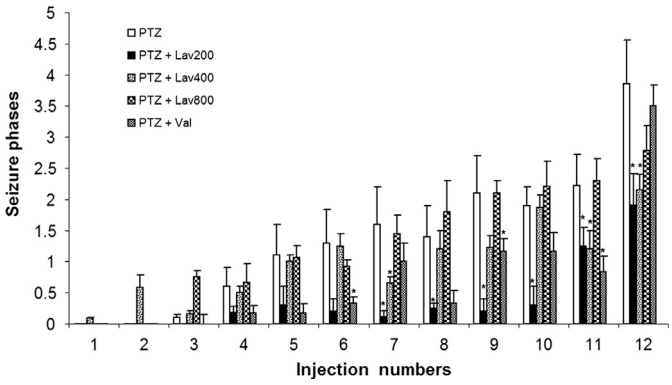

Fig. 1. Effect of Val and different doses of *Lavandula officinalis* pretreatment on the development of PTZ-kindled seizure. Kindling was produced by a total of 11 treatments with 35 mg/kg of PTZ i.p. on alternate days. Val pretreatment significantly reduced the course of kindling induced by 11 injections of PTZ. However, treatment with *Lavandula officinalis* significantly inhibited the seizures score, as none of the animals could achieve a score of 5 with 12 injections of PTZ. *Lavandula officinalis*-pretreated mice (200 mg/kg) revealed significant decreases in mean seizure scores on periods 7 to 12 relative to the PTZ group ($p < 0.05$). * Statistically significant compared with the PTZ group. PTZ, Val and Lav indicate pentylenetetrazol, valproate and *Lavandula officinalis*, respectively.

Table 1
Effect of Val and different doses of *Lavandula officinalis* on PTZ-induced kindling factors.

Experimental groups	Latency of 2th phase (min)	Duration of 2th phase (sec)	Latency of 5th phase (min)	Duration of 5th phase (sec)
PTZ	4.41 ± 0.52	27.19 ± 2.19	3.33 ± 0.86	4.11 ± 0.48
PTZ+Val	3.66 ± 1.17	*9.50 ± 1.55	2.12 ± 0.60	*2.15 ± 0.45
PTZ+Lav200	6.33 ± 2.53	*7.55 ± 1.48	–	–
PTZ+Lav400	6.55 ± 1.18	*10.40 ± 0.32	–	–
PTZ+Lav800	4.41 ± 0.52	*12.86 ± 1.65	–	–

Effect of Val (150 mg/kg) and three doses of Lavandula officinalis (200, 400 and 800 mg/kg) on the latency and duration of 2th and 5th phases of seizures, n = 10 in each group. PTZ, Val and Lav indicate pentylenetetrazol, Valproate and Lavandula officinalis, respectively.

* Statistically significant as compared to the PTZ group, $p < 0.05$.

Table 2
Effect of Val and different doses of *Lavandula officinalis* on latency and duration of 5th phase of seizure in PTZ test dose.

Experimental groups	Incidence of mortality (%)	Duration of 5th phase (sec)	Latency of 5th phase (min)	Chimney test % of rats that showed motor disturbance
PTZ	10	4.51 ± 0.58	3.86 ± 0.70	0
PTZ+Val	0	3.15 ± 0.45	3.50 ± 0.60	0
PTZ+Lav200	0	–	–	0
PTZ+Lav400	0	–	–	0
PTZ+Lav800	0	–	–	0

Effect of Val and different doses of Lavandula officinalis on latency and duration of 5th phase of seizure and mortality incidence in PTZ test dose. PTZ, Val and Lav indicate pentilentetrazol, Val and Lavandula officinalis, respectively.

3.2. Effect of Val and different doses of Lavandula officinalis on biochemical indexes of oxidative stress markers

Table 3 shows brain levels of oxidative stress markers in the kindled and non-kindled (control) mice. PTZ kindling produced a significant increase in the brain tissue MDA content, an index of lipid peroxidation, when compared with control group. Pretreatment with 400 mg/kg of *Lavandula officinalis* in kindled group significantly reduces brain tissue MDA content when compared with PTZ group. PTZ kindling produced a non-significant increase in the NO level in brain tissue when compared with control group. However, pretreatment with Val and three doses of *Lavandula officinalis* significantly suppressed brain tissue NO level when compared with control and PTZ groups, respectively. In addition, pretreatment with 200 mg/kg of *Lavandula officinalis* suppressed NO level when compared with Val group. On the other hand, PTZ kindling caused a non-significant decrease of the SOD level, whereas Val and *Lavandula officinalis* pretreatment non-significantly enhanced it.

Table 3
Effect of Val and different doses of *Lavandula officinalis* on biochemical indexes of oxidant/antioxidant stress markers.

Group test	NO (mg/g protein)	MDA (nmol/g protein)	SOD (U/g protein)
Control (non-kindled)	3.05 ± 1.29	10.96 ± 0.98	102.94 ± 21.30
PTZ	3.41 ± 1.74	27.42 ± 2.80*	83.81 ± 19.46
PTZ+Val	0.64 ± 0.20*,**	26.02 ± 1.94*	119.88 ± 19.92
PTZ+Lav200	0.05 ± 0.02*,**,***	21.32 ± 0.54*	135.41 ± 5.53
PTZ+Lav400	0.18 ± 0.92*,**	19.53 ± 0.34*,**	134.63 ± 8.19
PTZ +Lav800	0.11 ± 0.05*,**	22.98 ± 1.39*	115.99 ± 16.17

NO, MDA and SOD levels of brain in experimental groups. PTZ: Pentylenetetrazole, Val: Valproate, Lav: Lavandula officinalis

* *Significant difference versus control group, $p < 0.05$.*
** *Significant difference versus PTZ group, $p < 0.05$.*
*** *Significant difference versus Val group, $p < 0.05$.*

4. Discussion

In recent years, the medicinal properties of plants have been studied in the light of scientific developments throughout the world, due to their potent pharmacological effects, low toxicity and economic viability (Ihan et al., 2005). In traditional medicine, *Lavandula officinalis* (Ustu khuddoos) has been used in nervous disorders like epilepsy, dementia, depression, chest and rib pains etc. in extract form for internal use, and in form of ointment for external use (Sharafkandi, 1991). *Lavandula officinalis* has generally been considered as a sedative, antidepressive, antispasmodic, antiflatulent, and antiemetic, diuretic, anticonvulsant, antibacterial, and a general tonic (LaGow et al., 2004).

The present study demonstrated a potent anticonvulsant properties of repeated administrations of *Lavandula officinalis* (200 and 400 mg/kg) against the development of PTZ kindling. *Lavandula officinalis* was more effective as an anticonvulsant agent than Val, a drug for epilepsy treatment in the clinic, because *Lavandula officinalis* (but not Val) completely inhibited 5th phase of seizures. *Lavandula officinalis* was also more effective than Val against the effects of the test dose of PTZ (75 mg/kg) in mice. In addition, our results showed that NO suppressive effect of *Lavandula officinalis* was higher than Val. Also, our finding indicated that repeated administration of aerial part extract of *Lavandula officinalis* (200, 400 and 800 mg/kg) inhibits PTZ induced seizures in a dose dependent manner. While 800 mg/kg of the extract had a weaker effect on inhibition of PTZ induced convulsions, the most effective dose was 200 mg/kg. This may be due to excitatory or cytotoxic effects of the extract at higher doses.

Gilani et al. reported that pretreatment of mice with different doses of the *Lavandula stoechas* flowers extract (400 and 600 mg/kg, i.p.) 60 min before the subcutaneous injection of PTZ (90 mg/kg) causes a dose-dependent protection against PTZ induced convulsions (Gilani et at., 2000). It has also been shown that pretreatment of mice with hydroalcoholic leaves extract of the *Lavandula officinalis* (100, 200, 400, 600, and 800 mg/kg i.p) dose dependently inhibits nicotine-induced seizures. Meanwhile 600 mg/kg was the most effective dose (Arzi et al., 2011). Stem and flowers methanolic extract of *Lavandula officinalis* produced a significant sedative effect at doses of 200, 400, and 600 mg/kg (by oral route), and hypnotic effect at doses of 800 and 1000 mg/kg. Also, the treatment of mice with the aqueous extract at doses of 200 and 400 mg/kg via oral route significantly produced sedative effect in mice. However, following oral administration of *Lavandula officinalis* extract at doses of 500, 1000, 1500, 2000, 3000, and 5000 mg/kg, no toxicity and no significant changes in the body weight were observed in mice. Therefore, it is shown that the LD_{50} was higher than 5000 mg/kg for oral administration (Alnamer et al., 2012).

It seems that different effective doses in various studies are related to different animal models and also the kind of extract and its route of administration. Our findings demonstrate that chronic administration of *Lavandula officinalis* extract was possibly more effective than other applications. In Iranian traditional medicine, chronic administration of Ustu Khuddos is recommended for nervous disorders and extract dosage was about 90–230 mg/kg. Extraction has been performed by aerial part of plant in pure wine and it was used in the form of sirup (Sharafkandi, 1991). Recently, *Lavandula officinalis* flower oil is clinically used for mood disturbances such as restlessness or insomnia, functional abdominal complaints, nervous stomach irritations, Roehmheld syndrome, nervous intestinal discomfort. *Lavender* oil dosage (internal): 1–4 drops (ca. 20–80 mg), external use as bath additives: 20–100 g of drug for a 20 L bath. Drug contains at least 1.5% (v/w) essential oil (Blumenthal et al., 1998). However, CNS depression, constipation, respiratory depression, headache, meiosis, vomiting, and convulsions may occur in overdose (LaGow et al., 2004). Therefore, it seems that at higher doses, depressive or excitatory effects are appeared.

There are different suggestions about the mechanism of action of *Lavandula officinalis* extract in inhibition of PTZ induced seizures. The studies of Silva Brum showed that linalool (a monoterpen in *Lavender*) inhibits the NMDA, kinin and PTZ induced convulsion (Silva Brum et al., 2001). Gilani and colleagues reported calcium channel blocking effect of Ustu khuddoos (Gilani et al., 2000). Buyukokuro studies also demonstrated anticonvulsant effect of aqueous extract of *Lavandula angustifolia* flowers in glutamate dependent convulsion model. *Lavandula* extract prevented glutamate induced neurotoxicity of cerebellar granular cell culture of rat pups. Neuroprotective effect of *Lavandula* extract is mediated by blockade of calcium channel and its antioxidant properties (Buyukokuro et al., 2003). Therefore, it seems that antiepileptic activity of *Lavandula officinalis* is mediated by inhibition of glutamate release, NMDA receptors and/or calcium channel blockade.

Free radicals are involved in pathogenesis of many diseases such as epilepsy. The important effect of free radicals is membrane lipid per-oxidation and tissue injury which leads to cell membrane destruction and its dysfunction. Normally biological effects of free radicals in the body are controlled by a lot of antioxidants and via antioxidant enzymes like SOD (Sudha et al., 2001; Ilhan et al., 2006). Free radical production act on seizure via inactivation of glutamine synthesis that result in the enhancement of L-glutamate brain level (Alabad et al., 1999; Haliwell and Gutteridge, 1991). However, some researchers suggest that only NMDA receptor activation and NO production, without glutamine synthesize inhibition are involved in the seizure (Lapouble et al., 2002). Generalized epilepsy is accompanied by reversible convulsing and can induce production of reactive oxygen species in the brain (Haliwell and Gutteridge, 1991). Since it is supposed that free radicals mediate the convulsion development, nowadays searching for antiepileptic drugs with antioxidant and neuroprotective effects are of interest.

It has also been observed that antioxidants significantly inhibit PTZ-induced seizure and reduce seizure-induced oxidative stress (Ihan et al., 2005). Furthermore, in epileptic patients, the serum level of antioxidants reduces and Lipid per-oxidation increases that are correctable with antiepileptic drugs (Sudha et al., 2001). We especially preferred to use the PTZ kindling model in the present study, because some studies reported that PTZ induced kindling distinctively impaired antioxidative defense in mice brain. For instance, PTZ kindling caused a decrease of the SOD activity. The SOD itself is an antioxidant enzyme which catalyzes the conversion of superoxide to hydrogen peroxide and in this way protects the cell against the oxidative stress (Sudha et al., 2001).

In the present study PTZ kindling caused a non-significant decrease of the SOD brain levels. Val and *Lavandula officinalis* extract at all doses non-significantly increased SOD brain level.

It seems that PTZ is a starter of various processes such as membrane phosphorylation, proteolysis, and nuclease and consequently release of free lipid peroxides and free radicals (Obay et al., 2008). In the present study, a significant increase of brain MDA level as an index of lipid per-oxidation in the PTZ group leads to excess production of free radicals and oxidative stress in the brain. Therefore, this study supports the theory that in the PTZ-kindled animal, oxidative stress is possibly one of the factors that is involved in the pathophysiology of epilepsy. In the present study, *Lavandula officinalis* extract at a dose of 400 mg/kg significantly decreased MDA level relative to PTZ group. Therefore, it may be concluded that antiepileptic effect of *Lavandula officinalis* is partly mediated through its antioxidant property.

NO is a diffusible, mobile and membrane permeable molecule that has potential to influence a variety of biological functions (Itoh and Watanabe, 2009). Nowadays, NO is known as an important neurotransmitter that in addition to various physiological roles, is also related to synaptic plasticity, neuronal excitability regulation, and epileptic activity (Buisson et al., 1993). NO is formed from arginine by the action of three different nitric oxide synthase (NOS) isozymes, two calcium-dependent forms, neuronal (nNOS) and endothelial (eNOS) and one calcium-independent inducible (iNOS). NO in neurons is synthesized by nNOS, which is constitutively expressed in neurons (Itoh and Watanabe, 2009). Involvement of NO in epilepsy is approved via different experiments and systemic injection of NOS inhibitors (Starr M.S and Starr B.S., 1993). Controversial effects of NO on the PTZ-induced convulsions have been obtained. It has been shown that NOS inhibition in kindling model amplifies the 60 mg/kg PTZ-induced seizure intensity, but has a protective effect against 80 mg/kg PTZ-induced tonic seizure (Tsuda et al., 1997). So, it has been proposed that pre-convulsant or anticonvulsant activity of NOS and NO inhibitors is dependent on the PTZ dose and the seizure model. It has also been reported that PTZ-induced seizure is modulated by endogenous NO production and ionotropic glutamate receptors. Using nNOS (−/−) mice (lacking nNOS gene) and nNOS inhibitors, it has been shown that basic and enhanced levels of NO implies negative and positive modulatory effects, respectively (Itoh and Watanabe, 2009).

On the other hand, Kovacs et al. reported that NO deprivation by NOS inhibitors and NO scavengers prevent initiation of seizure-like events (SLEs) in 75% of slice cultures and 100% of hippocampal-entorhinal cortex slices. Additional evidence was obtained from knock-out animals because SLEs developed in a significantly lower percentage of slices from nNOS (−/−) mice and showed different characteristics, such as prolongation of onset latency and higher variability of SLE intervals. Enhancement of synaptic transmission by NO under epileptic conditions represents a positive feedback mechanism for the initiation of SLEs. For this reason, endogenous NO is regarded as a key promoting factor for initiation of seizures (Kovacs et al., 2009). Also, it has been reported that NOS inhibitors (i.e. 7-nitroindazole) markedly suppress the incidence of convulsions induced by enoxacin in mice pretreated with fenbufen. The suppression of the convulsions by 7-nitroindazole was not reversed by the pretreatment of L-arginine. It has been suggested that endogenous NO may be involved as a proconvulsant substance in the development of enoxacin-induced convulsions in mice pretreated with fenbufen (Masukawa et al., 1998).

PTZ via NMDA glutamate receptors activates calcium release via NMDA receptor that consequently activates calcium-calmodulin pathway to increase nNOS protein expression and NO level is able to increase the induction of generalized epilepsy (Itoh and Watanabe, 2009). In our study, PTZ non-significantly enhanced NO level in brain tissue while, Val and all doses of *Lavandula officinalis* significantly suppressed NO level as compared to control and PTZ groups. In addition, *Lavandula officinalis* extract at a dose of 200 mg/kg also suppressed NO level as compared to Val. Since antiepileptic effect of *Lavandula officinalis* (200 mg/kg) was higher than Val, it seems that higher suppression of NO level by *Lavandula officinalis* (200 mg/kg) may be responsible for this fact. Since 800 mg/kg of *Lavandula officinalis* also suppressed brain NO level and showed a low antiepileptic activity, it may be suggested that NO suppressing effect of the extract is partly responsible for antiepileptic property of *Lavandula officinalis*. However, NMDA receptor blockade and oxidative stress attenuation may also be involved in this respect.

Taken together, this is the first report to demonstrate NO suppressing and anti-epileptogenic effect of chronic administration of *Lavandula officinalis* extract on acquisition of epilepsy in PTZ kindling mice model.

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