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## Effect of Hydroalcoholic Extract of *Lavandula officinalis* on Nicotine-induced Convulsion in Mice

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**Abstract:** Epilepsy an important CNS (Central Nervous System) problem that about 1% of world's population suffer of it. The aim of study was to evaluate of anticonvulsant effect of hydroalcoholic extract of *Lavandula officinalis*. In this study, anticonvulsant activity of the hydroalcoholic extract of *Lavandula officinalis* (*L. officinalis*) was studied against chemoconvulsant-induced seizures in male mice. *Lavandula officinalis* (100, 200, 400, 600 and 800 mg kg<sup>-1</sup>), diazepam (0.15 mg kg<sup>-1</sup>) and normal saline (10 mL kg<sup>-1</sup>) were injected intraperitoneally, respectively in different groups of mice, 30 min before nicotine (5 mg kg<sup>-1</sup> i.p.). The onset time intensity and duration of convulsions and the percentage of death were recorded. Also the time-response (0, 15, 30, 45, 60 min before nicotine injection) for most effective dose of plant extract (600 mg kg<sup>-1</sup>) was investigated. The results showed that hydroalcoholic extract of *Lavandula officinalis* had anticonvulsant effect. The most effective dose of plant extract was 600 mg kg<sup>-1</sup>. In time-response study for the most effective dose of extract (600 mg kg<sup>-1</sup>), the onset, duration and intensity of convulsion significantly ( $p < 0.05$ ) increased, decreased and decreased, respectively for all tested times. The best response observed in 30, 45 and 60 min. The results showed significant anticonvulsant effect for hydroalcoholic extract of *Lavandula*.

**Key words:** *Lavandula officinalis*, anticonvulsant effect, Ca<sup>+</sup> channels, Na<sup>+</sup> channels, traditional medicine, herbal medicine, convulsion

### INTRODUCTION

A seizure is a transient alteration in behavior that is cause of disordered, rhythmic and synchronous firing of a group of neurons in brain (Brunton *et al.*, 2008). In fact, physical manifestation of abnormal electrical activity is called seizure (Kester *et al.*, 2007). About 1% of world's population suffers of convulsions and it is the most common neurologic disorder after stroke (Porter and Meldrum, 2009). Epilepsy more occurs among young children or adults over 65, however it is seen any time (Tripathi, 2008; Kumar *et al.*, 2011).

Nicotine is a natural liquid alkaloid. It is a colorless volatile base alkaloid that turns brown and acquires the odor of tobacco on exposure to air. The central effects of nicotine are complex. Nicotine stimulates nicotinic acetylcholine receptors by opening sodium channels and causes neuronal excitation (Luscher, 2009). This agent also, significantly stimulates CNS. Its low doses produce weak analgesia while in high doses induces tremors, leading to convulsions (Luscher, 2009).

There are a lot of self-medications around the world (Sarahroodi and Arzi, 2009; Sarahroodi *et al.*, 2010), that a large amount of it is made by herbal and traditional medicine (Sarahroodi *et al.*, 2009). *Lavandula officinalis* L. (Lamiaceae), locally known as 'Osto khuddous', is indigenous from the Arabic and Mediterranean Coasts to Asia Minor (Samsam-Shariat, 1990). Also it is cultivated throughout different parts of Iran and Europe (Wichtl and Bisset, 1994). This plant was used as bactericide to disinfect hospitals and sick rooms in ancient Persia, Greece and Rome (Zargari, 1999). Also it was used as an antiseptic in ancient Arabian, Greek and roman medicine. In Germany Lavender is licensed as a standard medicinal tea for sleep disorders and nervous stomach. Lavender flowers are often used in United States as a dietary supplement in aqueous infusions. *Lavandula* species are used for the extraction of oil (known as lavender oil) employed in perfumery and cosmetics (Gilani *et al.*, 2000). The essential oil which is obtained by the aerial part of *L. angustifolia* Mill and is called Lavander oil is predominantly used in

aromatherapy as a sedative, relaxant and carminative (Cavanagh and Wilkinson, 2002; Sanz *et al.*, 2004; Meftahizade *et al.*, 2011). Also another study indicated that lavender essential oil has antibacterial activity (Lodhia *et al.*, 2009).

More over, some researches have been shown antifungal activity of lavandula essential oil (Soylu *et al.*, 2005) and anti swarming activity of methanolic extract (Zaker and Mosallanejad, 2010).

This plant is used by traditional healers for central nervous system diseases, such as epilepsy and migraine (Hakeem *et al.*, 1991). It is also being used in folk medicine, as antispasmodic in colic pain (Usmanghani *et al.*, 1997). This plant has been traditionally used as antiseptic agent in wounds swabbing, burns and insects bite (Meftahizade *et al.*, 2011) and also hypnotic and anti migraine in traditional Palestinian medicine (Jaradat, 2005). Based on the above evidences, the present study was conducted to assess the anticonvulsant effects of hydroalcoholic extract of *Lavandula officinalis* in rats and to determine the possible mechanism of action of this plant.

## MATERIALS AND METHODS

**Animal:** Young wistar albino male mice from Razi Institute (Karaj, Iran), weighting 20-25 g used. Animals were housed eight per cage, in a room with a 12:12 h light/dark cycle and controlled temperature ( $23\pm 2^{\circ}\text{C}$ ) with related humidity of 45-55%. Animals had access to food (Khorakdam, Shoushtar, Iran) and water *ad libitum*. The animals were allowed to adapt to the laboratory conditions for at least 1 h before the test. All experiments were performed between 1 and 3 a.m. Each mouse was tested only once. A total number of 112 animals were used, eight animals were used in each group of experiments and all animal experiments were carried out in accordance with the regulations of the Ethics Committee of Ahwaz University of Medical Sciences. The study was performed in year 2010, in school of pharmacy, Ahwaz Jondishapour University of Medical Sciences.

**Plant material and preparation of extracts:** Dried leaves of *Lavandula officinalis* were purchased from Gol-Daru Co., Isfahan, Iran and identified at Department of Pharmacognosy, School of Pharmacy, Ahwaz University of Medical Sciences, Ahwaz, Iran. Plant leaves were powdered and 100 g of this powder was extracted using ethanol 80% (v/v) by maceration. The extract was separated and filtrated by Watman filter papers. The prepared extract concentrated by vacuum evaporation and then it was dried in low temperature.

**Chemicals:** Drugs used as follows: Nicotine (Merck, Germany), diazepam (Chemidaru, Iran), naloxone (Tolid Daru, Iran). Nicotine, diazepam and naloxone were dissolved in normal saline. All compounds were prepared freshly each time and administered intraperitoneally.

**Nicotine-induced seizures:** Following nicotine injection (i.p.) mice were placed individually in the observation cages. Nicotine produced an almost immediate response consisting of tonic-colonic seizures and sometimes tonic seizures followed by death. Based on initial experiments, a nicotine dose of  $5\text{ mg kg}^{-1}$  was selected as a reliably convulsive dose for the further interaction experiments. Seizures were scored at 0, 15, 30, 45 and 60 min before nicotine injection.

## Anticonvulsant activity

**Dose-response study:** The mice were divided into groups of eight animals each. Five groups of animals were pre-treated (i.p.) by different doses (100, 200, 400, 600 and  $800\text{ mg kg}^{-1}$  i.p.) of *Lavandula officinalis* extract, respectively, 30 min before the administration of nicotine ( $5\text{ mg kg}^{-1}$  i.p.). Two other groups were received diazepam ( $0.15\text{ mg kg}^{-1}$  i.p.) of and normal saline ( $10\text{ mL kg}^{-1}$ ), respectively, 30 min before the administration of nicotine ( $5\text{ mg kg}^{-1}$  i.p.).

Each animal was placed into an individual plastic cage for observation lasting 1 h. The onset of a general clonus was used as the endpoint. The general clonus was characterized by forelimb clonus followed by full clonus of the body. The time taken before the onset of clonic convulsions, the duration of clonic convulsions and the percentage of seizure and mortality protection were recorded.

**Time-response study:** In this study different groups of animals received most effective dose ( $600\text{ mg kg}^{-1}$  i.p.) of plant extract, 0, 15, 30, 45 and 60 min before injection of nicotine ( $5\text{ mg kg}^{-1}$  i.p.), respectively and time of onset, duration and intensity of intensity were recorded. Normal saline ( $10\text{ mL kg}^{-1}$ ) and diazepam ( $0.15\text{ mg kg}^{-1}$ ) were used as negative and positive controls (Arzi and Shafei, 2002). In the end of trail, percentage of death were calculated for all groups.

The intensity of tremor was scored as: 1: Mild jaw and head tremors; 2: Sever jaw and ear tremors; 3: Mild tremors of body and 4: Bursts of sever tremors over entire body.

**Statistical analysis:** Statistical analysis of data calculating was made by using One-way ANOVA

followed by Tukey test. A p-value less than 0.05 were considered the level of significance. Data were expressed as Mean $\pm$ SEM.

## RESULTS

### Dose response

**The effect of different doses of the *Lavandula officinalis* hydroalcoholic extract 30 min before nicotine injection on nicotine-induced convulsions:** Figure 1 shows the effect of different doses of *Lavandula officinalis* hydroalcoholic extract on the onset time of nicotine-induced convulsion in comparison with normal saline and

Diazepam, 30 min before nicotine injection. Figure 1 indicated that the doses 200, 400, 600 and 800 mg kg<sup>-1</sup> of the plant extract significantly (p<0.05) increased onset time of convulsions in comparison with normal saline. While increasing of onset time by diazepam was significantly (p<0.05) more than all doses of the extract.

Figure 2 shows the effect of all lavender extract doses on the convulsions duration of nicotine-induced convulsion in comparison with normal saline and positive control Diazepam groups 30 min before nicotine injection.

The doses 200, 400, 600 and 800 mg kg<sup>-1</sup>, i.p. of our extract significantly (p<0.05) decreased duration of

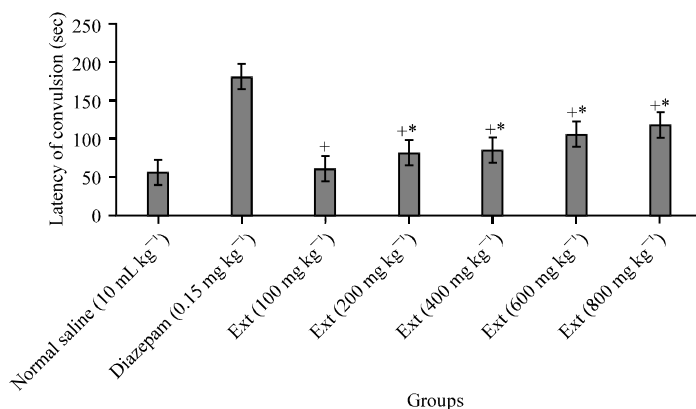


Fig. 1: Comparison of the effect of different doses (200, 400, 600 and 800 mg kg<sup>-1</sup> i.p.) of hydroalcoholic extract of *Lavandula officinalis*, 30 min before nicotine (5 mg kg<sup>-1</sup> i.p.) injection with normal saline (10 mL kg<sup>-1</sup> i.p.) and Diazepam (0.5 mg kg<sup>-1</sup> i.p.), on onset time of convulsion in mice. Values are Means $\pm$ SEM. Significant differences between test groups and normal saline and diazepam group indicated as \* and + at p<0.05, respectively. Each group contains 8 mice

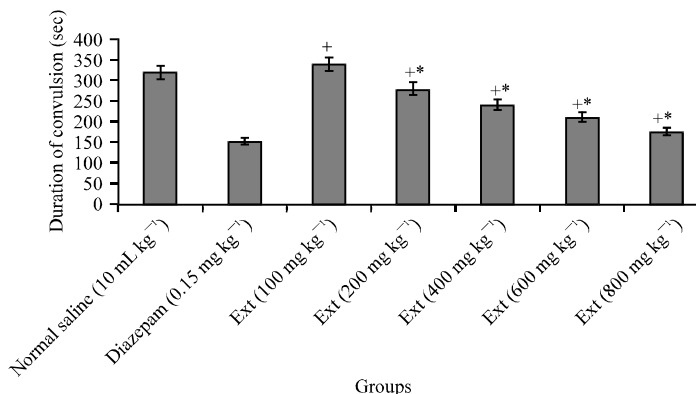


Fig. 2: Comparison of the effect of different doses (200, 400, 600 and 800 mg kg<sup>-1</sup> i.p.) of hydroalcoholic extract of *Lavandula officinalis*, 30 min before nicotine (5 mg kg<sup>-1</sup> i.p.) injection with normal saline (10 mL kg<sup>-1</sup> i.p.) and Diazepam (0.5 mg kg<sup>-1</sup> i.p.), on duration of convulsion in mice. Values are Means $\pm$ SEM; Significant differences between test groups and normal saline and diazepam group indicated as \* and + at p<0.05, respectively. Each group contains 8 mice

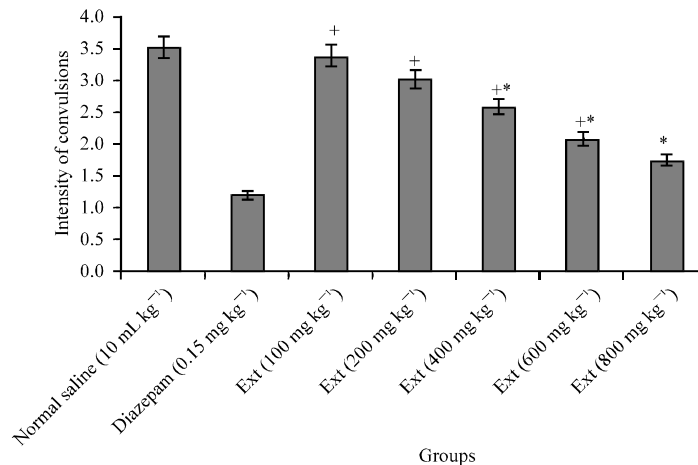


Fig. 3: Comparison of the effect of different doses (200, 400, 600 and 800 mg kg<sup>-1</sup> i.p.) of hydroalcoholic extract of *Lavandula officinalis*, 30 min before nicotine (5 mg kg<sup>-1</sup> i.p.) injection with normal saline (10 mL kg<sup>-1</sup> i.p.) and Diazepam (0.5 mg kg<sup>-1</sup> i.p.), on intensity of convulsion in mice. Values are Means±SEM; Significant differences between test groups and normal saline and diazepam group indicated as \* and + at p<0.05, respectively. Each group contains 8 mice

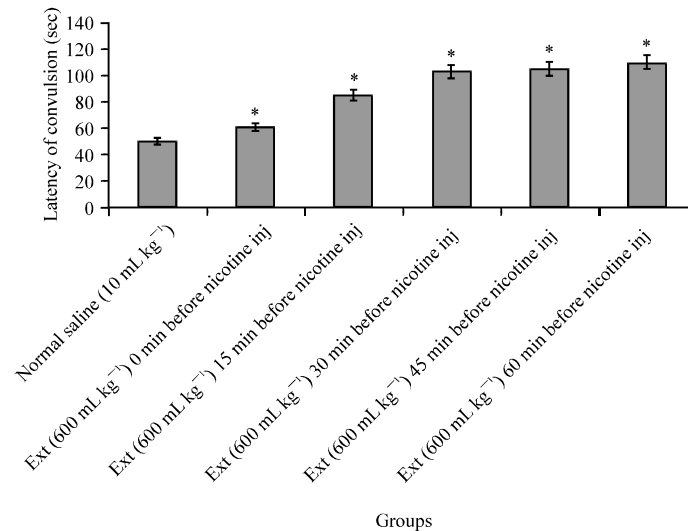


Fig. 4: Comparison of the most effective dose of hydroalcoholic extract of *Lavandula officinalis* (600 mg kg<sup>-1</sup> i.p.), 0, 15, 30, 45 and 60 min before nicotine (5 mg kg<sup>-1</sup> i.p.) injection with normal saline (10 mL kg<sup>-1</sup> i.p.), on onset time of convulsion in mice. Significant differences between test groups and normal saline group is indicated as at \*p<0.05. Values are means±SEM; Each group contains 8 mice

convulsions in comparison with normal saline. On the other hand diazepam decreased convulsions durations significantly (p<0.05) more than normal saline and all doses of extract.

Figure 3 reveals the effect of all *Lavandula officinalis* extract doses on intensity of convulsions in comparison with normal saline and Diazepam, 30 min before nicotine injection.

Figure 3 showed that the doses 400, 600 and 800 mg kg<sup>-1</sup> of lavender extract significantly (p<0.05) decreased intensity of convulsions in comparison with

normal saline. While Diazepam significantly (p<0.05) decreased intensity of convulsions more than all doses of lavender hydroalcoholic extract.

#### Time response

**The effect of *Lavandula officinalis* hydroalcoholic extract most effective dose (600 mg kg<sup>-1</sup>) 0, 15, 30, 45 and 60 min before nicotine injection on nicotine-induced convulsions:** Figure 4 shows the effect of *Lavandula officinalis* most effective dose (600 mg kg<sup>-1</sup>, i.p.) 0, 15, 30, 45 and 60 min before nicotine injection, on the onset time

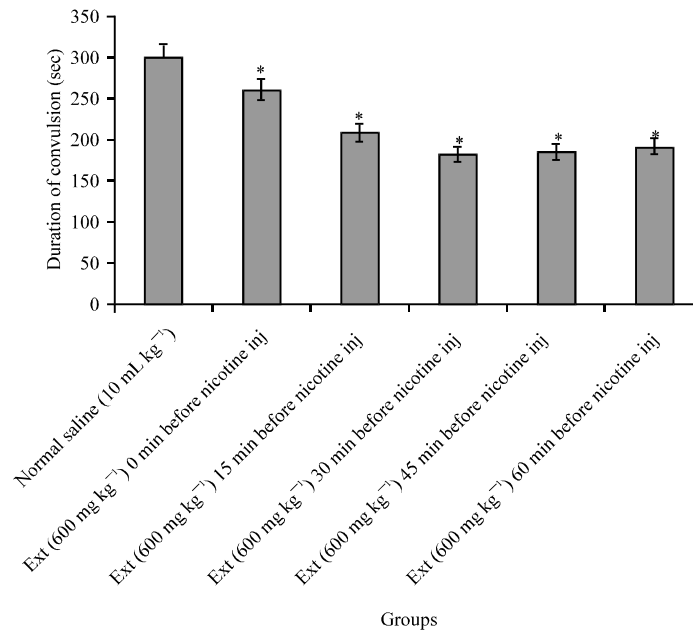


Fig. 5: Comparison of the most effective dose of hydroalcoholic extract of *Lavandula officinalis* (600 mg kg<sup>-1</sup> i.p.), 0, 15, 30, 45 and 60 min before nicotine (5 mg kg<sup>-1</sup> i.p.) injection with normal saline (10 mL kg<sup>-1</sup> i.p.), on duration of convulsion in mice. Significant differences between test groups and normal saline group is indicated as \*p<0.05. Values are Means±SEM; Each group contains 8 mice

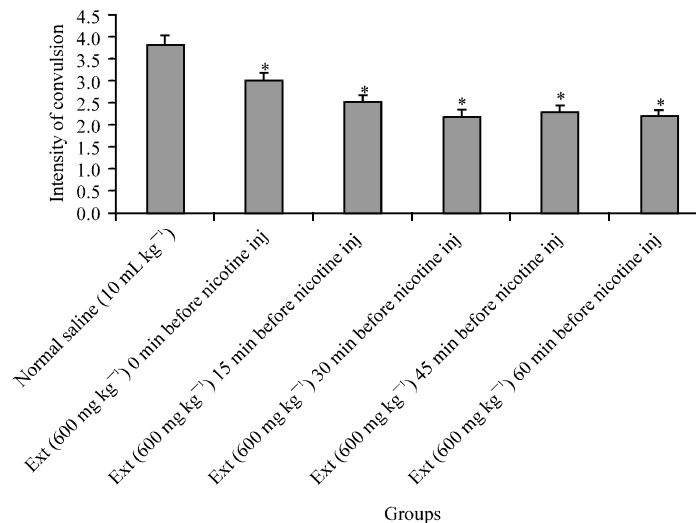


Fig. 6: Comparison of the most effective dose of hydroalcoholic extract of *Lavandula officinalis* (600 mg kg<sup>-1</sup> i.p.), 0, 15, 30, 45 and 60 min before nicotine (5 mg kg<sup>-1</sup> i.p.) injection with normal saline (10 mL kg<sup>-1</sup> i.p.), on intensity of convulsion in mice. Significant differences between test groups and normal saline group is indicated as \*p<0.05. Values are Means±SEM; Each group contains 8 mice

convulsions. One-way ANOVA revealed that 600 mg kg<sup>-1</sup> extract significantly (p<0.05) increased onset time in all tested times.

Figure 5 shows the effect of Lavander most effective dose (600 mg kg<sup>-1</sup>, i.p.) 0, 15, 30, 45 and 60 min before nicotine injection, on the duration of convulsions.

One-way ANOVA showed that duration of convulsions significantly (p<0.05) decreased duration of convulsions in the animals which received 600 mg kg<sup>-1</sup> of Lavander extract in all tested times.

Figure 6 shows the effect of *Lavandula officinalis* extract most effective dose (600 mg kg<sup>-1</sup>, i.p.) 0, 15, 30, 45

and 60 min before nicotine injection, on the onset time of convulsions. One-way ANOVA revealed that plant extract significantly ( $p < 0.05$ ) decreased intensity of convulsions in all tested times. As showed in Fig. 6, the best effect of this extract is seen 30 min before nicotine injection.

## DISCUSSION

The present study investigated the anticonvulsant effect of *Lavandula officinalis* using the nicotine-model. About 1% of world's population suffers from epilepsy (convulsions) that is the most common neurologic disorder after stroke (Porter and Meldrum 2009). According to the present study different doses of hydroalcoholic extract of *Lavandula* (200, 400, 600 and 800 mg kg<sup>-1</sup>) significantly ( $p < 0.05$ ) reduced intensity and duration of convulsions. While these doses increased latency (onset time) of convulsions. It seems that these effects increased dose dependently. These findings are in agreement with earlier studies. The study of (Yamada *et al.*, 2005) indicated the anti-convulsive effect of inhaling Lavender oil vapors. The other study in 2005, indicated anticonvulsant activity of Lavender oil and aqueous, alcoholic and acetonic extracts against pentylenetetrazol (PTZ) and Maximal Electroshock (MES) induced seizures in male mice (Shahriary *et al.*, 2005). The mechanism(s) by which *Lavandula* suppresses CNS has been investigated in some studies. Ghelardini *et al.* (1999) showed the inhibitory effect of lavender essential oil on Na<sup>+</sup> and Ca<sup>2+</sup> channels. Also, Gilani *et al.* (2000) believes that lavender's anticonvulsant and, hypnotic effect is cause of its calcium channel blocking.

On the other hand, some studies revealed that the calcium antagonists were also found useful in convulsive disorders (Gautam *et al.*, 2009; Cheong *et al.*, 2009; Caspi *et al.*, 2009; Edgerton *et al.*, 2010; Knierim *et al.*, 2011).

Over all we believes that anticonvulsant effect of *Lavandula officinalis* hydroalcoholic extract depends on it's calcium channel blocking effect.

## CONCLUSION

The present study provides evidence for anticonvulsant activity of *Lavandula officinalis* L. hydroalcoholic extract in the seizure of nicotine model. It seems that anticonvulsant effect of this plant depends on its ability in calcium channel blocking.

As the protective effects of this extract in colonic seizure it could be used in seizure treatment.

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