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Full Length Research Paper

Anticonvulsant effects of hydroalcoholic extract of *Melissa officinalis* on pentylenetetrazole (PTZ) model of convulsion in mice

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Extracts of *Melissa officinalis (Lemon Balm)* are extensively used in traditional medicines. This study examined the anticonvulsant activity of the hydroalcoholic extract *of M. officinalis* on pentylenetetrazole (PTZ)-induced seizures threshold in mice. The fresh leaves and the whole plant of *M. officinalis* were used by a routine method to produce an injectable solution for administration. *M. officinalis* and normal Saline were injected intraperitoneally (IP) at the doses 100, 300, 600 and 900 and 1200 mg/kg, i.p and 10 ml/kg i.p, respectively, 30 min before the administration of PTZ (90 mg/kg, i.p). For measurement of anticonvulsant activity of animals, convulsive symptoms were divided into 3 level (scores 1-3) which were C_1, C_2 and C_3. The time taken before C_1, C_2 and C_3 and the percentage of seizure and mortality protection rate were recorded. Statistical analyses of all treatment and control groups show that *M. officinalis* significantly can increases onset time of convulsive symptoms in all 3 levels of seizure in the PTZ model. On the other hand, onset time of convulsive symptoms increases by doses increasing which may indicate the dose-dependent effect of *M. officinalis*. It seems that it can be useful for treatment of seizure.

Key words: Anticonvulsant activity, *M. officinalis*, hydroalcoholic extract.

INTRODUCTION

Melissa officinalis (Lemon Balm) is a perennial, lemon-scented herb which originates from southern Europe, Anatolian region and southern parts of North America and all Mediterranean countries including the coastal regions of Turkey and northern Iran (Kennedy et al., 2004; Mrlianova et al., 2001). It has been used medicinally for over 2000 years. Traditional indications of Melissa officinalis are for its general central nervous system (CNS) effects like sedative, spasmolytic effects in nervous disorders and the reduction of excitability, anxiety, and stress and sleep disturbance (Kennedy et al., 2006; Perry et al., 1999). Although the mechanisms of action of *M. officinalis* are poorly understood, it has been suggested that the active components of extracts made from the leaves include monoterpenoid aldehydes, flavonoids, polyphenolic compounds including rosmarinic acid (Carnat et al., 1998), and monoterpene glycosides (Mulkens et al., 1985). These components may well underlie a number of effects seen *in vitro*, which include potent antioxidant properties and an affinity for binding to both nicotinic and muscarinic receptors in human brain cortex (Hohmann et al., 1999; Mantle et al., 2000). Extracts have been shown to have both nicotinic and muscarinic cholinergic receptor-binding properties in human brain homogenates (Wake et al., 2000; Perry et al., 1996) and essential oils have appreciable acetylcholinesterase inhibitory and antioxidant properties (Kennedy et al., 2003a; Ferreira et al., 2006; Lamaison et al., 1991; Tagashira et al., 1998) which are putatively attributable to their flavonoid content (Hohmann et al., 1999).

M. officinalis is most commonly used in combination with other herbs, most notably *Valeriana officinalis* to improve the sleep quality (Mantle et al., 2000). Several studies in rodents have also suggested a mildly sedative

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Abbreviations: PTZ, Pentylenetetrazole; **IP,** intraperitoneally; **SSRIs,** selective serotonin reuptake inhibitors; **SPSS,** statistical package for social science; **ANOVA,** analysis of variance; **CNS,** central nervous system

effect of *M. officinalis* with a reduction in spontaneous movement in mice after administration of both the volatile oil and isolated terpenes of *M. officinalis* (Cerny et al., 1999) and a reduction in behavioral parameters in mice due to hydroalcoholic extract of *M. officinalis* (Wagner et al., 1973).

The hydroalcoholic extract exhibited sedative effects on the central nervous system in animal studies. *M. officinalis* is approved by the German Commission E for nervous sleep disorders and functional gastrointestinal complaints. It does have some contraindications and possible side effects include palpitations, nausea, diarrhea, headache and EEG changes (Soulimani et al., 1991). There are some medications that may reportedly interact with *M. officinalis*, such as alcohol, sedatives, barbiturates, glaucoma medications, and SSRIs (selective serotonin reuptake inhibitors) which are used to treat depression and other psychiatric conditions (Blumental et al., 2000). The aim of the present study was to determine the effect of the hydroalcoholic extract of *M. officinalis* on pentylenetetrazole (PTZ) model of convulsion in mice.

MATERIALS AND METHODS

Herb

M. officinalis was used for the present study. First of all, it was collected freshly from cultivation farm of medicinal plants of Islamic Azad university of Ardabil. It was done in the September of 2009. Afterward the medicinal parts of plant, the fresh leaves and the whole plant (Ulbricht et al., 2005), were separated immediately and then were sent to department of chemistry for decoction.

Decoction of *M. officinalis*

At first step, the fresh leaves and the whole plant of *M. officinalis* were dehumidified at room temperature followed by grinding was done of 50 g of dried sample. This powder was soaked in 400^{cc} methanol (60%)-water (40%) mixture for 72 h. Afterward it was filtrated by gossamer and remaining slag also was washed with mentioned solvent to produce a semi clear solution. This solution was percolated with Buchner funnel and vaporized and condensed by rotary in low pressure to produce the final extract. The last stage was the dehumidification of extract by an oven in 60°C for at least 24 h. We got finally a waxy brown to black net solid extract.

Preparing of injectable solution of extract

To prepare the injectable solution from the extract, 2.5 g of extract was solved in 10 ml distilled water to produce 250 mg/ml injectable solution of extract.

Dose determination of extract

The initial dosage of solution of *M. Officinalis* extract was 100 mg/kg. Dose selection should have rational like $1/10^{th}$ of lethal dose (LD50 dose).

Animals

Albino male BALB/c mice (25–30 g) were obtained from the Razi Institute (Karaj, Iran). The animals were housed in colony rooms with 12/12 h light/dark cycle at 22 ± 2°C and had free access to food and water. There are should follow CPCSEA rules which had free access to tap water and food pellet mention the manufacturer. All animals 45 minute before examination were transferred to individual small cage and also were used only one time (Gruenwald et al., 2000).

Materials

Drugs used as follows: PTZ [Sigma], extract of *M. officinalis, normal saline* [Samen, Iran]. PTZ was dissolved in normal saline. All compounds were prepared freshly each time and administered intraperitoneally.

Anticonvulsant effects of herbal extract

In this study, animals (N=60) were divided to 5 groups of treatment (*M. officinalis*) and 1 group of control (The mice were divided into groups of ten animals each). In the five treatment groups, the mice were given *M. officinalis* solution at the doses (100, 300, 600, 900 and 1200 mg/kg i.p.) 30 min before the administration of PTZ (90 mg/kg i.p) and one group was injected normal saline 30 min before the administration of PTZ (90 mg/kg i.p.) (Gruenwald et al., 2000; Nassiri-Asl et al., 2007).

Response measurement method

Each animal is placed into an individual plastic cage for observation lasting 1 h. Convulsion symptoms of animals were divided into 3 levels (score 1-3) which are called "C_1 to C_3". C_1 was the onset of a general clonus which was characterized by forelimb clonus. C_2 was the onset of myoclonic convulsion and C_3 was the onset of the tonic-clonic convulsion. The time taken before the onset of each level of convulsions and the percentage of seizure and mortality protection were recorded

Statistical analysis

All statistical analyses were performed with using SPSS 11.5 (Statistical Package for Social Science) and One-way repeated measures analysis of variance (ANOVA) was used to determine the association of each factor and to be significant was considered at $p < 0.05$.

RESULTS

In this survey, the main purpose was to investigate the anticonvulsant activity of *M. officinalis* in three levels (C_1, C_2 and C_3) on the PTZ model of convulsion. *M. officinalis* at all the doses prolonged the onset times of three levels of seizure (C_1, C_2 and C_3) compared to saline group ($p < 0.05$; $p < 0.01$ and $p < 0.001$) (Table 1). *M. officinalis* at the dose of 100 mg/kg at level C_1 could not prolonged the onset time of seizure, compared to saline group ($p = 9764$) (Table 1). As they are shown in Figures 1, 2 and 3, *M. officinalis* exhibited its protection against seizure in a dose-dependent manner. Furthermore, the onset times

Table 1. Effects of Melissa officinalis on three levels of convulsion on PTZ-induced convulsion in mice.

Treatment (dose)	C_1 (s)	C_2 (s)	C_3 (s)
Normal Saline (10 ml/kg)	86.60 ± 1.62	173.50 ± 4.35	221.50 ± 6.67
Melissa officinalis (100 mg/kg)	86.50 ± 4.66	210.50 ± 5.02***	278.00 ± 16.04*
Melissa officinalis (300 mg/kg)	120.00 ± 7.99**	224.50 ± 4.62***	297.00 ± 11.70**
Melissa officinalis (600 mg/kg)	212.50 ± 6.80***	303.00 ± 9.29***	363.00 ± 14.44***
Melissa officinalis (900 mg/kg)	523.50 ± 9.01***	537.00 ± 39.67***	682.00 ± 14.26***
Melissa officinalis (1200 mg/kg)	862.00 ± 27.80***	937.00 ± 23.14***	1162.50 ± 54.25***

Normal saline and *Melissa officinalis* were administered i.p. 30 min before the injection of PTZ (90 mg/kg, i.p.); C1 (Slight strain of forelimbs), C2 (Myoclonic convulsion) and C3 (Tonic-Clonic convulsion) are the levels of convulsion. Values are the mean ± SEM for 10 mice. *p < 0.05** p<0.01; *** p < 0.001, compared to saline group, Paired t test.

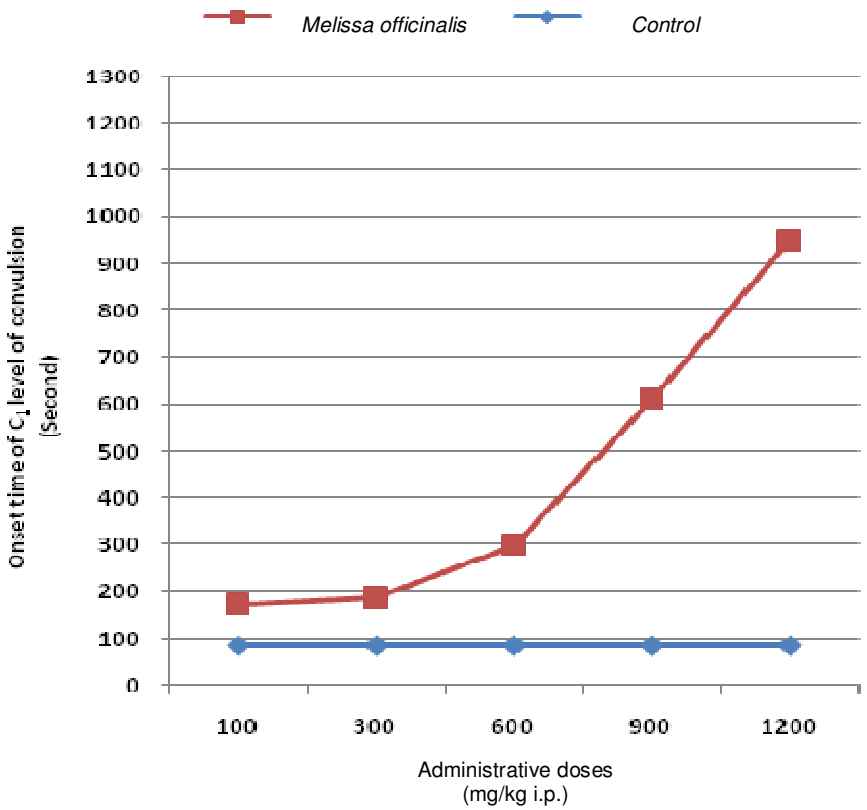

Figure 1. Effects of administration of *Melissa officinalis* in C_1 level of convulsion (Depending on the time) in mice. The number of mice used for each group of administrative doses was 10. Normal Saline and *Melissa officinalis* were administered i.p. 30 min before the injection of PTZ (90 mg/kg, i.p.)

of three levels of convulsion were prolonged concurrent by dose increase (Figures 1, 2 and 3).

Protection of seizure and mortality by *M. officinalis*

The percentage of seizure and mortality protection by *Melissa officinalis* has shown in Figure 4. Although, *M. officinalis* at lower doses (100, 300 and 600 mg/kg) exhibited its protection against seizure and mortality in a dose-dependent manner, these results were not seen in mentioned manner at higher doses (900 and 1200 mg/kg). Furthermore, seizure and mortality protection increased when doses increased, however, at high doses, these protections decreased.

DISCUSSION

The present study investigated the anticonvulsant effect

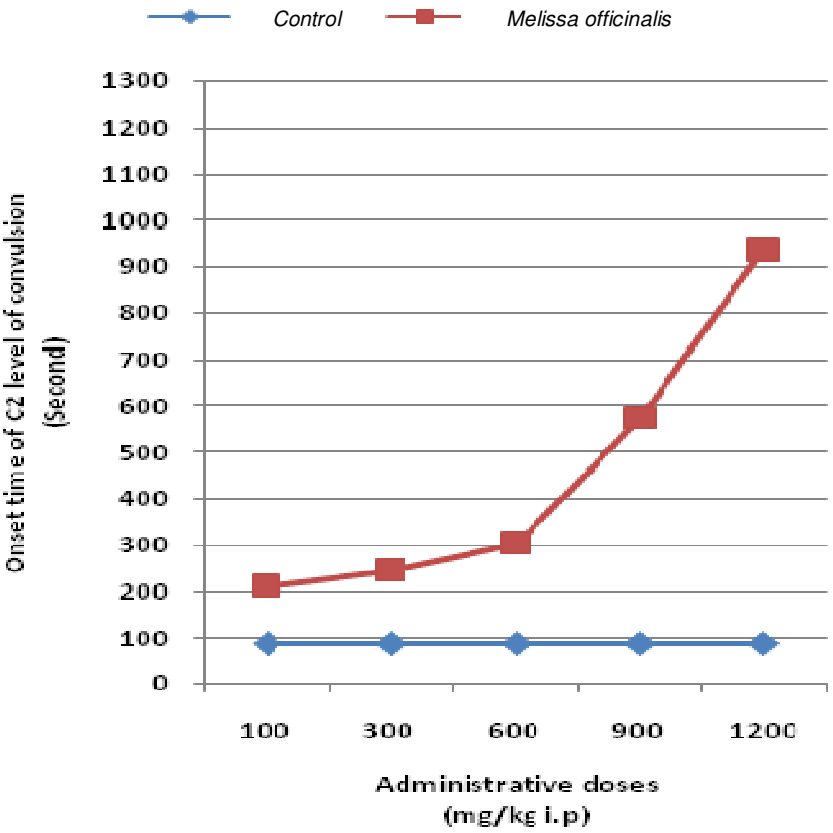

Figure 2. Effects of administration of *Melissa officinalis* in C_2 level of convulsion (Depending on the time) in mice. The number of mice used for each group of administrative doses was 10. Normal saline and *Melissa officinalis* were administered i.p. 30 min before the injection of PTZ (90 mg/kg, i.p.)

of *M. officinalis* using the PTZ-model. *M. officinalis* could suppress onset time of convulsion in PTZ model and it seems that this effect increased dose dependently. Seizure and mortality protection almost increased dose dependently but we could observe that at the high doses like 900 and 1200 mg/kg, the seizure and mortality protection decreased, all animals were not protected against seizure and mortality significantly. Our extract was the standard hydroalcoholic extract of aerial parts of herb which was prepared as the drug formulation. There are several controversial reports about the CNS effects of *M. officinalis.* In a study, 600 mg dose of standardized *M. officinalis* extract improved mood, calmness and alertness in human, and a 300 mg dose increased the subjects' mathematical processing speed (Vogel, 1997). Sedative properties of the aqueous alcoholic extract (in the mouse) at low doses have been reported, as well as peripheral analgesic activity with high dosage (Kennedy et al., 2006a).

In behavioral terms, a number of studies involving rodents suggest specific sedative effects following both essential oil (Kennedy et al., 2004) and a hydroalcoholic extract of *M. officinalis* (Wagner et al., 1973). Kennedy et al. (2003b) during a series of trials have also assessed the cognitive and mood effects of single doses of *M. officinalis* in humans. In the first single doses of *M. officinalis* extract (300 mg, 600 mg, and 900 mg) which were compared with placebo, it demonstrated a dose-dependent impairment of memory function, with concomitant reductions of alertness at the highest doses and increased 'calmness' following the lowest doses. Two lower doses (300 mg and 600 mg) were investigated using a multitasking laboratory stressor paradigm, with the highest dose again leading to reduced ratings of alertness and increased ratings of calmness during the stressor battery. The pattern of results from these two studies is broadly in line with the traditional mildly sedative and calming properties of these extracts.

To assess the effects of *M. officinalis* with cholinergic receptor-binding properties, a further experiment was conducted in two distinct phases. In the first phase, cholinergic receptor-binding properties were established across a number of samples, with the dried leaf with the highest nicotinic and muscarinic receptor-binding properties being administered in a placebo-controlled, double-blind, balanced crossover study assessing the

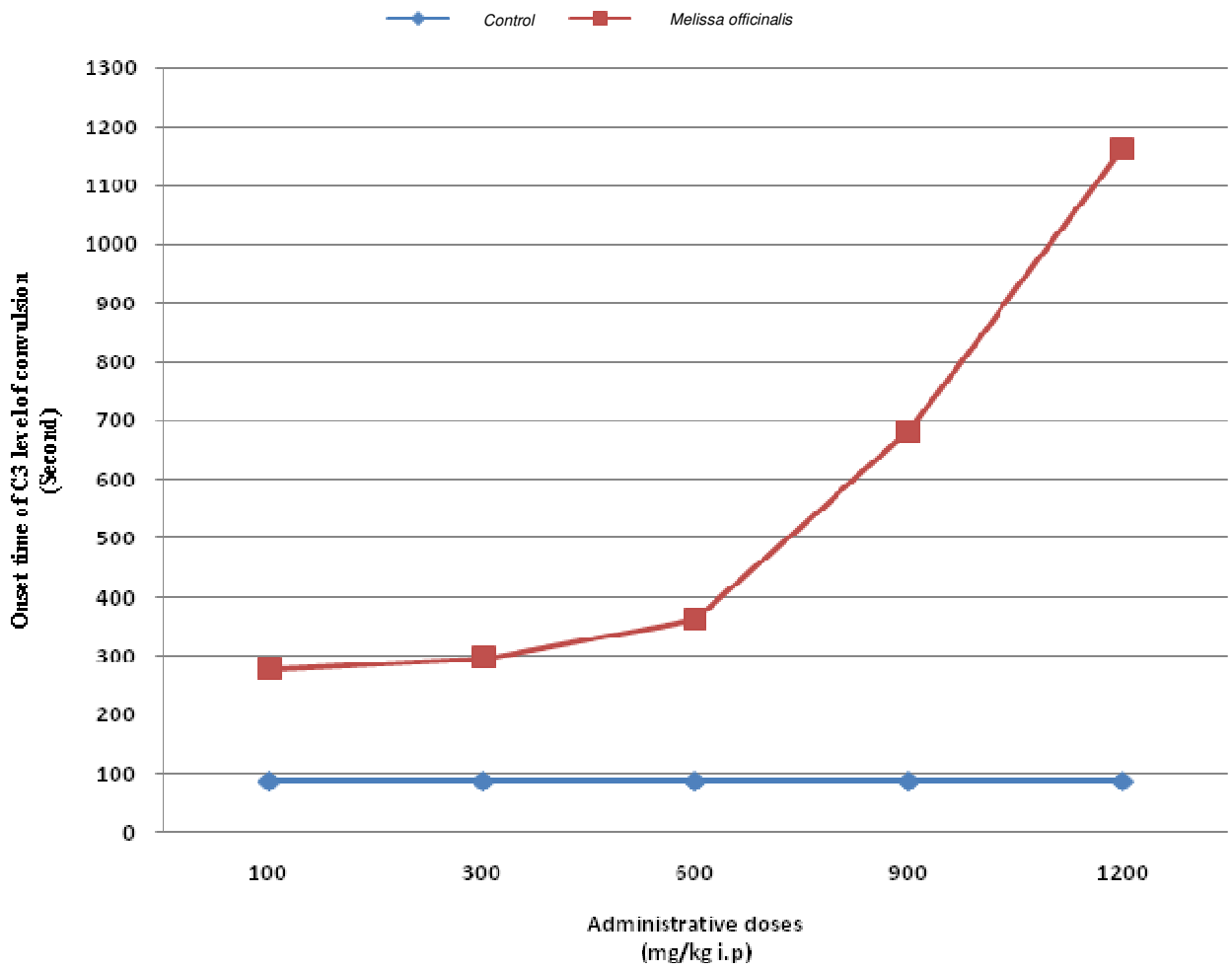

Figure 3. Effects of administration of *Melissa officinalis* in C_3 level of convulsion (Depending on the time) in mice. The number of mice used for each group of administrative doses was 10. Normal Saline and *Melissa officinalis* were administered i.p. 30 min before the injection of PTZ (90 mg/kg, i.p.)

cognitive performance and mood effects of several single doses (600, 1000 and 1600 mg dried leaf). In this case, while the lowest of the three doses evinced similar memory decrements as those seen in the previous study, the highest dose both increased 'calmness' and improved memory performance. The results from these three studies suggest that lemon balm owes its mildly sedative properties to something other than cholinergic receptor binding (Kennedy et al., 2002).

Two recent double-blind, placebo-controlled studies have also assessed the effects of *M. officinalis* in patients with dementia. Ballard et al. examined the effect of essential oil aromatherapy (in comparison with vegetable oil) on ratings of agitation and quality of life of 71 patients with severe dementia. After 4 weeks' treatment, patients in the active treatment group were rated, in comparison with the placebo group, as being less agitated, less socially withdrawn, and as engaging in more time spent in constructive activities (Kennedy et al., 2003b). Akhondzadeh et al. (2005) also assessed the effects of 60 drops/day of a tincture in 35 patients with mild-to-moderate dementia (20 verum, 15 placebo) who completed their 16-week trial. At the study endpoint, the results showed a clear cognitive advantage (ADAS-cog and Clinical Dementia Rating) and reduced agitation for the group taking the tincture.

Active components of *M. officinalis* may be related to anticonvulsant activity of it. It is found that many flavonoids which are one of the most important active components of extract could act as benzodiazepine- like molecules in the CNS and modulate GABA-generated chloride currents in animal models of anxiety, sedation and convulsion (Akhondzadeh et al., 2005). It is possible that anticonvulsant activities of *M. officinalis* related to its flavonoids. However further studies need to make clear which of these flavonoids or other compounds have anticonvulsant effects.

Our results show that *M. officinalis* could postpone the

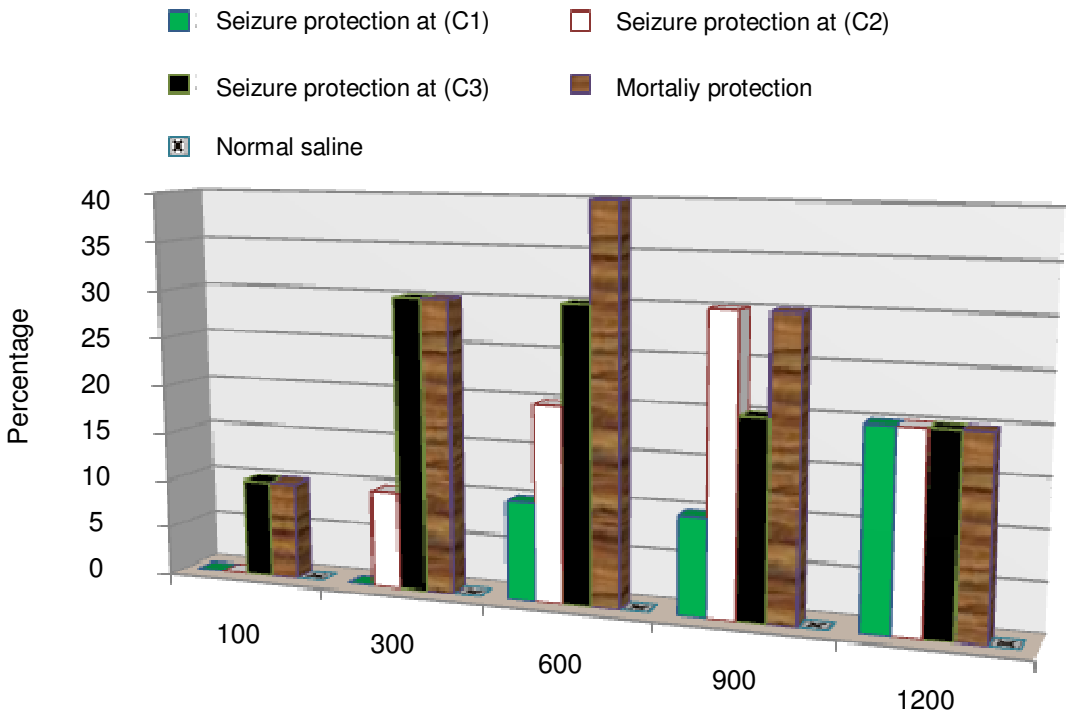

Figure 4. Effects of *Melissa officinalis* extract and normal saline on seizure protection and mortality protection on PTZ-induced convulsion in mice.

onset of each stage of a classic convulsion induced by PTZ. These results were significant in comparison with control group (normal saline). Although, *M. officinalis* could not protect against seizure and mortality completely, it could prevent the seizure and mortality partly.

Conclusion

In brief, the present study provides evidence for anticonvulsant activity of *M. officinalis* in the convulsion of PTZ model. Our experimental results show that *M. officinalis* extract increases the onset time of the classic tonic-clonic convulsion. On the other hand, there is a significant difference between each administrated dosage which may indicate the dose-dependent effects of *M. officinalis*.

Totally, as its protective effects, it seems that *M. officinalis* could be useful for treatment of the seizure, alone or with other classic drugs. Although, the anticonvulsant activity of *M. officinalis* is an interesting finding more studies are needed in order to investigate its exact mechanism.

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