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Spasmolytic Effects of Hydroalcoholic Extract of *Melissa Officinalis* on Isolated Rat Ileum

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A B S T R A C T

Lemon balm (*Melissa officinalis*) has spasmolytic activity and is used in gastrointestinal complains in traditional medicine. In this study, the effect and potency of different concentrations of hydroalchoholic extract of the plant were evaluated on isolated rat ileum. The extract of the leaves of lemon balm was prepared by maceration method and different concentrations of the extract were tested on isolated *N.Mari* rat ileum in an organ bath containing tyrode solution. To evaluate the potency, the relaxing effect of the extract was compared with the corresponding values for atropine and verapamil (10^{-6} and 10^{-7} M, respectively). The findings showed that the extract is able to inhibit contraction induced by carbachol (6.8×10^{-7} M) and KCl (20 M) at concentrations of 4 and 5.6 mg/ml, respectively, and leads to the relaxation of the smooth muscle of the intestine. It was also observed that 4 mg/ml concentration of the extract can exert an inhibitory effect similar to that of 10^{-7} M verapamil. The results show that the antispasmodic effect of *Melissa officinalis*' hydroalcoholic extract is associated with the involvement of muscarinic receptor and calcium channels and that the plant can be proposed as therapeutic agent for gastrointestinal spasms due to its potency.

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Introduction

Gastrointestinal spasm is an important disease of the gastrointestinal tract, which is associated with twitching and severe and involuntary contraction of muscles of digestive tract [1]. Increased bowel movements occur in various diseases such as irritable bowel syndrome, enteropathic and nephrogenic diabetes, progressive systemic sclerosis and thyrotoxicosis and lead to diarrhea [2]. These movements are usually associated with abdominal cramps, painful spastic contractions and increased defecation [3].

Today, because of the fewer side effects, the diversity of effective compounds and balancing of the effects of herbs, re-wide approach to the use of medicinal plants has been created [4]. *Melissa officinalis* are commonly used in traditional medicine in Iran and all over the world. In traditional medicine, this plant is used as a sedative and anti-spasmodic agent [5]. Lemon balm is an herbaceous, perennial and fragrant herb, which belongs to the Lamiaceae family and whose genus is Melissa. Lemon smell is the characteristic of this plant and that is why it is also called Lemon balm [6]. The most common therapeutic properties of lemon balm noted are the effect of its sedative , antidepressant, antioxidant, antispasmodic, carminative, antibacterial, antiviral and anti-inflammation properties [7-11]. Compounds identified in lemon balm include a number of monoterpenoid aldehydes (such as citrals) [12, 13], sesquiterpene [14], triterpenes [15-17], flavonoids [18-20], polyphenolic compounds [20-22]; especially rosmarinic acid [23] and glycosides monoterpenes [22]. Laboratory studies on digestive systems have provided different results. One study has shown that the essential oil of this plant has spasmolytic effects on rat duodenum, guinea pig jejunum and rabbits' ileum [24]. On the other hand, in guinea pigileum, in which histamine and acetylcholine have been used as spasmogenics, no significant spasmolytic effect was found in comparison with mint, cumin and chamomile [25]. In another study, it was found that the essential oil of lemon balm and its main compound, citral, have strong inhibitory effects on intestinal contractions induced by KCl, 5-HT, and Ach [5].

The aim of this study was to investigate the effect of lemon balm and determine the effective concentration of its extract on rat ileum contractions and provide scientific evidence for antispasmodic effects of this plant in irritable bowel abnormalities.

Materials and methods

In this experimental study, 68 male *N-Mari* rats (190-230 g) were used. All animals were housed in a room with controlled temperature (23±2°C) and luminosity (12 hours light, 12 hours dark). They had free access to food and water. 24 hours before the test, rats were deprived of food, but had free access to water. Ethical principles of work on animals were taken into consideration on the basis of the agenda of Ethics Committee of the Medical Sciences University.

Plant preparation and extraction

Dried leaves of lemon balm were prepared from the local market and were identified and approved by the Department of Plant Protection of Shahid Chamran University. Maceration method was used to prepare the extract. In each extraction, 80 g of the leaf powder were mixed with 400 ml of hydroalcoholic solvent (ethanol 70%) and the mixture obtained was kept in a closed container for 48 hours. After 48 hours, the contents of the container were filtered through a Buchner funnel and the extract was condensed by vacuum distillation at low pressure and a temperature of 35°C [26- 28].

Preparation of ileum portion

Having weighed the animals, they were mildly anesthetized with very small amounts of ether. After opening the abdomen of the animals, the appropriate segments of ileum with a length of about 1.5 - 2 cm were isolated and placed in a petri dish containing 10 ml of oxygenated tyrode (MERCK) (pH = 7.4, 37°C).

Measuring changes of bowel movements and activity

For this purpose, a bioscience isolated tissue perfusion device (Construction Palmer-Washington) was used. Ileal pieces were placed in an organ bath containing 50 ml of normal tyrode (specific solution for maintaining tissue, 1.04 mM, $NaH_2PO_4/2H_2O$: 0.4 Mm, 1.8 mM, $MgCl_2/6H_2O$: $CaCl_2$ KCl: 2.7 mM, NaCl: 137 mM, 12 mM, glucose/H_2O: 5 mM, $NaHCO_3$) at 37°C while oxygen gas was being passed through. Ileal segments with lengths of about 2 cm at one side were connected to a hook at the end of a hollow glass tube through which oxygen entered the organ bath , and a long thread connected to a hook at lever of isotonic transducer (Harvard, UK) at the other side. After connecting the isolated ileum to the device, a tension of 1 g was applied to the tissue. Under these conditions, the tissues were allowed to become stable. At this stage, tyrode was replaced every 15 minutes to prevent the accumulation of metabolites resulting from the activity in the organ bath. After giving the effect of drugs or extract, changes of the tissue were measured with handling the stability pen (Universal Osilograph, Harvard) as millimeter and based on the initial stretch created in the tissue (1000 mg =10 mm), these contractions were calculated in milligrams. At first, the effective doses of carbachol (ROCHE, Switzerland) and KCl (MERCK, Germany) were determined. The ,(Daroo Pakhsh, Iran) and concentrations of 1.6, 3.2, 6.4, 12.8 mg/ml of hydroalcoholic extract were investigated on the contraction caused by carbachol and potassium chloride immediately and after 15 minutes.

Statistical analysis

For evaluating the results and statistical comparison, Student's t-test was used.

Results and Discussion

Extraction yield

Extraction yield (mass of extract/mass of dry matter) was used as an indicator of the effects of the extraction conditions. The extraction yields of the leaves prepared by maceration methods using ethanol 70 was 14.37%. Previous studies have shown that ethanol 70 was a very suitable and efficient solvent for different bioactive phytochemical constituents from balm leaves [28].

The effect of different concentrations of carbachol and potassium chloride on isolated rat intestine

The findings of this study showed that carbachol and potassium chloride have contractile effects on the smooth muscle of intestine in a dose dependent manner. The effective doses of carbachol potassium chloridewere determined to be 6.8×10^{-7} and 20 mM/ml, respectively (Figures 1 and 2).

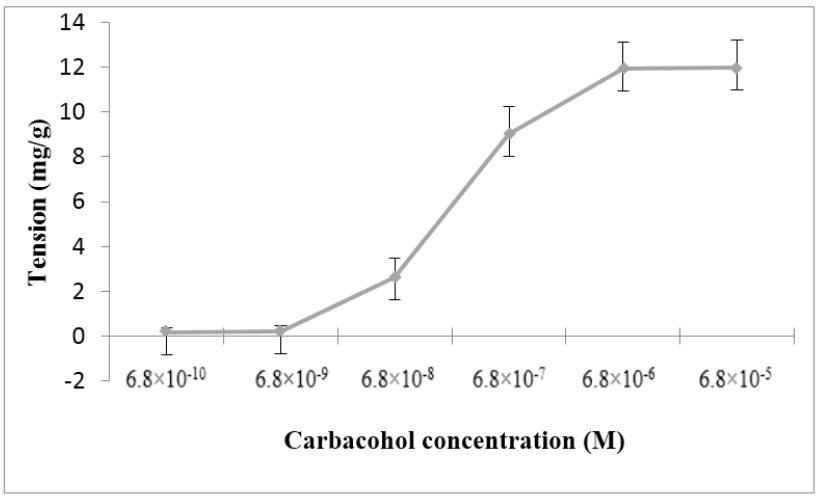

Fig. 1. Dose-response curve of carbachol on rat ileum tissue.

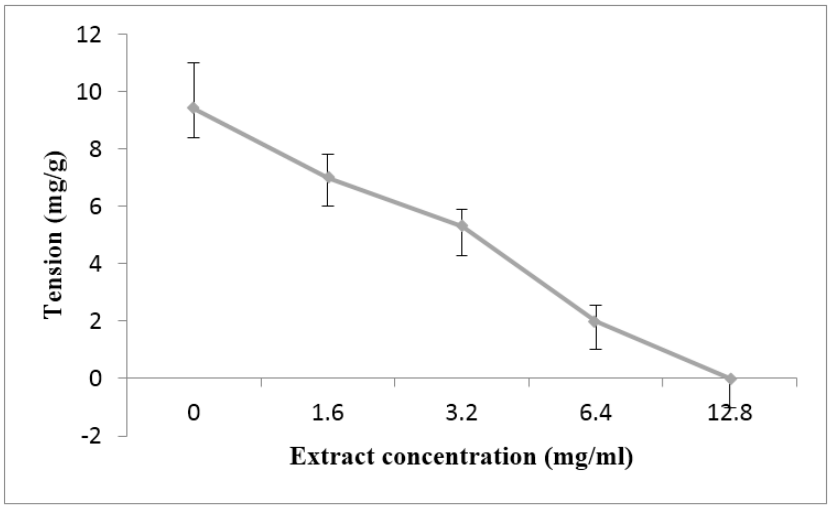

Fig. 2. Dose-response curve of potassium chloride on rat ileum tissue.

The effect of Melissa officinalis extract on ileal contractions induced by potassium chloride and carbachol

Different concentrations of the extract (1.6, 3.2, 6.4 and 12.8 mg/ml) were used versus carbachol (6.8×10^{-7} M) and potassium chloride (20 M). The extract reduced potency of contractions were induced by carbachol and KCl. The concentrations of the extract, which reduced the contractile effect of carbachol and KCl to 50 % (IC_{50}), were 5.6 and 4 mg/ml, respectively (Figures 3 and 4).

The effect of Melissa officinalis extract on ileal contractions induced by potassium chloride and carbachol

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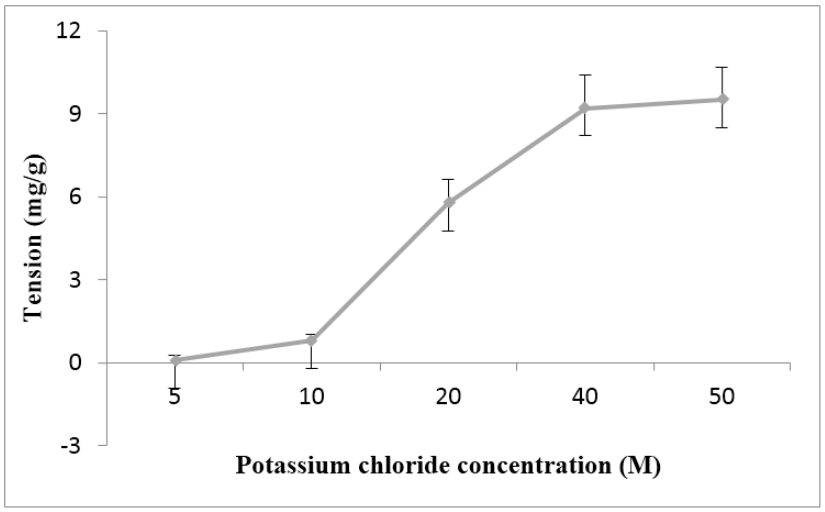

Fig. 3. The effect of different concentrations of *Melissa officinalis* extract on ileal contractions induced by carbachol (6.8×10^{-7}M)

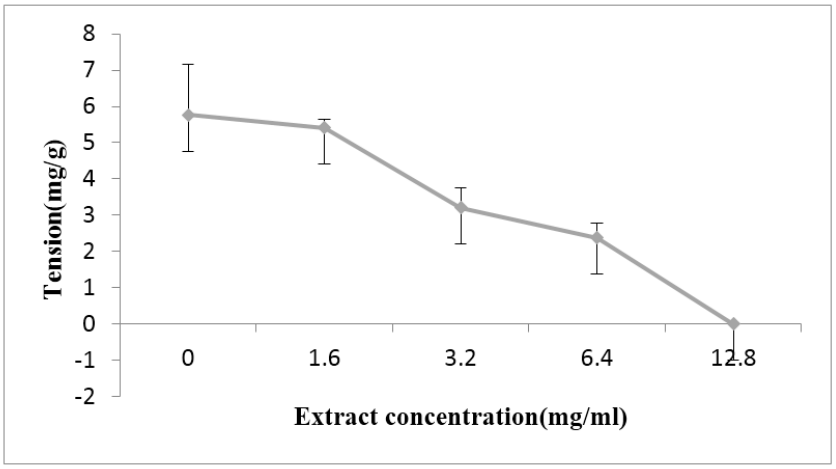

Fig. 4. The effect of different concentrations of *Melissa officinalis* extract on ileal contractions induced by potassium chloride (20 M)

Inhibitory effect of atropine and verapamil on contraction caused by carbachol and potassium chloride respectively

15 minutes before adding carbachol and potassium chloride, the desired doses of atropine and verapamil were added. The results showed that concentrations of 10^{-6} and 10^{-7} M of atropine decreased carbachol's potency contraction to 89±2.2% and 93±0.5%, respectively, ($P < 0.01$) and concentrations of 10^{-6} and 10^{-7} M of verapamil decreased potency contraction of KCl to 91±0.4% and 85± 0.7%, respectively ($P < 0.01$) (Figures 5 and 6).

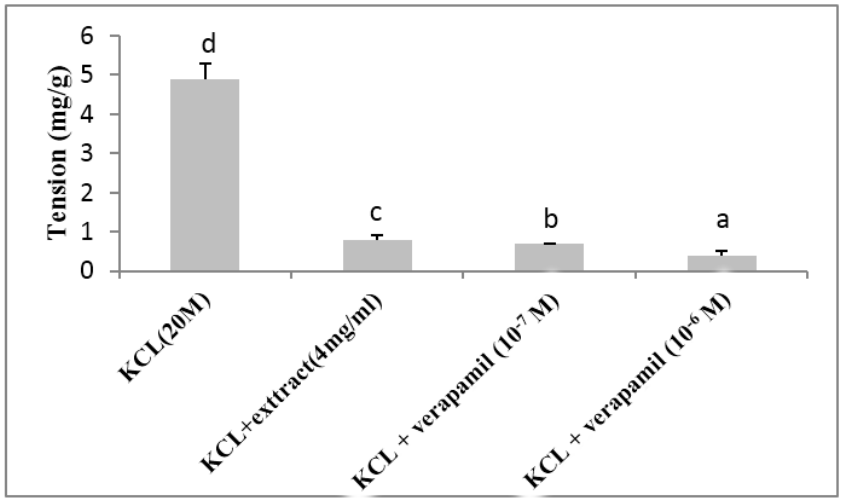

Fig. 5. Comparing the effect of *Melissa officinalis* extract (5.6 mg/ml) and atropine (10^{-6} and 10^{-7} M) on ileal contractions induced by carbachol (6.8×10^{-7}M). Means with the distinct letter are significantly different from each other.

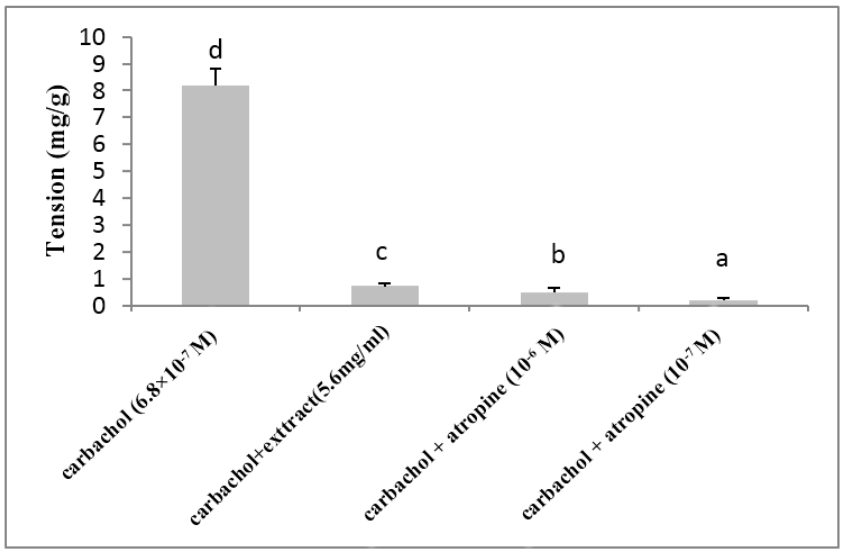

Fig. 6. Comparing the effect of *Melissa officinalis* extract (4 mg/ml) and verapamil (10^{-6} and 10^{-7} M) on ileal contractions induced by potassium chloride (20 M). Means with the distinct letter are significantly different from each other.

Inhibitory effect of extract on contraction induced by carbachol and potassium chloride

15 minutes before adding carbachol or potassium chloride, the desired concentrations of the extract were added. Dose of 5.6 mg/ml of extract versus 6.8×10^{-7} mM/ml carbachol reduced the potency of carbachol to 91±0.4% ($P < 0.01$) and concentrations of 4 mg/ml of the extract versus 20 mM/ml of potassium chloride reduced its contracting potency to 83±0.32% ($P < 0.01$) (Figures 5 and 6).

Comparing inhibitory effect of the extract (IC50) on carbachol and KCl induced contractions immediately and after 15 minutes

When the extract (5.6 mg/ml) and carbachol (8.6×10^{-7} M) were added to the environment at the same time, the extract inhibited 50±0.6% of contractions ($P < 0.01$) while the addition of the extract 15 minutes before carbachol inhibited 91±0.4% of contractions ($P < 0.01$). When 4 mg/ml concentration of the extract and 20 mM KCl were added to the environment at the same time, the extract inhibited 50±0.49% of contractions ($P < 0.01$) and when it was added 15 minutes before KCl, it inhibited 83± 0.32% of contractions ($P < 0.01$).

The findings of this study have shown that the extract of lemon balm can inhibit the contraction induced by carbachol and KCl and this inhibitory effect is comparable with those of atropine and verapamil. Since carbachol is one of acetylcholine derivatives, it is a mediator of parasympathetic nervous system, as expected, by affecting on the muscarinic receptors in the smooth muscle of the intestinal wall, causing its contraction. In addition, atropine (an acetylcholine antagonist) inhibits the contraction caused by carbachol via blocking muscarinic receptors. . The extract also inhibits the contractions induced by carbachol in a dose dependent manner. The active ingredients of the plant probably exert their effect through cholinergic muscarinic receptors and resolve the spasms in the digestive tract by inhibition.

On the other hand, it known that the antihistaminergic and antispasmodic effects of lemon balm on the isolated intestines of guinea pig are due to the presence of eugenol acetate [29]. With respect to the application of the contractile effect of acetylcholine and carbachol and histamine through the inositol triphosphate system (IP3), it may be suggested that the inhibitory effect of the active substance in the plant occurs by disrupting the effectiveness of this system. In some parts of this study, it was found that the effects of verapamil (10^{-7} M) and hydroalcoholic extracts of the plant (4 mg/ml) on the ileal contractions induced by potassium chloride (20 mM) are somewhat equal. A high concentration of potassium opens voltage gated calcium channels and causes the contraction of the intestinal smooth muscle' [30]. In a study, Cortés and colleagues reported that the contraction induced by potassium chloride is related to the calcium entry through voltage dependent calcium channels. Therefore, any substance, which can reduce the contraction induced by potassium chloride, probably acts by blocking these channels [31]. Since the extract inhibited contractions induced by potassium chloride, inhibiting calcium entry through calcium voltage gated channels possibly causes muscle relaxant. Verapamil is one of calcium channel blockers, which inhibits the entrance of extracellular calcium into the cells by affecting on intracellular segment of α_1 subunit of L-type voltage dependent calcium channel. There are large numbers of L-type voltage gated calcium channels in single unit smooth muscles of intestine. Furthermore, extracellular calcium also plays an important role in starting smooth muscle

contraction [30]. Existence of L-type channels in rat ileum has been demonstrated [32]. Bonded calcium to calmodulin causes activating myosin kinase, phosphorylating myosin heads, attaching actin to myosin and contraction [30]. Any interference in the function of these enzymes can disrupt contractile cycle. Therefore, the active ingredients in the extract may have acted so to inhibit contraction.

The results also showed that the effect of the extract shed on the tissue 15 minutes before carbachol or KCl is considerably different when it is shed at the same time. In the former case, it the extract inhibits contraction with much higher percentage. It can thus be concluded that the extract may act through the intracellular pathways and this time has been necessary for applying the effect of the extract.

Other possible mechanisms include stimulating the inhibitory non-adrenergic non-cholinergic (NANC) nervous system or inhibiting the stimulated NANC and opening potassium channels. Given that more than 94 different substances have been detected in *Melissa officinalis* , the extract may exert its effects through various mechanisms.

In agreement with the present study and other works, the relaxant effect of the essential oil of *Melissa officinalis* and its main component, citral, on rat ileum contractions was evaluated. The result of this study has shown that both essential oil and citral could inhibit the response to Ach, KCl and 5-hydroxytryptamine (5-HT) in a concentration dependent manner, indicating spasmolytic effects. In addition to the major role of citral in the inhibitory effect shown by *M. officinalis* essential oil (because of its contribution for 60% of the constituents of the essential oil), but the other constituents of the essential oil also have some contributions [5]. The hydroalcoholic extract of *M. officinalis* inhibits the response to KCl (IC_{50}=4 mg/ml) and carbachol (IC_{50}=5.6 mg/ml) in a concentration dependent manner. The essential oil of *M. officinalis* also inhibits rat ileum contractions in response to KCl and Ach with an IC_{50}=20 mg/ml. However, due to the difference in the kind and amounts of materials used to induce contractions, it cannot be concluded for sure that essential oil is more effective than hydroalcoholic extract and a comparison of the spasmolytic effects of both essential oil and extract under the same conditions is required.

However, the presence of more pure substances in the essential oil may lead to more potency than the extract. As the solvent in the hydroalcoholic extract is evaporated, it is likely that most of the essential oil is also lost. Thus, the inhibitory effect of the extract could not be due to the presence of the essential oil in the extract and other components could be responsible for the inhibitory effects [33]. However, it is known that the extracts have more stability than the oils and could be kept for longer periods of time. Secondly, the extract is preferred in some conditions because of the simplicity and cheapness of its production [34].

In general, the results of this study have shown that the extract of *Melissa officinalis* has an inhibitory effect on the contraction induced by carbachol and KCl, which is comparable with the effect of atropine and verapamil. Although a definitive statement about the mechanism of this plant needs more evidence, according to the results of this study, this plant can be recommended to treat gastrointestinal spasms. It is suggested that the effects of this plant, as a therapeutic agent, be examined by identifying its active ingredients and the mechanism of its action.

Conflict of interest

Authors certify that there is no actual or potential conflict of interest in relation to this article.

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