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Research Article

Analgesic Effect of Intrathecal *Melissa officinalis* in the Rat Model of Hot-Water and Formalin-Induced Pain

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Abstract
Melissa officinalis (MO) is one of the oldest herbal medicines commonly used in traditional medicine, which some studies have investigated for its analgesic effect. This study is an attempt to investigate the effects of intrathecal administration of *Melissa officinalis* on the pain induced by heat and formalin.

In this experimental study, 70 male Wistar rats with an average weight of 270-320 g were randomly divided into five groups: control; sham that received 25 μl of saline through the spinal catheter; and three experimental groups that received 5, 10 or 20 mg/kg *M. officinalis* via the spinal catheter respectively. Five days after catheterization of the spinal cord from the lumbar region under anesthesia, the effects of Intrathecal administration of *M. officinalis* on heat- and formalin-induced pain were evaluated. Data were analyzed by using one-way ANOVA. Intrathecal injection of *M. officinalis* blocked heat-induced pain compared to sham group ($p = 0.001$). Maximum analgesia was observed 30 min after the injection. Furthermore, intrathecal administration of MO alleviated both acute ($p = 0.007$) and chronic ($p = 0.001$) phases of formalin-induced pain.

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Motor block was not observed in any of the above mentioned groups. The results showed that intrathecal administration of MO could significantly improve hot-water and formalin-induced pain in male Wistar rats.

1. Introduction

Physiological injuries caused by accidents, diseases or surgical procedures are often accompanied by pain. Moderate to severe pain associated with surgery is very important and can cause sleep disturbances and restriction of movement, and lengthen the duration of hospitalization [1]. Followed by painful stimulation of peripheral tissues, pain messages are transferred to the posterior horn of the spinal cord through the primary afferent neurons (C, Aδ). This message can be stimulated or inhibited by mediators secreted from primary afferent fibers, interneurons or fibers coming down from the higher areas of the brain [2].

The increase of information about processes of the spinal cord has led to the identification of specific drugs that can inhibit pain transmission through the spinal cord. Intrathecal administration of these drugs is associated with hypotension, bradycardia, nausea and vomiting, urinary retention, rising levels of spinal anesthesia, cauda equine syndrome and the like [3]. Due to the side effects of conventional drugs, the use of new intrathecal drugs with fewer side effects is suggested.

Melissa officinalis (MO) is one of the oldest herbal remedies commonly used in traditional medicine that belongs to the Lamiaceae family. One characteristic of this plant is its lemon fragrance [4]. This plant is often found in the Mediterranean region, western Asia, southern Siberia and North-West Africa [5]. It has many properties of which some scientifically evaluated are: sedative [6,7], anti-inflammatory [8], antioxidant [9], liver [10] and nervous system [11] protection, antihyperlipidemia [12], anxiolytic [13], antibacterial [14], antiviral [15], anti-Alzheimer [16] and antidepressant properties [17]. Some studies have also examined the analgesic effects of MO. Oral administration of different doses of MO could heal visceral pain induced by acetic acid [8,9,18] and inflammatory pain induced by formalin test [5,8]. It seemed that MO exerted its analgesic effect through the cholinergic system and nitric oxide pathway [5]. However, the available researches showed that the effects of intrathecal administration of the plant on pain had not been evaluated. The advantage of formalin over other methods of inflammatory pain induction is that it provides both acute and tonic pain for an hour by a substance [19]. For that reason, this study aimed for an investigation into the effects of intrathecal administration of MO on the pain induced by heat and formalin.

2. Materials and Methods

2.1. Preparation of Hydro-Alcoholic Extract of *Melissa officinalis*

Dried leaves of the MO are milled (after approval by the botanist) and 50 g of powdered plant was stored in a container; 1000 ml of 75% ethanol was added to it and maintained for 48 h. Then, the extract was separated by Buchner funnel and filter paper. To prepare the powder, ethanol was isolated from the extract by a rotary device (vacuum distillation) [4].

2.2. Groups

The research project has received the confirmation by the Institution Ethics Committee according to National Ethics Committee for Biomedical Research. Seventy male Wistar rats with an average weight of 270-320 g were randomly divided into two subgroups of 35 rats: one subgroup for the evaluation of hot-water-induced pain and the other for the evaluation of formalin-induced pain. Each subgroup was again divided into five smaller divisions: control group without catheter to be used only for hot-water test or formalin test at designated times; the sham group, that received 25 μl of saline through the spinal catheter, and three experimental groups that received 5, 10 or 20 mg/kg MO dissolved in saline through the spinal catheter, respectively.

2.3. Intrathecal Catheterization

Catheterization of the spinal cord was commenced by anesthetizing of rats with the administration of ketamine and xylazine (72 and 8 mg per kg of body weight, respectively). Based on changes in the Pogatzki method [20], catheterization was performed in the fifth and sixth lumbar vertebrae levels. First, the animal's hair of the lumbar region was shaved and the area disinfected by betadine solution. The area between the fifth and sixth lumbar spine was transected finely and muscles were sidelined by forceps. Intervertebral soft tissue was gently cut until the cerebrospinal fluid was observed. Then, under a direct vision of 3-3.5 cm distance, polyethylene catheter 10 (which was marked by swelling of Parafilm with a total length of 18 cm) was placed into the spinal cord. The sudden movement of the mouse's legs or tail showed that the catheter had hit the spinal cord and confirmed the right direction of cannulation. Then with the aid of No. 20 needle, the other end of the catheter was passed through the lumbar muscle. The presence of a bulging section made the catheter stick behind the muscle and did not go out of the spinal cord. The catheter was inserted under the skin as much as its end exited from the top of animal's head. Next, a bump was created at a 2 cm distal of the catheter by using Parafilm. This procedure fixed the catheter in its place, in spite of the animal's attempt to move. At this stage, to ensure the openness of the catheter, 10 ml of saline was injected via catheter and the end of the cannula was closed by cautery to prevent the CSF exit and the entry of foreign material into the catheter. The duration of surgery was 10-12 min. Then, animals were kept in separate cages for

five days to repair the region. After catheterization, animals with motor problems were excluded from the study. To ensure the correct position of the catheter, one day after catheterization, 20 μl of 2% lidocaine with 5 ml of saline was injected via catheter. Bilateral paralysis of the lower extremity following the injection proved that the location of the catheter was correct. Paralysis caused by spinal injection of lidocaine was completely resolved after 24 h. Five days after catheterization and healing, animals were randomly divided into four groups: sham and three experimental groups.

2.4. Assessment of Hot-Water-Induced Pain

After cleaning and marking of 3-4 cm from the end of the tail, the rat was placed in the hot water with a temperature of 52°C. The length of time that took the rat to shake its tail was mentioned as the reaction time to pain or delay period to pain induced by hot water, which is in fact a measure of analgesia. The more the duration of analgesia lasted, the more the effects of extract on the induction of analgesia. To avoid damage to the animal's tail, the maximum time it was allowed to be immersed in water was limited to 20 s [21]. To obtain the start time, maximum time and end time of analgesia, the test was repeated at 10, 30, 60, 120, 240 and 1440 min after intrathecal administration of MO.

2.5. Evaluation of Formalin-Induced Pain

To study the formalin-induced pain, 30 min after the intrathecal injection of MO, 50 μl of 5% formalin was injected subcutaneously in the right hind paw and the animal was immediately passed into clear Plexiglas standard cage. A mirror was embedded in the rear part of the cage for better monitoring of animal behavior. To determine the pain score, the behavior of the animal was graded as follows:

Zero: the animal walked with perfect balance; its body weight was distributed on both feet.

One: the animal had difficulty walking; its body weight was not on the right leg.

Two: the animal's right foot was kept up and had no contact with the floor.

Three: the animal licked or bit or shook its right foot.

It should be explained that scores were given every 15 s for the entire time period. The time 0-10 and 15-60 min after injection of formalin were considered as the first and second phase, respectively [18].

2.6. Determination of the Motor Block

The amount of motor block was measured in groups for which hot-water-induced pain test was conducted. The motor block was scored as follows:

Zero: when the animal moves its legs easily and without restrictions.

One: when it was difficult for the animal to keep the balance of its body; its gait and leg movements were asymmetric. In other words, more weight was put on the left foot.

Two: when the animal failed to move its legs and couldn't respond to painful stimuli.

Three: when the legs were completely paralyzed [22].

2.7. Statistical Analysis

Results were presented as mean ± standard error of the mean (mean ± SEM). For comparison between groups by SPSS version 16 (SPSS Inc., Chicago, IL, USA), repeated measurement of ANOVA and one-way ANOVA with LSD *post hoc* test was used. A $p < 0.05$ was considered as a significant level.

3. Results

Different groups had no significant difference in weight and pain score before intrathecal injection of MO. According to Fig. 1, at various times after intrathecal injection of saline, there was no significant difference between the control and the sham group in pain score, which indicated that the catheterization and injection of saline did not affect the mean analgesia. Ten minutes after intrathecal injection of MO, analgesia was begun in the experimental group and showed a significant difference with control group ($p = 0.001$). Also, the 5 mg group was a significant difference ($p = 0.001$) with groups of 10 or 20 mg. No difference was observed between the concentrations of 10 and 20 mg. The maximum analgesic effect was observed in the experimental groups 30 min after intrathecal injection (Fig. 2). Analgesia decreased after 240 min in the 5 mg group so that showed a significant difference ($p = 0.001$) with 10 and 20 mg groups and sham group ($p = 0.01$). Analgesia decreased in the 5 mg group after 1440 min and showed no significant difference with sham or control groups. Analgesia in groups of 10 and 20 mg also reduced, although there was a significant difference ($p = 0.001$) with the control group after 1440 min.

As shown in Fig. 3, in the acute phase (up to 10 min after the injection of formalin) groups of 5 mg ($p = 0.04$) and 10 mg ($p = 0.007$) injections were significantly different from the sham group and no difference between the 20 mg, control and sham groups was observed. In the chronic phase (15 to 60 min after injection of formalin), the group 5 ($p = 0.001$) and 10 ($p = 0.001$) mg were significantly different from sham group. After intrathecal injection of 20 mg/kg, 6 of 18 rats developed severe convulsions and died an hour after injection.

None of the rats had a motor block in the experimental group after intrathecal injection of MO. In other words, rats easily walked without any problem.

4. Discussion

In this study, we have investigated the effects of intrathecal injection of MO on acute and chronic pain. Intrathecal injection of MO blocked heat-induced pain in male Wistar rats dose dependently. Maximum analgesia was observed 30 min after the injection. Furthermore, intrathecal administration of MO alleviated both acute and chronic phases of formalin-induced pain. On the other hand, motor block was not observed in the all groups. Lack

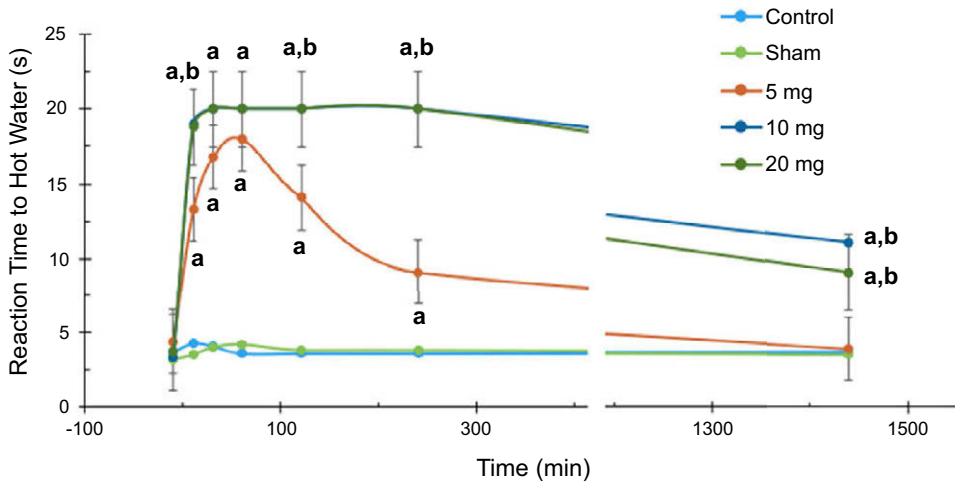

Figure 1 Average analgesia time (sec) at different times due to the intrathecal injection of Melissa Officinalis on hot water induced Pain in different groups. The average analgesia time in the rats treated with Melisa officinalis (0, 5, 10 and 20 mg/kg). Each data point represent mean and SEM from 6∼8 rats. The analgesia time was measured 10 min before and 10, 30, 60 120, 240 and 1440 min after intrathecal administration of Melisa officinalis. The letters, 'a' and 'b' indicate that the data point are significantly different from those of sham ($p = 0.001$) and 5 mg groups ($p = 0.001$), respectively.

of motor block in the sham group indicated that catheterization did not injure the spinal cord, and in the experimental groups showed that the values used in this study had no effect on the motor system.

These results are consistent with other studies [5,8] that have examined the effects of oral administration of MO on pain. Since the roots of the experimental group were not sedative, it can be said that MO has exerted through the spinal cord not through the brain [23]. Although the aim of this study was not to find analgesic mechanisms of MO, some mechanisms were predicted by other researches done on the issue that required further investigation to approve.

The heat-induced pain had central effect. Impulses of pain were transferred to the posterior horn of the spinal cord via primary afferent fibers and at this site modulated by interneurons or descending fibers [2]. Since the pain caused by the heat, in this study, was significantly reduced by MO, this effect may be considered as the results of a single mechanism or a set of them.

Formalin test is a standard method for measuring response to pain. Formalin injected was induced in two phases of pain: the first or acute phase lasted for 10 min due to direct stimulation of peripheral nociceptors known as neurogenic pain; the second phase or chronic phase lasted for 15-60 min after injection due to a combination of peripheral inflammatory reaction and central sensitization of spinal cord [18]. Reduction in both phases of formalin-induced pain following the administration of intrathecal MO suggested that this compound was effective against acute and chronic pain. According to Guginski et al [5], cholinergic, nitric oxide and glutaminergic system were involved in the analgesic effect of MO, and the opioid system is not involved. It has been shown

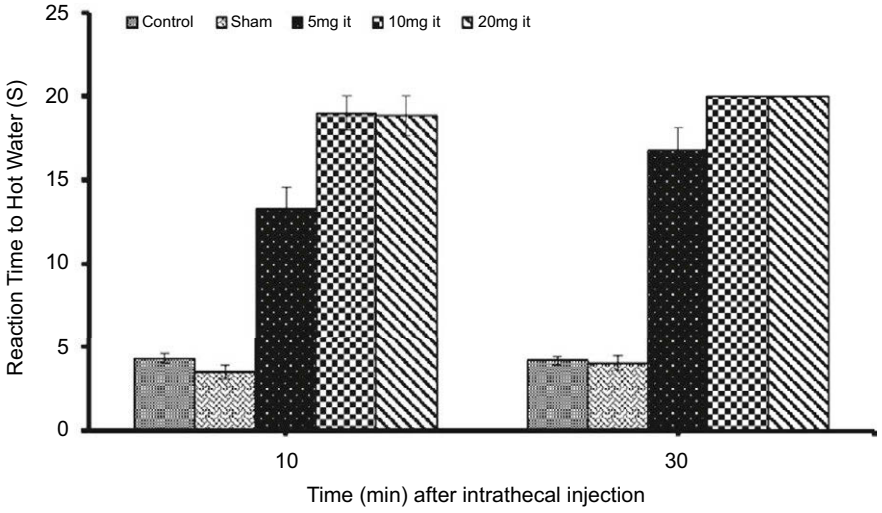

Figure 2 Bar diagram of 10 vs 30 min analysis for different doses of MO in hot-water test.

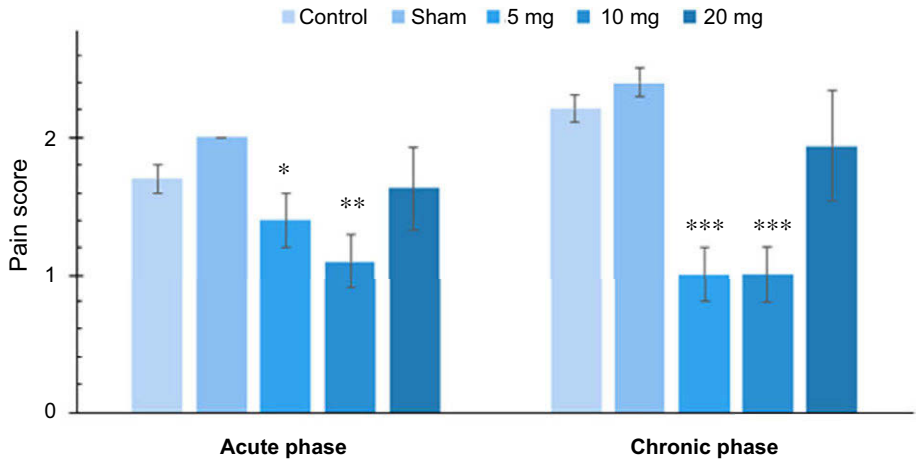

Figure 3 Average pain score due to the intrathecal injection of Melissa Officinalis in acute (0 - 10 min) and chronic (15 - 60 min) phases of formalin-induced pain in different groups. Pain scores of formalin-induced nociceptive behavior in the rats treated with Melisa officinalis (0, 5, 10 and 20 mg/kg). Pain scores were measured at 0∼10 min (acute phase) and 15∼60 min (slow phase) after formalin injection. Each data point represents mean and SEM from 6∼8 rats. *, ** and ***: different from the pain scores in the sham group ($p < 0.05$, $p < 0.01$ and $P < 0.001$), respectively.

that formalin causes pain by stimulating receptors of TRPA1 (transient receptor potential cation channel, subfamily A, member 1) [24]. The response of TRPA1 deficient mice and/or the mice that had received an antagonist of it was reduced to formalin-induced pain [24]. Since in the present study, formalin-induced pain was reduced by intrathecal administration of MO, it is possible that MO has an antagonistic effect on TRPA1 receptors.

Oxidative stress may also be involved in the pathogenesis of pain. Different antioxidants have improved the formalin-induced pain [25,26] and also the neuropathic pain [27,28]. It may be that the antioxidant properties of MO [9,29,30] are effective in their analgesic results.

5. Conclusion

The results showed that intrathecal administration of *Melissa officinalis* could significantly improve hot water- and formalin-induced pain in male Wistar rats.

Conflict of interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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