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Original article

A Comparative Study on the Analgesic Properties of Five Members of Lamiaceae Family Using Two Pain Models

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Abstract

Background and objectives: Alternative medicine is widely used to replace a variety of commonly prescribed synthetic drugs in order to achieve a state of substantial efficacy with considerably less adverse effects. The present work has focused on the comparative evaluation of the analgesic efficacy of five members of Lamiaceae family to prioritize their potentials to be used herein. **Methods:** Two common models of pain studies including the hot-plate and tail-flick tests were used to compare the analgesic properties of *Thymus vulgaris*, *Mentha piperita*, *Rosmarinus officinalis*, *Satureja hortensis*, and *Mentha pulegium* essential oils (EOs) at two doses of 0.5 and 1 cc per animal. **Results:** Significant increase in the response times of both tests were recorded compared to the control group following the administration of the EOs with the order of potency *T. vulgaris* 1 mL > *T. vulgaris* (0.5 mL) > *M. piperita* (1 mL) > *M. piperita* (0.5 mL) > *R. officinalis* (1 mL) > *R. officinalis* (0.5 mL). **Conclusion:** Although all studied EOs showed some extents of anti-nociceptive properties; however, *T. vulgaris* and *M. piperita* demonstrated the highest potential for pain management due to their rapid onset, long-lasting and steady mode of action. Their more potent anti-nociceptive effects in comparison to *R. officinalis* with previously proven analgesic efficacy, further supports this idea.

Keywords: *Mentha piperita*; *Mentha pulegium*; *Rosmarinus officinalis*; *Satureja hortensis*; *Thymus vulgaris*

Introduction

Herbal preparations have long constituted a major proportion of the prescriptions of folklore healers in almost every society. Today, despite the introduction of a vast variety of synthetic and at the same time effective medications for a great number of disorders and medical conditions, the interests of researchers and health care professionals have been once again attracted towards the use of natural resources for the development of new drugs. The fact that makes the priority for this reconsideration regarding the use of conventional medicines is the notable

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adverse effects exerted following their long term usage, an obstacle that could be resolved with the aid of alternative medicine [1]. Among the most commonly used drugs for management of inflammation and pain are non-steroidal anti-inflammatory drugs (NSAIDs) which despite being so effective, several side effects have been reported following their chronic usage [2-4]. The current focus on the development of new analgesic agents, has led to the identification of several plants with anti-nociceptive properties.

Thymus vulgaris and *Rosmarinus officinalis* are members of Lamiaceae family with already proven analgesic properties [1,5-7]. *Thymus vulgaris*, locally known as "Avishan", is widely used among Iranians as an aromatic food additive [8] and as folklore antitussive, antibacterial and antiviral remedy [1]. The essential oil (EO) of this plant mainly consists of monoterpenes the most important of which are thymol and cavacrol [9]. Pharmacological evaluation of the plant has also revealed its antimicrobial, antifungal, antispasmodic and potent antioxidant properties, as well as protective effects for the management of diabetes mellitus [10,11]. *Rosmarinus officinalis* is well known in the scientific literature for its anti-inflammatory and strong analgesic effects to an extent which can be introduced as a drug with therapeutic potential [6,12]. The major bioactive compounds present in the plant are carnosic acid, carnisil, and rosmarinic acid [13]; among them, the anti-inflammatory effect has been mainly attributed to the arnosic acid and carnosol [14]. Mechanistic evaluations of the analgesic effect of *R. officinalis* have revealed the involvement of the opioid pathway [15], and GABA receptors [5]. The plant has been traditionally used as a topical analgesic agent in Iran [16] and its ointment is commercially available for the alleviation of rheumatoid pain in combination with other plants. Among these plants are other members of Lamiaceae family including *Mentha piperata*, *Mentha pulegium*, and *Satureja hortensis* [17]. Numerous biological activities of these plants including the analgesic effects of only some of them have been previously reported using single models of pain studies [17-19]; however, there are few, if not any, inclusive studies in the literature to investigate the anti-nociceptive effects of these members of the Lamiaceae family. Our aim was to study the anti-nociceptive effect of these plants in a comparative fashion using two common methods of pain studies *i.e.* the hot-plate and the tail-flick methods in order to provide a substantial comparison of their analgesic efficacy based on the traditional usage.

Material and Methods

Plant material

The EOs of *T. vulgaris, M. piperita, R. officinalis, S. hortensis,* and *M. pulegium* were prepared according to the following procedure. Fresh aerial parts of the plants were collected from Urmia, West Azarbayjan province, Iran. *M. piperita, M. pulegium and S. hortensis* were collected in July, *T. vulgaris was obtained in* June, and *R. officinalis* was gathered in May (2016). A voucher number was deposited for each species in the herbarium of Mazandaran University of Medical Sciences, Sari, Iran (*T. vulgaris*: E2-36-4101; *M. piperita*: E2-36-4111; *R. officinalis*: E2-36-421; *S. hortensis*: E2-36-481; *M. pulegium*: E2-36-4112). The plants were dried in the shade and hydrodistilled using a Clevenger-type apparatus. The essential oils were kept at 4°C.

Animal studies

Sixty six male Wistar rats were randomly allotted to 11 groups of 6 each. After one week of acclimatization, a group of animals were labeled as the control group which remained untreated and 10 treatment groups each receiving one of the EOs mentioned above at a dose of at a dose of 400 or 800 mg/kg. The mentioned volumes contained 100 mg and 200 mg of each plant, respectively. These dosages were used to make a comparison between the effects of the same amounts of each plant in the animals since all the animals had the same weights of 250 g. During the whole experimental period, all the animals were kept in an isolated room under the standard cycle of 12 h light/dark and at a temperature of

23±1°C. Feed and water were given ad libitum. The Research was approved by the research committee of Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

Hot-plate and tail-flick tests

In order to perform the hot-plate test, after the intra-peritoneal treatment of the animals within each group with the corresponding EO, each rat was placed on a hot-plate apparatus surrounded with a cylinder-shaped wall exactly 30 min post-treatment. Simultaneously, a stopwatch was started to record the elapse time until the animal responded to pain by a jumping reaction. Various types of reactions have been previously considered as the response to heat stimuli in the hot-plate test including licking, stamping, jumping, *etc*. [20]. In this study, the jumping time; hereafter the "leg-out time" was recorded as the response time to heat stimuli. The same procedure was performed for the control group and was repeated 3 times with intervals of 30 minutes. A cut-off time of 20 s was maintained to prevent paw injury [21].

In a similar manner, the tail-flick latencies were recorded for all studied groups at intervals of 30, 60, 90, and 120 min post-treatment. In brief, the response latency was measured using a tail flick apparatus while the strength of the current was maintained at 2-3 Amps at a constant temperature of 55 °C during the test. A distance of 1.5 cm was considered between the heat source and the tail skin. The heat was applied to the tail at a width of 2.5 cm measured from the tail root. In order to avoid tissue damage, the cut-off reaction time was considered 25 s and the escape response was considered as the end pint of the test [22].

Results and Discussion

The results obtained from the hot plate test have been presented in figure 1. The leg-out time in both groups which had received *T. vulgaris* EO was dramatically higher than that of the control group ($p<0.0001$). This decline in the response time had initiated on the first examination (30 minutes post-treatment) and remained significant throughout the whole experimental period. The same results were recorded with both dosages of *M. piperita* and *R. officinalis* with the rank order of potency of *T. vulgaris* 1 mL> *T. vulgaris* (0.5 mL) > *M. piperita* (1 mL) > *M. piperita* (0.5 mL) > *R. officinalis* (1 mL) > *R. officinalis* (0.5 mL). The decrease in the response time exerted by *S. hortensis* EO (both 0.5 and 1cc groups) reached a significant level 60 minutes post-treatment and remained significant at both records of 90 and 120 minutes, in comparison to the control group ($p<0.001$). The effects observed in both *M. pulegium* treated groups was only significant at 120 minutes post-treatment ($p<0.01$).

As it is evident from figure 2, the strongest results were observed with the *T. vulgaris* EO in a way that the response time was significantly higher at all stages of the experiment, even when compared with the *M. piperita* and *R. officinalis* groups ($p<0.05$) (see figure 2). The leg-out time increased in the *T. vulgaris* (1 mL) and *M. piperita* (1 mL) groups in a steady fashion during the study period; however, the increase in the hot-plate response latency in the *T. vulgaris* (0.05 mL) and *R. officinalis* (1 mL) groups was accompanied with a later decline at 60 min post-treatment, still being significantly higher than the records of the control group ($p<0.0001$).

T. vulgaris, *M. piperita*, and *R. officinalis* groups, at both dosages of 0.5 and 1 cc, exerted a significant increase in the tail flick latency at all stages of the experiment (table 1). In parallel with the results of the hot plate test, the *M. pulegium* EO could not exert a significant anti-nociceptive effect in the tail-flick test either ($p>0.05$). In addition, the results obtained from the tail-flick test for the *S. hortensis* was in accordance with the hot-plate results for this plant except that the effects were initiated more rapidly (60 minutes post-treatment) in the hot-plate test, while the first significant increase in the tail-flick latency was recorded at 90 min post-treatment for this plant.

The comparison of tail -flick latencies between *T. vulgaris* (0.5 and 1 mL), *M. piperita* (1 mL), and *R. officinalis* (1 mL) showed that the two latter groups affected the tail-flick latency in a similar manner to the hot-plate test.

Table 1. Tail flick latencies (recorded as seconds) in different study groups

Groups	Dose (mg/Kg)	30 min	60 min	90 min	120 min
Control	-	3.00 ± 0.20	4.50 ± 0.50	3.50 ± 0.50	4.00 ± 0.30
Thymus vulgaris	400	7.50 ± 0.50 [a]	16.3 ± 0.65 [a]	14.4 ± 0.55 [a]	12.5 ± 0.50 [a]
Thymus vulgaris	800	10.0 ± 1.00 [a]	14.1 ± 0.90 [a]	18.6 ± 0.35 [a]	14.5 ± 0.50 [a]
Rosmarinus officinalis	400	7.75 ± 0.75 [a]	12.1 ± 1.10 [a]	10.5 ± 0.50 [a]	12.5 ± 0.50 [a]
Rosmarinus officinalis	800	10.5 ± 0.50 [a]	14.8 ± 0.20 [a]	12.0 ± 1.00 [a]	12.2 ± 0.25 [a]
Mentha pulegium	400	5.50 ± 1.50 [c]	4.50 ± 0.50	4.75 ± 0.25	3.50 ± 0.50
Mentha pulegium	800	4.45 ± 0.55	5.40 ± 1.10	4.85 ± 0.15	4.50 ± 0.50 [d]
Satureja hortensis	400	5.20 ± 2.00 [c]	5.90 ± 1.10	7.50 ± 0.50 [a]	5.65 ± 0.65 [c]
Satureja hortensis	800	3.95 ± 0.50	5.30 ± 0.20	6.60 ± 0.40 [b]	6.40 ± 0.40 [a]
Mentha piperita	400	7.40 ± 0.40 [a]	10.0 ± 0.45 [a]	12.6 ± 0.40 [a]	11.5 ± 0.55 [a]
Mentha piperita	800	10.5 ± 0.50 [a]	13.2 ± 0.45 [a]	15.2 ± 0.95 [a]	15.1 ± 0.15 [a]

[a] $p< 0.0001$; [b] $p< 0.001$; [c] $p< 0.01$; [d] $p<0.05$; were considered significant compared with the control (ANOVA followed by Dunnett's test). Values have been expressed as mean ± SEM.

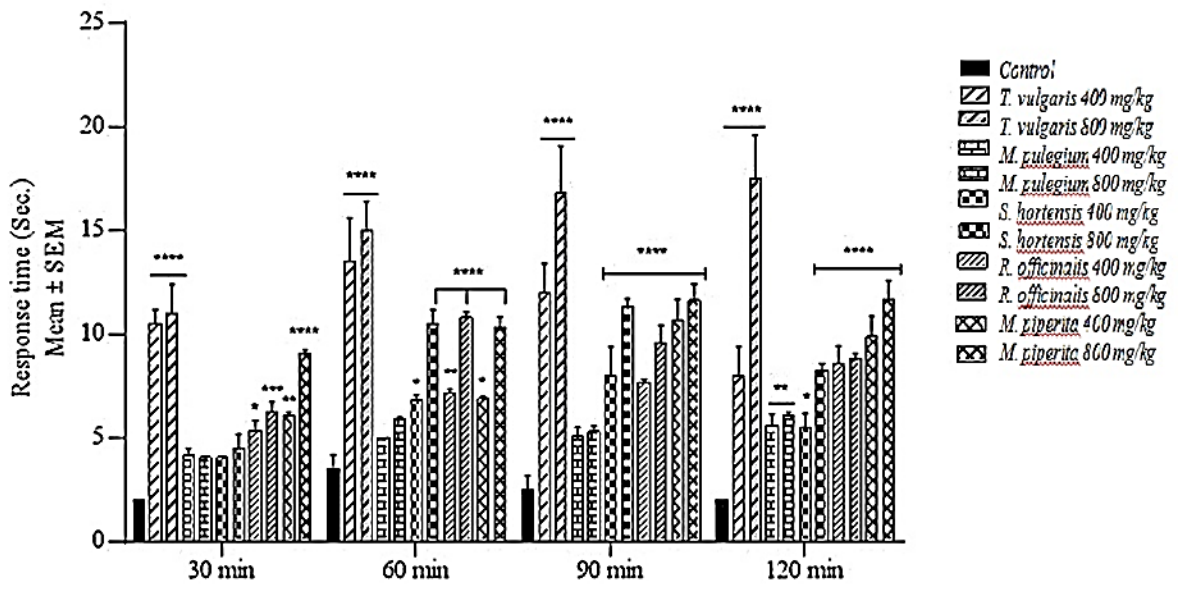

Figure 1. The comparison of response time recorded with the hot-plate test between different study groups. The values are expressed as mean ± SEM. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, and $^{****}p<0.0001$ were considered significant compared to the control group.

In contrast to the hot-plate model, the *T. vulgaris* group did not show a steady increasing pattern in the response latency of tail-flick, since it started to decrease after 90 minutes. Treatment with *T. vulgaris* exerted significantly higher latencies even in comparison with *M. piperita* (1 cc), and *R. officinalis* (1 cc) groups at the stage of 90 minutes ($p<0.05$), indicating a more potent anti-nociceptive activity of the plant (figure 3). The hot-plate and tail-flick tests are commonly used models for the study of acute pain in which the rapid response of the animal determines the elapsed time from the onset of a stimulus, indicating the extent of tolerance to peripheral pain following treatment with any compounds being studied for their probable analgesic properties [23].

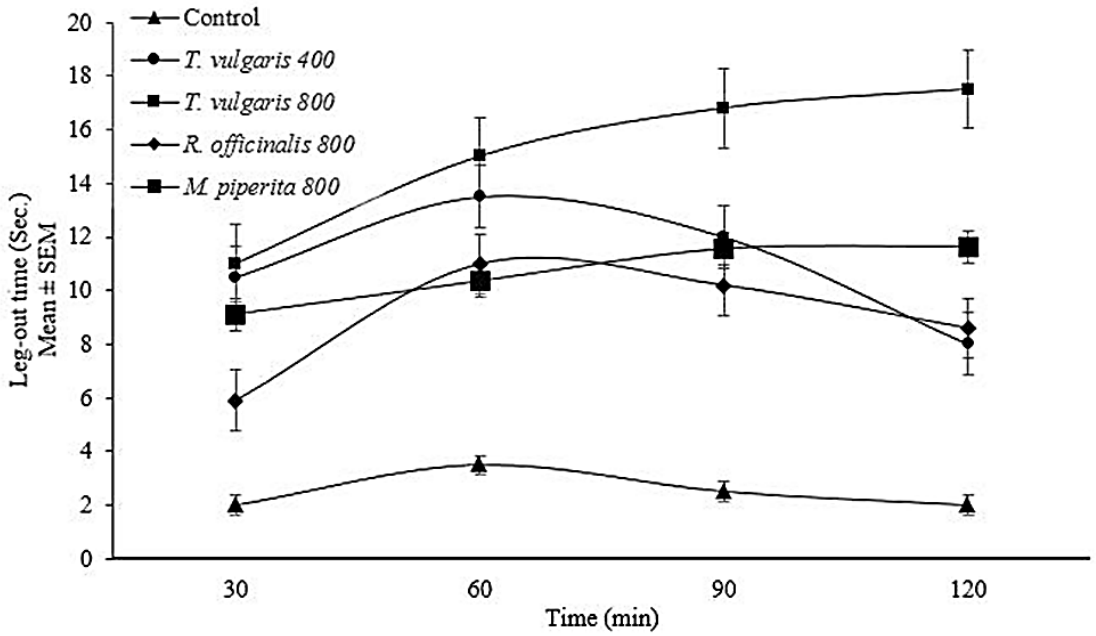

Figure 2. Comparison of the leg-out time at 30, 60, 90, and 120 min post-treatment between *T. vulgaris* (0.5 and 1 mL), *M. piperita* (1 mL), and *R. officinalis* (1 mL) groups. The values have been expressed as mean ± SEM.

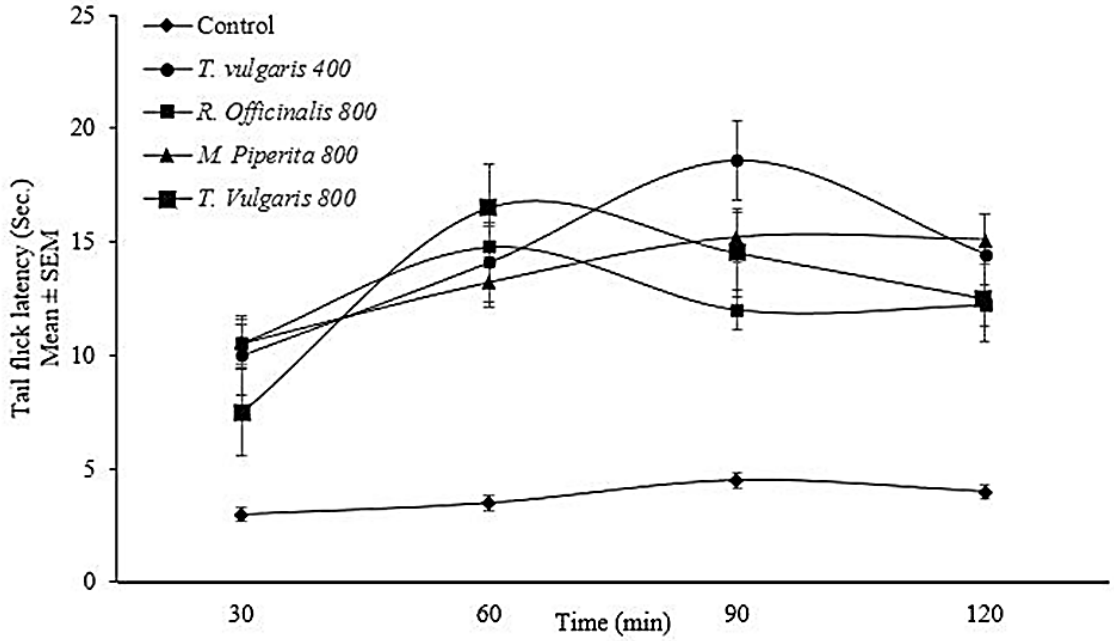

Figure 3. Comparison of the tail-flick latencies at 30, 60, 90, and 120 minutes post-treatment between *T. vulgaris* (0.5 and 1 mL), *M. piperita* (1 mL), and *R. officinalis* (1 mL) groups. The values have been expressed as mean ± SEM.

These two tests do not normally deliver similar results due to the different nature of spinal and supra-spinal responses to different types of stimuli; hence, the application of a single method is not recommended and a combination of these models would provide more reliable results [24]. On such a basis, in the present study, an attempt was made to evaluate the anti-nociceptive properties of five members of the Lamiaceae family, using both mentioned methods, in order to provide a comprehensive comparison of the observed effects.

The results obtained with the *T. vulgaris* EO in both pain models indicated that this plant possessed the strongest anti-nociceptive properties, not only in comparison with the control group, but also when individually compared with any of other tested EOs. The analgesic efficacy of *T. vulgaris* extract [7] and its essential oil [1] has been already shown using the single tail-flick model. Thymol, one of the main constituents of *T. vulgaris* essential oil, has been proposed to be responsible for its analgesic effects via acting on α_2-adrenergic receptors [25]. Since this effect of the plant was initiated 30 min post-treatment and lasted for the whole study in both methods, it can be postulated that thymol might have the potential to be isolated and used as a rapid-acting and long-lasting analgesic agent for the management of acute and chronic pain. The steady increasing pattern in the hot-plate response time recorded for the *T. vulgaris* (1 mL) might further support its desirable effect to be used herein. Nevertheless, such a steady increasing analgesic effect was only sustained up to 90 min from the onset of the experiment when tested with the tail-flick model. Although the results of *M. piperita* and *R. officinalis* were not as strong as that of *T. vulgaris*, these two essential oils had the second and third rank of potency, respectively. Anti-nociceptive effects for *M. piperita* methanol extract have been previously proven [26]. Interestingly, a more steady increasing fashion was observed in both hot-plate response time and tail-flick latency exerted by *M. piperita* (1 mL), compared to *T. vulgaris* (1 mL).

R. officinalis is well known for its potent analgesic properties and its efficacy has been shown in several pharmacological studies [5]. Although the anti-nociceptive activity shown in the present study for the EO of this plant is in harmony with the results of previous studies, the lower analgesic potency in comparison with *T. vulgaris* and *M. piperita*, was our major concern to be reported after the results were statistically analyzed. The response latencies recorded for *T. vulgaris* (1 mL) were significantly higher than both *M. piperita* and *R. officinalis* at 90 min post-treatment indicating its priority to be studied as a source of potent anti-nociceptive compounds. Another finding which could be of particular interest was the rapid onset of the analgesic effects of *T. vulgaris*, *M, piperita*, and *R. officinalis* essential oils at a dosage of 1 mL, at 30 min post-treatment in both tail-flick and hot-plate models.

Hydroalcoholic and polyphenolic extracts and the EO of *S. hortesis* have been shown to possess anti-inflammatory and analgesic effects (using formalin test), mainly due to the presence of polyphenols with the suggested mechanism of opioid receptor involvement [19,27]. Yet, its effects were not comparable to those of the above mentioned plants. Moreover, the plant may contain slow acting compounds since the onset of its analgesic effects was 60 min post-treatment in the hot-plate test and 90 minutes post-treatment in the tail-flick test.

M. pulegium EO did not exhibit potent analgesic properties neither in the hot-plate test nor the tail-flick test at least when compared to the other tested groups. This showed that despite being rich in polyphenols [28], the plant could not exert the same analgesic effects as exerted by the other species from the same genus (*M. piperita*). This might suggest that compounds other than polyphenols might also have a role for the analgesic effect of *M. piperita*.

Taken together, this study has provided the basis for other researchers to consider *T. vulgaris* and *M. piperita* as potential natural alternatives to be

used in pain management due to their rapid onset, long-lasting and steady mode of action. Their more potent anti-nociceptive effects in comparison to *R. officinalis* with previously proven analgesic efficacy, further supports this idea. Indeed, further studies are required to completely pave the way for other researchers in order to provide substantial data to introduce these natural resources as novel clinical medicines.

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Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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