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Short Communication

Effect of matricaria recutita on acute pain in the presence and absence of sex hormones

Mahnaz Kesmati, Neda Barfinejad**, Hadi Fathi Moghadam****

Abstract

BACKGROUND: Chamomile is a beneficial herbal drug that is used as an anti-inflammatory, sedative and anti-allergic agent. The mechanism of action of matricaria recutita (MR), a specious of chamomile, in nociception in male and female animals is not fully understood. In this study, the sedative effect of a species of chamomile, MR, on acute pain was investigated in both male and female adult mice in the presence and absence of sex hormones.

METHODS: Male and female NMRI mice weighing 28 ± 3 grams were used. Animals of each sex were divided into intact and gonadectomized groups. Intact group received saline or MR extract (10, 30, 50 mg/kg, intraperitoneally). Gonadectomized group contained two subgroups: a) group that received saline or MR hydro alcoholic extract (50 mg/kg, I.P.), and b) group that received sex hormones (testosterone in male mice and estradiol benzoate and progesterone in female mice), both with and without MR extract (50mg/kg, IP). The analgesia times in all groups were evaluated by hot plate test.

RESULTS: MR increased analgesia time both in intact and gonadectomized male and female mice, but had no effect in the presence of pharmacological doses of testosterone (2 mg/kg, subcutaneous) in male mice, and estradiol benzoate (0.1 mg/kg, SC) and progesterone (0.5 mg/kg, SC) in female mice.

CONCLUSIONS: It seems that MR can induce a pain-relieving effect with and without physiological doses of sex hormones in male and female mice, but sex hormones probably interact with its analgesic effect in their pharmacological doses.

KEY WORDS: Matricaria recutita, pain, testosterone, estradiol benzoate, progesterone, hot plate.

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Remarkable evidence indicates that sex-related differences in pain responses correspond to the effectiveness of various analgesia agents [1]. For example, male rodents display a significantly greater magnitude of antinociception than female rodents following exposure to stressors, such as cold-water baths and immobilization, which are mediated in part by circulating gonadal hormones [2]. Also, male rodents show a significantly greater magnitude of systemic morphine-induced antinociception compared to female rodents over a wide range of nociceptive tests and ages [3-5]. These differences elucidate the magnitude and importance of sex-related hormonal effects on the experience of pain [1,6]. It has been shown that estradiol and testosterone modulate both the sensitivity to pain and analgesia [7]. In addition, some researches have indicated that females are overrepresented in a variety of chronic pain disorders compared to males [8]. Thus, it seems that pain-modulating agents

*Assistant Professor, Department of Biology, Shahid Chamran University, Ahvaz, Iran. e-mail: mahnazkessmati@yahoo.com (Corresponding Author)
**MSc Graduate Student, Department of Biology, Shahid Chamran University, Ahvaz, Iran.
***Assistant Professor, Department of Physiology, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

with sex steroid-like compounds could have different effects on nociception in male and female subjects. Chamomile is an herb containing a weak sex hormone (phytoestrogen) and has a common use worldwide [9], while still widely used in the field of medicine. It has been reported that Matricaria chamomilla have sedative, anti-inflammatory, antiallergenic and spasmolytic properties [10]. Since there are differences in pain perception and analgesia between male and female, and some researches indicate that this difference is caused by sex hormones [1,6,8], we have evaluated the effect of methanolic extract of matricaria recutita (MR) in the presence/absence of physiological, pharmacological sex hormone concentrations in male and female mice.

Methods

Animals: Adult male and female NMRI mice weighing 25-31 grams were used in this study. Animals were housed in plastic cages in an animal room maintained at 23 ± 1^{oC} on a 12-hour dark cycle (light period, 7:00-19:00). Food and water were available at all times, except during experiments. Each animal was used only once and then sacrificed. In each experiment, seven animals were used.

Gonadectomy: The male and female mice were gonadectomized under ketamine (100mg/kg, IP) and xylazine (10 mg/kg, IP) anesthesia. The testes and ovaries of experimental animals were removed under surgical operation by induction of a midline scrotal incision in male, and an abdominal incision in female. Gonadectomy was performed between 8:00 and 14:00. All experiments were performed in accordance with national and institutional animal use guidelines.

Experimental procedure: Different experimental protocols were employed as shown in table 1.

Hot plate test: In the hot plate test, mice were placed on a metal plate heated to a mean temperature of 52 ± 0.2°C, and the reaction time was determined. The apparatus consists of a 25 X 25 cm Plexiglas cage that fits over the hot plate, and a foot-switch timer. Pain thresholds were measured by latency time (analgesia time) to nociceptive responses (paw lick), with a maximum exposure time of 30 seconds [11]. All animals were tested on the hot plate. Each mouse was placed in the center of the hot plate and subjects were tested individually, and only once, between 9:00 and 14:00. After each trial, the hot plate was cleaned to minimize lingering olfactory cues. Feces and urines were first removed with paper towels and the central platform was cleaned with 95% ethanol. After toweling off the solution, the apparatus was further allowed to air dry for about 2 minutes before another animal was introduced. One day before the hot plate tests, each animal was allowed to acclimatize and familiarize itself with the environment of the apparatus for about 2 minutes.

Preparation of the MR extract: Twenty grams of dried flower head powder of MR (Gol Darou, Iran) was suspended in 200 ml of methanol for 24 hours. The suspension, after filtration, was evaporated under vacuum. After drying, a certain amount of MR powder (10, 30 & 50 mg/kg) dissolved in 0.9% physiological saline and were injected intraperitoneally (IP). The control group received 0.9 % physiological saline. All injections were performed 30 minutes prior to testing on the hot plate.

Drugs: Testosterone, estradiol benzoate and progesterone were purchased from Aburaihan Drug Company, Iran, and applied subcutaneously (SC). Testosterone 2 mg/kg was administrated to male mice, while estradiol benzoate (0.1 mg/kg) and progesterone (0.5 mg/kg) were administered to female mice [12]. Each hormone was dissolved in 0.1 ml of sesame oil (Aburaihan, Iran) 15 minutes before MR administration.

Statistical Analysis: All results were presented as mean ± standard error of the mean for seven animals per group. A two-tailed student's t-test for independent samples was used to compare the mean frequency of the behaviors between groups, as shown in the results. Some data were assessed by analysis of variance (ANOVA). Following a significant F value, post-hoc analyses (Tukey test) were performed

for assessing specific group comparisons and differences, where P<0.05 was considered statistically significant. Calculations were performed using the SPSS (version 11) statistical package.

Results

Experiment 1: Treatment of MR extract (10, 30, 50 mg/kg, IP) in intact male mice with dose-response analysis

Figure 1 shows a dose response effect of MR on analgesia time. One–way ANOVA revealed a significant increase in analgesia time in intact male mice in a dose dependent manner of MR. Additionally, post hoc comparisons of Tuckey tests revealed that all doses of MR had more analgesic effect than the saline (P<0.05, P<0.01). Because 50 mg/kg of MR (figure 1) has the best effect on analgesia, we have decided to apply that concentration for the following experiments.

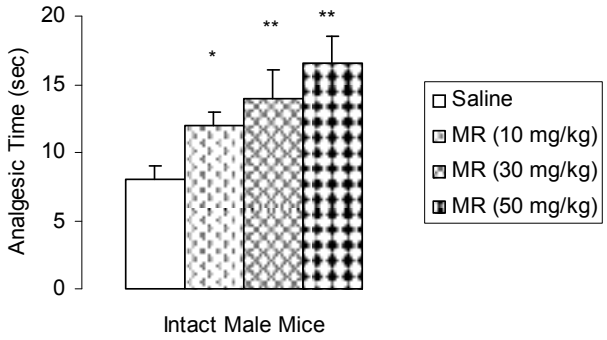

Figure 1. Analgesia time (mean ± SEM) in three groups of intact male mice (n = 7/group), 30 minutes after receiving MR (10, 30, 50 mg/kg, IP) at the hot plate test (P<0.05, P<0.01 compared to subjects received 0 mg/kg MR).

Experiment 2: Treatment of gonadectomized male mice with MR extract (50 mg/kg, IP)

Figure 2 shows the analgesia time of gonadectomized male mice 30 minutes after receiving the MR (50 mg/kg) at the hot plate test compared to the subjects receiving saline with 0 mg/kg MR. Unpaired t-test showed significant analgesic effect for MR in gonadectomized male mice (P<0.05). Removal of testes did not significantly affect analgesic response of MR in the hot plate test.

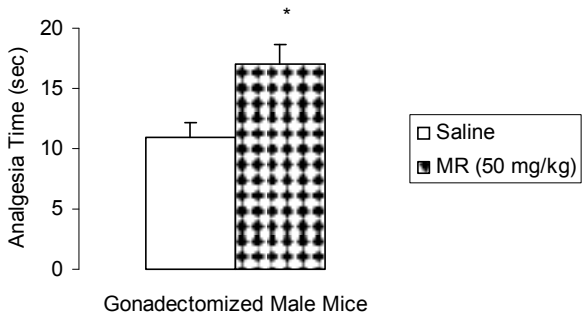

Figure 2. Analgesia time (mean ± SEM) of gonadectomized male mice (n = 7/group), 30 minutes after receiving MR (50 mg/kg, IP) at the hot plate test (P<0.05, compared to subjects received 0 mg/kg MR).

Experiment 3: Effect of co-administration of MR (50 mg/kg, IP) and testosterone (2 mg/kg, SC) on gonadectomized male mice

Figure 3 shows the analgesic response of gonadectomized male mice after 30 minutes MR (50 mg/kg, IP) and testosterone (2 mg/kg, SC) application in comparison with subjects that received testosterone and saline. Unpaired t-test did not show any difference between the groups.

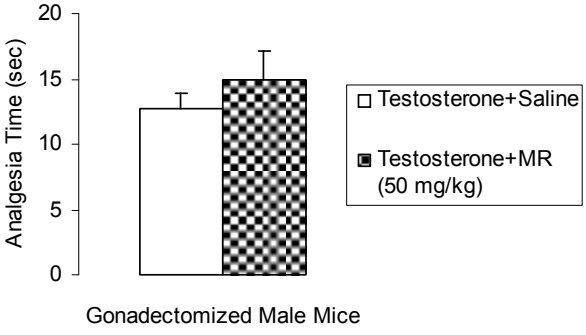

Figure 3. Analgesia time (mean ± SEM) of gonadectomized male mice (n = 7/group), 30 minutes after receiving MR (50 mg/kg, IP) and testosterone (2 mg/kg, SC) at the hot plate test. There was no significant difference between the two groups.

Experiment 4: Effect of MR (10, 30, 50 mg/kg, IP) on intact female mice

Figure 4 shows analgesic response of intact female animals after MR administration. One–way ANOVA and post hoc comparisons of Tuckey tests showed that a significant increase in effect occurred only when 50 mg/kg

(P<0.05) MR was applied. Therefore, the concentration of 50 mg/kg MR was selected for the following experiments.

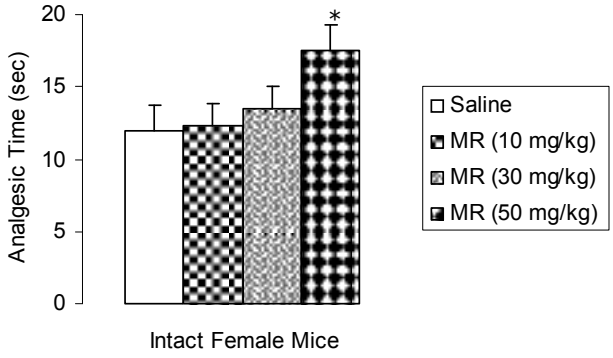

Figure 4. Analgesia time (mean ± SEM) in three groups of intact female mice (n = 7/group), 30 minutes after receiving MR (10, 30, 50 mg/kg, IP) at the hot plate test. (P<0.05 compared to subjects received 0 mg/kg MR).

Experiment 5: Treatment of gonadectomized female mice with MR (50 mg/kg, IP)

Figure 5 shows the analgesia time of gonadectomized female mice, 30 minutes after MR (50 mg/kg) administration using hot plate tests as compared to subjects who received saline without MR application. Unpaired t-test showed a significant effect of MR in gonadectomized female mice (P<0.001). Removal of ovaries did not significantly affect analgesic response of MR (50 mg/kg) in hot plate tests.

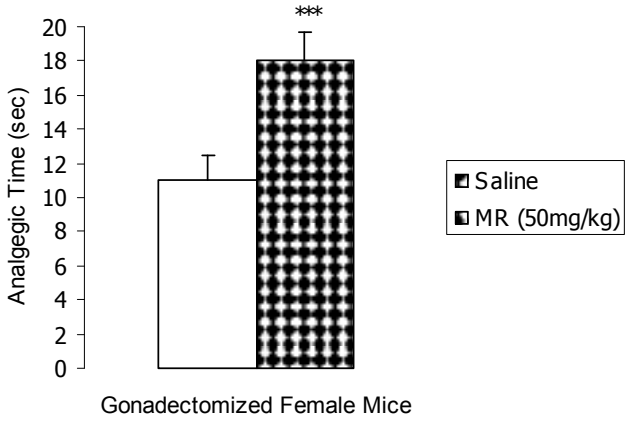

Figure 5. Analgesia time (mean ± SEM) of gonadectomized female mice (n = 7/group), 30 minutes after receiving MR (50 mg/kg, IP) at the hot plate test (P<0.001, compared to subjects received 0 mg/kg MR).

Experiment 6: Effect of co-administration of MR (50 mg/kg, IP) and estradiol benzoate (0.1 mg/kg, SC) on nociception in gonadectomized female mice

Figure 6 shows the analgesia time, 30 minutes after MR (50 mg/kg, IP) and estradiol benzoate (0.1 mg/kg, SC) administration compared to gonadectomized female mice that received estradiol benzoate and saline. Using statistical analysis of unpaired t-test has not shown any significant difference between the groups.

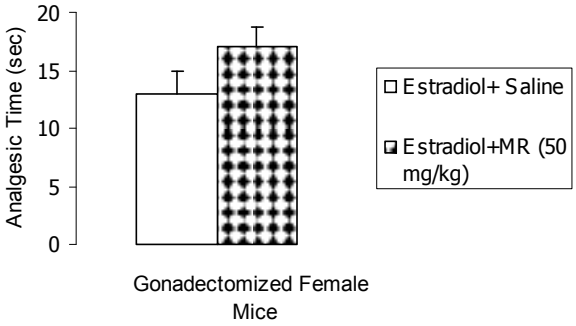

Figure 6. Analgesia time (mean ± SEM) of gonadectomized female mice (n = 7/group), 30 minutes after receiving MR (50 mg/kg, IP) and estradiol benzoate (0.1 mg/kg, SC) at the hot plate test. There was no significant difference between the two groups.

Experiment 7: Treatment of gonadectomized female mice with MR (50 mg/kg, IP) and progesterone (0.5 mg/kg, SC)

Figure 7 shows analgesic response, 30 minutes after MR (50 mg/kg, IP) and progesterone (0.5 mg/kg SC) administration compared to gonadectomized female mice that received progesterone and saline. According to statistical analysis of unpaired t-test, there were no significant differences between the groups.

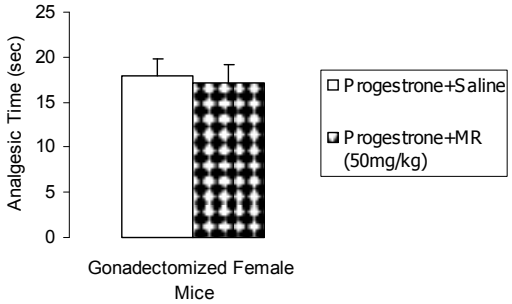

Figure 7. Analgesia time (mean ± SEM) of gonadectomized female mice (n = 7/group), 30 minutes after receiving MR (50 mg/kg, IP) and progesterone (0.5 mg/kg, SC) at the hot plate test. There was no significant difference between the two groups.

Table 1. Protocol of animal treatment and investigation.

Animal (mice)		Groups and Treatment	Investigation
male	intact	1-saline (I.P.) 2-MR (10 mg/kg, I.P.) 3- MR (30 mg/kg, I.P.) 4- MR (50 mg/kg, I.P.)	Analgesia time
	Gonadectomized	5-Saline (I.P.) 6-MR (50 mg/kg, I.P.) 7- Testosterone (2 mg/kg, S.C.)& saline (I.P.) 8-Testosterone (2 mg/kg, S.C.)& MR (50 mg/kg, I.P.)	
female	Intact	9-saline (I.P.) 10-MR (10 mg/kg, I.P.) 11- MR (30 mg/kg, I.P.) 12- MR (50 mg/kg, I.P.)	Analgesia time
	Gonadectomized	13- saline (I.P.) 14-MR (50 mg/kg, I.P.) 15- Estradiol (0.1 mg/kg, S.C.)& saline (I.P.) 16-Estradiol (0.1 mg/kg, S.C.)& MR (50 mg/kg, I.P.) 17- Progesterone (0.5 mg/kg, S.C.) & saline (I.P.) 18-Progesterone (0.5 mg/kg, S.C.)& MR (50 mg/kg, I.P.)	

MR: Matricaria recutita

Discussion

In herbal medicine, Matricaria chamomilla is widely used as a sedative, spasmolytic, anxiolytic and anti-inflammatory agent [13-17]. In the present experiments, we found that MR (especially in dose of 50 mg/kg) greatly decreased acute pain perception in both male and female mice, both in the presence and absence of testes and ovaries, respectively. The results of intraperitoneally administration of MR in male and female mice reported here support the recent investigation showing that a specious of Chamomile (extract of flower and leaf of tanacetum parthenium) has antinociception properties in formalin tests among male rodents [18]. On the other hand, MR has prevented the expression of withdrawal syndrome in morphine-dependent animals [19]. In addition, the dried flower heads of Matricaria chamomilla have been shown to exhibit spasmolytic and sedative properties, and although the active components responsible for the sedative activity have not been fully characterized [20] the sedative effect of MR is clearly indicated. Various studies indicated that Matricaria chamomilla contains several benzodiazepine receptor ligands, which exert benzodiazepine-like activity [20,21,22] as well as phytoestrogenic components [9]. Some studies suggested that the sedative effect of MR is related to flavonoid compounds such as apigenin and chrysin presented in their methanolic extracts [4] which exert benzodiazepine-like activity [16,23] as well as phytoestrogenic effects [9]. Benzodiazepines are known to be an inhibitory agent for anxiety and pain and potentiate GABA-induced chloride currents [16,24-26]. Therefore, it is possible that the inhibitory property of MR on the acute pain perception in the present study is related to the benzodiazepine-like activity of some of its constituents [19]. On the other hand, since estrogens may augment antinociception, MR's phytoestrogens could mediate the suppression of pain [27]. Although the mechanism of MR on nociception is not fully understood, it has been suggested that this herbal medicine is engaged in both central and peripheral analgesia mechanisms [18]. In addition, this study showed that MR has more analgesic effect in male than in female mice. Our data indicated that 10, 30 mg/kg MR extract has no effect on pain perception in female mice. Numerous studies of sex differences reported in the other studies on analgesic drugs in rodents revealed that

opioids are more potent and/or efficacious in males than in females [28,29]. Sex differences are also observed in analgesia produced by chemicals other than opioids and by environmental stimuli such as stress [28]. The observation of sex differences in analgesia implies that gonadal steroids modulate sensitivity to analgesic drugs as well as to environmental stimuli that engage neurobiological systems sub-serving pain perception [7]. On the other hand, the results of co-administration of MR and pharmacological doses of sex hormones such as testosterone in the male and estradiol and progesterone in the female mice indicated that sex hormones could prevent the analgesic properties of MR in gonadectomized mice. That is due to finding no difference in analgesia between two groups who received exogenous sex hormones, one with MR and the other without MR (figures 3, 6 and 7). The preventative effects of sex hormones such as testosterone, estradiol and progesterone on the analgesic effects of MR might be in part related to the common constituents present in MR. As mentioned above, there is some evidence that chamomile contains phytoestrogenic substances [30]. Phytoestrogens are estrogenic plant-derived nonsteroidal compounds comprised of isoflavonoids, coumestans and lignans [31-33]. MR contains apigenin, a flavone [20,21,34], showing both weak estrogenic and progestational activity at the highest concentration tested [9]. Current research suggests that phytoestrogens may be natural 'Selective Estrogen Receptor Modulators' (SERMs) [35] which means that they can bind to certain estrogen receptors in some tissues, either activating or down-regulating cellular responses [35]. The environmental estrogens are generally less potent than other endogenous estrogenic substances [17]. In addition, the specific actions of phytoestrogens in the presence of endogenous estrogen may be concentration dependent, given that agonist actions have been observed at low doses and antagonistic actions at high concentrations [36-38]. Therefore, depending on concentrations of endogenous estrogens on which receptor complexes are activated or down-regulated, phytoestrogens as SERMs can have either estrogenic or anti-estrogenic effects [35]. Testosterone converts to estrogen naturally in the body. In male animals that received testosterone, we have not observed alteration of the analgesic phenomena. Hence, in this experiment, testosterone probably produced estrogen and prevented analgesia through binding to estrogen receptors, which compete with phytoestrogens binding sites. It seems that pharmacological doses of sex hormones interact with phytoestrogenic compounds of MR and probably antagonize its analgesic properties [36,37]. Although the precise roles of gonadal steroid hormones in nociception are not well understood [7] there is evidence that sex hormones could modify the pain perception and analgesic effects of opioids [6,39-41]. Therefore, analgesic effects of MR may be modified by sex hormones like opioids. In summary, MR can modulate acute pain perception in the presence and in the absence of endogenous sex hormones in male and female mice, but this effect is modified by exogenous sex hormones in gonadectomized mice. Further research should be done to replicate and extend this work, and to identify the mechanisms underlying these findings.

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