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Research Article

MATRICARIA CHAMOMILLA EXTRACT DECREASES MORPHINE WITHDRAWAL SYMPTOMS AND MORPHINE SELF-ADMINISTRATION IN RATS

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ABSTRACT
Recent studies have demonstrated that the *Matricaria chamomilla* contains flavonoids which exert benzodiazepine-like activity and therefore it can influence both morphine dependence and the expression of morphine withdrawal symptoms. In this study the effects of *Matricaria chamomilla* extract (MCE) on both morphine withdrawal syndrome and self-administration of morphine were investigated. In the beginning, the development of morphine dependence was achieved by subcutaneous injection of rats with morphine sulfate twice daily for 7 days. The dose of morphine on day 1 and 2 was 2.5 mg/kg and doubled everyday for the next 5 days. An acute MCE administation (10, 25, and 50 mg/kg, i.p.) at day 7, 30 min before the naloxone injection greatly attenuated the withdrawal signs including rearing, jumping, climbing, ptosis, teeth chattering, and wet dog shaking in morphine-dependent rats in a dose dependent manner. In the second stage of the experiment, an acute central injection of MCE (10, 25, and 50 µg/µl into PGi, 1 µl/ 6 min) at day 7, 5 min before the naloxone injection also greatly attenuated the withdrawal signs in morphine-addicted rats in a U shape manner. In the third stage, the Wistar rats were allowed to self-administer morphine (1 mg/infusion) during 10 consecutive days for 2h/session. The number of lever pressings was recorded. An intrapritoneal MCE injection (50 mg/kg, i.p.), 30 min befor morphine self-administration for 10 days produced a significant decrease in the initiation of morphine self-administration during all sessions. Our results show that the MCE not only has the potential to attenuate the morphine withdrawal symptoms but it also has the ability to stop the morphine dependence by a decrease in morphine self-administration.

Keywords: *Matricaria Chamomilla, Self-Administration Morphine Withdrawal Signs*

INTRODUCTION
Considering the side-effects and detrimental properties of chemical drugs, a return to natural medicinal plants has gained more attention over recent years and a new insight into recognition of medicinal plants and their physiological, pharmacological, and cytological effects has been initiated within the last decades.
In this respect, the Matricaria chamomilla (MC); has a particular place as a herbal plant due to its therapeutical properties and the widespread clinical applications. MC is one of the most popular single ingredient herbal teas in Iranian traditional medicine.
Like many other herbal preparations used in traditional cultures, the therapeutic application and the health benefits claimed for chamomile are based largely on folklore rather than on scientific evidences. The flowers of MC are being used as demulcent, anti-inflammatory, analgesic, anxiolytic, and sedative in Iranian folk medicine.
The Chamomile tea, has been used as a traditional herbal remedy for its relaxation, anti-inflammatory, analgesic, anti-microbial, anti-spastic and calming effect (McKay and Blumberg, 2006; Gardiner, 2007).
MCE has been reported to exhibit anti-inflammatory, antioxidant, anticancer, anti-aging, antimicrobial, and cholesterol-lowering activities, as well as anxiolytic , spasmolytic, and sedative properties (Gardiner, 2007; Lee and Shibamoto, 2002; Babenko and Shakhova, 2005; Di Giorgio *et al.*, 2008; Skovgaard *et al.*, 2006; Srivastava and Gupta, 2007; Leung and Foster, 1998; Della Loggia *et al.*, 1982).

MCE contains several classes of biologically active compounds including phenolic compounds, flavonoids apigenin, quercetin, patuletin, luteolin and their glucosides, chamazulene (Avallone *et al.*, 2000; Viola *et al.*, 1995; Avallone *et al.*, 1996). Recently, it has been revealed that the MCE contains:
A) flavonoids, which exert benzodiazepine-like activity in the central nervous system and there for it can influence both morphine dependence and the expression of morphine withdrawal symptoms (Avallone *et al.*, 2000; Viola *et al.*, 1995; Avallone *et al.*, 1996; Capasso *et al.*, 1998; Gomaa *et al.*, 2003). B) quercetin that can reverse the development of morphine tolerance and dependence in mice through suppression of nitric oxide synthesis (Naidu *et al.*, 2003). C) Apigenin that has anxiolytic effects (Viola *et al.*, 1995). D) α-bisabolol that has anti-inflammatory and visceral antinociceptive effect (Leite *et al.*, 2011; Rocha *et al.*, 2011).
It has also shown that MC extract has analgesic effects in both phases of formalin test that indicate MC could have central and local effects (Abad *et al.*, 2011).
It has shown that MC extract has sedative and antinoniceptive and anti-anxiety effects, and it is also effective in morphine withdrawal symptoms (Viola *et al.*, 1995; Gomaa *et al.*, 2003). In this respect Gomaa *et al.*, (2003) showed that chronic co-administration of MCEwith morphine can prevent from dramatic increase of plasma cAMP induced by naloxone-precipitated abstinence (Gomaa *et al.*, 2003).
On the other hand, the nucleus paragigantocellularis (PGi), is a brain stem region that regulates homeostatic functions such as pain and opiate withdrawal syndrome. Several lines of evidence suggest that the PGi, is involved in the opiate physical dependence and withdrawal symptoms. 1) The PGi provides the major excitatory amino acid input to the locus coeruleus (LC) which involved in opiate dependence and withdrawal (Ennis *et al.*, 1992). In the opiate-dependent rats, there is a marked increase in the firing rate of LC neurons during opiate antagonist precipitated withdrawal. This increase in activity of LC neurons has been hypothesized to mediating in opiate withdrawal symptoms (Rasmussen and Aghajanian, 1989; Maldonado and Koob, 1993). 2) Lesions of the PGi (Rasmussen and Aghajanian, 1989) or administration of glutamate antagonists (Rasmussen *et al.*, 1996) attenuated the withdrawal-induced hyperactivity of LC., 3) electrical stimulation of the PGi in the nondependent rat produces a series of behaviors that are similar to those seen during opioid antagonist-precipitated withdrawal (Liu *et al.*, 1999). The present work was carried out to investigative the effect of peripheral and central hydro-methanolic extract of MCE on development of morphine dependence and withdrawal symptoms through changing morphine self-administration and morphine withdrawal symptoms.

MATERIALS AND METHODS

Extracting Method

MC flowers were purchased from Esfahan (Iran) Pharmaceutical Company, Identified by Dr Ebrahim Mirshekari Assistant Professor of agriculture and specialist in herbal plants, and drench method was used for extraction. For this purpose flowers were mildly powdered. 20 g of MC powder and 200 mL of 70% ethylic alcohol mixture and after 48 hour (the container was motivated for 5 minutes with 12 hours withdrawal time). The mixture leached and solvent extracted in rotary were adjusted in 70 ºC in medium round speed. The caliginous fluid was spread on a window and in 50 ºC oven and the powder was gathered and used in this experiment after drying (Avallone *et al.*, 2000; Avallone *et al.*, 1996).

Animals and Housing Conditions

100 Adult male Wistar rats (Razi Institute, Karaj, Iran), weighing 200–300 g were used for the experiment. Animals were kept in an animal house with a 12/12-h light–dark cycle and controlled temperature (22 ± 2 °C). Animals had free access to food and tap water except during the limited periods of experiments. After surgery, the animals were placed in individual home cage and allowed to recover from operation for 7 days before study. Efforts were made to minimize the animal suffering and the number of animal used. In order to avoid the bias of circadian rhythm, all experiments were performed between 9:00 a.m. to 12:00 a.m. Eight animals were used in each group; each animal was used once only and killed immediately after the experiment. Behavioral experiments were done during the light phase of the light/dark cycle (light on 07:00). All drugs were prepared immediately prior to use and given

intraperitoneally (i.p.) in a volume of 0.1 ml per 100 g body weight of rats. All experiments were executed in accordance with the Guide for the Care and Use of Laboratory Animals (National Institute of Health Publication No. 80-23, revised 1996) and were approved by the Research and Ethics Committee of Qazvin University of Medical Sciences.

Experimental Design

(A) Effects of Acute (Peripheral i.p. and Central PGi) Administration of MCE on Morphine Withdrawal Symptom Signs

To determine the effects of acute central and peripheral (i.p. and PGi) administration of MCE on morphine withdrawal signs, initially, 50 male Wistar rats were divided into two groups: control and morphine-treated rats. To develop morphine dependence, rats were injected subcutaneously with morphine sulfate (Temad, Tehran, Iran), (morphine dissolved in saline before injection) twice daily for 7 days. The initial dose of morphine on day 1 and 2 was 2.5 mg/kg while this dose was doubled every day thereafter to reach a maximum dose of 40 mg/kg on day 6. On day 7, the animals recived the last injection of morphine (50 mg/kg) and further divided into 5 sub-groups (Gomaa *et al.*, 2003):

(1) Morphine group, received morphine only

(2) Saline-morphine group (saline+morphine), received saline (0.5ml, i.p.) on day 7, 30 min before naloxone administration

(3, 4, 5) MCE-morphine group (MCE+ morphine), received MCE (10, 25, and 50 mg/kg, i.p.) on day 7, 30 min before naloxone administration. Control rats were treated with saline under the same conditions used for morphine group.

In the second step, one week prior to the experiments, rats were anesthetized with a combination of ketamine (100 mg/kg, ip) and xylazine (5 mg/kg, ip). The animal's head was fixed in the stereotaxic frame (stolting, USA).

The tooth bar was set at -3.3 mm. After local anesthesia with 2% lidocaine, the skull was surgically exposed and a stainless-steel guide cannula (15mm long, 0.65 mm outer diameter, 23 gauge needle) was implantated 1 mm above the PGi region. The coordinates for the PGi region were -11.8 caudal to bregma, +1.6 mm from the medial suture, and -8.6 mm below the skull surface (Rasmussen and Aghajanian, 1989). The guide cannula was fixed to the skull using two stainless-steel small screws anchored to the skull and dental acrylic cement. After the surgery, rats were allowed to recover for the next 7 days.

Microinjection of MCE was performed by removing the stylet from guide cannula and lowering a stainless-steel injector cannula (16 mm, 30 gauge needle) that extended 1.0 mm beyond the implanted guide cannula tip.

The injector cannula was connected to a 1-µl Hamilton microsyringe by a polyethylene 20 (PE-20) tubing and 1µl of drug solution or vehicle was infused over 6 min period. In order to minimize the drug back-flow into the injection track, the cannula was gently withdrawn 60s after the injection and followed by replacement of the stylet.

While filling the injection system, a small air bubble was introduced into the PE-20 tubing to monitor the movement of the fluid during the injection. Rats were made dependent to morphine 7 days after the stereotaxic operation as mentioned above (step1).

In this step 40 male wistar rats were divided into five experimental groups as follows: group 1, morphine treated rats without surgery and cannula implantation which received naloxone to assess morphine withdrawal signs; group 2, morphine treated animals with unilateral PGi-cannula implantation which received MCE vehicle (saline) into PGi,(1 ul/ 6 min) at day 7, 5 min before the naloxone administration and group 4, morphine treated rats with unilateral PGi-cannula implantation which received MCE (10, 25, and 50 µg/µl into PGi, 1 µl/ 6 min) at day 7, 5 min before the naloxone administration.

On completion of the sudy, 100 nl of pontamine sky blue (2%) was administerated into the injection site of groups 2 and 3, using the same injector to confirm the localization of the implanted cannula. Later, the animals were deeply anesthetized with an overdose of Ketamine and xylazine. They were intracardially perfused with 0.9% saline followed by 10% phosphate-buffered formalin (pH 7.4). Brains were removed from the skull and kept in 10% phosphate-buffered formalin for 24 h. Serial coronal sectioning was

performed under a light microscope and the neuroanatomical locations of the cannulae tips were confirmed using the rat brain atlas.

Animals showing a misplaced cannula were rejected and excluded from the final analysis. At the end of injecting day 7th Morphine withdrawal syndrome was precipitated by naloxone hydrochloride [Sigma, USA (3 mg/kg i.p.)] 4h after morphine administration in all groups. Thereafter, 10 distinct somatic signs of withdrawal (wet-dog shakes, teeth chattering, head tremor, sniffing, jumping, scratching, chewing, diarrhea, rearing and ptosis) were monitored in a clear Plexiglas cylinder test chamber (30cm diameter, 50cm height) during a 30-min period.

All animals' behaviors were evaluated by the same observer. The body weight was measured 1 and 24 h after the naloxone injection and the results were compared with the determined weight before the naloxone administration.

(B) Effect of MCE on Self Administration of Morphine

Surgery and procedure of self administeration task

Animals were anaesthetized as mentioned earlier and a cannula was inserted into the jugular vein. The cannula was guided subcutaneously up to the skull where it was fixed to acurred metal tube, which was secured onto the skull with small screws and fixed with dental acrylic cement.

The details of the procedure for self-administeration task have been described previously (Maldonado and Koob, 1993). Briefly, the testing was performed in a standard operant conditioning cage placed in a (21×21× 28cm) sound-attenuated room.

The test cage was equipped with two levers (active and passive levers), 2 cm above the floor, and a light located 4cm above the active lever. The i.v. cannula of animals was connected to an infusion pump. Pressing of the active lever marked by the red light resulted in an i.v infusion of 0.2 ml fluid (5 mg/ml morphine in saline solution or saline only) within 10 sec. A depression of the active lever during this time (10 sec) did not affect the infusion of the drug. Pressing of the passive lever had no programmed consequences.

The drug-naive animals were placed in the test cage and allowed to i.v self-administer the drug solution for 2 h a day (for 10 days). The number of the active and passive lever pressings was recorded by an oscillograph (Harvard).

In this step Thirty male wistar rats were divided into three experimental groups (n = 10 for each group) as follows: (1) Control group, received saline in self-administration sessions; (2) Morphine group, received morphine in saline solution (5 mg/ml) during the self- administration sessions; and (3) MCE-morphine group (MCE+morphine), received both MCE (50 mg/kg, i.p.) 30 min before each session and morphine in self- administration sessions.

Statistical Analysis

The results obtained during the self-administration session were analysed using analysis of variance (ANOVA) with repeated measures. The number of active and passive lever pressing during every session were calculated.

The difference in total number of active and passive lever pressings (sum of ten sessions) between the MCE and morphine groups were analysed by students t-test. This method of analysis was also used to analyze the data obtained for withdrawal signs.

The results obtained for each study group were analyzed by SPSS version12 using unilateral variance analysis (ANOVA) while a p value less than 0.05 was considered as significant. The results are expressed as Mean±SEM.

RESULTS AND DISCUSSION

Withdrawal Syndrome Signs

Non-dependent or saline treated rats (control group) in both steps 1 and 3 exhibited no withdrawal signs following naloxone administration. In comparison, morphine treated ones had pronounced withdrawal signs following the naloxone injection (groups 1 and 2) in step 1, 2 and group 2 in step3. Our results revealed that none of the members of the control groups which only received saline failed to show the

signs of morphine withdrawl syndrome; in other words, comparing the signs of morphine withdrawl between the case group which received morphine and the control group was indicative of a significant differece between two groups.

This implied that the administration of morphine for 7 successive days causes morphine dependence in expermental animals but to avoid the lengthening of the issue this comparison is not presented within the figures illustrated bellow. The 10 important signs of morphine withdrawl sydrome observed were jumping, climbing, rearing, wet-dog shakes, salivation, lacrimation, teeth-chattering, ejaculation, ptosis, and diarrhea.

Since there was no significant difference between group 1 (Morphine group, which received only morphine) and Saline-morphine group (MCE vehicle), this group was not included in the figures. Animals receiving MCE (10, 25, and 50 mg/kg, i.p.) 30 min before the expression of withdrawal syndrome by naloxone in morphine-dependent rats (group 3, 4, and 5) of the first experiment demonstrated a significant reduction in several signs of morphine withdrawal in a dose dependent manner compared to those which received vehicle (saline) prior to naloxone (group 2) (Table 1 & Figure 1).

Comparing the quantitative signs of morphine withdrawl in groups received the different doses of i.p. MCE and the morphine group demonstrated that all three doses of MCE affected the frequency of jumping, rearing, and climbing by animals in a dose dependent manner ($p<0.05$). Regarding the frequency of climbing, only two doses (25 and 50 mg/kg) were shown to have the ability to produce a significant decrease (Figure 1).

Comparison of qualitative signs of morphine withdrawl between the animals of these two groups demonstrated that the signs such as wet dog shaking, salivation, lacrimation, teeth chattering, paw tremors, ejaculation, ptosis, and diarrhea occurred in all eight members of the morphine group but the number of rats with these signs except the lacrimation in MCE group decreased in a dose dependent manner (Table 1).

Injection of naloxone caused a significant weight loss in all groups however, it was less obvious among those groups that received MCE. Also, as shown in Table 4, the 50 mg/kg dose of MCE could to a great extent prevent the weight loss 24 h after the naloxone injection and this indicates that the long-term effect of MCE in this particular case was higher than its short-term effect ($p<0.05$).

Also Injection of all three doses of MCE into the PGi could significantly decrease the frequency of jumping, rearing, and climbing in experimental animals as presented in Figure 2 ($p<0.05$). Injection of naloxone caused a significant decrease in weight loss among all groups except the MCE group with a dose of 25 µg/µl in which the MCE could to a large extent prevent the weight loss in rats especially over a long term (24 hr after naloxone injection) implying that the long-term effect of MCE in this particular case is higher than its short-term effect as shown in Figure 4 ($p<0.05$). Injection of all doses of MCE into PGi showed the ability to decrease the qualitative signs of morphine withdrawl which occurred in all six members of the morphine group yet the the number of animals showed these signs, except the lacrimationin, reduced in MCE group (Table 1). It is worth mentioning that the injection of 25 µg/µl of MCE into PGi produced a stronger effect on decreasing the signs of morphine wthdrawl in all cases associated with either the quantitative signs (frequency of jumping, rearing, and climbing), qualitative signs or weight loss (Figures 2 and 3).

Table 1 shows the effects of MCE (10, 25, and 50 mg/kg, i.p.) at day 7, 30 min before the naloxone injection and (10, 25, and 50 µg/µl into PGi, at day 7, 5 min before the naloxone injection on withdrawal syndrome signs in morphine-dependent rats. As demonstrated, an acute MCE administation (10, 25, and 50 mg/kg, i.p.) at day 7, 30 min before the naloxone injection in morphine-addicted rats, cosiderably attenuated the withdrawal signs like wet dog shaking, teeth chattering, ptosis, diarrhea, and salivation in a dose dependent manner, yet with no effect on lacrimation. Likewise, an acute central injection of MCE (10, 25, and 50 µg/µl into PGi, 1 µl/ 6 min) at day 7, 5 min before the naloxone injection greatly attenuated the withdrawal signs like wet dog shaking, teeth chattering, ptosis, diarrhea, and salivation in morphine-addicted rat in a U shape manner, however, it didn't affect the lacrimation too (Table 1 & Figure 2).

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Table 1: Effcts of i.p. Administration of MCE (10, 25, and 50 mg/kg, i.p.) at day 7, 30 min Before Naloxone Injection and (10, 25, and 50 µg/µl of MCE into PGi) 5 min Before Naloxone Administration on Naloxon-Precipitated Withdrawal Syndrom in Morphine-Dependent Rats

MCE 50µg/µl	MCE 25µg/µl	MCE 10µg/µl	MCE 50mg/kg	MCE 25mg/kg	MCE 10mg/kg	Control	Withdrawal Signs
4/6	2/6*	5/6	2/6*	3/6*	5/6	6/6	Wet-dog Shakes
2/6*	1/6*	4/6	2/6*	3/6*	3/6*	6/6	Teeth Chattering
2/6*	2/6*	3/6*	1/6*	3/6*	3/6*	6/6	Ptosis
3/6*	2/6*	4/6	3/6*	4/6	6/6	6/6	Diarrhea
3/6*	1/6*	4/6	3/6*	4/6	4/6	6/6	Salivation
4/6	4/6	5/6	4/6	5/6	5/6	6/6	Lacrimation

Numbers denote the number of rats showing positive signs relative to the total number of rats tested. *p<0.05 compared with the morphine withdrawal group by the Fisher ś exact test.

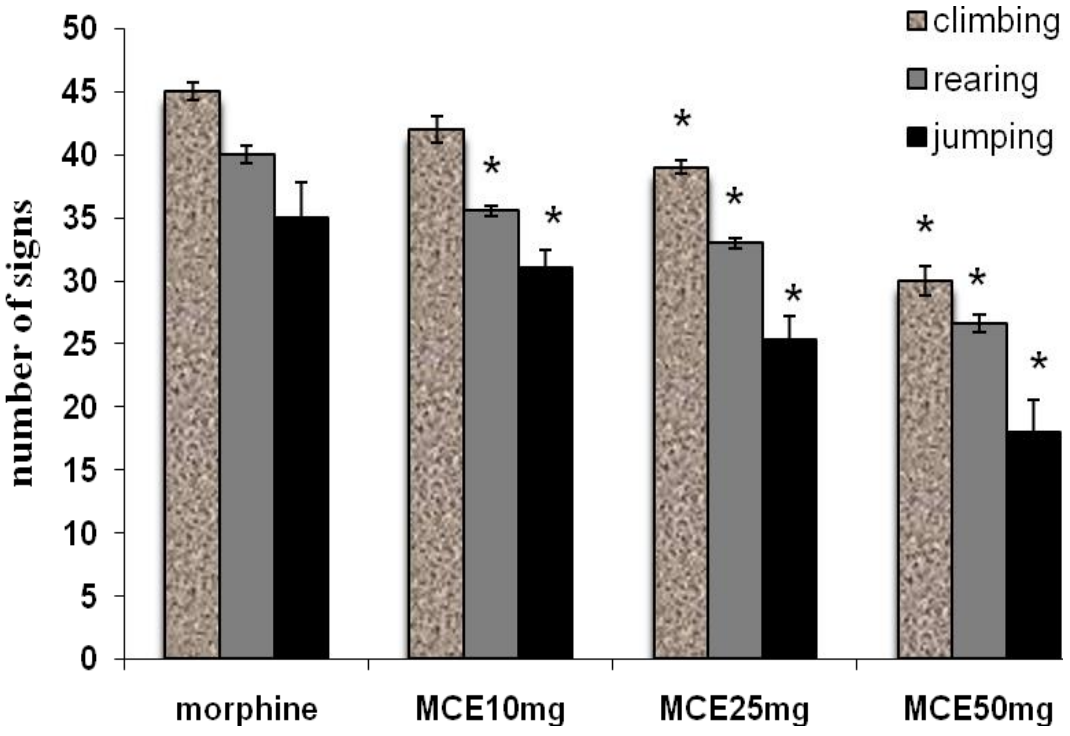

Figure 1: Effcts of an Acute MCE Administation (10, 25, and 50 mg/kg, i.p.) at Day 7, 30 min Before Naloxone Injection on Naloxon-Precipitated Withdrawal Syndrom in Morphine-Dependent Rats. MCE Greatly Attenuated the Withdrawal Signs (Rearing, Jumping, Climbing) in a Dose Dependent Manner

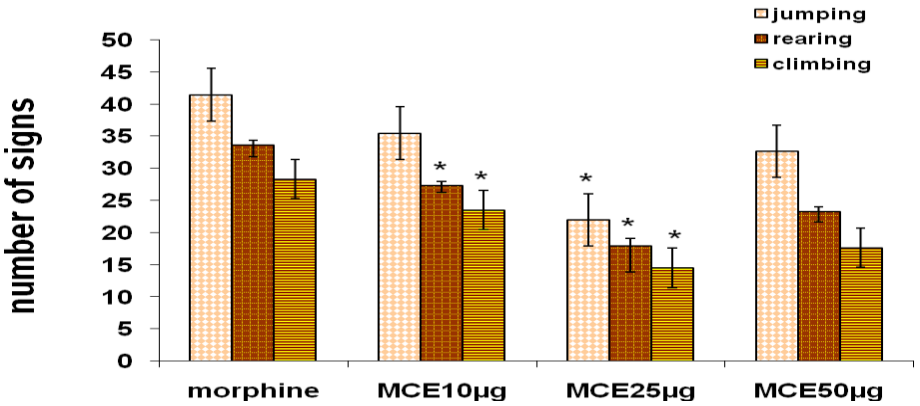

Figure 2: Effcts of an Acute MCE Administation (10, 25, and 50µg/µl of MCE into PGi) 5 min before Naloxone Injection on Naloxon-Precipitated Withdrawal Syndrom in Morphine-Dependent Rats. MCE Greatly Attenuated Withdrawal Signs (Rearing, Jumping, and Climbing) in a U Shape Manner

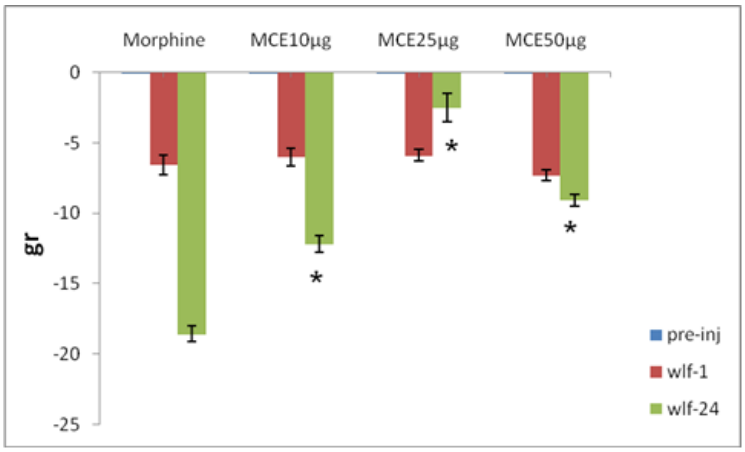

Figure 3: Effcts of an Acute MCE Administation (10, 25, and 50 mg/kg, i.p.) at Day 7, 30 min Before Naloxone Injection on Weight Loss in Morphine-Dependent Rats. Before Naloxone Injection and 1hr and 24 hr after Naloxone Injection

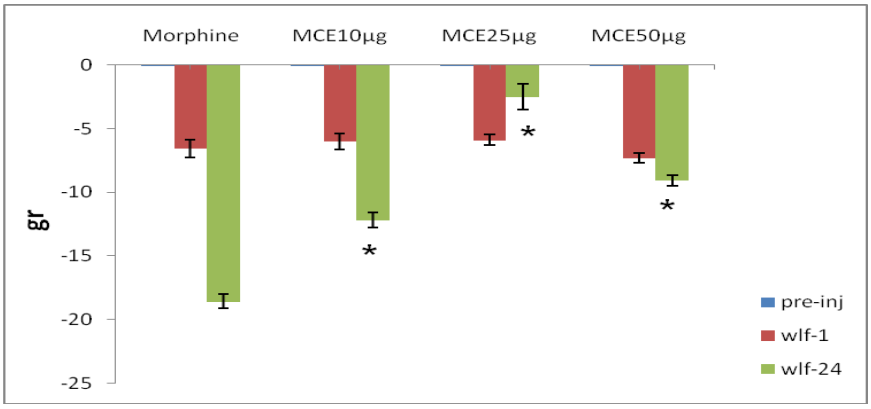

Figure 4: Effcts of an Acute MCE Administation (10, 25, and 50 µg/µl of MCE into PGi) 5 min Before Naloxone Injection on Weight Loss in Morphine-Dependent Rats. Before Naloxone Injection and 1hr and 24 hr After Naloxone Injection

Initiation of Drug Self-Administration

The self-infusion (SI) data during the initiation of morphine self-administration are presented in figure 5. The maximum values were obtained in a week. There was a significant difference between the number of self-infusions in three groups (ANOVA, $p<0.05$).

Self-infusion of morphine alone resulted in appearance of addiction in rats as shown by several folds higher pressing number of the active lever providing the morphine solution. The total number of active lever pressings in the morphine alone group was significantly higher (t-test, $p<0.01$) than two other (contol and morphine + MCE) groups (Figure 6).

There was no significant difference between the control (saline) and morphine+MCE (50 mg/kg, i.p.) groups, indicating that the MCE (50 mg/kg, i.p.) produced a significant decrease in the number of self-infusions (Figure 5 and 6).

Also, the total number of active lever pressings in the MCE group was significantly higher than the passive lever pressings in this group (t-test, $p<0.05$) (Figure 6).

The total number of passive lever pressings in MCE, vehicle, and morphine groups failed to reveal any significant difference between these groups (ANOVA, $p<0.05$) (Figure 7).

The i.p. administration of MCE (50 mg/kg) during all 10 days considerably attenuated most of the withdrawal signs specially the wet dog shakes, jumping, climbing, ptosis, and teeth chattering compared to those observed in morphine group. The data of morphine withdrawal signs of this group was not included in the figures.

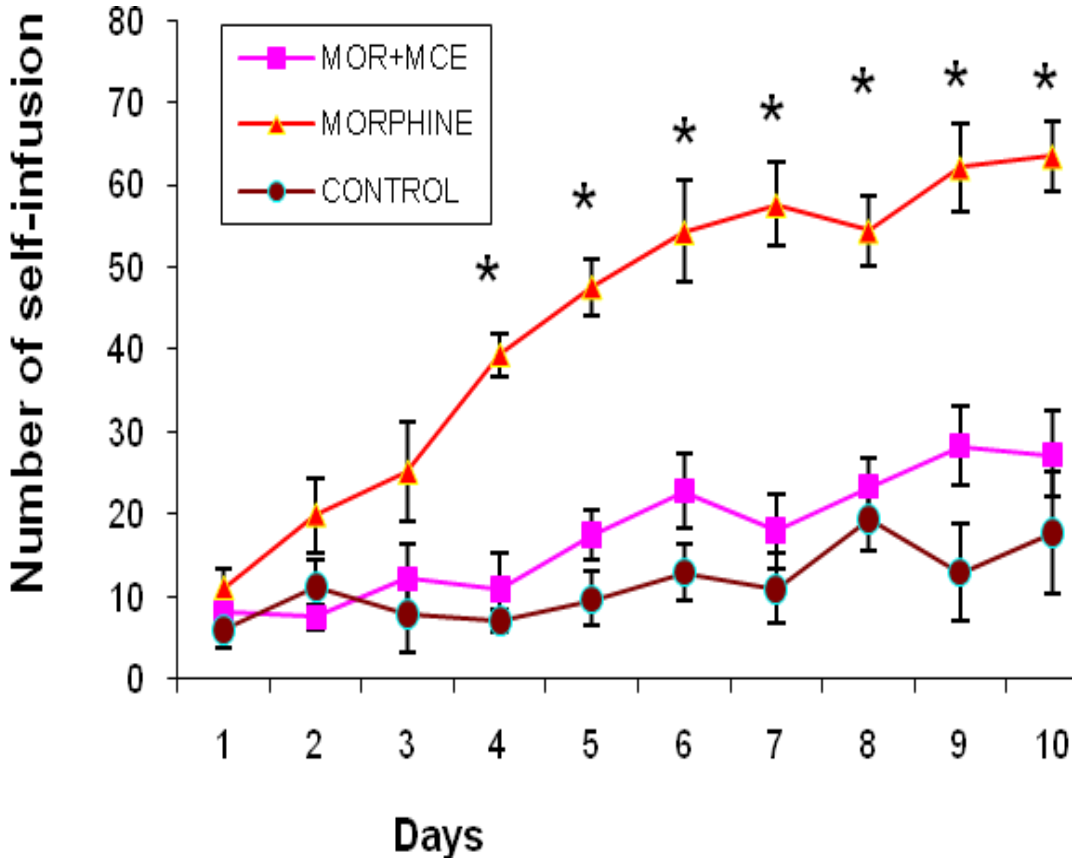

Figure 5: The Effct of Chronic i.p. Administration of MCE (50 mg/kg) during all 10 Days on Morphine Self-Administration Note to Increase in Self-Infusions of Morphine during the Experiment in Morphine Group

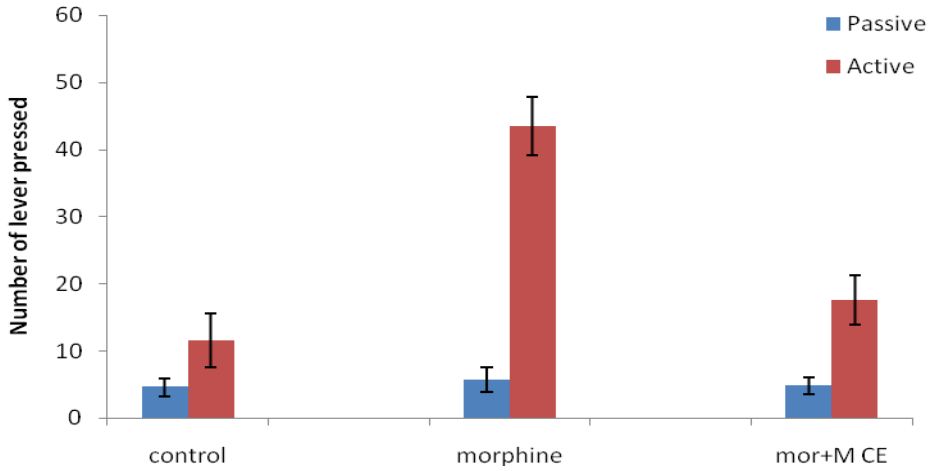

Figure 6: The Mean Number of Self-Infusions Indicated by Pressings of Active and Passive Levers in the Control, Morphine, and Morphine+MCE Groups during 10 Days of Self-Administration. The Active Lever Provided Saline for the Animals in Control Group while it Offered a Solution of Morphine Saline to Other Two Groups. In the Morphine Group, the Number of Pressings of the Active Reinforcement Lever Giving Morphine Solution was Several Times Higher Than that of the Passive Lever. There was a Significant Difference Between the Morphine Alone ($p<0.01$) and the Contol and Morphine+MCE (50 mg/kg) Groups. The Red Lamp Above the Active Lever Seemed to Slightly Increase the Use of this Lever Compared to the Passive Lever as Shown by the Control and Morphine+MCE (50 mg/kg) Groups Data. There was a Significant Difference Between the Number of Active and Passive Lever Pressings ($p<0.05$) in Morphine+MCE (50 mg/kg) Groups

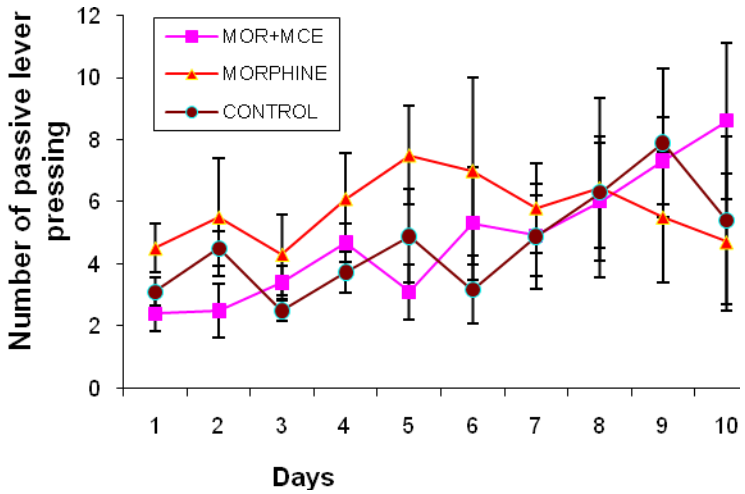

Figure 7: The Number of Responses of Passive Lever Pressings are Plotted vs. the Day of Experiment in the Control, Morphine, and Morphine+MCE Groups (recived MCE 50 mg/kg i.p.). There was no Significant Difference Between Three Groups ($p<0.05$)

Discussion

In international medicine, MC is widely used to obtain sedative, spasmolytic, and anti-inflammatory effects. Our results showed that the i.p. injection of MCE can prevent the occurrence of many signs of morphine withdrawal in rats in a dose-dependent manner and that the highest effect was observed at a

dose of 50 mg/kg. Also, the data of the present study revealed that the injection of all three doses of MCE into PGi could stop the incidence of most signs of morphine withdrawal in rats but not in a dose-dependent manner. Furthermore, the findings of the present study demonstrated that the injection of 25µg/µl of MCE into PGi produced a stronger effect compared to two other doses (10 and 50 µg/µl) leading to more decrease in signs of morphine withdrawal so that the injection of naloxone caused a significant reduction in weight loss among all groups except the one injected with 25µg/µl of MCE and this prevented the weight loss.

Since the injection of all three doses of MCE into PGi could prevent the incidence of most signs of morphine withdrawal in rats, it seems that part of i.p. injection of MCE effects in reducing the signs of morphine withdrawal to be associated with the center and through the PGi and that the injection of MCE through intra peritoneal route and into PGi nucleus produces diverse effects.

Several lines of evidence suggest that the PGi, is involved in the opiate physical dependence and withdrawal symptoms (Liu *et al.,* 1999). Hence, the reduction in signs of morphine withdrawal following the injection of MCE into the PGi nucleus could be both expected and justified.

Furthemor, it was revealed that both the chronic co-administration of MCE and morphine during self-administration session and the acute administration of MCE (i.p. and PGi) before the induction of withdrawal syndrome blocked the naloxone-precipitated morphine withdrawal syndrome in morphine-dependent animals indicating that MCE has an inhibitory effect on the expression of naloxone-precipitated morphine withdrawal syndrome.

Our findings also showed that the injection of a daily dose of 50 mg/kg of MCE, 30 min before morphine self-administration caused a significant decrease in initiation of morphine self-administration during the 10-day period of morphine self-administration.

This indicates that the MCE not only has the potential to prevent and decrease the signs of morphine withdrawal but it also has the ability to stop the morphine dependence by a decrease in morphine self-administration.

Our findings are consistent with those of Gomma *et al.,* (2003) in which the authors reported that the administration of MCE prevented both the morphine dependence and the occurrence of morphine withdrawal syndrome.

These findings are also in agreement with the results in which MCE is described to have the ability to reduce the pain (Ramos-e-Silva *et al.,* 2006; Srivastava *et al.,* 2010) and locomotor activity, and behavioral withdrawal response in morphine-treated rat (Gomma *et al.,* 2003; Ruckstuhl *et al.,* 1979). Moreover, our results show that the rats given MCE (50 mg/kg, i.p.) during morphine self-administration exhibited significant reduction in most morphine withdrawal signs compared with the morphine and saline groups. These results which highlight the inhibitory effects of chronic MCE on opiate withdrawal also confirm the previous findings in rats (Gomma *et al.,* 2003) in which the chronic co-administration of MCE with morphine was reported to have the potential to prevent the dramatic increase in plasma level of cAMP induced by naloxone-precipitated abstinence.

Furthermore, our result show that Injection of naloxone caused a significant weight loss in all groups however, it was less obvious among those groups that received MCE. Also the 50 mg/kg dose of MCE could to a great extent prevent the weight loss 24 h after the naloxone injection and this indicates that the long-term effect of MCE in this particular case was higher than its short-term effect, moreover the injection of naloxone caused a significant reduction in weight loss among all groups except the one injected with 25µg/µl of MCE in to PGi and this prevented the weight loss.

These findings are also in agreement with the results (Gomma *et al.,* 2003) in which chronic co-administration of MC with morphine is described to have the ability to prevent from the loss of weight 8 h after Naloxone injection.

Our findings shown that both the chronic co-administration of MCE with morphine during self-administration session and the acute administration of MCE (i.p. and PGi) before the induction of withdrawal syndrome blocked most of naloxone- precipitated morphine withdrawal signs in morphine-dependent rats. It is possible that the inhibitory effect of MCon the expression of Withdrawal syndrome

result from the benzodiazepine-like activity of some components of MCE. There are several reports in which a benzodiazepine-like activity for MCis considered (Avallone *et al.*, 1996; Salgueiro *et al.*, 1997).
Benzodiazepines are a group of drugs widely prescribed for treatment of anxiety and insomnia as they cause relief and tranquility through decreasing the neural firing in the brain (Avallone *et al.*, 2000; Salgueiro *et al.*, 1997).
Benzodiazepine is known to be an inhibitory agent for the development of dependence to opioids, and therefore, it is possible that the inhibitory property of MCon the expression of withdrawal syndrome is related to the benzodiazepine-like activity of some of its components (Capasso *et al.*, 1998; Gomaa *et al.*, 2003; Capasso *et al.*, 2000). It has been suggested that the co-administration of diazepam as a benzodiazepine and morphine may prevent some neurochemical changes in cortex leading to a decrease in morphine dependence (MiceSheu *et al.*, 1995). Also, some studies suggest that the benzodiazepine drugs inhibit the compatibility changes produced during the chronic addiction at the cAMP and met-enkephalin level causing a decrease in morphine dependence (Nestler, 2001; Smart and Lambert, 1996; Suzuki *et al.*, 1996).
Some reports suggested that benzodiazepine inhibited the morphine dependence by blocking the decrease of the met-enkephalin levels observed in morphine-dependent rats undergoing naloxone induced abstinence (Sribanditmongkol *et al.*, 1994).
Considering these properties for benzodiazepines, if the MC or some of its ingredients has such similar property they can produce comparable results and the findings of the present study will accordingly be justified. This implies that the MC with its benzodiazepine-like components through the relief and tranquility or preventing neurochemical changes in brain cortex prevents the morphine dependence and eventually the expression of Withdrawal syndrome.
Moreover, one of the outcomes of morphine consumption is a decrease in the level of endogenous opioids such as met-enkephalin which shows itself as hyperalgesia at the time of morphine Withdrawal and naloxone injection, while the benzodiazepines inhibit this decrease and also prevent the expression of G proteins and the protein compounds of the cAMP pathway which is a crucial issue in decreasing morphine dependence (Nestler, 2001; Smart and Lambert, 1996; Suzuki *et al.*, 1996; Sribanditmongkol *et al.*, 1994). So, the benzodiazepine drugs not only prevent the incidence of hyperalgesia during morphine withdrawal and thus reducing the severity and difficulty of morphine withdrawal symptoms but also, to some extent, inhibit the mechanisms involved in development of tolerance and dependence in which the cAMP plays a fundametal role. By accepting the viewpoint of those researchers who consider a benzodiazepine-like activity for flavonoids, of Matricaria chamomilla, the decrease in signs of morphine withdrawal could be justified. This indicates that the sedative and euphoric effects begin following the morphine consumption and quite the opposite, the state of instability and intranquility develops after naloxone administration. Considering the antianxiolytic role of MC mentioned earlier (Viola *et al.*, 1995) the decrease in some signs of morphine withdrawal such as anxiety, instability, and irritability could be true for MC and justifiable, accordingly.
In conclusion, the findings reported in this study indicate that MCmay contain novel bioactive principles with benzodiazepine-like activity properties with the ability to prevent both the development of dependence and the expression of Withdrawal syndrome.
In general, by considering the existing evidences, it seems that the MCE has a valuable ability in decreasing the painful and excruciating signs of morphine withdrawal. These results confirm the anxiolytic, spasmolytic, and sedative properties assigned to this plant in traditional medicine. However, more pharmacological and behavioral experiments are needed for routine administration of this plant as an approved herbal drug in clinical use. However, since the MCE has a phytoestrogenic property and in phenomena such as pain and anxiety acts in sex –dependent manner, more researches concerning the role of sex hormones and the differences between two genders are necessary.

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