Full Paper

***Matricaria chamomilla* Extract Inhibits Both Development of Morphine Dependence and Expression of Abstinence Syndrome in Rats**

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Abstract. The effect of *Matricaria chamomilla* (*M. chamomilla*) on the development of morphine dependence and expression of abstinence was investigated in rats. The frequencies of withdrawal behavioral signs (paw tremor, rearing, teeth chattering, body shakes, ptosis, diarrhea, and urination) and weight loss induced by naloxone challenge were demonstrated in morphine-dependent rats receiving *M. chamomilla* extract or saline. The withdrawal behavioral manifestations and weight loss were inhibited significantly by chronic co-administration of *M. chamomilla* extract with morphine. Administration of a single dose of *M. chamomilla* before the naloxone challenge in morphine-dependent animals abolished the withdrawal behavioral manifestations. The dramatic increase of plasma cAMP induced by naloxone-precipitated abstinence was prevented by chronic co-administration of *M. chamomilla* extract with morphine. These results suggest that *M. chamomilla* extract inhibits the development of morphine dependence and expression of abstinence syndrome.

Keywords: morphine dependence, withdrawal manifestation, *Matricaria chamomilla* extract, plasma cAMP

Introduction

The repeated use of opiates induces adaptive changes in the central nervous system leading to the development of dependence. The mechanism underlying opiate dependence is not fully understood (1, 2). However, there are several reports relating opioid dependence and opioid withdrawal syndrome to changes in the cAMP system and transcription factors (3–6). It has been reported that some phosphodiesterase inhibitors (3-150 butyl-1-methyl xanthine, enprophylline, rolipram, and nefiracetam) could attenuate the development of morphine dependence (6–9). Moreover, it was suggested that benzodiazepines inhibit morphine induced adaptive changes in cAMP levels and met-enkephalin associated with dependence (10–12).

Matricaria chamomilla (*M. chamomilla*) is an ancient as well as a modern herb with many uses. *M. chamomilla* has been reported to exhibit anti-inflammatory, spasmolytic, and sedative properties (13). Recently, it has been demonstrated that *M. chamomilla* contains flavo-

noids, which exert benzodiazepine-like activity (14, 15), and also phosphodiesterase inhibitory action, which leads to increased cAMP levels (16–19). Also, it has been reported that apigenin and chamazulene, the constituents of *M. chamomilla*, exert an inhibitory action on the protein up-regulation at the transcriptional level (20–23). These data are consistent with the finding of Capasso et al. (24) on flavonoids inhibition of morphine withdrawal in vitro.

In the present work, the ability of *M. chamomilla* to affect the development of morphine dependence and withdrawal symptoms through changing morphine induced up-regulation of the cAMP pathway as well as the expression of G protein and components of the cAMP system was investigated. To achieve this goal, we have examined the effect of chronic administration of *M. chamomilla* extract on the development of opiate dependence as well as the effect of acute administration of the extract on the induction of withdrawal syndrome in opioid-dependent animals. Since the plasma level of cAMP is considered a sensitive index for abstinence syndrome (25), the effect of *M. chamomilla* on the plasma cAMP in naive and dependent animals was

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examined.

Materials and Methods

Drugs

Morphine sulphate was purchased from Misr Com Pharma, Cairo, Egypt and naloxone hydrochloride from Sigma Chem. Co., St. Louis, MO, USA. *M. chamomilla* extract containing 0.3% apigenin was made by Draco, San Jose, CA, USA. Drugs were given at the doses reported to exert significant action; the appropriate references are given in brackets for each compound and dose. All drugs were dissolved in sterile saline.

Animals

Sixty adult male Wistar rats (150–200 g) were obtained from Laboratory Animal Center, Assuit University. The animals were housed at a controlled environmental temperature ($23 \pm 1^\circ\text{C}$, a 12-h light alternating with 12-h darkness cycle, lights on at 7:30 AM) and were allowed food and water ad libitum.

All experiments were performed in accordance with the Guiding Principles for Care and Use of Laboratory Animals of the Faculty of Medicine Assuit University. Rats were randomly assigned to 10 different groups of six animals each. The protocol of animal treatment and investigations is shown in Table 1.

Induction of morphine dependence

To develop morphine dependence, rats were injected subcutaneously with morphine twice daily for 7 days. The dose of morphine on days 1 and 2 was 2.5 mg/kg; this dose was doubled every day thereafter to reach on day 6 a total dose of 40 mg/kg. On day 7, the animals received the last injection of morphine, 50 mg/kg. (26).

Table 1. Protocol of animal treatment and investigations

Group No.	Treatment		Investigation
	for 7 days	4 h after	
1	Saline	Saline	Plasma cAMP
2	Saline	Naloxone	Plasma cAMP
3	Morphine	Naloxone	Plasma cAMP
4	Morphine	Saline	Plasma cAMP
5	Morphine + MC	Naloxone	Plasma cAMP
6	Morphine	SMC + Naloxone	Plasma cAMP
7	MC	Saline	Plasma cAMP
8	Morphine	Naloxone	Body weight
9	Morphine + MC	Naloxone	Body weight
10	Morphine	SMC + Naloxone	Body weight

MC: *Matricaria chamomilla*. SMC: Single dose of *Matricaria chamomilla* extract 30 min before naloxone injection.

Animals of groups 3, 4, 5, 6, 8, 9, and 10 were subjected to this treatment to develop morphine dependence.

Induction of withdrawal syndrome

Animals of groups 2, 3, 5, 6, 8, 9, and 10 received intraperitoneally 3 mg/kg naloxone 4 h after the last injection of morphine on the seventh day of morphine or saline treatment. Immediately after naloxone injection, each animal was placed in a transparent acrylic cylinder to observe the frequency of withdrawal manifestations (paw tremors, rearing, teeth chattering, body shakes, diarrhea, ptosis, and urination) for 30 min. N.B.: face washing was described as paw tremors.

Body weight determination

The body weight was measured 2, 4, and 8 h after naloxone injection in groups 8, 9, and 10 and compared with the determined weight before naloxone.

Effect of co-administration of *M. chamomilla* and morphine on the development of morphine dependence

M. chamomilla extract at 25 mg/kg, i.p. (14) was administered in group 5 and 9 concurrently with morphine, twice daily for 6 days; and on day 7, the animal received the last injection of *M. chamomilla* extract at 25 mg/kg, i.p. with morphine 4 h before the naloxone challenge. The effect of *M. chamomilla* extract on the development of morphine dependence was assessed by comparing the frequency of behavioral abstinence manifestations of animals in groups 3 and 5 and weight loss of animals in groups 8 and 9.

Effect of administration of *M. chamomilla* before naloxone on the expression of withdrawal syndrome

In groups 6 and 10, the morphine-dependent animals received only one dose of *M. chamomilla* extract (25 mg/kg) at 30 min before the naloxone injection. The effects of *M. chamomilla* extract on the expression of abstinence was determined by comparing the frequency of behavioral withdrawal signs of animals in groups 3 and 6 and weight loss of animals in groups 8 and 10.

Effect of *M. chamomilla* extract on plasma cAMP of naive and morphine-dependent animals after naloxone challenge

Plasma cAMP levels of animals in groups 5 and 6 were measured 30 min after naloxone injection to determine the effect of chronic and acute administration of *M. chamomilla* on plasma cAMP and compare it with that of morphine-dependent animals receiving saline and naloxone in groups 4 and 3. Animals in group 7 received only *M. chamomilla* extract (25 mg/kg, i.p.) twice daily for 6 days and one dose on day 7. Saline was given 4 h

after the last dose of *M. chamomilla* extract and 30 min later, blood samples were taken to demonstrate the effect of chronic administration of *M. chamomilla* on plasma cAMP in naive animals.

Plasma cAMP measurement

cAMP levels were determined in plasma using an enzyme immunoassay kit as described by the manufacturer (Amersham, Arlington Heights, IL, USA). In groups 2, 3, 5, and 6, blood samples were obtained by decapitation of the animal 30 min after naloxone injection. However, in groups 1, 4, and 7, blood samples were obtained 30 min after saline.

Statistical analyses

Statistical analysis was performed to compare withdrawal behavioral manifestations, weight loss, and plasma cyclic levels using one-way analysis of variance (ANOVA) and the *t*-test as post hoc analysis for the different groups. Data represent the mean $\pm$ S.E.M.

Results

Effect of repeated co-administration of *M. chamomilla* extract with morphine on the development of morphine dependence

Repeated administration of morphine produced physical dependence as assessed by a characteristic set of behavioral responses including paw tremor, rearing, teeth chattering, body shakes, diarrhea, ptosis, urination, and weight loss following naloxone challenge.

Repeated co-administration of *M. chamomilla* extract with morphine decreased significantly ($P < 0.01$) the frequencies of the signs of withdrawal syndrome compared with frequencies of withdrawal manifestations of morphine-dependent rats treated with saline (Table 2).

As shown in Table 2, naloxone induced a highly significant increase in the mean frequency of paw

tremors, rearing, teeth chattering, body shakes, diarrhea, ptosis, or urination in dependent rats treated with saline (group 3) compared to non-dependent naive rats (group 2). However, there were no significant differences between the means of frequency of these withdrawal signs induced by naloxone in morphine-dependent rats treated with chronic *M. chamomilla* extracts (group 5) and non-dependent naive rats treated with saline only (group 2).

Naloxone induced weight loss in morphine-dependent animals treated with saline (group 8): from 190 ± 0.2 g to 186.5 ± 0.15 g and from 194 ± 0.5 g to 187 ± 0.06 g after 2 and 4 h, respectively. Weight loss was marked after 8 h of naloxone injection: from 195 ± 0.5 g to 180 ± 0.17 g. The loss of weight was inhibited by chronic co-administration of *M. chamomilla* with morphine (Fig. 1): from 190 ± 0.1 g to 186 ± 0.24 g, from 180 ± 0.4 g to 174 ± 0.6 g, and from 185 ± 0.6 g to 177 ± 0.6 g after 2, 4, and 8 h, respectively. The loss of weight was significantly ($P < 0.05$) inhibited only after 8 h. These results prove that *M. chamomilla* extract prevents the development of morphine dependence.

Effect of acute administration of *M. chamomilla* extract on the induction of withdrawal syndrome

The effect of administration of *M. chamomilla* extract before expression of withdrawal syndrome by naloxone in morphine-dependent rats indicated the ability of *M. chamomilla* extract to inhibit the induction of withdrawal behavior syndrome by naloxone in morphine-dependent rats (Table 2). The mean frequency of teeth chattering, diarrhea, ptosis, urination, paw tremors, rearing, or body shakes after naloxone challenge was very significantly decreased ($P < 0.01$) in morphine-dependent rats treated by a single injection of *M. chamomilla* extract (group 6) compared with the mean frequency of the same manifestation after naloxone challenge of dependent rats treated with naloxone

Table 2. Effect of *Matricaria chamomilla* extract treatment on withdrawal manifestations induced by naloxone-challenge in dependent animals

Group No.	Treatment		Frequency of withdrawal manifestations during 30 min observation period						
	for 7 days	4 h after	Paw tremors	Rearing	Teeth chattering	Body shakes	Diarrhea	Ptosis	Urination
1	Saline	Saline	19.67 ± 02.32	7.33 ± 0.67	0.0 ± 0.0	2.33 ± 0.33	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
2	Saline	Naloxone	27.83 ± 0.95	12.83 ± 0.790	0.0 ± 0.0	2.33 ± 0.33	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
3	Morphine	Naloxone	$98.83 \pm 8.52^{**}$	$19.67 \pm 1.52^{**}$	$12.33 \pm 1.48^{**}$	$7.17 \pm 0.75^{**}$	$1.80 \pm 0.16^{**}$	$1.50 \pm 0.20^{**}$	$1.0 \pm 0.0^{**}$
4	Morphine	Saline	23.6 ± 2.29	8.17 ± 0.60	1.50 ± 0.50	2.00 ± 0.58	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
5	Morphine + MC	Naloxone	$39.67 \pm 6.36^{##}$	$7.00 \pm 1.06^{##}$	$4.17 \pm 0.65^{##}$	$3.17 \pm 0.31^{##}$	$0.0 \pm 0.0^{##}$	$0.0 \pm 0.0^{##}$	$0.0 \pm 0.0^{##}$
6	Morphine	SMC+Naloxone	$3.83 \pm 0.48^{##}$	$3.50 \pm 0.50^{##}$	$0.0 \pm 0.0^{##}$	$3.50 \pm 0.50^{##}$	$0.0 \pm 0.0^{##}$	$0.0 \pm 0.0^{##}$	$0.0 \pm 0.0^{##}$
7	MC	Saline	18.27 ± 0.01	9.5 ± 0.2	0.0 ± 0.0	1.4 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

** Significantly different from group 2 ($P < 0.01$). $^{##}$ Significantly different from group 3 ($P < 0.01$). Values represent the mean $\pm$ S.E.M. of 6 observations. Face washing was described as paw tremors. MC: *Matricaria chamomilla*. SMC: Single dose of *Matricaria chamomilla* 30 min before naloxone injection.

Fig. 1. Effect of *Matricaria chamomilla* extract treatment on weight loss in morphine-dependent and naive animals. MC: *Matricaria chamomilla* extract. SMC: Single dose of *Matricaria chamomilla* extract injection 30 min before naloxone. *Significantly different from group 8 (Morphine-Naloxone) ($P < 0.05$). ^{##}Significantly different from group 1 (Saline-Saline) ($P < 0.01$). Values represent the mean $\pm$ S.E.M. of 6 observations. % of weight loss = (weight before naloxone – weight after naloxone) $\times$ 100 / weight before naloxone.

Fig. 2. Effect of *Matricaria chamomilla* extract treatment on plasma cAMP of morphine-dependent and naive rats. MC: *Matricaria chamomilla* extract. SMC: Single dose of *Matricaria chamomilla* extract injection 30 min before naloxone. ^{**}Significantly different from group 3 ($P < 0.01$). ^{##}Significantly different from group 2 or 4 ($P < 0.01$). Values represent mean $\pm$ S.E.M. of 6 observations.

alone (group 3).

The weight loss induced by naloxone in dependent rats was reduced by a single injection of *M. chamomilla* extract, although this reduction was not significant (Fig. 1): from 170 ± 0.3 g to 167 ± 0.2 g, from 160 ± 0.5 g to 154 ± 0.1 g and from 190 ± 0.4 g to 179 ± 0.7 g after 2, 4, and 8 h, respectively. These observations indicate an inhibitory effect of a single dose of *M. chamomilla* extract on the appearance of most, but not all, withdrawal signs.

Effect of M. chamomilla extract on the plasma cAMP level of abstinent rats and naive rats

Morphine-dependent animals in group 3 going through naloxone-precipitated abstinence showed a 3-fold increase in plasma cAMP levels compared to morphine-dependent animals in group 4 treated with saline instead of naloxone (Fig. 2). Repeated co-administration of *M. chamomilla* extract with morphine abolished the increase in cAMP levels of animals undergoing naloxone precipitated withdrawal syndrome.

However, administration of a single dose of *M. chamomilla* extract (group 6) before induction of withdrawal syndrome did not significantly reduce the abrupt increase of plasma cAMP levels in abstinent rats.

Repeated administration of *M. chamomilla* extract induced a highly significant increase in plasma cAMP in the non-dependent rats (group 7) compared with the non-dependent rats treated with saline (group 1).

Discussion

In international medicine, *M. chamomilla* is widely used to obtain sedative, spasmolytic and anti-inflammatory effects. In this study, we found that co-administration of *M. chamomilla* extract with morphine greatly attenuated the development of dependence. Moreover, administration of *M. chamomilla* extract before induction of withdrawal syndrome by naloxone injection inhibited the expression of abstinence syndrome in morphine-dependent animals. These findings indicate that *M. chamomilla* has the ability to reduce both the development of dependence and expression of abstinence syndrome.

cAMP pathway is known to be an important accompaniment to morphine dependence and plasma cAMP level is considered to be a sensitive index of dependence to morphine (22). In this study, we examined the effect of *M. chamomilla* extract on plasma cAMP in dependent and naive animals, and the data obtained show that plasma cAMP was markedly increased by repeated administration of *M. chamomilla* extract in naive rats. These results are consistent with the observations that some flavonoids of *M. chamomilla* have phosphodiesterase inhibitory action resulting in an increase of cAMP in various tissues (8, 16, 17, 19, 27, 28).

Recent investigations have demonstrated that repeated co-administration of phosphodiesterase inhibitors like 3 isobutyl-methylxanthine, nefiracetam, or rolipram with morphine inhibited naloxone-precipitated withdrawal syndrome and increased cAMP in morphine-dependent animals (7–9, 29). Many investigators hypothesized that phosphodiesterase inhibitors may attenuate the morphine dependence by abolishing the up-regulation of the cAMP pathway and the modification of transcription factors associated with morphine dependence (6, 9, 30), where the up-regulated cAMP system could cause opioid receptor desensitization through phosphorylation of the receptor (31, 32).

We have shown that both the chronic co-administration of *M. chamomilla* extract with morphine and the acute administration of *M. chamomilla* extract before the induction of withdrawal syndrome blocked naloxone-precipitated morphine withdrawal syndrome in

morphine-dependent animals. However, only chronic co-administration of *M. chamomilla* extract prevented the increase of plasma cAMP level associated with abstinence syndrome. The hypothesis was, therefore, advanced that *M. chamomilla* may inhibit morphine dependence by the same mechanism reported for other phosphodiesterase inhibitors since it has phosphodiesterase inhibitory action (8, 19, 28) and has inhibitory action on the protein up-regulation at transcriptional level (20–23).

On the other hand, the effect of acute administration of *M. chamomilla* extract in preventing the expression of withdrawal syndrome in dependent animals was not attributed to its phosphodiesterase inhibitory action.

Since the acute administration of *M. chamomilla* failed to reduce significantly the increases of plasma cAMP associated with abstinence syndrome, it is probable that the inhibitory effect of *M. chamomilla* on the expression of abstinence syndrome may result from the benzodiazepine-like activity of some components of *M. chamomilla* extracts. Many studies demonstrated that *M. chamomilla* contains several benzodiazepine receptor ligands (14, 15, 33, 34). 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 *M. chamomilla* on the expression of abstinence syndrome is related to the benzodiazepine-like activity of some of its components. Several 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 (10) and by preventing the expressions of G proteins and protein components of the cAMP system (12).

In conclusion, co-administration of *M. chamomilla* extract with morphine not only inhibited dependence to morphine but also prevented the increase in plasma cAMP induced by naloxone-precipitated abstinence. Furthermore, naloxone precipitated morphine withdrawal behavior syndrome was abolished by acute *M. chamomilla* treatment before naloxone challenge, indicating that *M. chamomilla* extract has an inhibitory effect on the expression of naloxone-precipitated morphine withdrawal syndrome.

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