

STUDY OF BENZODIAZEPINE LIKE EFFECTS OF *MATRICARIA RECUTITA* ON MORPHINE WITHDRAWAL SYNDROME IN ADULT MALE RATS

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ABSTRACT

Some studies have shown that *Matricaria recutita* (*M. recutita*) have sedative effect on pain, anxiety and morphine withdrawal syndrome (MWS). Since some investigations have indicated the sedative effects of benzodiazepines in MWS, so the inhibitory factor of *M. recutita* may be related to some of its benzodiazepine-like components. In this study sedative properties of *M. recutita* in the presence and absence of flumazenil as a benzodiazepine receptors antagonist in MWS were investigated.

In this study Wistar male adult rats (250±20gr) were used. Using a vial morphine sulfate solution, morphine dependence induced with increasing doses injection subcutaneously at six days and on day seven the last dose was injected. Then four hours later Naloxone (3mg/kg, i.p.) was injected for induction of morphine withdrawal syndrome. In each group, the withdrawal signs of climbing, jumping and face washing were measured for half an hour immediately in presence and absence of *M. recutita* extract (25mg/kg) and Flumazenil (1mg/kg).

M. recutita decreased significantly the number of climbing in comparison to control group (P<0.001), but it had not significant effect on other signs. Flumazenil increased significantly the signs of jumping (P<0.01), face washing (P<0.05) in comparison to control group. *M. recutita* in the presence of flumazenil had not sedative effect and the climbing behavior increased significantly (P<0.05). The sedative effect of *M. recutita* on morphine withdrawal syndrome is probably related to its benzodiazepine like components that act on benzodiazepine receptors.

KEY WORDS: Benzodiazepine *Matricaria recutita*, Morphine withdrawal syndrome, Flumazenil.

Pak J Med Sci October - December 2008 (Part-I) Vol. 24 No. 5 735-739

How to cite this article:

Kesmati M, Abbasi ZZ, Fathi MH. Study of benzodiazepine like effects of *Matricaria recutita* on morphine withdrawal syndrome in adult male rats. Pak J Med Sci 2008;24(5):735-39.

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- * Received for Publication: January 8, 2008
- * Revision Received: March 25, 2008
- * Revision Accepted: July 25, 2008

INTRODUCTION

Addiction is a chronic reversible disorder that can be identified by means of neurobiological changes and leads to dependence to narcotic drugs. The base for developing addiction pharmacotherapy is to understand the neurochemical system involved in the changes during the dependence to narcotic drugs. The description of specific neuropharmacological changes involved in this disorder is still under discussion.¹ In relation to the side effects of chemical drugs for addiction treatment, in recent years a return to natural and plant

drugs have been in specific consideration, and *Matricaria recutita* (*M. recutita*) is widely used for its potential clinical and therapeutic benefits.

Some of the clinical benefits of *M. recutita* are anxiolytic, spasmolytic, sedative, anti-allergic, anti-inflammatory, anti-ulcer, anti-bacterial, anti-fungal and anti-viral besides other properties.^{2,3} Potentially active chemical components of *M. recutita* are flavonoids, terpenoids, coumarins and Spiro ethers.^{2,3}

Flavonoids of *M. recutita* have special place in different studies.^{2,4} It has become clear that *M. recutita* contains several benzodiazepine like ligand and it is an inhibitory factors that affects the development of opioid dependence.⁵⁻⁷ It is suggested that the inhibitory property of *M. recutita* on morphine withdrawal syndrome express is related to the benzodiazepine like activity and some of the constituents of *M. recutita*.⁵⁻⁸ Therefore, in this research the effect of *M. recutita* on morphine withdrawal signs as a result of naloxone along with administration of effective factors on benzodiazepine receptors has been investigated.

METHODOLOGY

Drugs: Morphine sulphate was purchased from Temad-Iran Company. Naloxone hydrochloride was obtained from Tolid Daroo Company/Iran, and Flumazenil (Roche Company/Switzerland). All drugs were dissolved in normal saline. *M. recutita* leaves were purchased from Gol darou Company/Iran to make hydro alcoholic extract.

M. recutita flowers were obtained from Gol Daru/Iran. Twenty gram grounded *M. recutita* flowers were added to 200 ml alcohol ethelic (70°) and kept for 48 hours and it was shaken every 12 hours. Finally using a filter paper the solution was filtered. The filtered extract was concentrated by rotary equipment. The concentrated *M. recutita* extract was spread on a glass (40cm by 40cm) to be dried in room temperature. The extract (powder) was kept in dry bottle and dissolved in saline in the case of application.

Animals: In this study Wistar adult male rats (250±20gr) were used. The animals were housed and grouped randomly and kept at room temperature 22±2°C with 12 hours light/dark cycle alternatively. Food and water was available for those animals.

Induction of morphine dependence: To develop morphine dependence, rats were injected subcutaneously with morphine twice daily for seven days. The doses of morphine were 2.5, 2.5, 5, 10, 20, 40 mg/kg and on day seven, the animals received the last injection of morphine, 50 mg/kg (8). (this need clarification either 2.5mg per day or twice daily)

Induction of withdrawal syndrome and its sign evaluation

Animals received intraperitoneally (IP) three mg/kg naloxone 4 hours after the last injection of morphine on the seventh day of morphine or saline administration. Immediately after naloxone injection, each animal was placed in a transparent acrylic cylinder to observe the frequency of withdrawal signs (climbing, jumping and face washing) for 30 minutes.⁸

Grouping animals: In this study first of all morphine dependency using morphine sulphate was induced and then animals were divided as following (Table-I):

1. Control group: This group received saline before morphine withdrawal test.
2. The group which received *M. recutita* extract: In this group the animals received only one dose of *M. recutita* extract 25mg/kg, i.p.^{8,9} at 30 minutes before the naloxone induced morphine withdrawal phenomena.
3. The group which received Flumazenil (benzodiazepine receptors antagonist): In this group animals received (i.p.) only one dose of Flumazenil 1mg/kg¹⁰ twenty minutes before the naloxone induced morphine withdrawal phenomena.
4. The group which received Flumazenil and *M. recutita* extract: In this group the animals at first received Flumazenil and 15 minute later *M. recutita* and 20 minutes later Naloxone before the morphine withdrawal test.

Table-I: Protocols of animal treatment and investigations

Group	For 7 days	4 hour after	Investigation
1	Morphine	Saline+Naloxone	Morphine withdrawal syndrome (MWS)
2	Morphine	<i>M. recutita</i> +Naloxone	MWS
3	Morphine	Flumazenil+Naloxone	MWS
4	Morphine	Flumazenil+ <i>M. recutita</i> +Naloxone	MWS

Statistical analysis: The results of these experiments were analyzed using t-test and one-way analysis of variance (ANOVA) and Post Hoc LSD. In all cases the significant level of difference was $p < 0.05$.

RESULTS

Effect of *M. recutita* extracts on signs of morphine withdrawal syndrome: Fig-1 Shows that *M. recutita* (25mg/kg) decreased significantly the sign of climbing in morphine withdrawal rats in comparisons to control group ($p < 0.001$), but it does not change other signs significantly.

Effect of Flumazenil on signs of morphine withdrawal syndrome: Fig-2 Shows that Flumazenil (1mg/kg) have increased the signs of jumping ($p < 0.01$), face washing significantly ($p < 0.05$) but it has no effect on the climbing sign in morphine withdrawal rats in comparisons to control group.

Effect of both Flumazenil and *M. recutita* on signs of morphine withdrawal syndrome Fig-3 shows that *M. recutita* extract in the

presence of flumazenil has not sedative effect and the withdrawal symptom but it has increased climbing behavior significantly ($p < 0.05$).

DISCUSSION

In this study *M. recutita* extract has reduced rat's climbing behavior significantly after morphine injection in intact animals, but has no significant effect on the other behaviors. Many reports have indicated the sedative effects of *M. recutita* such as showed both the chronic co-administration of *M. chamomilla* extract with morphine and also the acute administration of *M. chamomilla* extract before the induction of withdrawal syndrome blocked by naloxone-precipitated morphine withdrawal syndrome in morphine dependent animals.⁸ In the other studies investigators have showed that flavonoids, such as quercetin¹¹ flavone, catechin and chrysin were capable of blocking naloxone-induced contracture after exposure to morphine in a concentration dependent

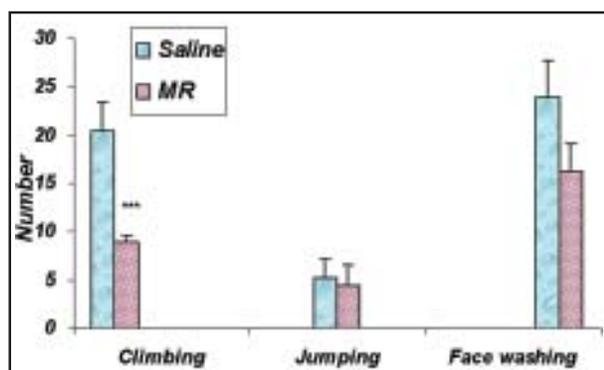


Fig-1: Effect of Matricaria recutita (MR) extract (25 mg/kg) on morphine withdrawal signs in adult male rats. All data are shown as mean \pm S.E.M.

*** indicates significant difference from saline group, $p < 0.001$ by post hoc LSD after one-way ANOVA and t-test.

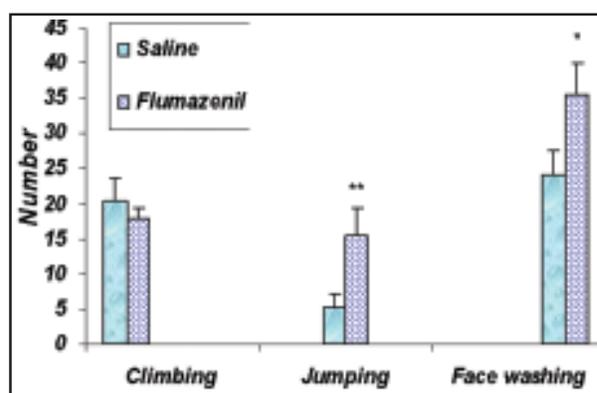


Fig-2: Effect of Flumazenil (1mg/kg) on morphine withdrawal signs in adult male rats. All data are shown as mean \pm S.E.M.

* indicates significant difference from saline group, $*p < 0.05$, $**p < 0.01$ by post hoc LSD after one-way ANOVA and t-test.

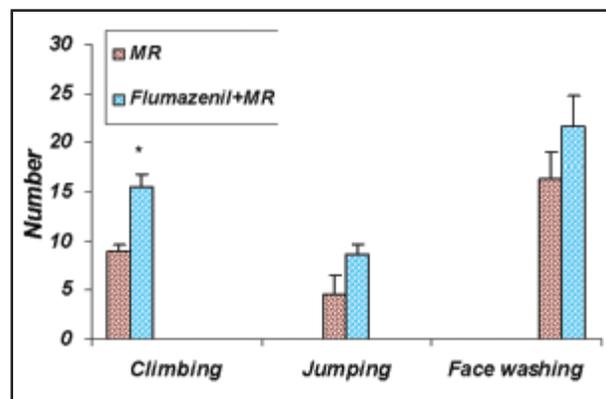


Fig-3: Effect of *Matricaria recutita* (25mg/kg) extract in the presence of Flumazenil (1mg/kg) on the morphine withdrawal signs in adult male rats. All data are shown as mean \pm S.E.M.

* indicates significant difference from MR group, $P < 0.05$ by post hoc LSD after one-way ANOVA and t-test.

fashion.^{7,12} These results suggested that flavonoids might play an important role in the control of morphine withdrawal behavior. Moreover, apigenin i.p. administration in rat reduced locomotor activity, but did not demonstrate anxiolytic, myorelaxant, or anticonvulsant activities.⁴ In the other study Zanolli, Avallone have demonstrated that apigenin and chrysin contained in *Matricaria chamomile* were equally able to reduce locomotor activity when injected in rats at a minimal effective dose of 25mg/kg.⁹ However chrysin exhibited a clear anxiolytic effect whereas apigenin 1mg/kg injected failed to exert this activity.

In connection with sedative effects of *M. recutita* several mechanism have been assumed.^{5,8,13} We have also seen that *M. recutita* (MR) can induce a pain relieving effect with and without physiological doses of sex hormones in mice.¹⁴ Gomaa and his colleagues have showed that repeated co-administration of *M. chamomile* extract with morphine abolished the increase in cAMP levels of animals under going naloxone precipitated withdrawal syndrome.⁸ However a single dose administration of *M. chamomilla* extract before induction of withdrawal syndrome did not significantly reduce the abrupt increase of plasma cAMP level in abstinent rats.⁸ Therefore, *M. chamomilla*

may inhibit morphine dependence by the same mechanism reported for other phosphodiesterase inhibitors since it has phosphodiesterase inhibitory action as well.⁸ On the other hand, the effect of acute administration of *M. chamomilla* extract may result from the benzodiazepine-like activity of some components of *M. chamomilla* extract. Many studies have demonstrated that *M. chamomilla* contains several benzodiazepine receptor ligands.^{5,8,13}

In this study the benzodiazepine-like activity of some components of *M. recutita* extract on morphine withdrawal syndrome have been investigated. Our results showed that flumazenil has increased significantly the withdrawal symptoms of jumping and face washing, and *M. recutita* extract in the presence of flumazenil has no sedative effect but it has the other withdrawal symptoms specially climbing behavior which has increased significantly. Other investigators have studied the effects of different benzodiazepine-receptor ligands on morphine withdrawal, and showed that the activation of the benzodiazepine receptor by agonists or high doses of partial agonists decreases jumping and increases wet dog shake behaviors, while the antagonists or the partial inverse agonists enhanced jumping and decreased wet dog shakes.¹⁵ Moreover, effects of flumazenil on ethanol withdrawal syndrome in rats have been investigated.¹⁶ Behavioral ethanol withdrawal syndrome symptoms appeared during the first six hours of ethanol withdrawal. Flumazenil increased horizontal and vertical locomotor activity significantly and also precipitated abnormal gait and agitation at the beginning of ethanol withdrawal syndrome in a dose dependent manner.¹⁶

As regards benzodiazepine-like effect of *M. recutita* several results could be identified. It has been showed that 6-Methylflavone acts as a positive modulator of recombinant GABA_A receptors at sites independent of flumazenil-sensitive benzodiazepine sites.¹⁷ By radioreceptor binding assays, Avallone and Zanolli has demonstrated the ability of the flavones to displace a specific radioligand, [3H] Ro 15-1788, from

the central benzodiazepine binding site.⁴ Electrophysiological studies have showed that apigenin reduced GABA (gama aminobutyric acid) activated Cl⁻ currents in a dose-dependent fashion.⁴ The pharmacological effects of 5, 7-dihydroxyflavone (chrysin), a naturally occurring monoflavonoid that displaces [3H] flunitrazepam binding to the central benzodiazepine receptors, were examined by Wolfman and his colleagues. These data suggest that this natural monoflavonoid is a partial agonist of the central benzodiazepine receptors.¹⁸

These results show that the *M. recutita* probably can decrease the dependence and morphine withdrawal syndrome express by benzodiazepine receptors. It has been shown benzodiazepine bind to GABA_A receptors subunits in neuronal-membrane of central nerve system has been shown. Therefore, the sedative effect of *M. recutita* on morphine withdrawal syndrome is probably related to benzodiazepine like components that act whereby GABA_A receptors. Since benzodiazepine receptor is a ligand-gated ion channel, activated by the neurotransmitter GABA and increase the GABA effect of benzodiazepine component on channel, therefore normally hyperpolarization of neurons leading to reduced action potential firing and thereby a reduction in neuronal activity and result in a sedative effect.^{5,6, 8,19,20}

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