



## Preventive effects of hydroalcoholic extract of *Passiflora incarnata* on morphine withdrawal syndrome and its comparison with clonidine in mice

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### ABSTRACT

Chronic use of morphine lead to dependence and discontinuation of the drug causes physical and emotional changes which needs pharmacological intervention. In the present study, the preventive effects of various doses of *Passiflora incarnata* extract on morphine withdrawal syndrome was evaluated in comparison to clonidine as a standard method. Total withdrawal score (TWS) was calculated based on behavioral sign of naloxone precipitated withdrawal syndrome in 56 adult male mice divided in 7 groups; in 6 groups morphine dependency was induced by chronic injection of morphine for 6 consecutive days. *Passiflora incarnata* hydro alcoholic extract was administered in doses 100, 200, 400, 800 mg/kg. Clonidine and normal saline were used in the control group. Withdrawal syndrome was induced by injection of naloxone. Total withdrawal score was decreased by chronic administration of *Passiflora incarnata* and clonidine. This study showed that clonidine (0.4mg/kg i.p.) and *Passiflora incarnata* extract significantly decrease TWS in the all used doses and in comparison to normal saline with a preventive effect comparable to clonidine. The data obtained in this study suggest *Passiflora incarnata* hydro-alcoholic extract therapeutic potential in prevention and management of opiate withdrawal syndrome which is comparable to the effect of clonidine, particularly at high doses of the plant extract.

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### Keywords

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### INTRODUCTION

Morphine dependency is a major problem that develops in people all around the world and is a major task to face with in many countries. Chronic use of opiates like morphine cause structural and functional alterations in central nerve system, leading to Morphine dependency. Retreat from the opiate cause physical and emotional trauma and the withdrawal syndrome management has not been satisfactory so far. In addition, animal models of addiction have shown a developing tendency of the withdrawal syndrome which

exact mechanisms is not yet clear [1, 2]. The plant *Passiflora incarnata* (*P. incarnata*), a member of Passifloraceae family, is a well-known plant that is widely distributed in many parts of Asia [3]. This traditional herbal medicine is widely used as a sedative, spasmolytic and anti-allergic agent in Asia. This plant has been used in Iranian folkloric medicine for central nervous system disorders. *Passiflora incarnata* contains indole alkaloids such as Harman, harmol, harmin, and harmalo, flavonoids such as ornitine isornitine, vitexin,

isovitexin and glosides, phenol, sterol and other volatile compounds [3, 4]. *Passiflora incarnata* sedative and anticonvulsant effects has made the plant a good choice to treat insomnia and nervous reinforcement. It has been demonstrated that flavonoid compounds such as chrysin in this plant are responsible for its anxiolytic effects [5]. *Passiflora incarnata* also act as an antioxidant. In vitro studies showed that chrysin bound to GABAA receptor in CNS and act like benzodiazepines and another study demonstrated that chrysin can be used for morphine abandonment therapy [6, 7]. There is evidence in literature that clonidine can diminish the opiates' withdrawal syndrome and the medication has been beneficial for attenuation of morphine withdrawal syndrome in addict people [8, 9]. The aim of the present study was to evaluate the effects of different doses of crude hydro alcoholic extract of plant *Passiflora incarnata* on withdrawal syndrome of opioids in male adult mice in comparison to clonidine as standard morphine withdrawal syndrome therapy.

## MATERIAL AND METHOD

### Animals

Fifty six adult male mice (25-30 grams) were purchased from Iran Razi Institute (Tehran, Iran). All animals were maintained at standard condition (22°C±2 and 12/12 hours dark and light cycle). The animals had free access to food and water. Animals were randomly divided into 7 groups. All procedures were performed at Razi Drug Research Center, Iran University of Medical Sciences.

### Preparation of extract

The plant *Passiflora incarnata*, was collected from areas around Alborz Mountain in north of Iran in May 2009. The sample was identified in botanical department, Pharmacy faculty, Tehran University of Medical Sciences (TUMS). The plant was dried and pulverized by a mechanical grinder and passed through sieve to get the fine powder. The powder was macerated in 70% ethanol as solvent for 48 hours with occasional shaking at room temperature (25°C±1). This procedure was done twice. The filtrate was collected and evaporated under vacuum and then was lyophilized. Finally, the extract with the yield of about 13% w/w was prepared. The extract was stored in a cool and dry place until use. For injection to animals, 30 mg of the extract was dissolved in 10 ml of normal saline and the required volumes were administered to animals.

### Drugs

Morphine sulphate (Themad Co, Tehran, Iran), naloxone and clonidine hydrochloride were purchased from Tolid Daru Co (Tehran, Iran).

### Induction of morphine dependency

To induce morphine dependency, in 6 groups (out of 7); the animals were injected morphine (20-45mg/kg) subcutaneously with increasing dosage during 6 days. Animals in the control groups received normal saline for 6

days.

### Treatments

Control group 1 received morphine for 6 days to induce dependency but no treatments afterwards and control group 2 just received normal saline for 6 days. *Passiflora incarnata* was injected to groups 4 to7 in doses of 100, 200, 400 and 800 mg/kg, respectively. These groups received morphine concurrently once a day from days 1 to 6. Group 3 received clonidine hydrochloride injections concurrently with morphine once a day from days 1 to 6.

### Induction and Evaluation of withdrawal syndrome

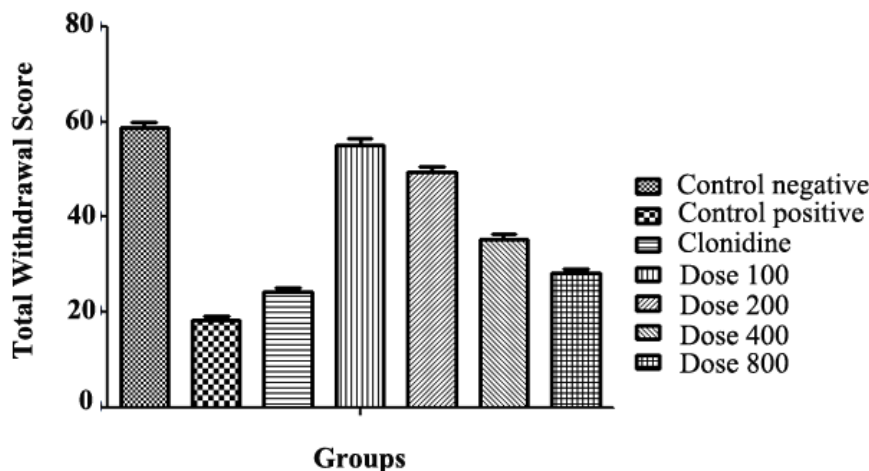
In day 7, animals of all groups were injected naloxone (3mg/kg) and their 14 behaviors (jumping, head shake, wet dog shake, for paw tremor, writhing, walking sniffing, sniffing, penile liking, rearing, chewing, body grooming, face wiping, swallowing, and teeth chattering) were recorded by camera. After computation of the recorded data, each behavior was divided to its weighing factor and a digit was obtained. The summation of the obtained digits gives the Total Withdrawal Score (TWS).

## RESULTS

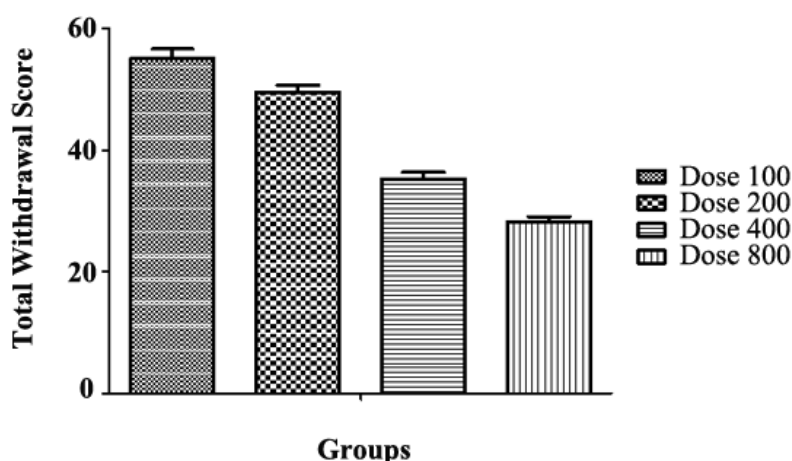
Morphine-dependent mice challenged with naloxone reliably displayed a profound withdrawal syndrome, consisting of jumping, paw tremors, diarrhea, weight loss, etc. The weighing factors for different withdrawal signs of morphine in mice are shown in Table 1. The aqueous extract of *Passiflora incarnata* attenuated naloxone-induced morphine withdrawal syndrome in a dose-dependent manner (Figs. 1 & 2). Treatment of animals with normal saline in negative control group (with no dependency) had a significant reduction in TWS compared to positive control (dependent) group (18.2±0.8 and 58.8±1.1 respectively), ( $p \leq 0.05$ ). The co-administration of various doses of *Passiflora incarnata* attenuated TWS to 55.1±1.5, 49.5±1.2, 35.2±1.2 and 28.2±0.8 for 100, 200, 400 and 800 mg/kg of the extract, respectively. This decrease is statistically

Table 1. Weighing factors for different withdrawal signs of morphine in mice

Number	Behavior	Weighing factor
1	Jumping	4
2	Head shake	5
3	Wet dog shake	5
4	Paw tremor	5
5	Writhing	5
6	Walking sniffing	5
7	Sniffing	5
8	Penile liking	5
9	Rearing	20
10	Chewing	20
11	Body grooming	10
12	Face wiping	10
13	Swallowing	10
14	Teeth chattering	10



**Figure 1.** Total withdrawal score (TWS) in mice negative control group versus positive control group and various doses of *P. incarnata* extract (n=8). Symbols above the bars denote statistic difference in comparison with the positive control group (\* $p < 0.1$  and \*\* $p < 0.05$ ) or with negative control group (\*\* $p < 0.05$ )



**Figure 2.** Total withdrawal score (TWS) in mice at various doses of *P. incarnata* extract (n=8). Symbols above the bars denote statistic difference in comparison with the positive control group (\* $p \leq 0.05$ ) or with negative control group (\* $p \leq 0.05$ )

significant ( $p \leq 0.05$ ) in comparison to positive control group. Also co-administration of clonidine with morphine decreases TWS to  $24.1 \pm 0.9$  and this attenuation is significant ( $p \leq 0.05$ ) in comparison with positive dependent control group. The result of administration of 800 mg/kg of *Passiflora incarnata* had no significant difference with clonidine and was comparable to TWS of this drug.

#### DISCUSSION

Opioids are the most commonly used drugs for relief of pain across the world and their chronic use is associated with development of dependence, which abrupt cessation of the drug causes precipitation of a severe withdrawal syndrome. None of the several available approaches could treat

abstinence syndrome. In last few years medicinal plants have been used widely for treatment of diseases by central nervous system origin across the world [8]. *Passiflora incarnata* extract is an effective agent for management of central nervous system disorders and has been used widely in folkloric medicine in many countries. There are scientific evidence that indicate *Passiflora incarnata* can be used for generalized anxiety disorder [9]. It has been mentioned in literature to be therapeutically effective on insomnia and can be used as sedative, anticonvulsant, anxiolytic and antioxidant [5, 10, 11]. Some studies suggest that *Passiflora incarnata* can be used as a therapeutic agent for the treatment of Attention Deficit Hyperactivity Disorder

(ADHD) [12]. The benzoflavone moiety of *Passiflora incarnata* significantly attenuate the expression of withdrawal effects of alcohol. Previous studies showed that chronic administration of *Passiflora incarnata* with alcohol had significantly better effects in comparison to single acute treatment in alcohol-dependent mice [7]. Both protocols were equally effective in treating the physical symptoms of withdrawal syndromes. Akhondzadeh et al showed that *Passiflora incarnata* extract could be an effective adjuvant agent in attenuation of opiate withdrawal [9]. There are other studies showing the effect of folkloric herbal medicine in management of drug addiction in mice [13]. The present study showed that *Passiflora incarnata* extract in various doses of 100, 200, 400 and 800 mg/kg, when used concurrently with morphine, can prevent and attenuate naloxone precipitated withdrawal syndrome and this happens in a dose-dependent manner. Also clonidine administration reduced morphine withdrawal symptoms (Fig. 1). This finding is in agreement with previous reports on rhesus monkeys [14], rodents [15, 16] and humans [17] that clearly demonstrate clonidine effectiveness to attenuates some opiate withdrawal signs and symptoms. Chen et al have shown that clonidine did not dramatically alter the time-course of the abstinence symptoms and is the only one able to attenuate its acute manifestations [18].

This study indicated that *Passiflora incarnata* (800mg/kg) has not been significantly different from clonidine (0.4mg/kg) considering total withdrawal score in mice. Also it has been demonstrated that *P. incarnata* (100 mg/kg) is not significantly different from negative control (independent) group. Several mechanisms have been proposed for management of morphine withdrawal signs such as NF- $\kappa$ B and C-C chemokine receptor 2 activation pathway linked mechanisms which takes place potentially in an interdependent manner [19], involvement of spinal cholinergic neurons which exert significant effects upon the cardiovascular system during opiate withdrawal [20]. Decher et al have shown that norharman significantly attenuated naloxone-precipitated withdrawal syndrome in rats. Some specific symptoms such as teeth-chattering, chewing, penile licking, diarrhea, Grooming and rearing response were reduced by norharman [21]. Several effective substances, flavonoids, chrysin, harman, harmalin have been found in *Passiflora incarnata* which are useful in morphine, alcohol, nicotine and other drugs withdrawal syndrome [22]. Presence of harman in *Passiflora incarnata* can explain the attenuating effect of the extract on withdrawal signs observed in this study. This study suggests *Passiflora incarnata* has great potential in management of morphine dependency. Since clonidine therapy has its own limitations and side effects as mentioned earlier; there is increasing evidence that extracts of *Passiflora incarnata* have sedative-hypnotic, anxiolytic and other properties makes it a suitable candidate for treatment of signs of morphine withdrawal syndrome without inducing dependence.

The authors declare that this research does not have any conflict of interest with anyone or any institute.

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