

## Effect of ethanolic extract of Safflower on naloxone-induced morphine withdrawal signs in mice

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### ABSTRACT

**Background and aims:** Safflower (*Carthamus tinctorius* L.) from Asteraceae family has different biological activities including analgesic, antidepressant, anti-inflammatory, antispasmodic and neuroprotective effects. This study designed to evaluate its effect on naloxone -induced morphine withdrawal signs.

**Methods:** In this experimental study, male NMRI mice (25-30 g) were randomly divided into 5 groups of 8: control groups received morphine and normal saline (10 ml/kg) and other groups received fluoxetine (20mg/kg) and different doses of ethanolic extract of Safflower (100, 200 and 400mg/kg). Morphine dependency was induced by intraperitoneal (i.p.) injection of increasing doses (50-75 mg/kg) of morphine. Withdrawal signs were elicited by naloxone (5 mg/kg, i.p.) and number of jumpings and also presence of climbing, writing, wet dog shakes, teeth chattering, diarrhea, grooming and rearing during a 30 min period. The data were expressed with one-way ANOVA followed by Tukey test and Mann-Whitney U test for comparison of checked signs data and they were analyzed with SPSS 19 software.

**Results:** The ethanolic extract at all doses (100, 200 and 400 mg/kg, i.p.,  $P < 0.001$ ) and fluoxetine (20 mg/kg, i.p.,  $P < 0.01$ ) significantly inhibited the number of jumps. Additionally, all doses of extract reduced the grooming and writing ( $P < 0.05$ ). Only 200 and 400 mg/kg of extract reduced the other checked signs including climbing, rearing and teeth chattering. All doses of extract couldn't reduce wet dog shake and diarrhea significantly ( $P > 0.05$ ). Fluoxetine significantly inhibited the other signs except wet dog shakes and diarrhea.

**Conclusion:** These findings indicated that Safflower extract has therapeutic potential in management of opiate withdrawal signs and this is comparable to the effect of fluoxetine. However, further studies need for clarify their exact mechanism of action.

**Keywords:** Safflower, Morphine, Dependence, Withdrawal signs, Naloxone.

Original article

### INTRODUCTION

Opiate analgesic such as morphine, and some other types are among the most extensively prescribed and efficient medications for the managing of chronic pain clinically. However, the consumption of these compounds due to the agile

development and physical dependency has been so relentlessly troubled.<sup>1</sup> It is well known that conventional way of detoxification is treating the patients with tapering doses of opioid agonists (methadone or buprenorphine) and/ or with

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clonidine or lofexidine ( $\alpha_2$ -adrenergic-receptor agonists) that can relieve the symptoms of withdrawal.<sup>2</sup> In addition to conventional drugs, several medicinal herbs including *Avena sativa*, *Rosa damascena*, *Cymbopogon citratus* (Lemon grass), *Crocus sativus*, *Rosmarinus officinalis*, *Otostegia persica* and *Aloe vera* have been investigated for their suppressive effects on opioid withdrawal signs in animal models of opioid withdrawal. In other hand, some herbal drugs used for opioid dependence because of their effects on lessening signs of withdrawal syndrome.<sup>3-9</sup>

Safflower or *Carthamus tinctorius* L. is a member of the family Compositae or Asteraceae. It is believed to have originated in southern Asia and is known to have been cultivated in China, India, Iran and Egypt almost from prehistoric times. It is cultivated mainly for its seed, which are used for making edible oil and as birdseed. There are records that it is used for reducing ailments from the neurotropic, cardiotropic, hemopoietic, and diaphoretic systems. Many clinical and laboratory studies support the use of the medicine properties of safflower for menstrual problems, cardiovascular disease, pain, antidepressant, sedative, swelling associated with trauma, anti-inflammatory and anti-tumor activities.<sup>10-14</sup> In other hand, Safflower has been used in traditional medicine to treat pain, inflammatory conditions and neuropsychological disorders. Since Safflower has antidepressant, anti-inflammatory and nociceptive effects, the aim of present study was to investigate the effect of Safflower ethanolic extract on morphine withdrawal signs in male mice.

## METHODS

This experimental study was conducted in 2015 in Urmia Branch, Islamic Azad University. The following drugs were used for the tests: morphine sulfate (Temad, Iran),

naloxone HCL (Tolidaru, Iran) and fluoxetine HCL (Arya Pharmaceutical Co, Iran). All drugs and extracts were dissolved in saline and were injected intraperitoneally (i.p.) at volumes of 10 ml/kg.

The dried flower of Safflower was bought from grocery and was identified by an expert botanist at Islamic Azad University of Pharmaceutical Science Branch (Tehran, Iran). After identification, the dried flowers were powdered with a blender and the ground samples were extracted twice with 70% ethanol for 24 h at room temperature. Finally, the extract was filtered through Whatman No.2 filter paper and evaporated under the vacuum at 40°C and then further dried to a powder using a freeze-dryer at -50°C.<sup>15</sup>

Male NMRI mice (weighing between 25-30 g) were obtained from animal house of Faculty of Medicine, Urmia University of Medical Science, Iran. The animals were housed in standard laboratory conditions and allowed access to water and food *ad libitum*. They were maintained under constant temperature (21±2°C) and in a 12h light-dark cycle. The experimental protocol was approved by the animal care review committee of UUMS (Urmia University of Medical Sciences).

In present study, for induction of morphine dependence, the mice were treated intraperitoneally (i.p.) with morphine three times a day (10 a.m., 1 p.m. and 4 p.m.) for three days, and the doses of morphine were 50, 50 and 75 mg/kg, respectively. The higher daily dose, injected at 4 p.m., was aimed at minimizing any overnight withdrawal. On day 4 they received a last dose of morphine (50 mg/kg, 10 a.m.).<sup>6</sup>

In animal grouping, forty male mice randomly divided into 5 groups of 8: negative control group received morphine and normal saline (10 ml/kg), positive control group received fluoxetine (20 mg/kg) and other groups received

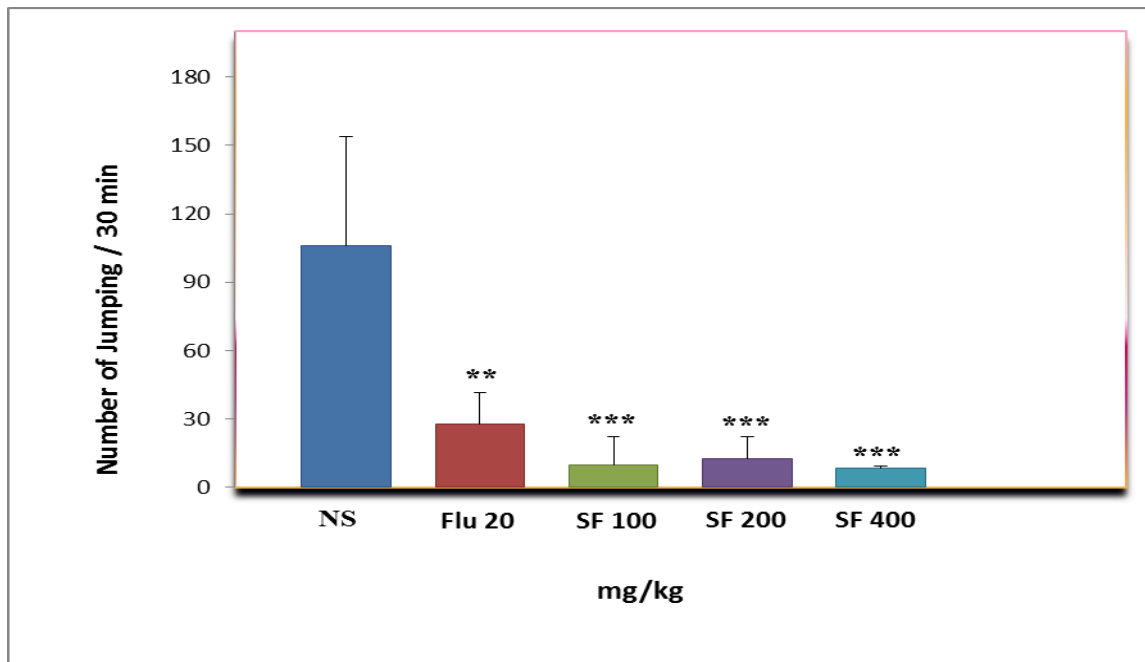
different doses of ethanolic extract of Safflower (100, 200 and 400 mg/kg), respectively. Morphine was administered to mice in all groups as discussed above. Withdrawal signs were elicited by i.p. injection of naloxone hydrochloride (5 mg/kg) 2 h after the last injection of morphine. Counted and checked signs were evaluated during a 30 min period starting just after naloxone injection. Jumping's were counted and checked signs including diarrhea, grooming, climbing, rearing, teeth chattering, writing and wet dog shakes were evaluated over 30 min with one point given for the presence of each sign during each period (range of scores: 0-3).<sup>16,17</sup>

The data were expressed as mean  $\pm$  SEM. One-way ANOVA followed

by Tukey test was used for comparison of data and P- values less than 0.05 were considered significant. The Mann-Whitney U test was used for comparison of checked signs data. All statistical calculations were done with SPSS SPSS software.

## RESULTS

The administration of all doses of Safflower extract (100 to 400 mg/kg) after the last dose of morphine on 4<sup>th</sup> day, significantly decreased the number of jumping ( $P < 0.001$ ). The maximum effect was observed at dose of 400 mg/kg. Additionally, all doses of extract better than fluoxetine reduced the number of jumping (Figure 1).



**Figure 1:** The effect of fluoxetine and different doses of Safflower on number of jumping during 30 min

Normal Saline: NS; Flu: Fluoxetine; Safflower: SF ( $n = 8$ , Mean  $\pm$  SEM, \*\* $P < 0.01$  and \*\*\* $P < 0.001$  compared to NS group, Tukey test).

Results relieved all doses of extract reduced the grooming and writing behaviors ( $P < 0.05$ ). Only 200 and 400 mg/kg of extract reduced the other checked signs including climbing, rearing and teeth

chattering. The extract also could not reduce wet dog shake and diarrhea significantly ( $P > 0.05$ ). Fluoxetine also significantly inhibited the other signs except wet dog shakes and diarrhea (Table 1).

**Table 1:** The effect of different doses of Safflower on morphine withdrawal signs

Treatment/Group	Grooming	Teeth chattering	Climbing	Rearing	Wet dog shakes	Writhing	Diarrhea
Control (NS)	2(2-3)	2(2-2)	2(1-2)	2(1-3)	1(1-2)	2(1-2.5)	3(2-3)
(Flu 20 mg/kg)	0(0-0.5)	0.5(0-1.75)	1(1-1)	0.5(0.5-1)	1(1-1)	0(0-0.5)	1(1-2)
	**M-WU=1	*M-WU=14	*M-WU=7.5	*M-WU=13	M-WU=20.5	*M-WU=24.5	M-WU=18
SF 100 mg/kg	1.5(0.25-2)	1(1-2)	1(1-2)	1(1-1)	1(1-2)	1(0-1)	2(1-3)
	*M-WU=6	-WU=13	M-WU=10.5	M-WU=20	M-WU=21	*M-WU=14	M-WU=17.5
SF 200mg/kg	1(0.25-1.75)	0.5(0-1.75)	1(1-1)	1(0.5-1)	1(0.25-1)	1(0.5-1.5)	2(2-2)
	*M-WU=9	*M-WU=8.5	*M-WU=6	*M-WU=11	M-WU=17.5	*M-WU=10	M-WU=21
SF 400mg/kg	1(1-1)	0.5(0-1.75)	1(1-1)	1(0.5-1)	1(0.25-1)	1(0.5-2)	2(2-2)
	*M-WU=5	*M-WU=6.5	*M-WU=6	*M-WU=8	M-WU=12	*M-WU=10	M-WU=15.5

The upper numbers show the median, and the numbers in the parenthesis show the range of scores. Normal Saline: NS; Fluoxetine: FLU; Safflower: SF (n= 8, Median, \*P<0.05 and \*\*P< 0.01; statistically significant between test groups and NS group, respectively; Mann-Whitney test: M-WU).

## DISCUSSION

The present study findings revealed that ethanolic extract of Safflower reduced the withdrawal signs of morphine. In line with our findings, previous studies showed that selective serotonin reuptake inhibitors (SSRIs) including fluoxetine attenuate morphine withdrawal syndrome and serotonin-mediated transmission play a significant role in the expression of acute morphine withdrawal syndrome.<sup>18,19</sup> In other hand, acute systemic administration of morphine enhances brain 5-HT synthesis and tryptophan hydroxylase activity.<sup>20,21</sup> Acute morphine also enhances the turnover and release of 5-HT and dopamine (DA) in widespread areas of the forebrain.<sup>22</sup> The effect of morphine on 5-HT release appears

to be mediated by activation of dorsal raphe neurons and may reflect disinhibition caused by direct inhibition of GABA transmission. A similar mechanism has also been proposed for the excitatory effects of opiates on DA neurons in the ventral tegmental area.<sup>23</sup>

The present study findings revealed that intraperitoneal administration of different doses of Safflower reduced the number of jumps in morphine dependent mice. Different studies showed that jumping is one of the main signs of opioid withdrawal.<sup>24,25</sup> Additionally, results revealed all doses of extract reduced the grooming and writing behaviors. Only middle (200 mg/kg) and high (400 mg/kg) doses of extract reduced the other checked signs including climbing,

rearing and teeth chattering. The extract also couldn't reduce diarrhea significantly. These findings supported previous studies and demonstrated the cathartic activity of Safflower. Hence, the applied doses could not attenuate diarrhea in morphine dependent mice.<sup>26</sup> Also, the extract could not reduce wet dog shake behavior.

The exact mechanism of action of the Safflower extract could not be predicted from present study findings. However, phytochemical studies of the species revealed the presence of sesquiterpene glycosides, aromatic acids, serotonin, flavonoids (especially quercetin and Kaempferol), sterols and triterpenes.<sup>27-30</sup> Between these ingredients, quercetin is a flavonoid found in plant foods and herbal medicines. It possesses antidepressant-like effects in forced swimming test-loaded rodents. In this regard, it showed that serotonin and quercetin bind in the same region of the active site with a similar binding energy but quercetin has a much bigger inhibition constant. Furthermore, Safflower has been clinically used in Chinese medicine for treating major depression and its possibly antidepressant activity is mediated by inhibition of 5HT reuptake in CNS<sup>14</sup>. Zhao *et al.* reported that novel coumaroyl spermidine analog (N10-(E)-tri-p-coumaroyl spermidine) has been isolated from Safflower and proved to be a novel selective serotonin transporter inhibitor.<sup>31</sup>

Therefore, the Safflower extract might be due to the presence of serotonin derivatives, flavonoids (especially quercetin) and coumaroyl spermidine analog could attenuate morphine withdrawal signs. Hence, it seems Safflower extract probably acts by enhancing serotonergic neurotransmission.

## CONCLUSION

These findings indicated that Safflower extract has therapeutic potential in management of opiate withdrawal signs and

this is comparable to the effect of fluoxetine. However, further studies need for clarify their exact mechanism of action.

## CONFLICT OF INTEREST

The author declares that there was no conflict of interests.

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