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Evaluation of the Effects of *Artemisia austriaca* on Morphine Withdrawal Syndrome in Rats

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ABSTRACT

Background: Opioidal analgesics are one of the most important drugs that have been widely used in attenuating moderate to severe pain. Unfortunately, the problems of long term use of opiods are tolerance, dependence, and ultimately addiction to mentioned drugs. Glial cells (microglia and astrocyte) and pro-inflammatory cytokines are important factors in morphine tolerance and withdrawal symptoms. It has been demonstrated that *Artemisia austriaca* extract has antinociceptive and anti-inflammatory properties. In this study the effect of total methanolic extract of aerial parts of Artemisia austrica on withdrawal syndrome of morphine in male rats has been evaluated. ***Methods:*** Adult male Wistar rats were rendered morphine-dependent by injection of additive doses of morphine subcutaneously twice daily for 9 days. To determine the effect of *Artemisia austriaca* extract on morphine withdrawal syndrome, 12 hours after the last injection of morphine, different doses of the methanolic extract of *Artemisia austriaca* (100, 200, 400 mg/kg, i.p.) dissolved in (%25DMSO in saline), was injected and after 30 minutes, naloxone (4 mg/kg, i.p.) was injected and withdrawal signs were recorded for 45 minutes. ***Results:*** The results showed that methanolic extract of *Artemisia austriaca* could reduce the morphine withdrawal symptoms in a dose-dependent manner. ***Conclution:*** The results of the present study indicate that *Artemisia austriaca* has beneficial effects in reducing withdrawal syndrome of morphine. The underlying mechanisms of this effect may consist of reduction of inflammatory response and attenuating of pro-inflammatory cytokines activation by its alkaloids content.

Introduction

The main active component in opium, morphine, has been used for thousands of years in order to pain controlling related to various clinical conditions. Development of physical dependence is one of major problems associated with the chronic use of morphine. The other issue is withdrawal of opioids which emerges as withdrawal syndrome and is considerd a big obstacle in treatment of addicted patients. The exact mechanism of opioid dependence and withdrawal syndrome are still not well known. Studies have shown the role of immune system in morphine tolerance and withdrawal symptoms.[1,2] Long-term usage of morphine causes increasing of inflammatory cytokines expression such as interleukin 12 (IL 12), Tumor necrosis factor alpha (TNFα) and also increases the activity of microglia and astrocyte,[1] In addition, analysis during morphine withdrawal syndrome, indicates increased number of glial cell activator markers, cytokines, and neurotrophic factors. Suppressing the inflammatory response of glial cells or antagonizing the activity of pro-inflammatory cytokines of interleukin 1β, interleukin 6 and TNFα reduce hyperactivity and weight loss caused by morphine withdrawal in rats.[3] Herbs have been used for a long time and in recent years their use for disease treatment has been considered and lead to more research and study in field of herbs efficacy.[4,5]

Artemisia is belonging to the Asteraceae family and mostly can be found in the northern hemisphere. The Artemisia different species have several chemical compounds such as sesquiterpene lactones, flavonoids, phenyl alkynes, coumarin, alkamides, and monoterpenes.[6,7] Aerial parts of different species of this plant are traditionally used in the treatment of inflammatory pains.[8,9] Due to presence of compounds such as coumarin, flavonoids, acetophenone, monoterpenes, sesquiterpenes and cinnamic acid derivatives in Artemisia species and analgesic and anti-inflammatory effects of this plant, in this study we evaluate the effect of methanolic total extract of the *Artemisia austriaca* on morphine withdrawal syndrome in male rats.

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Materials and methods

Animals

Male wistar rats (220-280 g) were housed in cages, four per cage, under standard environmental conditions such as 12 hrs light-dark cycle and 22 ± 2 temperature. All rats had free access to food and water.

Preparation of Artemisia extract

Artemisia austriaca plant that were used in these experiments were collected from Payam highlands of Marand city in East Azerbaijan and in order to be identified, botanic study was done and mentioned species were confirmed.

After cutting aerial parts, it was dried away from direct light and powdered by electric mill. Then 200 gram of the powder put in soxhlet extractor and initially it was extracted with 500 ml of n-hexan. Then remained powder extracted by 500 ml of dichloromethane and finally it extracted by 500 ml of methanol which we used the methanol extract.

Experimental procedure and groups

56 male wistar rats were allocated into 7 different experimental groups (n=8) randomly. The rats were included in 2 saline treated groups (non-dependent group) and 5 morphine treated groups (morphine dependent groups). The procedure of the morphine administration is as follow: day 1: 5 mg/kg/12h, days 2 and 3: 10 mg/kg/12h, days 4 and 5: 15 mg/kg/12h, days 6 and 7: 20 mg/kg/12h, and days 8 and 9: 25 mg/kg/12h. Injections were performed at 8 am and 8 pm. Under the same condition, the rats in non-dependent groups were received only normal saline.

On the tenth day of the experiment, one of morphine-dependent group was injected saline, and one of morphine dependent group (the control group) was injected extract vehicle (%25 DMSO mixture in saline) and three morphine-dependent groups were injected *Artemisia austriaca* methanolic total extract intraperitoneally (100, 200 and 400 mg/kg) thirty minutes before naloxone injection. One of non-dependent groups was treated with saline (twice daily, s.c.). The other non-dependent group only recieved the highest dose of extract (400 mg/kg) thirty minutes prior naloxone injection. In all groups in order to evaluate and observe symptoms of morphine withdrawal syndrome, one dose of naloxone (4 mg/kg, i.p.) was administrated and markers of dependence evaluation (signs of withdrawal syndrome) were recorded for 45 minutes.

Study of the withdrawal behaviors

For recording withdrawal related behavior, the animal was placed in a glass chamber in a quiet environment and symptoms of withdrawal syndrome such as Jumping, Rearing, teeth chattering, paw tremor, head shakes, wet dog shake, body grooming, face wiping, genital grooming, swallowing and writhing were recorded for 45 minutes and then based on adjusted Rasmussen method,[10] total withdrawal score (TWS) achieved, so that the values obtained for each parameter divided by standard values according to the table 1, then numbers were summed and an average was calculated for each group and these symptoms altogether reported as total withdrawal score.

Statistical analysis of each data set was performed by SPSS software (version 11.5). All the results were presented as mean ± SEM for 8 rats. Statistical comparisons among the experimental groups were made by the one way analysis of variance (ANOVA) followed by Tukey test where differences with values less than 0.05 were considered significant.

Table 1. Weighting factors of morphine withdrawal symptoms.

Behavior	Weighting factor
Jumping	4
Wet-dog-shakes	5
Head shakes	5
Paw tremor	5
Genital grooming	5
Body grooming	10
Face wiping	10
Teeth chattering	10
Swallowing	20
Rearing	20

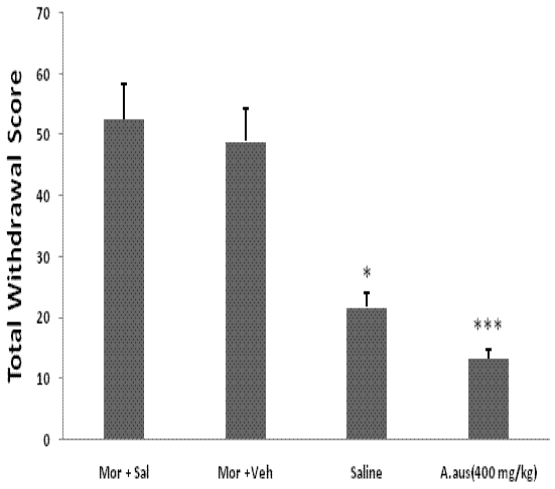

Fig 1. Comparison of total withdrawal score between the control group (morphine+ vehicle treated group), morphine+ saline treated group, saline treated group and *Artemisia austriaca* (400 mg/kg) treated group is shown. Data are expressed as mean ± SEM (n=8 in each group). Shown statistical differences are (* p <0.05, ** p <0.01 and *** p <0.001) compared to control group (morphine + vehicle).
Mor= morphine; Sal= saline; Veh= vehicle; A.aus= Artemisia austriaca.

Results

The TWS in the control group (morphine+ vehicle treated group) was increased significantly in

comparison to that of the saline group ($p<0.05$) and the group received highest dose of extract alone ($p<0.001$). In addition results showed that there is no significant difference between the control group and morphine with saline treated group.

Administration of methanolic total extract of *Artemisia austriaca* reduced the naloxone precipitated TWS in a dose dependent pattern and administration of 200 and 400 mg/kg of *Artemisia austriaca* extract made a significant difference versus control group ($p<0.05$ for A. austriaca 200 mg/kg and $p<0.001$ for A. austriaca 400 mg/kg) as is depicted in Fig 2.

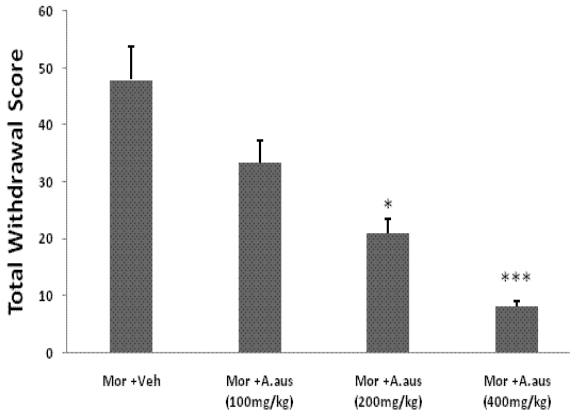

Fig 2. Effect of *Artemisia austriaca* on naloxone (4 mg/kg, i.p.) precipitated total withdrawal score. *Artemisia austriaca* (100, 200, 400 mg/kg) injected 30 minutes prior naloxone. $p<0.05$ was considered statistically significant. Data are expressed as mean ± SEM (n=8 in each group). Shown statistical differences are (* $p<0.05$, ** $p<0.01$ and *** $p<0.001$) compared to morphine+ vehicle treated group.
Mor= morphine; Sal= saline; Veh= vehicle; A.aus= Artemisia austriaca.

The results of this study indicated attenuation in morphine withdrawal syndrome through Artemisia extract. The methanolic extract of *Artemisia austriaca* significantly attenuated withdrawal symptoms except swallowing and teeth chattering as are depicted in table 2.

Discussion

Naloxone induced morphine withdrawal resulted in emerging of withdrawal signs such as jumping, wet dog shakes and other well-known behaviors in the rats. In this study it was resulted that acute administration of *Artemisia austriaca* methanolic total extract could attenuate the naloxone precipitated withdrawal syndrome dose-dependly in the rat. The underlying mechanisms involved in the induction of opioid tolerance and dependency are a very complicated issue, but recent evidences had implicated a glial inflammatory response in the pathogenesis of dependence.[2] Astrocytes and microglia respond to repeated opioid administration in a pro-inflammatory manner, with up regulation of markers of activation and expression of pro-inflammatory cytokines.[11] During the inflammatory response, at least two transcription factor, nuclear factor-kappaB (NF-kappaB) and Activator protein 1 (AP-1) were involved which were activated by pro-inflammatory cytokine such as IL1, IL6 and TNFα.[2] A study on human umbilical cord endothelial cells has shown that different species of Artemisia extract such as Artemisia alba, and asiatica because of having a compound named Artemisolide significantly decrease the activation of these inflammatory factors.[12] It is well known that such pro-inflammatory cytokines can enhance excitatory synaptic transmission and potentiate N-methyl-D-aspartate (NMDA) induced currents.[13] In addition, it was extensively showed that the NMDA receptor antagonists such as the MK-801 can inhibit dependency.[14] Artemisia possesses anti-inflammatory properties, visceral pain and headache analgesic properties, antipyretic, anti-cough and antiseptic properties that most of these properties are related to flavanoid, coumarin, monoterpene and sesquiterpene present in these plants.[5,6] Eucalyptol, which is a monotrepnoid considerably, exists in aerial parts of Artemisia austriaca.[6] According to a study on cultured human lymphocytes and monocytes Eucalyptol considerably has reduced the production of cytokines IL1β and TNFα.[15] Eucalyptol has considerable anti-inflammatory and analgesic effects in the rats.[16,17]

Artemisia is an important source of flavanoids.[6] The flavonoids, suggested as having different biological roles. The anti inflammatory actions of flavonoids in vitro or in cellular models involve the inhibition of the synthesis and activities of different pro-inflammatory mediators such as eicosanoids, cytokines adhesion molecules and C-reactive protein. Molecular activities of flavonoids include inhibition of transcription factors such as NF-kB and AP-1, as well as activation of nuclear factor-erythroid 2-related factor 2 (Nrf2).[18]

7-Hydroxycumarin, which is the major metabolite of coumarin has shown strong analgesic effects on animal pain models. This chemical appears to inhibit the secretion of proinflammatory cytokine IL1 and TNFα. it also inhibits the production of prostaglandin E2 which is a pain process mediator. It also reduces the pain caused by inflammation stimuli through preventing the entry of neutrophils into damaged tissue.[19] Monoterpenes such as borneol, camphor, eucalyptol, β-pinene, and camphene have anti-inflammatory and analgesic effects. Borneol shows its anti- inflammatory effects by inhibiting the production of nitric oxide (NO) and prostaglandin E2. Since mentioned monoterpene exist in Artemisia austriaca, these effects can be attributed to this plant.[20]

Conclusion

Our results suggest that the methanolic extract of the *Artemisia austriaca* can significantly reduce the withdrawal syndrome of morphine. It seems that acute administration of *Artemisia austriaca* methanolic total extract suppresses NMDA receptor activation and

prevents morphine withdrawal signs by its inhibitory effects on immune system and pro-inflammatory cytokines. Investigations of the pharmacology of natural products are necessary to gain evidence concerning the usefulness of medicinal plants in phytotherapy. Our experiments therefore contribute to our knowledge of the pharmacology of Artemisia austriaca.

Table 2. Mean value of the morphine withdrawal signs induced by naloxone (4 mg/kg) in different groups during the 45-min observation period.

Groups	*Signs*								
	Jumping	**Swallowing**	**Paw tremor**	**Genital grooming**	**Body grooming**	**Face wiping**	**Teeth chattering**	**Head shakes**	**Rearing**
Mor + Sal	36.4±9	44.7±9.2	36±8.1	22.4±3.3	21.3±5.8	36.9±6.3	21.1±4.7	17.2±3.9	42±8.1
Mor +Veh	39.6±6	56.2±21.4	27.3±6.8	17.3±6.4	20±6.2	31.3±11	19.4±4.6	14.6±7.4	32.3±7.2
Mor +A.aus(100mg/kg)	7.2±1.1***	45.2±11.6	31.3±9.5	8.2±2.4*	14.3±4.7	19.1±6*	24.4±5.3	14.2±5	20±6.2*
Mor +A.aus(200mg/kg)	3.4±1.2***	30.2±9.4	21.4±6.1	10.±2.3	11.2±3.9	8.3±3***	11.2±5.1	4.3±2**	18.2±5*
Mor +A.aus(400mg/kg)	1.2±0.5***	11.3±4.1**	9.2±3***	4.2±2.5**	3.4±2**	5.2±3***	7.4±3.1	5.6±1.4*	11±3**
Saline	2.1±0.7***	26.2±11.4*	12±4.3**	6.1±2.3*	4.16±1.2**	18.2±7.4*	13.7±4.5	5.1±2.3*	19±4*
A.aus(400 mg/kg)	1.1±0.2***	13.2±7.3**	2.3±0.4***	1.3±0.2***	6.2±2.5*	4±1.1***	6.3±2.9	2±0.6***	8.1±3***

Sal= saline; Veh= vehicle; Mor= morphine; A.aus = Artemisia austriaca. All data are expressed as mean ± SEM.
Shown statistical differences are (* p <0.05, ** p <0.01 and *** p <0.001) compared to control group (Mor +Veh).

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