

The Effects of Hydroalcoholic Extract of *Satureja khuzestanica* on Naloxone-Induced Morphine Withdrawal Symptoms in Wistar Rats

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Abstract

Introduction: The inflammatory system, oxidative stress, and cholinergic pathways are some important factors in long-term opioid dependence withdrawal. The adverse effects of some adjunctive medications on withdrawal symptoms treatment limit their clinical efficiency and finding compounds with inhibitory effects on opioid dependence can be helpful. The antioxidant, antinociceptive, and anti-inflammatory properties of *Satureja khuzestanica* extract (SKE) have been noted. Additionally, it could reduce morphine analgesic tolerance. Given that no study has assessed the effect of SKE on morphine-induced withdrawal symptoms, we have tried to investigate it in this study.

Methods: Male Wistar rats were used in this study. The rats were treated with morphine for 7 days. The control group received saline, and intervention groups received SKE intragastrically by gavage (100 mg/kg, 50 mg/kg, and 25 mg/kg) 15 minutes before morphine injections. Five hours after the last injection, naloxone was used and withdrawal symptoms were assessed for one hour. SPSS software version 16.0 was used for statistical analysis, and $P \leq 0.05$ was considered statistically significant.

Results: SKE diminished weight loss and jumping ($P \leq 0.001$). It decreased grooming behaviors and cramps ($P \leq 0.01$). SKE was found to decrease morphine withdrawal symptoms and 100 mg/kg was the most effective dose. Additionally, different doses of SKE were able to eliminate ptosis, diarrhea, and teeth chattering in animals; however, different doses had different effects on withdrawal symptoms.

Conclusion: Naloxone significantly increased the frequency of jumping, cramps, weight loss, grooming, and induced ptosis, diarrhea, and teeth chattering. Treatment with SKE can significantly reduce morphine withdrawal symptoms. This can be done through its ability to decrease inflammation.

Keywords: Morphine, *Satureja khuzestanica* extract, Dependence, Withdrawal symptoms, Opioids



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Introduction

Opioids are the most current analgesics for acute and chronic pain treatment.^{1,2} Since twenty years ago, fatal opiate overdoses have increased all over the world and those who have experienced non-fatal overdoses are expected to experience it again.^{3,4}

Continual usage of an opioid can induce long-lasting behavioral sensitization, which is shown to be associated with

neuroplasticity that is generated by exposure to addictive drugs. Continued opioid use induces a variety of complications such as constipation,⁵ respiratory depression,⁶ and neuropathic pain.⁷ Patients with prolonged opioid exposure can have an increased risk of overdose, depression, and opioid-induced hyperalgesia.^{7,8} Cessation of opioid or any other drug administration after the continuous use of them is named

withdrawal syndrome.⁹

Long-term agonist maintenance is a common clinical treatment option for patients' withdrawal.^{10, 11} Under these situations, non-opioid adjunctive medications can reduce patients' withdrawal symptoms.¹² However, the adverse effects of these medications restrict their use.¹³ In this regard, finding drugs and natural compounds with possible inhibitory effects on opioid dependence can be helpful. In the last years, medicinal herbs are more attractive due to fewer complications.^{14, 15}

In recent years, some experimental studies have indeed evaluated Iranian medical herbs using modern scientific methods for finding an effective treatment for different diseases.¹⁶⁻¹⁹ *Satureja khuzestanica* Jamzad (Lamiaceae) appears in small populations in the southern part of Iran, which is used as a muscle and neuropathic pain reliever in Iran traditional medicine.²⁰ The anti-inflammatory, antimicrobial, and antinociceptive properties of the *Satureja khuzestanica* extract (SKE) have been shown in previous studies.²¹ This plant has antihyperalgesic effects on diabetic neuropathy and antinociceptive effects on morphine tolerance.^{22, 23} SKE exerts its effect on tolerated rats through an increase in the level of glial fibrillary acidic protein (GFAP) and also tumor necrosis factor- α (TNF α).²³ Based on the antioxidant, antinociceptive, and anti-inflammatory properties of SKE and its ability to induce morphine antinociceptive tolerance,²¹⁻²³ this study was designed for investigating the effect of SKE on the morphine-dependent withdrawal syndrome in Wistar rat.

Materials and Methods

Animals

Male (200–250 g) Wistar rats were kept in isolation under specified pathogen-free conditions, controlled temperature ($22\pm 0.5^{\circ}\text{C}$), and a 12/12 h light/dark cycle. Food and water were supplied ad libitum. Three days

before the experiment, the animals were handled daily to minimize stress. The animals were randomly divided into 5 groups ($n=8$). The experiments were done in accordance with ethical guidelines for investigations of experimental pain.²⁴

Preparation of *Satureja khuzestanica* Extract

The SKE (ethanolic extract) was prepared in Razi Herbal Medicines Research Center, Lorestan, Iran. Leaves were dried and stored as a powder at the herbarium of the Razi Herbal Medicines Research Center. Air-dried leaves (200 g) were ground into a powder and then extracted twice, each time with 1 L ethyl alcohol (80%). The extract was concentrated in a rotary evaporator under reduced pressure and then it was freeze-dried. Based on the results of gas chromatography-mass spectroscopy analysis, SKE contains carvacrol (78.3%), 9-octadecenoic acid (13.5%), hexadecanoic acid (6.7%), bis (2-ethylhexyl) phthalate (1.0%), and beta-bisabolene (0.5%).²²

Study Design

For this study, rats were divided into 5 different groups: control, morphine alone, morphine with normal saline (0.9%), morphine with three different doses of SKE (100 mg/kg, 50 mg/kg, and 25 mg/kg). Morphine was injected intraperitoneally (i.p.) 15 minutes after that the extract was given intragastrically (i.g.) by gavage. To assess morphine dependence, morphine was injected into the animals ($n=8$) twice a day, for a week (an interval of 12 hours between injections). The used dosage and the work protocol are described in Figure 1. Five hours after the last morphine injection, 3 mg/kg naloxone was injected (i.p.). Withdrawal behaviors were then observed as described by Way et al.²⁵ The animals were kept within a clear Plexiglas cylinder (a day before the experiment) and were observed for an hour.^{26, 27}

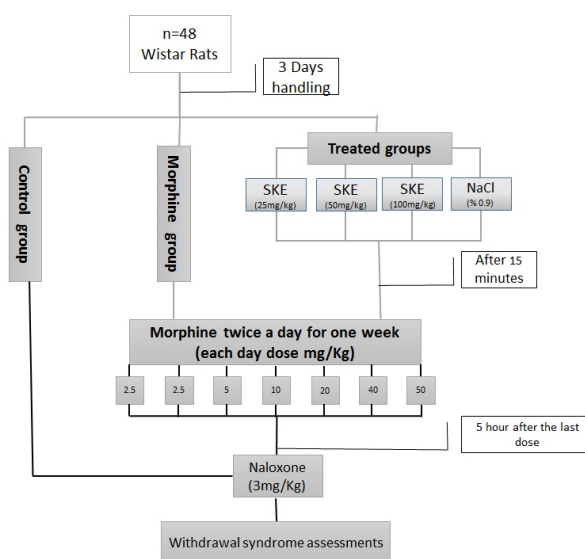


Figure 1. The Flowchart of the Study Design.

Withdrawal Symptoms Assessment

A trained observer (blind to treatment) used a 7-item rating system which included autonomic, gastrointestinal, musculoskeletal, and mood symptoms. The scores varied across items for grooming, jumping and cramps, and their numbers per 1 hour were recorded. The rats were weighed before the naloxone injection, after 30 minutes, and after one hour of injection. Other factors, ptosis, diarrhea, and teeth chattering were scored either 0 (no indication of symptoms) or 1 (an indication of symptoms) and then all the responses of rats were summed.

Statistical Analysis

The data were presented as mean values \pm SD and analyzed using SPSS software version 16.0. Data comparisons were performed by analysis of variance (ANOVA). $P < 0.05$ was considered statistically significant.

Results

Results of Weight Loss Assessment

To assess weight loss, rats were weighed directly before injection, 30 minutes after injection, and one hour after injection. In the morphine group, the weight significantly decreased after 1 hour compared with the initial weight. Weight loss with time was not significant in SKE treated rats and the control group (Figure 2).

Results of Jumping Assessment

The number of jumping increased after using naloxone in the morphine group in comparison with the control group. Using different doses of SKE could significantly decrease the mean number of jumping; however, there was no difference between the groups treated with different doses (Figure 3A).

Results of Grooming Assessment

Morphine and morphine +100 mg/kg or 50 mg/kg SKE groups had a significantly higher level of grooming

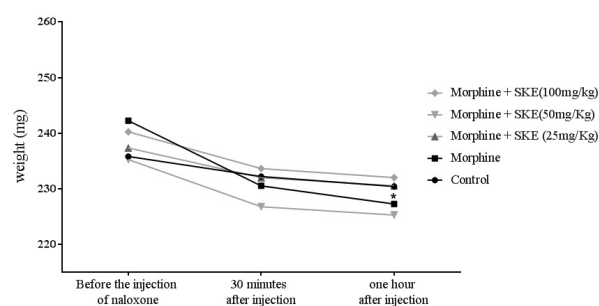


Figure 2. Effect of Different Doses of *Satureja khuzestanica* Extract on Weight Loss in Morphine-dependent Rats. A significant difference was observed between before naloxone injection and one hour after injection. Results were expressed as means \pm SD. $P \leq 0.05$ was considered significant.

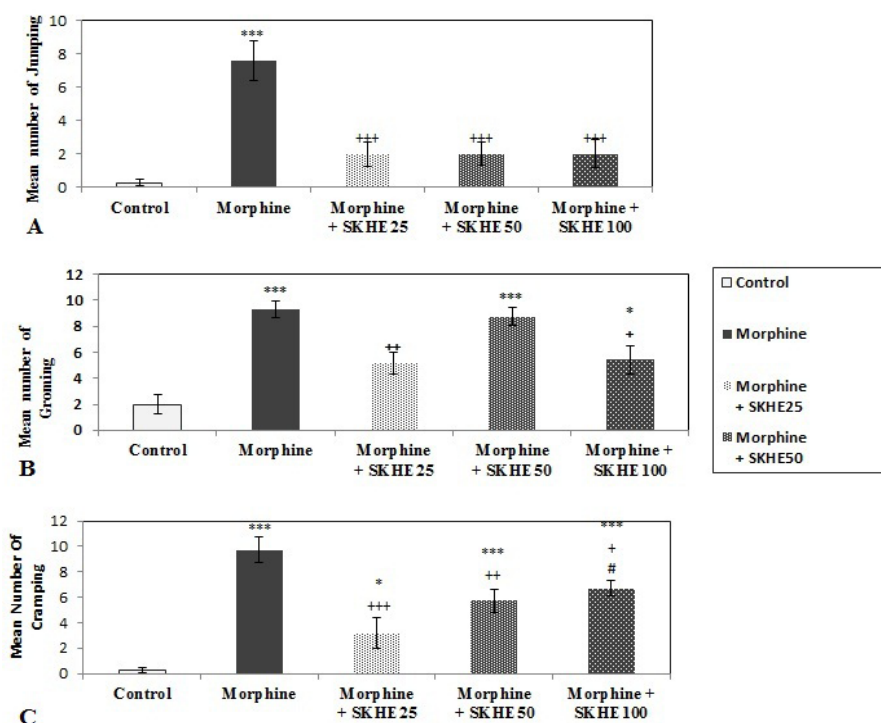


Figure 3. Effect of Different Doses of *Satureja khuzestanica* extract on Jumping (A), Grooming (B), and Cramps (C) in Morphine-dependent Rats. Results are expressed as means \pm SD. Comparison with control group: * $P \leq 0.05$, ** $P \leq 0.01$; comparisons with other group: + $P \leq 0.05$, ++ $P \leq 0.01$, +++ $P \leq 0.001$.

compared with the control group. Using 100 mg/kg and 25 mg/kg SKE could significantly decrease this side effect in comparison with the morphine group (Figure 3B).

Results of Cramp Assessment

The number of cramps after using naloxone in all treatment groups was higher in comparison with the control group. Using different doses of SKE significantly decreased the number of cramps, and the most effective dose of SKE was 50 mg/kg (Figure 3C).

Ptosis, Diarrhea, and Teeth Chattering Assessments

Ptosis, diarrhea, and teeth chattering were observed in the morphine group; however, using SKE decreased them in the last two quarters (after 30 minutes). The best effects belonged to the morphine+100 mg/kg SKE group. Regarding ptosis, the effects of other doses were not significant (Table 1).

Discussion

Naloxone is an opioid receptors antagonist that prevents opioids from acting and induces the addiction withdrawal symptoms in opioid-addicted patients.²⁸ Physical symptoms like diarrhea, grooming, ptosis, cramp, jumping, weight loss, and teeth chattering are the main withdrawal syndrome symptoms in rats that can be assessed.²⁵ Pharmaceutical drugs can stop morphine withdrawal; however, their adverse side effects can lead to the suggestion of using medicinal plants with natural effective substances which can cause fewer side effects and lower costs.^{12,14,15} SKE has shown the ability to reduce pain^{21,22} and morphine tolerance.²³

As this study showed, using SKE could decrease the symptom of morphine withdrawal and 100 mg/kg of SKE extract was the most effective dose. Using SKE could decrease weight loss, jumping, grooming, and cramps after the injection of naloxone. In a number of rats, different doses of the extract especially 100 mg/kg were able to eliminate ptosis, diarrhea, and teeth chattering.

Studies established that jumping during morphine withdrawal could be mediated by muscarinic or nicotinic cholinergic receptors.^{29,30} According to some previous studies, diarrhea occurs due to the effect of morphine on the cholinergic system.³¹ Previous studies have shown

that an increased acetylcholine release during withdrawal can inhibit jumping. This release may be a secondary response to some primary withdrawal effector.³² The primary mechanism for the withdrawal is not known; however, a rapid increase in brain dopamine levels which occur after naloxone injection in morphine-dependent mice can be an example.³³ Drugs affecting cholinergic and dopaminergic pathways in central nervous system (CNS) can affect the naloxone-induced jumping behavior.^{29,30,34} Some medicinal plants with flavonoid and phenolic compounds could reduce withdrawal syndrome by preventing the function of the cholinergic system through inhibiting cholinesterase.³⁵ In a study, *Borago officinalis* flowers, which contain large amounts of flavonoids and phenolic compounds, were reported to inhibit the function of the cholinergic system and decrease withdrawal symptoms.¹⁴ A new monoterpene-flavonoid and twelve known flavonoids were isolated from an ethyl acetate extract of *S. khuzestanica* Jamzad,³⁶ therefore, SKE can exert its effects through its flavonoids efficacy on the function of cholinergic system.

On the other hand, in the cholinergic anti-inflammatory pathway which receives signals from the efferent vagus nerve, there are interactions between these nerves, macrophages, and other immune cells through nicotinic acetylcholine receptors.³⁷⁻³⁹ In addition, brain cholinergic associations with the vagal-mediated cholinergic anti-inflammatory pathways take a role in regulating systemic inflammation.³⁹ This cholinergic modulatory effect can be used as a novel therapeutic modality for controlling excessive inflammation.^{37,40} According to these studies, using anti-inflammatory components can reduce withdrawal symptoms. It is proven that *S. khuzestanica* has an anti-inflammatory effect on inflammatory pain²¹ and can also reduce the morphine-induced elevation of TNF α level.²³ Therefore, it seems that the reduction of morphine-induced cytokine-mediated neuroinflammation and the subsequent neuronal plasticity by SKE are the possible mechanisms for its effects on morphine dependence.

In this experimental project, the clinical symptoms of cramps and diarrhea, which were observed during naloxone-induced withdrawal, decreased after using different doses of SKE. Another study showed that the pharmacological modulation of glial activity with

Table 1. The Effect of Different Doses of *Satureja khuzestanica* Extract on Ptosis, Diarrhea, and Teeth Chattering in Morphine-Dependent Rats

Groups	0-15 minutes			15-30 minutes			30-45 minutes			45-60 minutes		
	Ptosis	Diarrhea	Teeth Chattering	Ptosis	Diarrhea	Teeth Chattering	Ptosis	Diarrhea	Teeth Chattering	Ptosis	Diarrhea	Teeth Chattering
Morphine	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8
Morphine+ NaCl	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8
Morphine+ SKE (25 mg/kg)	7/8	6/8	6/8	8/8	4/8 *	4/8*	8/8	1/8**	1/8**	8/8	1/8**	1/8**
Morphine+ SKE (50 mg/kg)	8/8	6/8	6/8	7/8	6/8	6/8	5/8	2/8**	2/8**	6/8	3/8*	3/8*
Morphine+ SKE (100 mg/kg)	8/8	5/8	5/8	6/8	6/8	6/8	2/8**	1/8***	1/8**	2/8**	3/8*	3/8*

* Significant difference with morphine group ($P \leq 0.05$), ** Significant difference with morphine group ($P \leq 0.01$). *** Significant difference with morphine group ($P \leq 0.001$). Results are expressed as means \pm SD.

ibudilast decreased the opioid withdrawal symptoms in humans.¹³ This study showed that two ibudilast groups had lower ratings of withdrawal symptoms on SOWS items (“anxious”, “perspiring”, “restless”, and “stomach cramps”).¹³ Astrocyte cells express GFAP in CNS which is involved in some of the important processes such as chronic morphine-treatments.^{13,23} Our previous study showed that SKE prevented chronic morphine-induced GFAP activation.²³ Moreover, withdrawal symptoms can be mediated by this function of SKE.

Conclusion

In conclusion, this study showed that the ethanolic extract of *S. khuzestanica* can significantly reduce morphine withdrawal symptoms. This can be done through its ability to decrease inflammation. Further pharmacological studies on traditional herbal drugs are needed to obtain evidence about the usefulness of medicinal plants in phytotherapy.

Ethical Approval

The experiments were approved by the Animal Experimentation Ethics Committee of Kerman Neuroscience Research Center (EC/KNRC/91).

Conflict of Interest Disclosure

None.

Authors' Contribution

SEM designed the study, BE performed the data collection and data analysis. All authors have contributed to the conception of the research, drafting the article, or revising it and approved the final version.

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