

## An anti-inflammatory and anti-nociceptive effects of hydroalcoholic extract of *Satureja khuzistanica* Jamzad extract.

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**ABSTRACT PURPOSE:** *Satureja khuzistanica* Jamzad (Lamiaceae) is an endemic plant that widely distributed in the southern parts of Iran. This plant has been used as analgesic and antiseptic among the inhabitants of southern parts of Iran. **METHODS.** The *Satureja khuzistanica* hydroalcoholic extract was prepared and its anti-inflammatory and anti-nociceptive effects were investigated using the carrageenan-induced rat paw edema and formalin test. **RESULTS.** A similar anti-inflammatory activity was seen between *S. khuzistanica* hydroalcoholic extract (150 mg/kg; i.p.) and indomethacin (4 mg/kg; i.p.) in carrageenan test. The extract showed anti-nociceptive activity in a dose-dependent (10-150 mg/kg; i.p.) manner at the second phase of formalin test which was comparable with morphine (3 mg/kg; i.p.). **CONCLUSION.** This study confirms that anti-inflammatory and anti-nociceptive properties of *S. khuzistanica* are comparable to those of indomethacin and morphine. Presence of flavonoids, steroids, essential oil, mainly carvacrol and tannin might be responsible for anti-inflammatory and anti-nociceptive activities of this plant.

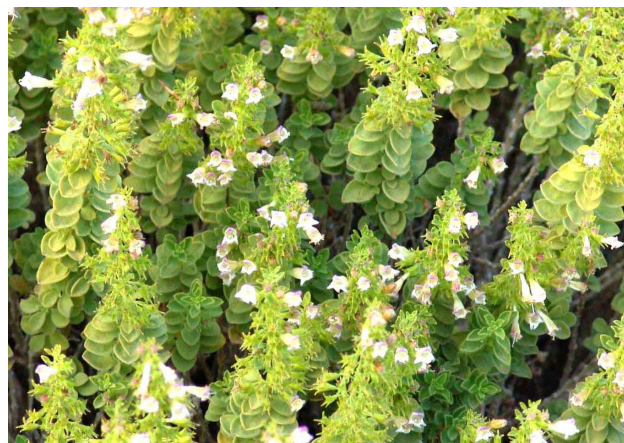
### INTRODUCTION

Inflammation is a pathophysiological response of living tissues and a defense mechanism (1). Numerous studies have been carried out to develop more powerful anti-inflammatory drugs with lesser side effects. *Satureja khuzistanica* Jamzad (marzeh khuzistani in Persian, family of Lamiaceae) is an endemic plant that widely distributed in the southern parts of Iran (2). It is a subshrub, branched stem  $\pm$  30 cm high, densely leafy, broadly ovate-orbicular covered with white hairs. Base of the leaves is attenuate and petioliform.

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Each verticillaster has 2-8 flowers, shortly pedunculate and remote.

Botanically this species is close to *S. edmondi* Briquet but differs from it in having erect and branched stems, verticillasters are shortly pedunculate (2, picture 1).



**Picture 1.** *Satureja khuzistanica* Jamzad

This plant has been used as analgesic and antiseptic among the inhabitants of southern parts of Iran. Composition of the essential oils of wild and cultivated *S. khuzistanica* and the antioxidant, antidiabetic, anti-hyperlipidemic and reproduction stimulatory properties of this plant have been recently reported from Iran (3-5).

No work has been carried out on the anti-inflammatory and anti-nociceptive effects of this species. Keeping this in view, the present study has been undertaken to evaluate the anti-inflammatory and anti-nociceptive effects of the hydroalcoholic extract of this species using the carrageenan-induced rat paw edema and formalin test respectively.

## MATERIALS AND METHODS

### *Plant material and extraction procedure*

*S. khuzistanica* aerial parts were collected from Khoramabad, Lorestan Province, Iran in August 2002 at an altitude of 1170 m. The plant was authenticated by Herbal Museum of the Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran. A voucher specimen (No. 58416) has been deposited at the Herbarium of the Research Institute of Forests and Rangelands (TARI), Tehran, Iran.

The aerial parts of *S. khuzistanica* were chopped and air-dried under shade and then powdered and stored in an airtight container. *S. khuzistanica* hydroalcoholic extract (SKHE) were prepared with aqueous ethanol (50%; v/v) by percolation at room temperature for 24 h and concentrate at low pressure to constant weight (yield: 14.5 %).

### *Animals*

Male albino Wistar rats (190-210 g) were obtained from the central animal house of the Razi Institute in Iran. They were kept at standard environmental conditions (12/12-h light/dark cycle) at the animal house of the Department of Pharmacology in the Faculty of Medicine, Tehran University of Medical Sciences (TUMS) and were allowed free access to food and water. Before each test, the animals were fasted for 24 h with free access to water. The rats were randomly divided into test and control groups, each group consisted of age and weight matched rats ( $n = 9$ ). Each animal tested once. The experimental protocol was approved by the animal care review committee of TUMS.

### *Formalin test*

Male Albino Wistar rats, 190–210 g, were kept in Plexiglas cages with free access to food and water. Testing took place in the middle of the light period of a 12:12-h light:dark cycle. The SKHE and/or morphine sulfate were suspended in vehicle (1% carboxymethylcellulose (CMC) in saline) and administered interaperitoneally (i.p.) at a dose of 10, 100, 150 mg/kg for SKHE and 3 mg/kg for morphine in a volume of 1.5–2 ml for each rat (6). Control group received vehicle (2 ml; i.p.).

The anti-nociceptive activity of the tested compounds was determined using the formalin test as described by

Dubuisson & Dennis (7). One hour before testing, the animal was placed in a standard cage (30x12x13 cm) that served as an observation chamber. A total of 20  $\mu$ l of 1% formalin was injected into the plantar surface of the left hindpaw. The rat was observed for 60 min after the injection of formalin, and the amount of time spent licking the injected hindpaw was recorded. The first 5 min post formalin injection was considered as the early phase and the period between 15–60 min as late phase. The test compounds were administered (i.p.) 30 min before injection of formalin.

### *Carrageenan test*

The anti-inflammatory activity of SKHE was determined by the carrageenan-induced edema test in the hindpaws of rats using the technique described by Niemegeer et al. (8). Male Wistar rats weighing 190–210 g were fasted for 24 h before the experiment with free access to water. Fifty  $\mu$ l of a 1% suspension of carrageenan (Sigma-Aldrich, St. Louis, MO, USA) in saline prepared 1 h before each experiment was injected into the plantar side of hindpaw of the rats.

The SKHE and indomethacin were suspended in vehicle (1% CMC) and administered i.p. at a dose of 100 and 150 and 4 mg/kg respectively in a volume of 1.5–2 ml for each rat (6). Control group received vehicle (1 % CMC, 2 ml; i.p.). Tested compounds and vehicle alone were injected 30 min before the carrageenan injection and the degree of the carrageenan-induced swelling of the paw was measured for 3 h after carrageenan injection using a mercury plethysmograph (Ugo Basil, Italy).

The degree of swelling induced was evaluated by the ratio  $a/b$ , where  $a$  and  $b$  are total volumes of both hindpaws before and after carrageenan treatment respectively. A ratio smaller than 1.5 after drug administration was considered as a significant inhibitory effect of the drugs (8).

### *Statistical analysis*

The data are expressed as mean  $\pm$  SEM. Student  $t$ -test followed by Tukey-Kramer multiple comparisons test were used to determine significant differences between groups.  $p$ -values less than 0.05 were considered as indicative of significance.

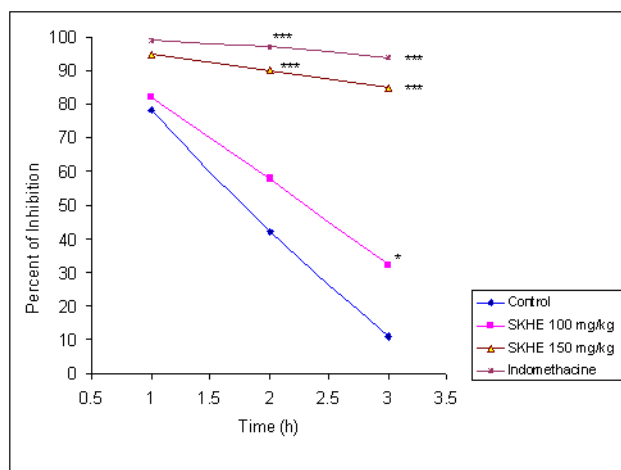
## RESULTS

The results of this study for formalin and carrageenan tests are presented in Table 1 and Figure 1 respectively. As displayed in Table 1, no significant difference ( $p > 0.05$ ) is seen between animals treated with SKHE (10-150 mg/kg) and those treated with vehicle in the first phase of formalin test. The same result is seen for morphine sulfate (3 mg/kg) and control group. No significant was seen between morphine (3 mg/kg) and SKHE (10-150 mg/kg).

**Table 1: Anti-nociceptive activity of hydroalcoholic extract of *S. khuzistanica* on formalin test in rats<sup>a</sup>**

Group	Dose (i.p., mg/kg)	Pawlicking time (s)	
		Phase (0-5 min)	Phase (15-60 min)
Control	2 (ml/kg)	71.23±7.45	321.31±46.62
SKHE	10	55.74±6.63	161.59±21.73***
SKHE	100	53.12±9.85	131.21±11.83***
SKHE	150	45.93±9.03	127.75±9.01***
Morphine	3	47.32±7.19	46.82±8.28***

<sup>a</sup>Values are mean±s.e.m (n=9), \*\*\* means that the difference between treated and control group is significant at  $P < 0.001$ .



**Figure 1: Effect of the *S. khuzistanica* hydroalcoholic extract (SKHE) on carrageenan-induced edema in rats.**

Values are means±s.e.m (n=9); \*\*\*  $P < 0.001$  compared with control group, \*  $P < 0.05$  compared with control group.

This table revealed also a significant difference ( $p < 0.001$ ) between all dose of SKHE and morphine with control group in second phase of formalin test. Never-

theless, at the same time there were a significant differences ( $p < 0.001$ ) between morphine and all dose of SKHE in this phase. Different dose effects in formalin test showed a dose-dependent manner over the range of 10-150 mg/kg ( $y = -0.2531x + 162.12$ ;  $r^2 = 0.9304$ ) especially in second phase of formalin test.

The result of carrageenan test (Fig 1) showed a significant difference ( $p < 0.001$ ) between both SKHE (150 mg./kg) and indomethacin (4 mg/kg) with control group. These results also revealed the similarity SKHE (150 mg/kg) with indomethacin effect ( $p > 0.05$ ).

In formalin test, the animals which received SKHE more than 200 mg/kg showed sleepy or some kind of immobility phenomena which interfere with the test.

## DISCUSSION

In this study, the anti-nociceptive and anti-inflammatory effects of SKHE were investigated. The formalin test is believed to represent a significant model of clinical pain and formalin produced a distinct biphasic response to pain stimulus and different analgesic compounds may act differently in the early and late phases of this test. The early phase is the result of direct chemical activation of nociceptive primary afferent fibers, while the factors that contribute the late phase are not well defined (9,10). Therefore, this test can be used to clarify the possible mechanisms of anti-nociceptive effect of a test compound (9). Centrally acting drugs such as opioids inhibit both phases equally (11) but, peripherally acting drugs, such as cyclooxygenase inhibitors (aspirin and indomethacin) and corticosteroids only inhibit the late phase (12-14).

The effect of SKHE on the first and second phases of formalin test suggests that its activity may be resulted from its peripheral action when compared with morphine activity in this respect to suggest any mediator merits further investigation.

Among the tests most widely used for the screening of new anti-inflammatory agents is the carrageenan-induced edema in the rat hind paw (14). This edema depends on the participation of kinins and polymorphonuclear leukocytes with their pro-inflammatory factors, including prostaglandins (16, 17). Isolation and purification of different fractions of the hydroalco-

holic extract and assaying anti-inflammatory/analgesic activity of each fraction was not goal of this study. Nevertheless, based on the results of this study, we can suggest that the anti-inflammatory effect of SKHE may be attributed to inhibition of prostaglandin release and similar mediators involved in this test (15-17). It may also be related partly to the presence of flavonoids, steroids, essential oil and tannin (3) that have been shown to exert analgesic effects in animal models of nociception (18,19).

Carvacrol is one of the most important components of many species including those belong to *Satureja* genus (20-23). This phenolic compound has shown antiseptic, antibacterial, antifungal as well as anti-nociceptive and anti-inflammatory properties in *Satureja* spp. (20,22,24-25).

Furthermore, humoral reactions resulting in the antioxidative action effect and anti-inflammatory activity of major constituents of this species may attribute to the anti-inflammatory effect of this plant. In any case, the consistent of the result of this study with indomethacin effect can take into account to confirm the traditionally use of this plant as an analgesic and anti-inflammatory agent.

Thus, it seems that the extract relieved pain through both central and peripheral mechanisms. Further studies are necessary to elucidate the exact mechanism behind its traditional effects.

#### ACKNOWLEDGMENTS

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