

Original Article

Use of *Satureja khuzestanica* Essential Oil (SKEO) in the Treatment of Diarrhea: Modes of Action on Intestinal Function in Animal Model

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Abstract

Background and Aim: *Satureja khuzestanica* is an annual herb that is used in Iranian traditional medicine to treat intestinal disorders such as abdominal pain and diarrhea. *Satureja khuzestanica* essential oil (SKEO) was tested to elucidate the mechanism(s) of its gastrointestinal activity in two animal models.

Materials and Methods: The in vitro test of the effect of SKEO on the contractions induced by different spasmogens in isolated rat ileum preparations was carried out. The impacts of SKEO on intestinal transit, diarrhea, and intestinal secretion in mice were investigated with the charcoal meal, castor oil-induced diarrhea, and entropooling assays.

Results: Pretreatment with SKEO at doses of (5-20 µg/mL) had a significant and concentration-dependent relaxant effect on contractions induced by acetylcholine, nicotine, histamine and serotonin in isolated rat ileum preparations. Moreover, it had an inhibitory effect on both K⁺- and Ca²⁺-induced contractions in the same tissue. At doses of 100 and 200 mg/kg, SKEO decreased intestinal transit, fecal weight and intestinal secretion.

Conclusion: SKEO showed anti-diarrheal, anti-motility and anti-secretory activities that might prove beneficial in the treatment of gastrointestinal disorders.

Keywords: Anti-spasmodic, Diarrhea, *Satureja khuzestanica*, Intestinal transit, Intestinal secretion, Rats

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Introduction

Certain illnesses such as abdominal cramps and diarrhea are treated almost exclusively by the use of traditional medicines in developing countries. Hence, it could be potentially useful to test alternative natural remedies to identify antispasmodic and antidiarrheal components with insignificant adverse

effects (if any) (1). *Satureja khuzestanica* is a plant endemic to and widely distributed throughout southern Iran. This annual herb is used in Iranian traditional medicine as a remedy for stomach disorders such as cramps, nausea, indigestion and diarrhea (1). The genus *Satureja* belongs to the family Lamiaceae, subfamily Nepetoidea. Carvacrol (95%) was found to be the main component of the oil (2).

Recent studies have indicated that *Satureja khuzestanica* is capable of having a variety of activities including antiparasitic (3), antioxidant (4), antidiabetic and antihyperlipidemic effects (5, 6). Moreover, this plant has been found to be effective against the inflammatory bowel disease (7), ulcerative colitis (8), and hemorrhagic cystitis (9). It is also efficient in pain relief (10), and the stimulation of reproductive functions without apparent toxicity or adverse effects (11).

Despite the extensive use of *Satureja khuzestanica* essential oil (SKEO) in Iranian traditional medicine for gastrointestinal problems, there are apparently no published studies concerning the antispasmodic and antidiarrheal effects of this plant either in humans or in animal models. The present study was undertaken with the aim of testing the antispasmodic and antidiarrheal properties of SKEO, and shedding light on the possible mechanism(s) of these activities. We tested the effect of SKEO on rat ileum contractility induced by different spasmogens in vitro, and its impact on diarrhea induced by castor oil in mice. The effects of SKEO on small intestinal transit and intestinal fluid accumulation were also evaluated in mice to shed light in the probable mechanisms of the anti-diarrheal effect of SKEO.

Materials and Methods

Plant Material

The aerial parts of the plant were collected during the flowering period from May to September, 2009 from Khorramabad in Lorestan province. The specimens were identified and authenticated by our team members, and voucher specimens were deposited (No. 58416) at the herbarium of Tehran University, Iran.

Preparation of the Essential Oil

The aerial parts were air dried at ambient temperature in the shade, and distilled in water with a Clevenger type apparatus for 5 h. The result was yellow oil at 0.9% yield, which was dried over anhydrous sodium sulfate, and stored at 4°C.

Animals

Adult male Wistar rats (Pasture Institute, Iran) weighing 200-250 g were used for ileum contractility tests, and male NMRI mice (Pasture Institute) weighing 25-30 g were used for anti-diarrheal tests

after 1 week of acclimation to housing conditions. The animals were housed in polypropylene cages in a temperature-controlled room ($22\pm2^\circ\text{C}$) with a humidity of 50% to 60% and a 12-h light-dark cycle (light on at 07:00). Food (normal laboratory chow) was withdrawn 12 h before the experiments, but free access to drinking water was allowed. There were seven or eight animals per group in each experiment. The procedures were performed in accordance with institutional guidelines for animal care and use.

Isolated Tissue Experiments

The rats were killed by a blow on the head followed by exsanguinations. A terminal portion of 3 cm after the 10 cm nearest to the ileocecal junction of the rat ileum was isolated and placed in oxygenated Tyrode's solution at room temperature. The connective tissue was carefully trimmed away, and isolated and cleaned preparations were placed vertically in a 50-mL thermostatically controlled organ bath at 37°C containing Tyrode's solution aerated with 95% O₂ and 5% CO₂. The force of contraction was recorded on a Narco-Biosystem physiograph equipped with a previously calibrated F60 isometric force transducer. The influence of the different doses of SKEO on the contraction induced by serotonin, acetylcholine, histamine and nicotine was studied in preparations pre-incubated for 1 min.

Determination of Calcium Antagonist Activity

To assess whether the spasmolytic activity of SKEO occurred via calcium channel blockade, K⁺ (50 mM) was used (12). The high-potassium solution was obtained by equimolar replacement of NaCl by KCl in Tyrode's solution, which produced sustained contraction. The SKEO was then added to the tissue bath in a cumulative fashion to obtain concentration-dependent inhibitory responses (13). Relaxation of the ileum preparations that had been precontracted with K⁺ (50 mM) was expressed as a percentage of the control response mediated by K⁺.

To confirm the calcium antagonist activity of SKEO, the ileum was allowed to stabilize in normal Tyrode's solution. This was then replaced with Ca²⁺-free Tyrode's solution, which in turn was replaced with K⁺-rich (50 mM), Ca²⁺-free Tyrode's solution. After an incubation period of 20 min and when the absence of spontaneous contractions of the ileum was confirmed, Ca²⁺ was added in a cumulative fashion (1×10^{-4} to

3×10^{-2} M) every 3 min to obtain control concentration-response curves for Ca^{2+} . The concentration-response curves for Ca^{2+} were repeated after 10 min of incubation with different doses of SKEO (5 to 20 $\mu\text{mol}/\text{mL}$).

Castor Oil-Induced Diarrhea in Mice

Diarrhea was induced in mice by the oral administration of castor oil (14). Briefly, mice were treated orally with SKEO (50, 100 or 200 mg/kg, 0.1 mL/10 g body weight) or vehicle (0.1 mL/10 g body weight) and atropine [0.1 mg/kg intraperitoneally (i.p.)]. After 30 min, a dose of 0.2 mL castor oil was administered to each animal by gavage, and the animals were immediately placed in individual cages in which the floor was lined with clean white filter paper. Food was removed to avoid the effects of food intake. Four hours later, the cages were individually inspected by an observer unaware of the treatment each animal had received. The total defecates for each animal were weighed (fecal output) and the presence of characteristic diarrheal droppings was checked. The percentage of fecal output (%FOP) was calculated with the formula $\% \text{FOP} = \text{ft/fc} \times 100$, where ft is the mean fecal weight of each treatment group and fc is the mean fecal weight of the control group. The collected feces were graded into three levels depending on consistency (diarrhea score) as follows: 0, normal feces; 1, swollen, moist feces; 2, wet, shapeless feces.

Intestinal Transit Measuring

The effect of SKEO on small intestinal transit was measured after a charcoal meal, as described by Rajan *et al* (14). Briefly, mice received an oral aqueous suspension containing 10% activated charcoal in 5% Arabic gum (0.3 mL/mouse). SKEO (50, 100 or 200 mg/kg, 0.1 mL/10 g body weight) were given orally and atropine (0.1 mg/kg) was given i.p. 15 min before the charcoal meal. After 20 min, the animals were killed and the gastrointestinal tract was rapidly and carefully removed and then laid out on filter paper. The distance traveled by the charcoal was assessed visually, and expressed as a percentage of the total length of the small intestine from the pyloric sphincter to the ileocecal junction of each animal.

Intestinal Fluid Accumulation

Intestinal secretion was indirectly analyzed with

entropooling assays (14). Briefly, the mice received SKEO (50, 100 or 200 mg/kg, 0.1 mL/10 g body weight) or carvacrol (200 mg/kg) orally, or and atropine (0.1 mg/kg) i.p., 40 min before the oral administration of castor oil (0.3 mL/mouse). The animals were killed after a further 30 min and the entire small intestine was removed and weighted. Tyrode's solution was made up in double-distilled water with the following composition in g/L: NaCl 8; KCl 0.2; MgSO₄ 7; H₂O 0.25; NaH₂PO₄.H₂O 0.05; glucose 1; NaHCO₃ 1; CaCl₂, 2H₂O 0.26 (pH 7.4). The KCl solution was made up as a 2M solution in double-distilled water. Acetylcholine chloride (10^{-9} - 10^{-1} M), histamine dihydrochloride (10^{-9} - 10^{-1} M), atropine sulphate, nicotine (10^{-9} - 10^{-1} M), and serotonin (10^{-9} - 10^{-1} M) solutions were made up in double-distilled water. All chemicals were purchased from Merck, drugs were purchased from Sigma and SKEO was prepared as described above. Contractions were measured as the maximum changes in tension from the pre-drug baseline during the period of exposure. The data were shown as the mean \pm standard error, and were analyzed by one-way ANOVA followed by the Tukey's test. The non-parametric data were analyzed by Chi-squared test. A p value <0.05 was considered significant.

Results and Discussion

Antispasmodic Effect of SKEO on Ileum

Pretreatment with SKEO obtained from the aerial parts of the plant, at doses from 5 to 20 $\mu\text{g}/\text{mL}$, reduced the contractions induced by acetylcholine, nicotine, histamine and serotonin in a concentration-dependent manner in isolated rat ileum preparations. The spasmolytic effects of SKEO were more pronounced in preparations contracted with histamine than with acetylcholine, serotonin or nicotine. The concentration-response curves to histamine in the presence of SKEO (5-20 $\mu\text{g}/\text{mL}$) were shifted to the right, and histamine-induced contractions were almost completely blocked with SKEO at a dose of 10, 20 $\mu\text{g}/\text{mL}$ even with higher doses of histamine.

At the same concentrations of SKEO (5-20 $\mu\text{g}/\text{mL}$), the concentration-response curves for acetylcholine, serotonin and nicotine also decreased. Compared to control assays, the percent reduction in the maximum response of SKEO (20 $\mu\text{g}/\text{mL}$) solution was

$77.91 \pm 3.89\%$ with acetylcholine, $72.93 \pm 2.32\%$ with histamine, $42.06 \pm 2.4\%$ with nicotine and $69.36 \pm 4\%$ with serotonin (Figure 1).

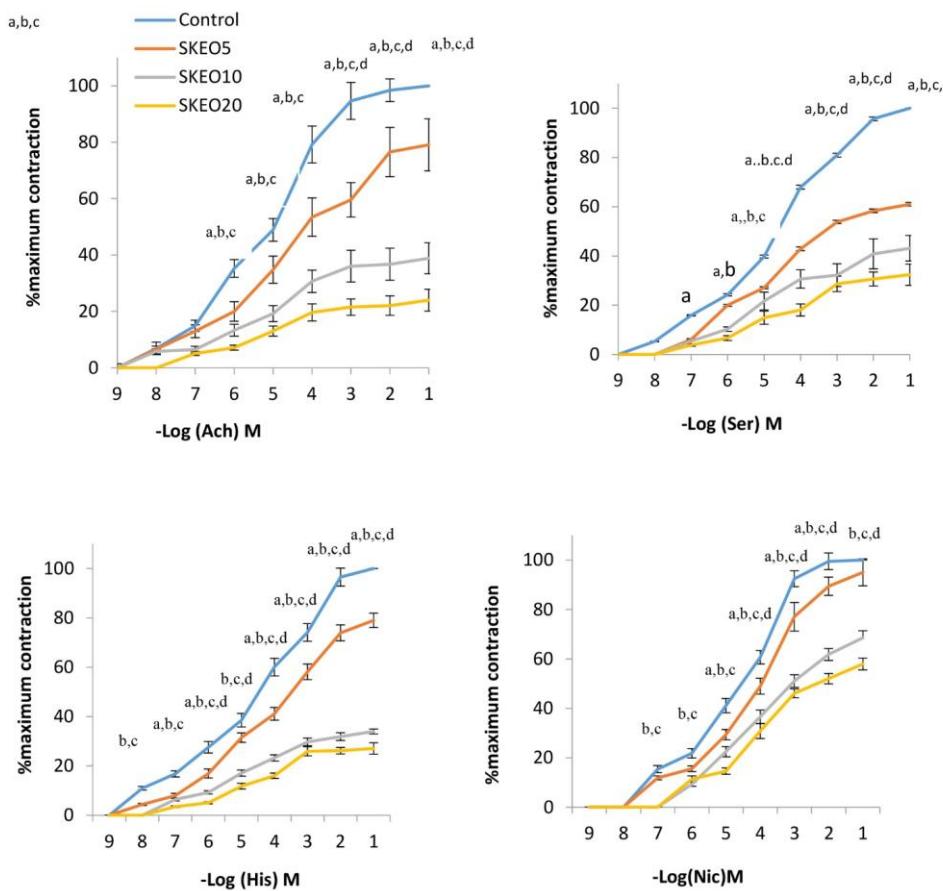


Figure 1. Concentration-response curves of the inhibitory effect of *Satureja khuzestanica* essential oil (EO) for acetylcholine (Ach), serotonin (Ser), histamine (His) and nicotine (Nic). Values shown are the mean \pm SE for $n=8$. ^aSignificant difference between control and SKEO 5,

^b Significant differences between control and SKEO10, ^c significant differences between control and SKEO20, ^d Significant differences between SKEO5 and SKEO10.

When tested in isolated rat ileum preparations, SKEO showed a spasmolytic effect in tissues pretreated with a high K^+ concentration (50 mM) with dose-dependent manner. In the $20\text{ }\mu\text{g/mL}$ bath, the response to KCl was completely abolished by SKEO. Adding Ca^{2+} to a high- K^+ preparation led to a contractile response. Under the same conditions, but in the presence of SKEO, the contractile response was diminished, with a shift to the right in the Ca^{2+} curves (Figure 2). Maximum inhibition (81.8% of maximum contraction in the control group) was obtained at an SKEO concentration of $20\text{ }\mu\text{g/mL}$.

Antidiarrheal Effect of SKEO

At concentrations of 100 and 200 mg/kg , SKEO reduced fecal output in mice by 22% and 73% , respectively. The reduction in fecal output by atropine

was 60% compared to the control group. Mean fecal weight correlated well with diarrhea score (Table 1).

Effect of SKEO on Gastrointestinal Transit and Entropooling

At a dose of 200 mg/kg , SKEO inhibited small intestine motility of the charcoal marker in mice by 59% . Treatment with atropine inhibited transit by 32% (Table 2). After pretreatment with SKEO (100 or 200 mg/kg) and atropine (0.1 mg/kg) reduced intestinal weight (Table 3).

In this study, the spasmolytic effect of SKEO was evaluated in ileum of rat. We demonstrated for the first time a non-selective spasmolytic effect of SKEO

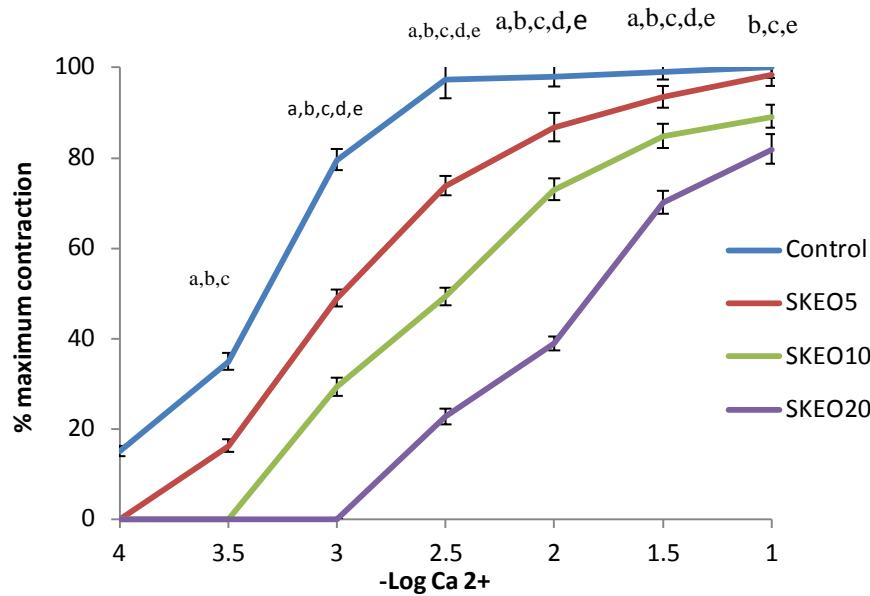


Figure 2. Effect of different concentration of *Satureja khuzestanica* essential oil on the cumulative concentration-response curve to CaCl_2 in isolated rat ileum preparations. Values shown are the mean \pm SE for $n=8$. ^a Significant difference between control and SKEO 5,
^b Significant differences between control and SKEO10, ^c Significant differences between control and SKEO20, ^d Significant differences between SKEO5and SKEO10, ^e Significant differences between SKEO10 and SKEO20.

Table 1: Effect of *Satureja khuzestanica* essential oil (SKEO) on fecal output in mice with castor oil-induced diarrhea Values shown are the mean \pm SE for $n=7$. ^a $p<0.05$, ^b $p<0.01$ compared to Tween 20 group.

Treatment	FOP	% FOP	Diarrhea score	(% protection)
Tween 20 (1%)	470.21 ± 163.43	100	2 ± 0	7/7 (0%)
Atropine (0.1 mg/kg)	188.84 ± 39.25^a	40 ^a	0.5 ± 0.2^b	1/7 (15%)
SKEO				
50 mg/kg	451.23 ± 112.36	95	1.8 ± 0.1	7/7 (0%)
100 mg/kg	369 ± 55.9	78	1.1 ± 0.2^a	5/7 (70%)
200 mg/kg	129.7 ± 29.78^b	27 ^b	0 ± 0^b	7/7 (100%)

FOP: Fecal Out Put

induced by various spasmogens on ileal contractions in a dose-dependent manner, including acetylcholine, nicotine, histamine and serotonin, shifting the curves to the right with E_{max} reduction and slope of reduction, suggesting a noncompetitive antagonism. The non-selective and noncompetitive spasmolytic effect of SKEO on ileum of rats does not involve receptor blockade. Hence, SKEO action could be explained by several mechanisms: i) stabilization of the muscle membrane, ii) interference with Ca^{2+} at one or more steps in the contraction sequence after membrane activation, iii) interference with the

normal function of the regulatory proteins (e.g., troponin and tropomyosin) involved in contraction and relaxation, or iv) inhibition of actomyosin ATPase, and the subsequent inhibition of chemo mechanical transduction. Haj hashemi *et al.* (2002) indicated the antispasmodic effect of *Satureja Hortensis* essential oil (SHEO), other species of *Satureja*, induced solely by acetylcholine. Nevertheless, the possible mechanism(s) of action were not addressed (15).

The oil also relaxed the ileum pre-contracted by KCl in a concentration-dependent manner. This effect was probably due to the inhibition of Ca^{2+} influx through

voltage-gated calcium channels (CaV) (17). However, other mechanisms are not discard.

Furthermore, our data demonstrate that SKEO had a marked antidiarrheal activity, which might be related

Table 2: Effect of *Satureja khuzestanica* essential oil(SKEO) on gastrointestinal transit and secretion in mice. Values shown are the mean \pm SE for n=7. ^ap<0.05, ^bp<0.01, compared to Tween 20 group.

Treatment	%Distance traveled by marker	Intestinal weight (mg/20 g body weight)
Tween 20 (1%)	72.6 \pm 14.42	1178 \pm 65.1
Atropine	49.28 \pm 11.84 ^a	905 \pm 54.8 ^a
SKEO		
50 mg/kg	78.2 \pm 10.2	1060 \pm 84.0
100 mg/kg	68.57 \pm 12.85	975 \pm 91
200 mg/kg	30.48 \pm 22.23 ^b	862 \pm 52 ^a

To confirm the interaction of the oil with voltage-dependent Ca²⁺ channels in this study, the tissue was pretreated with high-K⁺ solution. The contractions induced by KCl were inhibited by SKEO. The contractions induced by KCl are dependent on the entry of Ca²⁺ into the cells through voltage-dependent calcium channels. Hence, a substance which can inhibit high K⁺-induced contraction is considered to be a Ca²⁺ channel blocker (16). In this research, SKEO inhibited nicotine-induced contraction in a noncompetitive manner as an antagonist. Nicotine at low doses is an autonomic ganglion stimulant, and causes contraction by preganglionic acetylcholine release. This is followed by a postganglionic parasympathetic nerve action potential and the release of the cholinergic neurotransmitter at the neuromuscular junction (13). According to these findings, SKEO might inhibit the generation of action potentials or the release of the neurotransmitter acetylcholine.

In the present study, SKEO inhibited castor oil-induced diarrhea, small intestine secretion and small intestine motility of a charcoal marker in mice. Castor oil is reported to cause diarrhea via raising the volume of the intestinal contents, and preventing water reabsorption. In fact, ricinoleic acid induces irritation and inflammation of the intestinal mucosa, leading to the release of prostaglandin, which in turn stimulates secretion (17). At doses of 100 and 200 mg/kg, SKEO reduced intraluminal fluid accumulation, and significantly altered small intestinal secretion.

to the inhibition of muscle contractility and motility. This explanation is supported by the decreased intestinal transit of the charcoal meal and consequent reduction in intestinal propulsion.

Earlier studies demonstrated that nitric oxide (NO) might be involved in the diarrheal effect of castor oil. This oxide increases the permeability of the epithelial layer to calcium ions, leading to an increase in intracellular Ca²⁺ and enhanced calmodulin stimulation of NO synthase activity. Nitric oxide might also stimulate intestinal secretion (18). Amanlou et al. indicated that the water/alcohol extract of *Satureja khuzestanica* had an anti-inflammatory effect on edema in rats, which might be attributed to the inhibition of the release of prostaglandin and similar mediators shown to exert anti-inflammatory and analgesic effects in animal models (10).

Carvacrol is one of the most important components of *Satureja khuzestanica*, and had a strong anti-inflammatory effect on edema in rats as well as an inhibitory effect on prostaglandin biosynthesis. Nitric oxide and prostaglandins are known to be crucial mediators contributing to the inflammatory response to castor oil. Alternatively, the effect of castor oil might be attributed to disordered motility, and consequently to an increase in intestinal transit of intraluminal material. In this connection, castor oil might alter the coordination of intestinal motility and promote greater fluid loss from the intestine (19).

The antidiarrheal effect of SKEO might also appear through its antimicrobial activity. The antimicrobial activity of *Satureja khuzestanica* extracts has already

been reported (11). This effect is likely to contribute to the antidiarrheal properties of the plant during infective diarrhea.

Conclusion

It appears that *C. longa* extract is an effective anti-In conclusion; the present study indicated that the use of SKEO had anti-diarrheal, anti-motility and anti-secretory activities, which might prove beneficial in the treatment of gastrointestinal disorders. Further experiments are required to elucidate the cellular mechanism(s) involved in the anti-diarrheal properties of SKEO, and to identify the active principle(s) responsible for this effect.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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