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Original Research

Phytochemical Properties and Antidepressant Potential of *Satureja khuzestanica* Jamzad Essential Oil in Mouse Models of Depression

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Abstract

Oxidative stress may play a pivotal role in the pathogenesis of depression. Furthermore, antioxidants are also believed to have antidepressant properties. Previous studies have reported the antioxidant effects of *Satureja khuzestanica* Jamzad. Therefore, this study examined the antidepressant potential of *Satureja khuzestanica* essential oil (SKEO) in male mice based on a forced swim test (FST) and tail suspension test (TST). The GC-MS was used to evaluate the phytochemistry of SKEO. In behavioral studies, 72 male mice were allocated to twelve groups of six and intraperitoneally received the vehicle (10 mL/kg), fluoxetine (20 mg/kg), imipramine (30 mg/kg), or SKEO (25, 50, and 100 mg/kg). Immobility time in TST and immobility, swimming, and climbing times in FST were measured. In the open-field test (OFT), the number of crossings and rearings was recorded. According to GC-MS results, carvacrol, γ-terpinene, cymene, and 2-pinene were the most abundant compounds in SKEO. In FST and TST, all doses of SKEO (except for 25 mg/kg in FST), fluoxetine, and imipramine reduced the immobility time compared to the control group. Moreover, 50 and 100 mg/kg doses of SKEO and fluoxetine increased the swimming time without significantly changing the climbing time. However, imipramine increased the climbing time without significantly changing the swimming time. None of SKEO doses caused a significant change in the number of crossings or rearings in OFT. According to our findings, the antidepressant-like effects of SKEO are similar to those of fluoxetine. While the compounds in SKEO seem to induce their effects through the serotonergic mechanism, further studies are warranted to clarify their exact mechanism of action.

Keywords: Phytochemical; Antidepressant; *Satureja khuzestanica*; Mouse models

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Introduction

Depression is a common mental disorder with critical impacts on the quality of life of patients. According to available statistics, the frequency of this condition has been increasing throughout the world in the recent decades [1,2]. Various studies have shown the significant role of the monoaminergic system in the pathophysiology of depression [3,4]. Moreover, common antidepressants such as tricyclic antidepressants (e.g., imipramine) and selective serotonin reuptake inhibitors (SSRIs e.g., fluoxetine) work by increasing brain monoamines like noradrenaline (NA) and serotonin (5HT) [5]. While SSRIs have been the treatment of choice in patients with depression for 30 years, they are less effective or even ineffective in two-thirds of the patients [6]. Furthermore, their application might be limited due to the occurrence of some complications such as anxiety, tachycardia, tremor, sedation, insomnia, serotonin syndrome, parkinsonism, postural hypotension, and blurred vision [7]. Therefore, it is essential to find new antidepressants with fewer side effects. Plant extracts or essential oils may be an ideal choice for the treatment of mental disorders because they have shown therapeutic effects in a wide range of animal models [8]. In this regard, the antidepressant-like activity of certain plants such as *Citrus sinensis* L., *Canarium resiniferum* Bruce ex King, *Plinia trunciflora* (O.Berg) Kausel, *Mentha x piperita* L., *Satureja bachtiarica* Bunge and Satureja hortensis L. have previously been demonstrated [9-14].

Satureja khuzestanica Jamzad (SK), native to and widely distributed in the southern regions of Iran, belongs to the Lamiaceae family. In traditional medicine, SK is believed to posses antispasmodic, analgesic, anti-inflammatory, antithyroid, antibacterial, antiseptic, diuretic, and appetizing properties [15]. In recent years, the antihypercholesterolemic, antidiabetic, antibacterial, antioxidant, and anti-inflammatory effects of SK have also been reported [16-18]. Antioxidants are also known to have antidepressant properties [19]. On the other hand, synthetic antidepressant agents (e.g. fluoxetine) [20] and herbal remedies could also induce their therapeutic effects by suppressing the production of reactive oxygen species (ROS) or rescuing the antioxidant defense [10-14]. Furthermore, carvacrol, a main component of SK essential oil (SKEO), has been reported to have antidepressant effects in forced swim test (FST) and tail suspension test (TST) as two common mouse models of depression [21].

FST is sensitive to the effects of most antidepressants affecting the serotonergic and noradrenergic systems [22]. TST is less stressful and has more pharmacological sensitivity compared to FST [23]. The open-field test (OFT) is also used to estimate the locomotor activity of rodents [24].

A study reported that SKEO contains the largest amount of carvacrol and other monoterpenes [25]. However, the antidepressant-like effect of SKEO has not been investigated, which encouraged us to study the effects of SKEO on depression for the first time. Hence, this study aimed to investigate the phytochemical and antidepressant properties of SKEO by using FST and TST in male mice.

Methods

Preparation of essential oil and drugs

This study used imipramine hydrochloride (Marhamdaru, Iran) and fluoxetine hydrochloride (Jalinous, Iran), and commercially available SKEO (Tabib Daroo Company, Kashan, Iran; batch number TBQ99). The animals received intraperitoneal (i.p.) injections of 10 mL/kg of the drugs or SKEO. The control or vehicle group received a combination of the Tween 80 (5%) and normal saline.

Gas chromatography-mass spectrometry (GC-MS)

The Agilent gas chromatograph GC-MS 5975,7890 (Agilent, USA) equipped with an MS detector and a capillary column (H5-5MS) was used in this study. The column length, inner diameter, and layer thickness of the device were 30 m, 0.25 mm, and 0.5 µm, respectively. The injection volume was 1 µL and the carrier gas was helium with a flow rate of 1 mm/minute. The injection chamber temperature was 260 °C. SKEO components were identified using Kovats retention index. MS was performed according to standard compounds and Wiley Library guidelines [26].

Animals

This experimental study used eight-week-old male NMRI mice. The animals were obtained from Urmia University of Medical Sciences, Urmia, Iran and weighed 20-30 g. They were kept in separate cages under standard laboratory conditions (22 ± 2 °C, 12:12 light-dark cycle, and 50% humidity). During this time, they were provided with commercial food and water. All experiments were performed during the light period. All ethical principles in the treatment of laboratory animals were observed according to the code of ethics provided by the Ethics Committee of the Islamic Azad University of Urmia (Ethics Code: IR.IAU.URMIA.REC.1400.002).

Forced swim test (FST)

In this test, immobility time was recorded as depression and its reduction indicated an antidepressant effect in mice. After receiving the drugs or SKEO, the animals were individually placed in a glass container (8 × 12 × 25 cm) containing 25 °C water. The test took six minutes but during the first two minutes, the

animals were allowed to adapt to the environment. A chronometer (Citizen, Japan) was used to measure the immobility time, swimming time, and climbing time (in seconds) in the last four minutes of the test [22].

Tail suspension test (TST)

In TST, 70 cm metal bars were vertically fixed and connected with a 50 cm string. The mouse's tail was fixed to the string with a tape and the test began by striking the animal. The animal first tried to escape but ultimately became completely immobile, passive, and unresponsive. The immobility time (in seconds) was recorded with a chronometer. Similar to FST, the TST lasted for six minutes but the immobility time was recorded during the last four minutes as the animal was allowed to adapt to the environment during the first two minutes [23].

Open-field test (OFT)

OFT is used to evaluate locomotor activities and measures the number of square crossings and rearing. The test lasted for five minutes but the first minute was for the animal to adapt to the environment. Animal behaviors were thus monitored in the last four minutes and the mentioned behaviors were measured using a counter [27].

Animal grouping and experimental design

In order to perform FST and TST, a total of 72 mice were randomly allocated to twelve groups of six as follows:

Groups 1 and 2 (negative controls or vehicle): The 12 mice in these two groups received the vehicle (10 mL/kg) separately for both tests.

Groups 3-6 (positive controls): The 24 mice in these four groups received fluoxetine (20 mg/kg) and imipramine (30 mg/kg) separately for both tests.

Groups 7-12 (SKEO): The 36 mice in these groups separately received different doses of SKEO (25, 50, and 100 mg/kg) for both tests.

In all groups, FST and TST were performed 30 minutes after receiving the vehicle, drugs, or SKEO, and animal behaviors were recorded by a person blinded to the group classifications. To use fewer animals (mice used in FST), OFT was performed five minutes before FST (Figure 1).

All doses of SKEO and drugs were determined based on previous studies and available references [12,29].

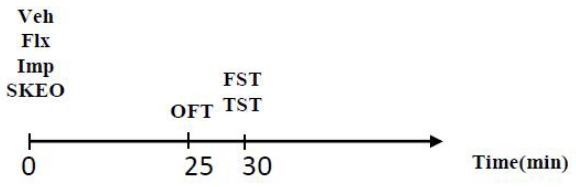

Figure 1. Shematic diagram of experimental desigen Imp,Imipramine;Flx,Flouxetine;FST;Forced swimming test; OFT.Open filed test;SLEO, *Satureja Khuzestanica* essential oil;TST,Tail suspension test;Veh,Vehicle.

Data analysis

One-way analysis of variance (ANOVA) and subsequent Newman-Keuls test were conducted and the mean ± SD values for each group were reported. GraphPad Prism 9 (GraphPad Software, San Diego, CA,

Table1. GC/MS analysis of SKEO (*Satureja Khuzestanica* Jamzad essential oil)

No	Compound	RT (min)	Percentage(%)
1	A-Thujene	6.435	0.47
2	**2-Pinene**	**6.682**	**3.35**
3	Camphene	7.059	0.16
4	β-Pinene	8.024	0.59
5	β -Myrcene	8.653	0.96
6	l-Phellandrene	9.124	1.51
7	α-Terpinene	9.659	1.72
8	**Cymene**	**9.984**	**24.10**
9	β -Phellandrene	10.126	0.49
10	Limonene	10.22	1.92
11	**γ-Terpinene**	**11.63**	**26.96**
12	β -Phellandrene	12.852	0.06
13	Isoborneol	15.729	0.18
14	p-Menth-1-en-4-ol	16.296	0.29
15	Carvol	19.32	0.56
16	l-Carvone	19.472	0.61
17	Thymol	22.34	1.59
18	**Carvacrol**	**23.1**	**31.18**
19	2,4,6-Trimethylanisole	24.567	0.09
20	trans-Caryophyllene	27.45	0.45
21	Zingiberene	28.184	0.08
22	Ledene	30.113	0.06
23	β -Bisabolene	30.669	0.60
24	δ -Cadinene	30.989	0.06
25	(+) spathulenol	32.467	0.12
26	Caryophyllene oxide	32.551	0.26
27	Dillapiole	33.657	0.32
28	Rosefuran	42.096	0.29
29	P-Cymen-α-Ol	44.319	0.09
30	Phenol, 2,3,5,6-tetramethyl-	45.09	0.31
31	Isodurenol	46.647	0.22
Total			**99.66**

USA) was used to analyze the data and plot the charts. P values less than 0.05 were considered significant.

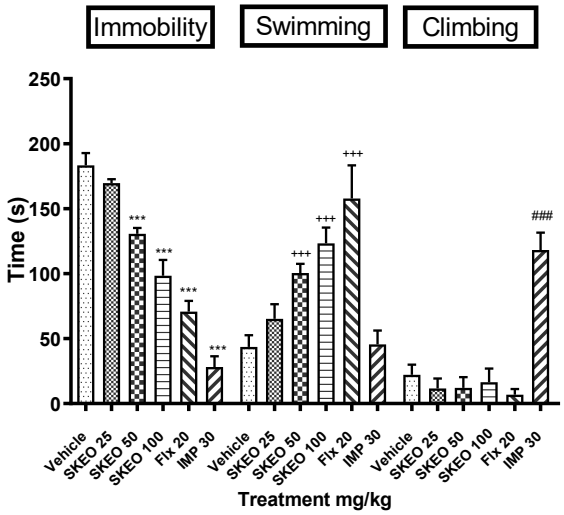

Figure 2. The effects of SKEO (*Satureja Khuzestanica* Jamzad essential), Flx(fluoxetine) and IMP (imipramine) on the mean immobility, swimming and climbing times in FST. Data are presented as mean± S.D. (n=6) mice/group. ***, +++, and ### show significant differences between the vehicle (control) group at P<0.001, P<0.001 and P<0.001 in immobility time, swimming time, and climbing time, respectively. Results were analyzed by one-way ANOVA followed by Newman-Keuls test.

Table 2. The effects of SKEO (Satureja Khuzestanica Jamzad essential oil) on the number of crossings and rearing's in OFT.

Group	Dose	Number of crossings	Number of rearing's
Vehicle	10 ml/kg	35.33 ± 6.37	10.67 ± 2.09
SKEO	25 mg/kg	35.67 ± 4.95	16.83 ± 4.17
SKEO	50 mg/kg	54.83 ± 3.65	23.68 ± 3.48
SKEO	100 mg/kg	52.00 ± 6.04	21.33 ± 4.90

Data are presented as mean± S.D. (n=6) mice/group.

control (vehicle) group ($F_{5,30}$ = 315.3, p <0.0001). These doses of SKEO also increased swimming time compared to the control group ($F_{5,30}$ = 68.83, p <0.0001). However, no significant increase was observed in climbing time following the administration of SKEO at any dose (P >0.05). Both fluoxetine and imipramine reduced immobility time compared to the control group (p <0.0001 for both). While fluoxetine only caused a significant increase in swimming time (p <0.0001), imipramine only caused a significant increase in climbing time (p <0.0001). Therefore, the antidepressant effects of different doses of SKEO are more similar to fluoxetine.

Results

GC-MS

The analysis of SKEO compounds showed the presence of carvacrol (31.18%), γ-terpinene (26.96%), cymene (24.10%), and 2-pinene (3.35%). Other compounds had concentrations below 2% and were not, hence, included (Table 1).

Effects of SKEO on immobility time, swimming time, and climbing time in FST

As seen in figure 2, only 50 and 100 mg/kg doses of SKEO reduced immobility time compared to the

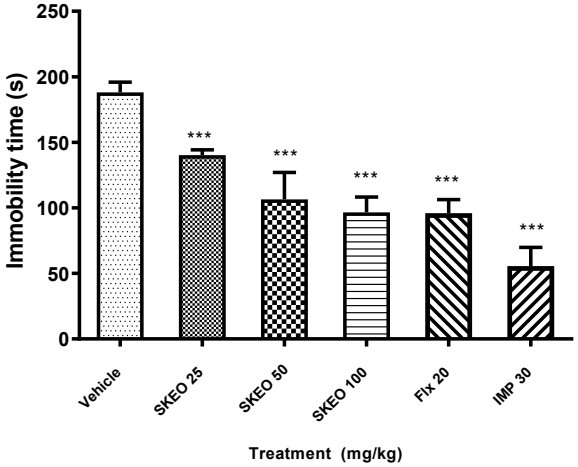

Figure 3. The effects of SKEO (Satureja Khuzestanica Jamzad essential), Flx(fluoxetine) and IMP (imipramine) on the mean immobility time in TST. Data are presented as mean± S.D. (n=6) mice/group. *** show significant differences between the vehicle (control) group at P<0.001 in immobility time. Results were analyzed by one-way ANOVA followed by Newman-Keuls test.

Effects of SKEO on immobility time in TST

According to figure 3, all three doses of SKEO (25, 50, and 100 mg/kg) reduced immobility time compared to the control (veheicle) group ($F_{5,30}$ = 77.82, p <0.0001). Moreover, fluoxetine and imipramine, as two standard drugs, decreased immobility time compared to the control group (p <0.0001, for both).

Effects of SKEO on the number of square crossing and rearing in OFT

As table 2 shows, none of the different doses of SKEO significantly changed the number of square crossings or rearings compared to the control group (P >0.05).

Discussion

The results of this study showed high concentrations

of carvacrol, γ-terpinene, cymene, and 2-pinene in SKEO. Similar findings were reported by previous research [18,25,29]. Behavioral tests showed that all doses of SKEO (except for 25 mg/kg in FST), as well as fluoxetine and imipramine, reduced immobility time compared to the control group. Moreover, 50 and 100 mg doses of SKEO and fluoxetine increased the swimming time but did not significantly change the climbing time. Conversely, imipramine increased the climbing time without significantly changing the swimming time in FST.

In line with our findings, previous studies reported that selective norepinephrine reuptake inhibitors (such as imipramine) significantly decrease the immobility time and increase the climbing time [9,30]. On the other hand, SSRIs (e.g., fluoxetine) significantly reduce the immobility time and increase the swimming time, but fail to increase the climbing time. In other words, while swimming is sensitive to serotonergic antidepressants (e.g., fluoxetine), climbing is sensitive to tricyclic antidepressants or selective inhibitors of the noradrenergic system (e.g., imipramine) [9,31,32]. Our findings confirmed the fluoxetine-like effects of SKEO. That is, SKEO also caused a significant decrease in immobility time and a significant increase in swimming time; while the climbing behavior did not show a significant change. All these findings are completely consistent with the results of the present study. According to the OFT results, none of the SKEO doses significantly changed the number of square crossings or rearings. OFT was used because research has shown that some compounds, such as antihistamines and psychotropic stimulants may cause a false positive FST result. This finding is completely consistent with other studies indicating the ineffectiveness of antidepressants on animals' performance in this test [33,34].

Although our findings cannot clarify the exact mechanism of SKEO's effects, they highlighted the beneficial effects of SKEO in acute models of depression. However, we failed to evaluate the effects of SKEO on chronic depression and determine the antidepressant effects of each SKEO component. Nonetheless, a simillar study demomnstrated the anxiolytic and antidepressant-like effect (400 mg/kg) of hydroalcoholic extract of Satureja hortensis (from the Lamiaceae family) in rats exposed to chronic restraint stress [14]. The GC-MS results introduced carvacrol as the most important compound in SKEO. Carvacrol is a monoterpenoid phenolic compound with various biological and pharmacological properties including antibacterial, antioxidant, and anticancer effects [35]. Previous studies have reported the antidepressant effects of carvacrol and emphasized the role of the dopaminergic system in such effects. Carvacrol has been found to decrease immobility time and induce antidepressant effects in both FST and TST. However, the compound could not significantly change the number crossings or rearings in OFT [24]. Zotti et al. administered carvacrol for seven consecutive days and conducted FSO. They found that carvacrol increased serotonin and even dopamine levels in the frontal cortex and hippocampus [36]. Morover, a study demonstated that rosmarinic acid and carvacrol are the main components of Satureja montana extract. They showed rosmarinic acid and carvacrol induced their anxiolytic effect through serotonergic and GABAergic systems[37]. This is consistent with our findings and confirms the role of the serotonergic system in the antidepressant-like effects of SKEO.

In addition to carvacrol, other monoterpenes, including γ-terpinene, cymene, and 2-pinene, were also found in large quantities in SKEO. Previous research has documented the antidepressant properties of monoterpenes. The antidepressant effects of monoterpenes, such as linalool and β-pinene, have been attributed to the monoaminergic mechanism [38]. The results of another study showed that R-limonene, γ-terpinene and citral as major components of lemon essential oil increase the release of monoamines from rat brain slices [39].

In one of our previous studies, the crude extract of *Mentha* x *piperita* (other Lamiaceae familiy), exerts its antidepressant-like activity, at least in part, via serotonergic pathways. In support of this view, pre-treatments of animals with WAY100135 (selective 5-HT_{1A} receptor antagonist), ketanserin (a selective $5HT2_{A/C}$ receptor antagonist) and p-chlorophenylalanine (pCPA) (an inhibitor of serotonin synthesis) blocked the antidepressant-like effect of the *M. piperita* extract (400 mg/kg, i.p.) in FST [12].

Apart from the role of monoaminergic system, oxidative stress may play a pivotal role in the pathogenesis of depression. Accordingly, the use of antioxidants is a new target for the treatment of neuropsychiatric disorders including depression [40,41]. In patients with depression, the levels of antioxidants (e.g. vitamin E) and antioxditvie enzemes [e.g. catalase and superoxide dismutase (SOD)] were decresesd. In addition, high levels of ROS may also affect the functions of serotonergic receptors [42]. Antioxidants are also believed to inhibit serotonin reuptake (5-HT) and induce antidepressant effects [43,44]. Hence, antidepressants normalizes the levels of SOD and nurotransmitters (e.g serotonin) in patients with depression. Previous studies have also described the in vitro and in vivo antioxidant properties of SKEO [17,45].

All these findings confirm the results of the present study and the role of the serotonergic system in the antidepressant effects of SKEO. Hence, SKEO compounds would induce antidepressant effects via the serotonergic mechanism.

Conclusion

According to our findings, the antidepressant-like effects of SKEO are similar to those of fluoxetine. However, further studies are warranted to understand the exact mechanism involved.

Conflict of Interests

Authors have no conflict of interests.

Acknowledgments

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