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RESEARCH PAPER

Chemical composition, acute and sub-acute toxicity of *Satureja khuzestanica* essential oil in mice

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ABSTRACT

To date, herbal medicines are the important source in the development of new drugs to remedy various diseases. However, it is crucial to provide scientific justification of these drugs to determine their side effects in treatments. This study was aimed to evaluate the chemical composition, acute and sub-acute toxicity of *Satureja khuzestanica* essential oil (SKEO) in a mice model. Plant materials of *S. khuzestanica* were collected from the rural regions of Lorestan Province, west of Iran. Gas chromatography/mass spectrometry (GC/MS) analysis was performed to determine the main components of SKEO. To assess the acute toxicity, 0.5, 1, 2 and 4 ml/kg doses of SKEO were injected intraperitoneally into four groups of six mice. The number of deaths was counted at 24 h after the treatment. Sub-acute toxicity of the effects of SKEO was evaluated by determine the clinical chemistry and hematological parameters of the treated mice after oral administration of SKEO at the doses of 0.2, 0.4 and 0.6 ml/kg, respectively, for 14 consecutive days. Twenty-three compounds were identified in SKEO by GC/MS analysis, in which the main component was carvacrol (64.4%). The LD50 value of intraperitoneal injection of SKEO was 1.79 ml/kg and the maximum nonfatal dose was 1.13 ml/kg. No death was observed at the doses of 0.2 and 0.4 ml/kg, while in the group receiving 0.8 ml/kg of SKEO, only one mouse died (12.5%). There was no significant difference between the biochemical and hematological parameters following the oral administrations of SKEO at the used doses of 0.2, 0.4 and 0.8 ml/kg and the controls ($P>0.05$). The obtained results in this work showed that SKEO at the tested doses had no significant toxicity on the liver and kidney tissues as well as on the hematological parameters in the mice. Therefore, SKEO could be safe for the mammalian host at the used doses.

Keywords: GC/MS; Acute and sub-acute toxicity; *Satureja khuzestanica* essential oil; Mice

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Introduction

Since the last centuries, traditional remedy serves people in all countries around the world.

The employ of herbal medicines is generally widespread for the avoidance and treatment of a wide spectrum of diseases such as infectious ones in addition to maintaining the public health (1). Hence, a broad range of medicinal plants is accessible and it is essential to give scientific validation for these drugs for identifying their side effects in treatments (2, 3). One of these attractive herbs is *Satureja khuzestanica* Jamzad (family Lamiaceae). *S. khuzestanica* (SK) is a remedial plant that is frequently grown in various parts of Iran such as Lorestan Province, West of Iran (4). Different parts of SK such as root, leaves, and branches have been broadly applied as analgesic, anti-diarrhea, and antiseptic in the folk medicine (5). Furthermore, a range of pharmacological activities including antioxidant, anti-inflammatory, hepatoprotective and antimicrobial have been related to this plant (5-11).

Essential oils are originating in aromatic plants and have volatile fractions that are responsible for biological activity, smell, and taste (12). Reviews have reported that the main constituents of *S. khuzestanica* essential oil (SKEO) in the majority species are phenols, carvacrol, thymol, *p*-cymene, b-caryophyllene, linalool, monoterpenes, sesquiterpenes, alcohols and phenolic acids (7, 12). On the other hand, it has been previously proven that the chemical composition of essential oils is directly depended on some factors such as the geographical source and harvesting time (13, 14). Previous investigations on laboratory animals have demonstrated that the liver and kidney are the key target organs in the evaluation of toxicity of drugs (15). Damage to the constructional entirety of the liver is evaluated by the elevated serum levels of some important enzymes for example alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin. On the other hand, the creatinine blood test (Cr) and blood urea nitrogen (BUN) are used to evaluate the function of kidneys.

To the best knowledge of the authors, there is no study on the toxicity effects of SKEO. Therefore, the present study was aimed to evaluate the chemical composition, acute and sub-acute toxicity of SKEO in the mice model by assessing some biochemical and hematological parameters.

Materials and methods

Plant materials

Plant materials (aerial parts) of SK were collected from rural regions of Lorestan Province, Western Iran, in May 2015. They were identified by a botanist of the Botany Department of Lorestan University, Khorramabad, Iran. Voucher specimens have been deposited in the herbarium of Research Center for Agriculture Sciences, Khorramabad, Iran (No.3215).

Isolation of the essential oil

Air-dried plant material was subjected to hydrodistillation for 3 h using an all-glass Clevenger type apparatus. The obtained essential oil was dried over anhydrous sodium sulfate and stored in darkness at 4°C until testing (16).

Gas chromatography/mass spectrometry (GC/MS)

GC analysis

GC analysis was carried out by Hewlett-Packard 6890 (Hewlett-Packard, Palo Alto, CA) with a HP-5MS column (30 m×0.25mm, film thickness 0.25 mm). The column temperature was maintained at 55°C for 3 min, programmed to 180°C in the rate of 5°C per min, and kept constant at 220°C for 5 min. The injector and interface temperatures were 220 and 290°C, respectively. The flow rate of helium as a carrier gas was (1 mL/ min C.F). The percentageswere calculated by the electronic integration of FID peak areas without using response factor correction. Linear retention indices for all the components were determined by the co- injection of the samples with a solution containing homologous series of C8-C22 n-alkanes

GC/MS analysis

GC/MS analysis was performed using a Thermoquest-Finnigan gas chromatograph equipped with fused silica capillary DB-5 column (30 m×0.25 mm, film thickness 0.25 mm) coupled with a TRACE mass (CAS, Manchester, UK). Helium was used as the carrier gas with an ionization voltage of 70 eV. Ion source and interface temperatures were 220 and 290°C, respectively. Mass range was from 40 to 400 u. Temperature program of the oven was as mentioned above for the GC.

Identifying the essential oil components

The components of the essential oil were identified by comparing their retention indices and mass spectra with the standards, Wiley 2001 library data of the GC/ MS system, or those reported in the literature data (17).

Animals

Sixty-four male NMRI mice (3-4 months old, 30-35 g) were obtained from the Animal Breeding Stock Facility of Razi Institute of Iran (Karaj, Iran). Animals were housed in a colony room with a 12:12 h light/ dark cycle at 21 ± 2°C and were handled according to standard protocols for the use of laboratory animals.

Acute toxicity

To determine the acute toxicity, various doses of SKEO (0.5, 1, 2, and 4 ml/kg) were administrated as orally into four groups of six mice. The number of deaths was counted at 24 h after treatment. LD50 values were determined by the Probit test in SPSS software (18).

Sub-acute toxicity

Sub-acute toxicity of SEKO was evaluated by determination of clinical chemistry and hematological parameters of treated

mice after orally administration SKEO at the doses of 0.2, 0.4, and 0.6 ml/kg, for 14 consecutive days. Forty mice were randomly divided into five groups with 8 mice in each group.

Group 1 was administrated normal saline orally (orogastric gavage).

Groups 2-4 were orally administrated SKEO at the doses of 0.2, 0.4, and 0.6 ml/kg, respectively, for 14 consecutive days.

Group 5 did not receive any drug.

After two weeks, animals were fasted overnight and anesthetized using Ketamine (100 mg/kg) and Xylazine (10 mg/kg) combination. Sodium pentobarbital (70 mg/kg, ip.) was used as euthanasia agent and then the abdomen was opened, and blood samples were obtained from the heart. In order to assess the hematological parametrs, total blood was collected into tubes containing ethylene diamine tetraacetic acid (EDTA) anticoagulant, and hematological parameters, including hemoglobin (HGB), hematocrit (Hct), white blood cell (WBC) counts, red blood cell (RBC) counts, and platelet (PLT) counts were measured. To measure clinical chemistry parameters in serum, blood was collected into tubes containing no anticoagulant, allowed to clot, and serum was separated by centrifugation at 2000*g* for 20 min. The assays of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatinine (Cr), blood urea nitrogen (BUN), and bilirubin (direct and total), were performed using Roche diagnostics kits (Mannheim, Germany) (19, 20).

Ethical approval

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Lorestan University of Medical Science (Permit Number: 91/27, 2013).

Statistical analysis

Obtained results are expressed as the mean ± SEM. Data analysis was carried out by using SPSS statistical package version 17.0 (SPSS Inc., Chicago, IL, USA). One-way ANOVA with Tukey's post-hoc test was used to assess differences between experimental groups. In addition, $p<0.05$ was considered statistically significant.

Results

GC/MS analysis of SKEO

The major components of SKEO were found by GC/MS analysis. Table 1 indicates the obtained findings by GC/MS

Table 1. Essential oil composition of *S. khuzestanica* identified by GC-MS

No	Components	RT	% Composition
1.	β-Myrcene	9.47	0.1
2.	1-Phellandrene	9.89	0.2
3.	α-Terpinene	10.31	0.2
4.	ρ -Cymene	10.41	0.8
5.	Limonene	10.73	0.2
6.	γ- Terpinene	11.69	0.3
7.	α- Terpineolene	12.67	0.2
8.	Linalool	12.88	0.5
9.	Borneol	14.85	0.5
10.	Terpinene-4-ol	15.27	0.9
11.	α- Terpineolene	15.86	0.3
12.	Thymol	18.53	7.5
13.	Carvacrol	18.96	64.4
14.	Carvone	19.16	20.2
15.	3-Methyl-4-isopropylphenol	19.36	0.2
16.	Eugenol acetate	20.16	0.2
17.	β-Caryophyllene	22.43	0.12
18.	Geranyl aceton	22.78	0.1
19.	β-Bisabolene	24.57	1.2
20.	α-Bisabolene	25.23	1.2
21.	Cis-Alpha-Bisabolene	25.32	0.2
22.	Isoterpinolene	26.00	0.13
23.	3-Ethyl-2,5-dimethylpyrazine	26.42	0.07
	Total		99.74

a Retention indices on non-polar DB-5 ms column in reference to n-alkanes

analysis of SKEO. Twenty-three compounds were identified, which indicated 99.74% of the total oil. The main components were carvacrol (64.4%), carvone (20.2%) and thymol (7.5%), respectively.

Acute toxicity

Acute toxicity effects of SKEO were evaluated on the male NMRI mice. The LD50 value of oral administration of SKEO was 1.79 ml/kg and the maximum nonfatal dose was 1.13 ml/kg.

Clinical chemistry and hematological parameters

In accordance with the acquired results of LD50, the doses of 0.2, 0.4 and 0.6 ml/kg were chosen to evaluating the sub-acute toxicity of SKEO. There is no death at the doses of 0.2 and 0.4 ml/kg, while in the group receiving 0.6 ml/kg of SKEO, one mouse died (12.5%). As shown in Tables 2 and 3, no significant difference (*P>0.05*) was observed between the biochemical and hematological parameters subsequently the oral administrations of SKEO at the used doses of 0.2, 0.4 and 0.6 ml/kg and the control.

Discussion

A rising number of people are using plants and their derivates for protective and curative purposes around the world. Approximately, twenty percent of all the plants are applied for therapeutic use, whereas nearly 10% of them are applied for commercial goals. Moreover, natural products are measured as a key resource in the pharmaceutical industry and probing for new possible sources of bioactive molecules (1, 2). This study was designed to evaluate the acute and sub-acute toxicity of SKEO in the mice model by assessing some chemical and hematological parameters. Liver function tests which are indicator of enzymes function are a collection of blood experiments that recognize inflammation and injury including hepatitis and cirrhosis to the liver. BUN examination is mainly applied in company with the creatinine test to assess the kidney function in a broad spectrum of conditions, to make possible diagnosing kidney diseases and to monitor people with acute or chronic renal dysfunction or failure (21). The obtained findings demonstrated that the LD50 value of intraperitoneal injection of the SKEO was 1.79 ml/kg and the maximum nonfatal dose was 1.13 ml/kg body weight. We found no significant difference (*P>0.05*) between the biochemical parameters of ALT, AST, ALP, and bilirubin for assessment the liver function as well as Cr and BUN for renal function, following the oral administrations of SKEO at the used doses of 0.2, 0.4 and 0.6 ml/kg, and the control.

In line with our results, Abdollahi *et al.* (2003) demonstrated that the oral administration of SKEO to the rats induced the noticeable antioxidant, antidiabetic, antihyperlipidemic and duplicate stimulatory effects without the incidence of any toxic or unfavorable effects (22). Assaei *et al.* (2014) demonstrated that the administration of SK had a hepatoprotective effect in hyperthyroid rats during normalizing the enzymatic activities of serum AST and ALT and was more efficient when being applied with vitamin E (23). Previously, it has been proven that the main monoterpenic phenol of SKEO such as carvacrol has antioxidant, anti- inflammatory and hepatoprotective properties (24). Recently, Palabiyik *et al.* (2016) reported that thymol and carvacrol sheltered against acetaminophen-induced toxicity in HepG2 cells by increasing antioxidant action and diminishing pro inflammatory cytokines, for example tumor necrosis factor a and interleukin 1b (25). A previous study conducted by Ahmadvand *et al.* (2015) demonstrated that carvacrol enhanced the loss of leukocyte infiltration (9.42%) and tubular necrosis and indicated helpful properties on the kidney function examination in gentamicin- induced nephrotoxicity in rats (26). Samarghandian *et al.* (2016) also

Table 2. Clinical chemistry parameters in mice sera (Mean±SD).

Parameters	*S. khuzistanica* essential (mL/kg)		Contro
	0.2	0.3	
AST (U/L)	159.6± 10.5	162.3 ± 19.3	124 ± 11.5
ALT (U/L)	85.2 ± 6.6	102 ± 9.6	80 ± 12.3
ALP (U/L)	186.2 ± 23.4	212.1 ± 23.5	280 ± 16.5
Cr (mg/dL)	0.45 ± 0.07	0.42 ± 0.08	0.4 ± 0.05
BUN (mg/dL)	64.3 ± 6.1	60.1± 1.51	61.4 ± 2.15
TB (mg/dL)	0.1 ± 0.2	0.11 ± 0.15	0.89 ± 0.1
DB (mg/dL)	0.04± 0.01	0.06 ± 0.02	0.1 ± 0.015

AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; Cr, creatinine; BUN, Blood urea nitrogen; TB, Total bilirubin; DB, Direct bilirubin.

Table 3. Hematology parameters in whole blood (Mean±SD).

Parameters	*S. khuzistanica* essential (mL/kg)		Control
	0.2	0.3	
RBC (×l0^6/µL)	5.82 ± 0.24	5.7 ± 0.29	5.4 ± 0.23
HGB (g/dL)	9.4 ± 1.23	9.7 ± 1.07	11.3 ± 0.71
Hct (%)	35.3 ± 2.5	38.2 ± 1.25	41.3 ± 1.18
WBC (×l0^3/µL)	2.7 ± 0.22	2.9 ± 0.61	2.5 ± 0.2
PLT (×l0^3/µL)	291.4 ± 33.1	275.3 ± 25.56	319.3 ± 28.2

RBC, red blood cell; HGB, hemoglobin; Hct, hematocrit; WBC, white blood cell; PLT, platele

reported that carvacrol be able to hold back repeated stress-induced oxidative injure in the brain, liver, and kidneys so that it might be measured as a new pharmacological drug to decrease chronic stress-induced oxidative damage (27). Thus, it should be suggested that SKEO may protect the constructional entirety of the liver and kidney by carvacrol as the main component of essential oil.

Here, we found that SKEO had no significant change in hematological parameters such as HGB, Hct, WBC, RBC, and PLT counts. Hashemipour *et al.* (2013), demonstrated that the hematological parameters were not affected by means of the combination of thymol and carvacrol, while this mixture amplified antioxidant enzyme activities, retarded lipid oxidation and enhanced immune response in broiler chickens (24).

The GC/MS analysis of SKEO demonstrated that the main components were carvacrol (64.4%), carvone (20.2%) and thymol (7.5%), respectively. Consistent with our study, Kheirandish *et al.* (28) and Farsam *et al.* (29) have demonstrated that the main component of SKEO is carvacrol. However, it should be mentioned that the chemical composition of essential oils is dependent on species, type of weather, gathering time and growth phase, indicating the change of the studied biological activities (30, 31).

Conclusion

The obtained results in this work showed that SKEO at the tested doses had no significant toxicity on the liver and kidney tissues as well as on hematological parameters in NMRI mice for 14 days; however further investigations are required to evaluate other toxicity aspects of the SKEO such as genotoxicity, chronic toxicity. Therefore, SKEO could be safe for the mammalian host at the used doses.

Declaration of Interest

The authors declare that there is no conflict of interest in this study.

Satureja khuzestanica uçucu yağının kimyasal bileşimi, farelerde akut ve sub-akut toksisitesi

ÖZ

Bitkilerin çeşitli hastalıkların tedavisi için ilaç geliştirme amacıyla kullanılan önemli kaynaklar olduğu bilinmektedir ancak bitkisel ilaçların tedavi amacıyla kullanılabilmesi için yan etki profillerinin belirlenmesi gereklidir. Bu çalışmada, *Satureja khuzestanica* uçucu yağının (SKEO) kimyasal bileşiminin tespiti ve bir fare modelinde akut ve sub-akut toksisitesinin gösterilmesi amaçlanmıştır. *Satureja khuzestanica* örnekleri İran'ın batısındaki Lorestan bölgesinin kırsal alanlarından toplanmıştır. SKEO'nun kimyasal bileşimi gaz kromatografisi/kütle spektrometrisi (GC/MS) yöntemiyle belirlenmiştir. Akut toksisitesinin belirlenmesi amacıyla SKEO, 0.5, 1, 2 and 4 ml/kg dozlarda 6'şar fareden oluşan 4 gruba intraperitoneal enjeksiyonla uygulanmıştır. Uygulamadan 24 saat sonra ölen fareler sayılmıştır. SKEO'nun sub-akut toksik etkilerinin belirlenmesi için 14 gün boyunca oral yoldan 0.2, 0.4 and 0.8 ml/kg dozlarda SKEO uygulanan farelerin biyokimyasal ve hemotolojik parametreleri incelenmiştir.

GC-MS yöntemiyle yapılan çalışmada SKEO içeriğinde bulunan 23 bileşik tespit edilmiş ve bu bileşikler içerisinde en yüksek miktarda bulunan türevin %64.4 oranıyla karvakrol olduğu görülmüştür. İntraperitoneal enjeksiyon yoluyla uygulanan SKEO'nun LD50 değeri 1.79 ml/kg, maksimum öldürücü olmayan dozu ise 1.13 ml/kg olarak bildirilmiştir. SKEO'nun; 0.2 and 0.4 ml/kg dozlarda uygulandığı gruplarda ölüm tespit edilmezken bileşiğe 0.8 ml/kg dozda maruz falan farelerden sadece biri ölmüştür (%12.5). Oral yoldan 0.2, 0.4 and 0.8 ml/kg dozlarda SKEO uygulanan farelerin biyokimyasal ve hemotolojik parametreleri kontrol grubuna ait değerlerle karşılaştırıldığında anlamlı bir farklılık tespit edilmemiştir ($P>0.05$). Bu çalışma sonucunda elde edilen veriler; SKEO'nun, farelerin karaciğer, böbrek dokuları ve kan parametreleri üzerinde belirgin bir toksistesi olmadığını göstermiştir. Bu nedenle SKEO'nun belirtilen dozlarda diğer memeliler için de güvenli olduğu düşünülmektedir.

Anahtar kelimeler: GC/MS; Akut ve sub-akut toksiste; *Satureja khuzestanica* uçucu yağı, fare

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