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Original Article

Effects of *Satureja khuzestanica* on Serum Glucose, Lipids and Markers of Oxidative Stress in Patients with Type 2 Diabetes Mellitus: A Double-Blind Randomized Controlled Trial

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Satureja khuzestanica is an endemic plant of Iran that is widely distributed in the Southern part of the country. It has antioxidant properties and thus it seems to be useful in diseases related to oxidative stress such as diabetes and hyperlipidemia. The present study investigates the effect of *S. khuzestanica* supplement in metabolic parameters of hyperlipidemic patients with type 2 diabetes mellitus. Twenty-one hyperlipidemic patients with type 2 diabetes mellitus were randomized in a double blind, placebo controlled clinical trial to receive either *S. khuzestanica* (tablets contain 250 mg dried leaves) or placebo once a day for 60 days. Blood samples were obtained at baseline and at the end of the study. Samples were analyzed for levels of glucose, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglyceride, creatinine, thiobarbituric acid reactive substances (TBARS) as marker of lipid peroxidation and ferric reducing ability (total antioxidant power, TAP). Treatment of patients by *S. khuzestanica* for 60 days induced significant decrease in total cholesterol ($P = 0.008$) and LDL-cholesterol ($P = 0.03$) while increased HDL-cholesterol ($P = 0.02$) and TAP ($P = 0.007$) in comparison with the baseline values. *S. khuzestanica* did not alter blood glucose, triglyceride, creatinin and TBARS levels. In comparison with baseline values, no significant change was observed in blood glucose, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglyceride, creatinine, TBARS and TAP in placebo-treated group. Usage of *S. khuzestanica* as a supplement to drug regimen of diabetic type 2 patients with hyperlipidemia is recommended.

Keywords: hyperlipidemia – oxidative stress – *Satureja khuzestanica* – type 2 diabetes mellitus

Introduction

Satureja khuzestanica Jamzad (*Marzeh khuzestani* in Persian, family of Lamiaceae, sub family of Nepetoidae) is an endemic plant that is widely distributed in the Southern parts of Iran. It is a subshrub, branched stem ~30 cm high, densely leafy, broadly ovaiate-orbicular covered with white hairs (1). Base of the leaves is attenuate and petioliform. It is famous for its therapeutic value as an analgesic and antiseptic in folk medicine (2).

In the recent years, antimicrobial (3–5), antiviral (6,7), antioxidant (8–10), antiproliferative (11), antiprotozoal

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(12,13), antifungal (14,15), anti-inflammatory and antinociceptive (16,17), antidiarrheal (18) and vasodilatory (19,20) activities were reported from different species of *Satureja*.

It has been shown that hydroethanolic extract of *S. khuzestanica* has anti-inflammatory and antinociceptive properties in carrageenan-induced rat paw edema and formalin test comparable with those of indomethacin and morphine (21). The methanolic extract of the aerial parts of *S. khuzestanica* has been investigated for its antimicrobial activity. The maximum antibacterial and antifungal activities has been observed against *Staphylococcus aureus* and *Candida albicans* (22). Significant decrease in fasting blood glucose and triglyceride levels were observed with essential oil of *S. khuzestanica* in diabetic and hyperlipidemic rats. A significant decrease in the normal lipid peroxidation and an increase in body antioxidant power were reported while the cholesterol level did not change significantly in hyperlipidemic rats (23). It has been reported that essential oil from *S. khuzestanica* does not affect blood glucose concentrations but decreases hepatic phosphoenolpyruvate carboxy kinase activity and increases hepatic glycogen phosphorylase in rats (24). Study on the effect of oral administration of essential oil from *S. khuzestanica* in male rat fertility has revealed significant improvement in all parameters of libido such as potency, fecundity, fertility index and litter size. Moreover, concentrations of FSH and testosterone and the weights of testes, seminal vesicles and ventral prostate weights were significantly increased. Histopathological analysis showed that the number of spermatogonium, spermatid cords, Leydig cells and spermatozoids was increased and Sertoli cells were hypertrophic (25). Essential oil from *S. khuzestanica* has protected mice from experimentally induced inflammatory bowel disease and significantly decreased lipid peroxidation in bowel of animals (26). Primary acute toxicity and teratogenicity tests were also performed and confirmed plant's safety (23).

One of the specific identifying characteristics of the subfamily Nepetoidae is that its representatives contain $<0.5\%$ essential oil (27). The main component of essential oil of *S. khuzestanica* is carvacrol (28). Other constituents identified in this plant are flavones, triterpenoids, steroids and tannins (29). Both carvacrol and flavonoids have been found to have antioxidant properties (30–33). Lipid peroxidation and consequent oxidative stress are increased in diabetes and hyperlipidemia, and have been proposed to play roles in the pathogenesis of these diseases (34–37). The above-mentioned studies in animals confirmed the safety of *S. khuzestanica*. In the present study, the antidiabetic, antihyperlipidemic and antioxidant effects of *S. khuzestanica* were investigated in patients with type 2 diabetes mellitus.

Methods

Plant Collection and Tablet Preparation

The aerial parts of the *S. khuzestanica* were collected during the flowering stage in June 2000 from Khorramabad in the Lorestan province of Iran. The plant was identified by the Department of Botany of the Research Institute of Forests and Rangelands (TARI) in Tehran. A voucher specimen (No. 58416) has been deposited at the TARI Herbarium. The plant was cultivated in Khorramabad and the aerial parts of the plant were collected during the flowering stage. The aerial parts were air dried at ambient temperature in the shade. Tablets were prepared from dried leaves of plant (each tablet contained 250 mg of dried leaves). Other ingredients of tablet were lactose, microcrystalline cellulose, starch, poly vinyl pyrollidone (PVP) and magnesium estearate. These ingredients were pressed with hardness of 5–7 kg and the final mean weight of tablet was 375 mg. The pressed tablets were film coated with hydroxy propyl methyl cellulose, poly ethylene glycol 4000, talc, titanium dioxide, PVP, distilled water and ethanol.

Patient Selection

In this randomized, prospective, double-blind controlled clinical trial, the database contains details of patients' age, sex, clinical history, previous pharmacologic therapies, hospitalizations and specialist consultations. We recruited 21 (12 male and 9 female) patients with type 2 diabetes mellitus with a mean age of 50.65 from outpatient clinic of a University Hospital. Diabetic patients were chosen according to American Diabetes Association (ADA) criteria (38). The patients were controlled by diet and oral antidiabetic drugs. Diabetics with hyperlipidemia who met the criteria for pharmacologic treatment according to National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) guideline for diabetic patients were recruited (39). All patients were receiving antidiabetic and antihyperlipidemic medications at least 3 months before beginning the experiment.

Exclusion criteria were the followings: uncontrolled hypertension (systolic blood pressure >140 mm Hg and/or diastolic blood pressure >90 mm Hg); uncontrolled hypothyroidism; obstructive hepatic or biliary disease; alcoholism, smoking; autoimmune disease; chronic pancreatitis; active liver disease or hepatic dysfunction [i.e. alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin higher than 2-fold the upper limit of normal]; nephritic syndrome or renal dysfunction (i.e. serum creatinine concentration $>1.5\,\mathrm{mg\,dl^{-1}}$); concomitant participation in other clinical studies; use of heparin and/or oral anticoagulants; pulmonary infections; gangrene; diabetic foot ulceration; treatment with insulin and/or use of multivitamins and

herbal traditional supplements in last 3 months. Patients treated with steroids (unless administered topically or for post-menopausal hormone replacement therapy), cyclosporine A, erythromycin, or ketoconazole were also excluded; as well as patients with a history of myocardial infarction, angioplasty, or major surgery in the 6 months before the beginning of the study. Pregnant, possibly pregnant, or breastfeeding women were excluded from the study. Women of childbearing age were required to use an effective method of birth control throughout the study.

The subjects were completely informed of the purpose, procedure and hazards of the trial and were free to leave the trial at any time. All patients signed informed written consent before being included in the study. The research followed guidelines of the Declaration of Helsinki and Tokyo for humans, and was approved by Ethics Committee on Human Experimentation of PSRC/TUMS.

Patients who met the eligibility criteria were randomly assigned to one of the two treatment groups according to a simple randomization scheme. Group I ($n = 11$) received *S. khuzestanica* tablets (250 mg) once a day for 60 days and group II ($n = 10$) received placebo with the same regimen. The subjects were asked not to alter their usual diets and physical activity throughout the study and any changes in their medication were avoided whenever possible.

Sample Collection and Handling

After 12–14 h overnight fasting, between 08:00 and 10:30 h and before taking any oral hypoglycemic agent(s), 10 ml blood sample was collected from each subject in tubes containing heparin, before beginning of experiment and after 60 days administration of *S. khuzestanica* or placebo. After centrifugation of blood at $3000\times g$ for 30 min at 4°C, the plasma supernatant fluid was separated and stored at −80°C until analyzed further.

Body mass index, blood pressure, serum total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), triglyceride (TG), fasting blood glucose and serum creatinine were measured at the start and after 60 days of treatment.

Total Antioxidant Power (TAP) Assay

Antioxidant power of plasma was determined by measuring their ability to reduce Fe^{3+} to Fe^{2+} established as named (ferric reducing ability of plasma, FRAP) test that described previously (40). Briefly, in this test, the medium is exposed to Fe^{3+} and the antioxidants present in medium start to produce Fe^{2+} as an antioxidant activity. The FRAP reagent prepared freshly, contained 25 ml of 300 mM acetate buffer (pH 3.6) plus 2.5 ml of 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl and 2.5 ml of 20 mM ferric chloride ($FeCl_3$-6H2O). The complex between Fe^{2+} and TPTZ gives a blue color with absorbance at 593 nm.

Lipid Peroxidation Assay

The method based on the reaction of malonedialdehyde (MDA) as the end product of the oxidation of polyunsaturated fatty acids and its concentration in the medium is an established measure of lipid peroxidation extent. In this test the reaction of MDA with TBA creates a complex which is determined spectrophotometrically while lipid peroxidation in samples are assessed in terms of thiobarbituric acid (TBA) thiobarbituric acid reactive substances (TBARS) produced. Briefly, the samples were diluted by buffered saline (1 : 5) and 800 µl of trichloroacetic acid (TCA, 28% w/v) was added to 400 µl of this mixture and centrifuged in $3000\times g$ for 30 min. Then, the precipitation was dissolved in sulfuric acid and 600 µl of the mixture was added to 150 µl of TBA (1% w/v). The mixture was then incubated for 15 min in a boiling water bath. Following incubation, 4 ml of *n*-butanol was added, the solution was centrifuged, cooled and the absorption of the supernatant was recorded at 532 nm using a UV-160-A Shimadzu double beam spectrophotometer (Japan). The calibration curve of a 1,1,3,3-tetraethoxypropan standard solution was used to determine the concentrations of TBA-MDA adducts in samples (41).

Statistical Analysis

The statistical analysis was performed with the use of StatsDirect version 2.6.2. Continuous data are expressed as mean ± SE. Statistical analysis was initially performed by Kolmogorov-Smirnov normality test. Unpaired two-sample student's *t*-test was used for analysis of continuous data and Chi-square test was used for categorical data if applicable. A P-value < 0.05 was considered to be statistically significant.

Results

Patients' Characteristics

Twenty-one patients with type 2 diabetes mellitus (12 male and 9 female) who fulfilled the enrolment criteria entered into the study between July 2006 and January 2007. Eleven patients were randomized to receive *S. khuzestanica* and 10 to receive placebo. No patients were withdrawn from the study. Demographic characteristics did not differ significantly between two groups (Table 1). No adverse events were reported from the subjects throughout the study period.

Patients' Blood Glucose and Lipid Profile and Oxidative Stress Biomarkers

Table 2 shows changes found on metabolic parameter values in both groups between baseline and end of therapy. Patients in the *S. khuzestanica* group showed significant decrease in total cholesterol ($P = 0.008$), LDL-C ($P = 0.03$) and increase in HDL-C ($P = 0.02$) and TAP ($P = 0.007$) measured from baseline to 2 months while patients in the placebo group showed no changes. The other parameters including blood glucose, triglyceride, creatinin and TBARS were not significantly altered in *S. khuzestanica* group.

Discussion

Recently, there has been a considerable interest in finding natural antioxidants from plant materials to replace synthetic ones. Data from both scientific reports and laboratory studies show that plants contain a large variety of substances that possess antioxidant activity (42). Phytochemicals with antioxidant effects include some cinnamic acids, coumarins, diterpenes, flavonoids, lignans, monoterpenes, phenylpropanoids, tannins and triterpenes (43). Therefore, it seems that plants particularly those with high levels and strong antioxidant compounds have an important role in improvement of disorders involving oxidative stress such as diabetes mellitus (37). *S. khuzestanica*, an endemic plant of Iran, possess antioxidant properties (23,26) and is expected to be useful in diabetes and its complications.

The results of this study, show the significant effects of *S. khuzestanica* in improving patients' total cholesterol, LDL-C, HDL-C and TAP. In previous study, it was revealed that *S. khuzestanica* significantly decrease fasting blood glucose and triglyceride levels in diabetic and hyperlipidemic rats. It also improved the lipid peroxidation and TAP levels (23). In another investigation, it was reported that *S. khuzestanica* does not affect blood glucose concentrations of rats (24). The main constituents of *S. khuzestanica* are isopropanoids such as carvacrol, thymol and flavonoids. It has been shown that thymol and carvacrol significantly decrease the serum cholesterol levels. They increased microsomal geranyl pyrophosphate pyrophosphatase activity by 2-fold. The structural diversity of the isopropanoids which suppress cholesterol synthesis may be reconciled by their ability to increase pyrophosphatase activity, thus leading to the production of the endogenous, post-transcriptional regulator of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity (44). Flavonoids have also shown antioxidant and anti hyperlipidemic properties. Soy isoflavones significantly reduced serum total and LDL-C but did not change HDL-C and triacylglyceride (45). Covas *et al.* (46) reported that consumption of polyphenol-rich olive oil increases HDL-C levels and lowers levels of oxidative stress markers and oxidized LDL-C.

Table 1. Baseline characteristics of subjects

	Satureja khuzestanica ($n = 11$)	Placebo ($n = 10$)
Age (year)	50.65 ± 2.11	51.71 ± 2.05
Male/female (*n*)	6/5	6/4
SBP (mmHg)	128.60 ± 3.67	127.58 ± 3.71
DBP (mmHg)	80.70 ± 3.03	81.08 ± 3.60
BMI ($kg\,m^{-2}$)	26.35 ± 1.70	27.63 ± 2.44
HbA_{1c} (%)	7.25 ± 1.42	7.65 ± 1.50
Concomitant therapy		
Glibenclamide (*n*)	8	6
Metformin (*n*)	5	5
Statins (*n*)	9	9
fibrates (*n*)	2	1

SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; HbA_{1c}, glycated hemoglobin; *n*, number of patients.
The values are expressed as mean ± SE.

Table 2. Levels of blood glucose, triglyceride, total cholesterol, LDL-C, HDL-C, creatinine, TAP, and lipid peroxidation before and after administration of *Satureja Khuzestanica* or placebo

	Satureja khuzestanica ($n = 11$)			Placebo ($n = 10$)		
Parameters	Before	After	*P*-value	Before	After	*P*-value
Glucose ($mg\,dl^{-1}$)	163 ± 10.4	160 ± 8.9	0.7	165.9 ± 17	201 ± 12	0.37
Triglyceride ($mg\,dl^{-1}$)	147.2 ± 22.7	167 ± 37	0.22	155 ± 17.1	230.9 ± 15.1	0.12
Total cholesterol ($mg\,dl^{-1}$)	202.8 ± 3.5	156.2 ± 4.2	0.008	189.1 ± 6.5	214 ± 9	0.1
LDL-C ($mg\,dl^{-1}$)	120 ± 4.5	103 ± 3.9	0.03	123 ± 6	122 ± 6.6	0.8
HDL-C ($mg\,dl^{-1}$)	45.3 ± 3.2	50 ± 1.6	0.02	45 ± 2	44.4 ± 3.2	0.3
Creatinine ($mg\,dl^{-1}$)	1.02 ± 0.04	1.07 ± 0.05	0.42	1.24 ± 0.1	1.24 ± 2.2	1
TAP ($mmol\,l^{-1}$)	336 ± 48	477 ± 24	0.007	381 ± 0.45	417 ± 15.8	0.1
TBARS ($\mu mol\,l^{-1}$)	152 ± 17.3	164 ± 19.1	0.51	151 ± 12.5	164 ± 19.1	0.1

HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; TAP, total antioxidant power; TBARS, thiobarbituric acid reactive substances.
The values are expressed as mean ± SE.

Recently, *S. khuzestanica* has been found to prevent from malathion-induced toxicity through improvement of oxidative stress parameters (47). Since free radicals has a pivotal role in pathogenesis and complications of diabetes (37), every natural or synthetic compound that can reduce oxidative stress may be beneficial in the management of diabetes (48–53). One of our expectations in the present study was to observe significant reductions in blood glucose and level of lipid peroxidation by *S. khuzestanica* treatment but this did not happen. The first thing that comes to our mind is that dose of *S. khuzestanica* was low and duration of the study was small. On the other hand, disturbed lipid profile is usually observed in diabetic patients and most of diabetics usually receive lipid lowering agents mostly statins that accompany with some adverse effects and also costs a lot for diabetic patients (54,55). Introduction of new herbal medicines like *S. khuzestanica* as a drug with low price and safe can be helpful.

Conclusion

In summary, it seems that *S. khuzestanica* have a lipid lowering and antioxidant properties in human and since it has not shown any adverse effects, it could be used as a supplement in diabetic patients with hyperlipidemia. The exact role of this herbal supplement in management of hyperlipidemic type-2 diabetic patients remains to be elucidated by larger sample size and longer term clinical trials.

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