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Amelioration of Glomerulosclerosis by Satureja khozestanica Essential Oil in Alloxan-Induced Diabetic Rats

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Abstract

***Background*:** *Satureja khuzestanica*, an endemic plant of Iran, has been reported to be used traditionally to treat diabetes. We examined possible protective effect of *Satureja khozestanica* essential oil (SKE) on glomerulosclerosis in alloxan-induced type 1 diabetic rats.

***Materials and Methods*:** In this experimental study, 30 Sprage-dawley male rats were divided into 3 groups randomly; group 1 as control, group 2 diabetic untreated, and group 3 treatments with SKE by 500 ppm in drinking water, respectively. Diabetes was induced in the second and third groups by alloxan injection subcutaneously. After 8 weeks, animals were anaesthetized; livers and kidneys were then removed immediately. Kidney paraffin sections were prepared and stained by periodic acid Schiff method. Glomerular volume and leukocyte infiltration were estimated by stereological rules and glomerular sclerosis was studied semi-quantitatively.

***Results*:** Flow treatment of diabetic animals with SKE could significantly inhibit glomerular hypertrophy (22%) leukocyte infiltration (31%) and glomerulosclerosis (20%) in comparison with the diabetic untreated group.

***Conclusion*:** The findings showed that SKE alleviates loss of glomerular volume, leukocyte infiltration, and glomerulosclerosis and exerts beneficial effects on the lipid peroxidation in alloxan-induced type 1 diabetic rats.

Introduction

Free radicals are generated continuously in the body due to both normal metabolism and disease [1]. Oxidative stress is the imbalance between oxidant and antioxidant systems in favor of the former. Antioxidant systems include antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX), in addition to low molecular agents and dietary antioxidants. Clinical and experimental studies have shown that disturbing of oxidant–antioxidant balance system is involved in the pathogenesis of chronic diseases such as cancer [2], coronary heart disease, diabetes and many diabetic complications [2]. Hyperglycemia is confounded for the complications of diabetes because hyperglycemia directly causes glycation of proteins, lipids and nucleic acid, then injures cells and induces lipid peroxidation [3]. Also antioxidant and antioxidative enzyme activities reduce due to glycation or increase of lipid peroxidation products [4]. A number of natural antioxidant such as vitamin E and phenolic compunds are known to have hypoglycemic, hypolipidemic or both activities [5]. Chemical drugs have many side effects; therefore, screening for new antidiabetic sources from natural antioxidants is still attractive because they are safe and good alternrtive for treatment of diabetes mellitus. A growing body of research indicates that nutritional deficiencies such as antioxidants contribute to the development of diabetes. Recently, much attention has been focused on the central and key role of oxidative stress in the pathogenesis of different diabetic complications [6]. Several studies have shown that antioxidant treatment reduces diabetic complications [7]. Because of increasing demand of patients for the use of natural products and other herbal drugs with anti-diabetic activity, the general trend now is to use the natural products for medicinal application in their natural available form [8]. Polyphenols, well-known antioxidants, have also been showed to function as antidiabetic by reducing blood glucose levels [9]. Researchers are recently interested in investigation and research into extraction of natural antioxidants from medical herbs to replace synthetic antioxidants [10]. Therefore, the research into the determination of the natural antioxidant source is very important to promote public health [10]. *Satureja khuzestanica*, an endemic plant of Iran, decreases glucose and malondealdehyde in serum diabetic patients [10, 11]. The components of this extract were analyzed with gas chromatography/mass spectrometry (GC/MS) in Research Center of Lorestan University as reported in our previous paper [11]. The main component of this extract is carvacrol as a good antioxidant [11]. Since the protective effects of *Satureja khozestanica* essential oil (SKE) on glomerulosclerosis in alloxan-induced type 1 diabetic rat have not previously been reported; the objectives of the present study were to

investigate amelioration of altered loss of glomerular volume, leukocyte infiltration, glomerulosclerosis, by *Satureja khozestanica* essential oil in alloxan-induced type 1 diabetic rats.

Materials and Methods

Isolation of the essential oil from *Satureja khozestanica*: *Satureja khozestanica* essential oil was prepared from cultivated *satureja khozestanica* in Khoramabad (Lorestan province, western Iran). The aerial parts of the plants were collected during flowering stage and were air-dried at ambient temperature in the shade. The aerial parts were hydro-distilled using a Clevenger apparatus for 4 hours, giving yellow oil in 0.9% yield. The oil was dried over anhydrous sodium sulfate and stored at 4ºC. The plant was previously identified by the department of Botany of the Research Institute of Forests and Rangelands (TARI) in Tehran, Iran. A voucher specimen (No. 58416) has been deposited at the TARI Herbarium [10].

Animals: This is an experimental study. Thirty male mature Sprague-Dawley rats (180-200 g) were obtained from Pasteur Institute of Tehran and were allowed to adapt themselves with the new location for one week. The rats were kept at 12/12 dark-light period in 21±3ºC. All animals were allowed free access to food and water ad libitum during the experiment. This study was approved by the Animal Ethics Committee of the Medical University of Lorestan with accordance to the national health and medical research council guidelines. The rats were divided to 3 groups (10 per each). The studied groups were as follows: group 1 as control, group 2 as diabetic without treatment and 3rd group as diabetic treatment with SKE by 500 ppm in drinking water.

Diabetes induction: Diabetes was induced after overnight fasting in the second and third groups by injection of alloxan monohydrate (120 mg/kg) subcutaneously [10]. Beta cell degradation by alloxan leads to release of more insulin. Because of acute hypoglycemia, the rats received 10% sucrose solution for 48 h instead of drinking water. Five days after induction of diabetes, blood samples were gathered from the end part of tails. Blood glucose was measured by glucometer and the rats with blood glucose level of ≥300 mg/dL (16.7 mmol/L) were considered as diabetic [10]. During the first 5 days after diabetes induction, 1-3 rats per group died because of alloxan toxicity. The third group was treated with SKE by 500 ppm in drinking water [10]. The treatment was begun at the first day of diabetes induction. After 8 weeks treatment, animals were anesthetized (nesdonal 50 mg/kg, IP), kidneys were removed immediately and used fresh or kept frozen until the analysis [10].

Mean glomerular volume estimation: The kidneys were fixed in formal saline solution and routinely were processed. Random sections (5 µm thick) of the renal cortex were stained with periodic acid-Schiff (PAS). The surface areas of 100 glomeruli were measured with the motic image plus (version 2) software program on PAS stained tissue sections at 400 time magnification via motic microscope equipped by motic camera. The mean glomerular volume (VG) was calculated according to the method of Weibel and Gomez. VG=Area 1.5×1.38/1.01 where 1.38 represents the shape coefficient, and 1.01 represents the size distribution coefficient [12].

Glomerulosclerosis assessment: The severity of glomerulosclerosis was studied semiquantitatively. This part of study was performed by an experienced histologist in a blinded fashion. Severity in tissue sections was assessed by assigning a score 1-4 to each glomerulous according to the tuft demonstrating sclerosis: normal glomeruli=0; up to 25% involvement=1; 25-50% involvement=2; 50-75% involvement=3 and more than 75% involvement=4. The glomeruli were selected for the assessment that appeared randomly in microscopic fields. At least 150 glomeruli were assessed in kidney sections of each group of animals [12].

Leukocyte infiltration assessment: The glomeruli were selected for the assessment that appeared randomly at 400 time magnification in microscopic fields. Severity in infiltration was assessed by assigning a score 1-4 to each glomerulus according to the tuft demonstrating leukocyte infiltration: normal=0; up to 25% involvement =1; 25-50% involvement=2; 50-75% involvement=3 and more than 75% involvement=4 [13].

Statistical analysis: All values are expressed as mean±SD. The data were compared between groups by Mann-Whitney *U* test. Statistical analyses were performed using the SPSS-13 for windows software. $p<0.05$ was considered statistically significant.

Results

Effect of SKE on glomerular volume of diabetic rats: The level of the glomerular volume is shown in figure 1. Our study demonstrated that the value of glomerular volume in untreated diabetic rats was significantly (2.3 fold) higher than that of the control animals. Treatment of diabetic animals with SKE could inhibit glomerular hypertrophy (22%) in comparison with untreated diabetic group (Table 1).

Effect of SKE on leukocyte infiltration of diabetic rats: The level of the glomerular volume is shown in figure 2. Our study showed that leukocyte infiltration in untreated diabetic group were significantly (14 fold) higher than that of control animals and the level of leukocyte infiltration in the diabetic rats that were treated with SKE was significantly lower than that of diabetic untreated animals. Treatment of diabetic animals with SKE could significantly inhibit leukocyte infiltration (31%) in comparison with untreated diabetic group (Table 1).

Effect of SKE on glomerulosclerosis in diabetic rats: Glomerulosclerosis increased significantly in the diabetic untreated group by 4.9 fold in comparison with the control group. Treatment of diabetic animals with SKE could significantly (20%) inhibit glomerulosclerosis in comparison with the diabetic untreated group (Table 1).

Tabel 1. The effect of SKE on glomerular volume, glomerulosclerosis and leukocyte infiltration in alloxan induced diabetic rats

Experimental groups	Glomerular volume (mm^3) mean±SE	Glomerulosclerosis (Score 0-4) mean±SE	LeuKocyte infiltration (Score 0-4) mean±SE
Control	27400±2170	0.40±0.07	0.21±0.0195
Diabetic	62000±3220*	1.623±1.243*	1.84±0.197*
Diabetic treated	44100±3050*#	1.20±0.072*#	1.36±0.112 *#

Values represented as mean±SE, *p <0.05 as compared with control group. #p <0.05 as compared with diabetic without treatment group

Discussion

This study showed that SKE could significantly inhibit leukocyte infiltration glomerular hypertrophy and glomerulosclerosis in comparison with untreated diabetic group. There is much evidence that oxidative stress play a key role in the most pathogenic pathway of diabetic injuries. Free radicals such as superoxide can induce cell and tissue injuries throughout lipid peroxidation and increase carcinogenesis, inflammation, early aging, cardiovascular diseases and tissue damage in diabetes [12]. Herbal extracts such as *Artemisia afra Jacq*, *Aframomum melegueta* and Aloe vera gel extracts [14] and antioxidants such as vitamin E, coenzyme Q10 and antioxidant enzymes such as SOD, GPX and CAT protect the cells against oxidative stress mediated cellular injuries by converting the toxic free radicals to non-toxic products [15]. Therefore use of antioxidant as complementary therapy is useful for diseases that related to oxidative stress. Our study with designed base stereological methods showed that the treatment of diabetic animals with SKE prevented glomerular hypertrophy, leukocyte infiltration and glomerulosclerosis significantly in comparison with untreated diabetic animals. There are reports that vitamin C, alpha lipoic acid, coenzyme Q10 and vitamin E could inhibit or decrease glomerulosclerosis in diabetic rats [13]. There are reports that vitamin E has protective effect against coronary heart disease, atherosclerosis and vascular complication [16]. Lipoic acid as antioxidant has protective effect against central and peripheral nervous system defects, neuropathic defects [13]. Many studies indicated that various herbal extracts decrease diabetic complication such as nephropathy in diabetic patients. The rhizomes of *Picrorhiza scrophulariiflora* with potential anti-inflammatory activity improve diabetic nephropathy and attenuate kidney inflammation in animal model [17]. Methanolic extract of *Litsea deccanensis* decrease necrosis, less tissue damage and reduce infiltration of inflammatory cells in the isoproterenol induced myocardial infarction in rats [18]. The extract *Rhodiola crenulata* protects nervous system from apoptosis or necrosis, and improves neurogenesis [19]. Previous our study showed that rosmarinic acid and coenzyme Q10 reduce glomerular hypertrophy, glomerulosclerosis and loss of glomerular number in gentamicin induced nephrotoxicity and untreated diabetic nephropathy rats [12]. Results of our study are in accordance with others researcher's study that showed SKE could reduce glomerular hypertrophy and glomerulosclerosis. Therefore natural antioxidant with protective effects on glomerular hypertrophy and glomerulosclerosis could prevent or be helpful in reducing the complications in diabetes patients. Also researchers reported the role of oxidative stress as a central factor in onset and progression of diabetic complications such as vascular defects and nephropathy [5, 20]. According to numerous reports, our results showed efficacy of antioxidative supplements administration in the prevention of diabetic complications. Antioxidant therapy is one of the most important treatment strategies for diabetic patients for the prevention and slowing of diabetic complications progression such as hyperlipidemia, hepatic damage. In diabetic nephropathy, structural injury develops over years before clinical and laboratory abnormalities such as albuminuria, hypertension, or declining glomerular filtration rate (GFR) appear. Thus, waiting for clinical or laboratory manifestation of renal disease before initiating treatment may hinder efforts that prevent progression to end stage of renal disease. Although the detailed molecular protective mechanisms of SKE can not be fully explained by our results, our results are satisfactory. SKE as a source of potent antioxidants, with multi beneficial properties can be introduced to diabetic patients without diabetic nephropathy for inhibition and progression of diabetic nephropathy. This study showed that SKE has protective effects on glomerular hypertrophy, glomerulosclerosis and leukocyte infiltration in alloxan-induced-diabetic rats. Hence, attenuation of glomerulosclerosis can decrease diabetic complication such as nephropathy in diabetic patients.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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