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Research Article

Essential Oil of Cultivated *Satureja khuzestanica* Jamzad Improves Activities and Genes Expression of Hepatoglycoregulatory Enzymes in Diabetic Rats

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Abstract

Objective: The aim of the present study is to evaluate the effect of cultivated *Satureja khuzestanica* essential oil (SKEO) on activity and gene expression of hepatic glucokinase (GK), glycogen phosphorylase (GP) and phosphoenolpyruvate carboxykinase (PEPCK) in normal and diabetic rats.

Methods: Thirty two wistar male rats were divided into four groups randomly; group one as control, group two as diabetic untreated, group three as sham treated with cultivated SKEO (100 mg/kg/d) in aqueous solution orally for 21 days, and group four as diabetic treated with cultivated SKEO (100 mg/kg/d) in aqueous solution orally for 21 days. After 21 days, animals were anaesthetized, liver were then removed immediately and used fresh or kept frozen until analysis of GK, GP and PEPCK activity and gene expression of GK, GP and PEPCK by using the quantitative real-time RT-PCR technique.

Results: Hepatic GK activity and gene expression of GK in diabetic treated groups compared with diabetic untreated groups were significantly increased. Hepatic GP and PEPCK activity and gene expression of GP and PEPCK in diabetic treated groups compared with diabetic untreated groups were significantly decreased. Also, hepatic activity of GK, GP and PEPCK activity correlated positively with their gene expression.

Conclusion: This study showed that SKEO might be a exert beneficial effects on activities of GK, GP and PEPCK and gene expression of GK, GP and PEPCK in diabetic rats. Therefore, SKEO may contribute to reduction of serum glucose, which seems to be related to its antioxidant properties.

Keywords: *Antihyperglycemic; Gene Expression; Glucokinase; Glycogen Phosphorylase; Phosphoenolpyruvate Carboxykinase; Satureja khuzestanica Essential Oil*

Introduction

Diabetes mellitus, one of the leading metabolic syndromes, accounts for highest morbidity and mortality worldwide [1]. Diabetes mellitus is characterized by abnormalities in carbohydrate, lipid and protein metabolism due to complete or relative insufficiency of insulin secretion from pancreatic β-cells and/or defect in insulin action [2]. Hyperglycemia is confounded for the complications of diabetes because hyperglycemia directly causes glycation of proteins such as hepatoglycoregulatory enzymes, lipids and nucleic acid and their functions, then injures cells and induces lipid peroxidation [3]. Also, hepatoglycoregulatory enzymes enzyme activities reduce due to glycation

or elevation of lipid peroxidation products [4]. A number of natural antioxidant such as vitamin E, coenzyme Q10 and herbal medicine are known to have hypoglycemic, insulin secretion inducer, hypolipidemic and protection of altered hepatoglycoregulatory enzymes and lipid peroxidation *in vivo* [5]. Chemical drugs have many side effects; therefore, looking for new antidiabetic drugs from natural sources is still attractive because they are safe and good alternative for treatment of diabetes mellitus. A growing body of research indicates that nutritional deficiencies such as natural antioxidants contribute to the development of diabetes [5,6].

Satureja khuzestanica Jamzad is an endemic plant of Iran that is widely distributed in the Southern part of Iran has been employed as an analgesic and antiseptic in the southern parts of Iran. Crude of the aerial parts of *Satureja khuzestanica* Jamzad is credited in folk medicine as a supplement agent for diabetic patients [1,2]. Researchers have been reported that SKEO has a wide range of effects such as: anti-inflammatory, antinociceptive [1]. antioxidant, antihyperlipidemic [3]. Hypolipidemic, antiatherogenic, hypoglycemic and antimicrobial activities [4]. Also, researchers have been reported that SKEO has beneficial effects on male rat fertility [5] on stimulating reproduction [3]. Liver glucokinase (GK) plays an essential role in glucose homeostasis. In the liver its activity is critical for taking up glucose in the fed state [7]. Glycogen phosphorylase (GP) is a key regulatory enzyme that catalyzes the breakdown of glycogen and in the post-absorptive state is an important mechanism in the control of blood glucose homeostasis. Phosphoenolpyruvate carboxykinase (PEPCK) catalyzes one of the rate-limiting steps of gluconeogenesis. Studies in diabetic animals have shown that gluconeogenesis is a major factor in the increase plasma glucose that appears in fasting and post-absorptive state [8]. The natural habitats of each site for any plant are distinguished by geographic, abiotic and biotic features. Plants of a single species growing in different habitats have different oil composition [9]. The cultivated *Satureja khuzestanica* Jamzad grows in a different natural habitat than that of the wild plant. In previous studies, streptozotocin (STZ)-induced diabetic rats exposed to SKEO have shown significantly decreased levels of plasma glucose [3]. Normal rats treated with SKEO have also shown significantly decreased PEPCK activity and increased GP activity in the liver [10]. Since the effects of SKEO on hepatic activities of GK, GP and PEPCK and gene expression of GK, GP and PEPCK. in normal and diabetic rats have not previously been reported; the objectives of the present study were undertaken to evaluate the effect of cultivated SKEO on activities and gene expression of hepatic GK, GP and PEPCK in normal and diabetic rats.

Material and Methods

Essential oil preparation

Satureja khuzestanica Jamzad, family of lamiaceae, was collected from Khorramabad (Lorestan Province in Iran) at the flowering stage during October. The plant was indentified by the Department of Botany of the Research Institute of Forests and Rangelands in Tehran. A voucher specimen (No. 58416) has been deposited at the TARI herbarium. Aerial parts of the *Satureja khuzestanica* Jamzad were chopped, air-dried, powdered and then hydrodistilled using a Clevenger type apparatus for 5h, giving a yellow oil in a 2.1% (w/w), yield. The oil was dried over anhydrous sodium sulfate and stored at 4ºC [2].

Gas chromatography/mass spectrometry

Fid- GC was carried out using a Hewlett-Packard 6890 with HP-5 capillary column (phenyl methyl siloxane. 25m, 0.25 mm i.e. ratio, 1:25 and flame ionization detector. Temperature programmer: 60ºC (2 min) rising to 240ºC at 4ºC/min: injector temperature 250ºC, detector temperature, 260ºC. GC-MS was performed using a Hewlett-Packard 6859 with a quadruple detector, on a HP-5 column, operating at 70 eV ionization energy, using the same temperature programmer and carrier gas as above. Retention indices were calculated by using retention times of n-alkanes that were injected after the oil at the same chromatographic [11].

Identification of components

The linear retention indices for all the compounds were determined by conjunction of the sample with a solution containing the homologous series of C8-C22 n-alkanes. The individual constituents were identified by their identical retention indices, referring to known compounds from the literature and also by comparing their mass spectra with either the known compounds or with the Wiley mass spectral database [11,12].

Animals

Male albino rats of the Wistar strain, with initial weights between 180 and 230g, were made diabetic by STZ injection. Diabetes was induced with STZ (Sigma-Aldrich Inc., USA), which was administered intravenously in 0.1 mol/l cold citrate buffer, pH 4.5, at a single dose of 45 mg/kg body weight. All the animal experiments were conducted according to the guide for the care and use of laboratory animals of the National Institutes of Health. Whole blood glucose > 18 mmol/l and the presence of glycosuria confirmed that these animals could be considered diabetic and thus used for further experimentation.

The animals were randomly divided into four groups of *eight* as follows; Group I: Normal control rats; Group II: Diabetic control rats; Group III: Normal rats that received cultivated SKEO (100 mg/kg/d); Group V: Diabetic rats that received cultivated SKEO (100 mg/kg/d) in aqueous solution orally for 21 days. At the end of the experimental period, in the fed state, animals were killed by decapitation and the serum was separated for determination of glucose and insulin levels. The liver was rapidly removed, sliced into very small pieces, washed with cold saline, frozen in liquid nitrogen and finally stored at -80ºC.

Measurement of fasting blood glucose (FBS) and insulin serum levels

Glucose concentration was measured by the glucose oxidase method (Parsazmun Co., Tehran, Iran) and insulin was assayed using a rat insulin ELISA kit (Mercodia AB, Uppsala, Sweden).

Measurement of activity of hepatoglycoregulatory enzymes

For all of the enzymes, portions of liver tissue were homogenized in 4 volumes of cold 20 mM Tris- HCl (pH 7.4) buffer containing: 1 mM EDTA for PEPCK; 4 mM EDTA and 0.5 mM dithiothreitol for GP; and 100 mM KCl, 2 mM $MgCl_2$, 1 mM EDTA and 1 mM dithiothreitol for GK, using a polytron homogenizer (Kinematica AG, Littau, Switzerland) for 45 sec pulse. This crude homogenate was centrifuged for 60 minutes at 105,000×g 4ºC, using Beckman Ultracentrifuge Type 90 Ti rotor (Palo Alto, CA, USA). The crude supernatant was used for enzyme assays. GK and hexokinase activities were measured by modification of a previously described method [13]. Assays were performed in 100mM Tris-HCl buffer (pH 7.5) containing 100 mM KCl, 8 mM $MgCl_2$, 5 mM ATP, 0.5 mM $NADP^+$, 1 mM dithiothreitol, 0.1 g/l bovine serum albumin, 0.5 U/ml glucose -6- phosphate dehydrogenase, 0.5 or 100 mM glucose, and 50 µl crude supernatant in a total volume of 1 ml. Hexokinase activity was measured at a glucose concentration of 0.5 mM and GK activity was estimated as the difference between activities at 0.5 and 100 mM glucose. PEPCK activity was determined as previously described [14]. GP activity was assayed spectrophotometrically using the crude supernatant by modification of a previously described method [15]. AMP at 5 mM concentration was added instead of caffeine to the reaction mixture. Total protein concentration was determined by the method of Lowry [16]. Enzyme activities are expressed as nmoles of substrate converted by 1mg hepatic supernatant protein per minute.

Measurement of genes expression of hepatoglycoregulatory enzymes

RNA extraction and cDNA synthesis

Total RNA was extracted from each rat liver using high pure RNA tissue kit (Roche Diagnostics, Mannheim, Germany) according to the protocol provided by the manufacturer. The absorption ratio 260/280 nm all of preparations ranged between 1.8 and 2.0 and RNA integrity was assessed by gel electrophoresis using an agarose-ethidium bromide gel. Total RNA was reverse transcribed using a transcriptor first strand cDNA synthesis kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions.

Real-time RT-PCR

Real-time PCR was performed with a Lightcycler 2.0 system (Roche Applied Science, Indianapolis, USA) using lightcycler faststart DNA master SYBR Green I kit (Roche Diagnostics, Mannheim, Germany). The sequences of the specific primers for hepatic GP, PEPCK and β-actin (as an internal standard) gene expression are shown in table 1. Samples were incubated in the Lightcycler apparatus for a predenaturation at 95ºC for 10 minutes followed by 45 PCR cycles. Each amplification cycle was 95ºC for 10 seconds, primer Tm (Table 1) for

15 seconds and 72ºC for 20 seconds. Amplification of specific transcripts was confirmed by melting curve profiles generated at the end of each PCR. Product length and PCR specificity were checked further by 2% agarose gel electrophoresis and ethidium bromide staining. The relative quantity was calculated from a standard curve for each gene generated from diluted normal cDNA samples. Relative expression mRNA levels were normalized against β-actin.

Gene name	Genbank accession	Primer sequences	Amplicon size	Tm
GK	NM_012565	Forward:5-'ACTGACTATCCGGCTACATG-3' Reverse: 5'-GATTCCTGCTTGAATAGTGC-3'	199bp	47°C
GP	NM_022268	Forward: 5'-CCCGAGCACCCAATGACTTTAACC -3' Reverse: 5'- GCGAGTGCGGGATGTGTGTCA -3'	298 bp	66°C
PEPCK	NM_198780	Forward: 5'-GTCACCATCACTTCCTGGAAGA -3' Reverse: 5'-GGTGCAGAATCGCGAGTTG-3'	84 bp	55°C
β actin	NM_03114	Forward: 5'-ATGGATGACGATATCGCTGC-3' Reverse: 5'-CTTCTGACCCATACCCACCA-3'	150 bp	57°C

Table 1: *Sequences of primers for GK, GP, PEPCK and β actin gene expression.*

Statistical analysis

To detect changes in variables between control and treated groups, we performed a one-way ANOVA followed by post-hoc multiple comparisons by Tukey's test. Bivariable correlations between enzyme activities and their mRNA levels in experimental groups were performed using Pearson correlation (r). A p-value ≤ 0.05 was considered as statically significant.

Results

Chemical composition of cultivated *Satureja khuzestanica* Jamzad aerial parts essential oil

The yield of the essential oils obtained from cultivated *Satureja khuzestanica* Jamzad *aerial parts* was 2.1% (W/W) respectively. Results of the GC-MS analysis of the oils are shown in table 2. Thirty five compounds of SKEO were identified (98.8% of the total oils respectively). The main constituents found in the cultivated *Satureja khuzestanica* Jamzad *airal parts* were carvacrol (63.17%); para cymene (5.61%); 4-Terpineol (4.1%); B-Bisabolene (3.77%); Linalool (3.32%); γ-Terpinene (2.77%) and Myrcene (2.43%).

Effect of the cultivated SKEO on FBS and insulin serum levels

The level of FBS of diabetic rats were significantly increased compared with control groups, while the levels of insulin decreased significantly ($p < 0.001$) (Table 3). The level of FBS of diabetic rats receiving SKEO compared with those of diabetic control rats were significantly decreased ($p < 0.016$), although the level FBS in diabetic rats treated with SKEO was still markedly higher than that in normal control rats ($p < 0.001$). Serum insulin also was not significantly changed in diabetic rats treated with SKEO compared with that of diabetic control rats ($p > 0.05$). Similarly, the FBS and insulin of normal rats treated with SKEO were not significantly altered compared with the those levels in normal control rats ($p > 0.05$).

Effect of the cultivated SKEO on hepatic GK activity and its mRNA levels

Effect of 21 days oral administration of the cultivated SKEO on hepatic GK activity and its mRNA levels is shown in figure 1 and 2, respectively. Activity of the hepatic GK and its mRNA levels of diabetic rats were significantly reduced compared with those of normal control rats ($p < 0.015$). Hepatic GK activity and its mRNA levels in normal rats treated with SKEO were not significantly altered compared with levels of the normal control group ($p > 0.05$). Diabetic rats treated with SKEO showed a 48% elevation in hepatic GK activity and a

Number	Compound Name	Retention Time (min)	Yield %
1	3-Methyl butanal	3.5	0.14
2	α-Thujene	9.5	1.26
3	α-Pinene	9.8	0.99
4	Camphene	10.4	0.14
5	iso Amylpropionate	11.0	0.23
6	β-Pinene	11.3	0.32
7	Myrcene	11.5	2.43
8	trans-2-Carene-4-ol	11.7	0.73
9	isoButyl-2-methyl butyrate	11.9	0.19
10	3-Carene	12.2	0.36
11	α-Terpinene	12.5	0.73
12	para Cymene	13.0	5.61
13	β-Phellandrene	13.1	0.31
14	1,8-Cineole	13.2	0.24
15	γ-Terpinene	14.0	2.77
16	cis Sabinene hydrate	14.5	0.68
17	Terpinolene	14.8	0.22
18	Linalool	15.4	3.32
19	Nonanal	15.5	0.24
20	4-Terpineol	18.2	4.1
21	α-Terpineol	18.6	0.42
22	Thymyl methyl ether	19.7	1.21
23	trans Dihydrocarvone	21.0	0.26
24	Carvacrol	22.5	63.17
25	Eugenol	23.9	1.33
26	β-Caryophyllene	25.5	0.7
27	Geranyl acetone	25.8	0.5
28	α-Franesene	27.1	0.7
29	β-Bisabolene	27.4	3.77
30	α-Bisabolene	28.2	0.51
31	Caryophyllene oxide	29.8	1.53
32	β-Udesmol	30.0	0.32
33	Heptadecane	31.7	0.19
34	α-Bisabolol	32.0	0.27
35	Musk ambrette	38.5	0.08

Table 2: *Chemical composition of the Satureja khuzestanica Jamzad area parts essential oil (SKEO).*

Group	Plasma glucose (mM)	Plasma insulin (µg/l)
Normal control	8.29 ± 0.99	0.99 ± 0.46
Normal + 100 mg/kg SKEO	7.42 ± 0.62	0.88 ± 0.31
Diabetic control	22.31 ± 2.5*	0.22 ± 0.047*
Diabetic +100 mg/kg SKEO	19.86 ± 1.12*,#	0.28 ± 0.068*

Table 3: *Effect of oral administration of the cultivated SKEO on the plasma levels of glucose and insulin for 21 days.*

24% elevation in the level of its mRNA compared with diabetic control rats although this difference is not significant ($p > 0.05$). As shown in figure 3, there was a significant correlation between the hepatic GK activity and the hepatic GK mRNA levels in all experimental groups of rats ($r = 0.977$ at $p < 0.01$).

Effect of cultivated SKEO on hepatic GP activity and its mRNA levels

Effect of 21 days oral administration of the cultivated SKEO on hepatic GP activity and its mRNA levels is shown in figure 4 and 5 respectively. Activity of the hepatic GP and its mRNA levels of diabetic rats were significantly reduced compared with those of normal control rats ($p < 0.05$). Diabetic rats treated with SKEO showed a 20% reduction in GP activity and a 19% reduction in its mRNA level

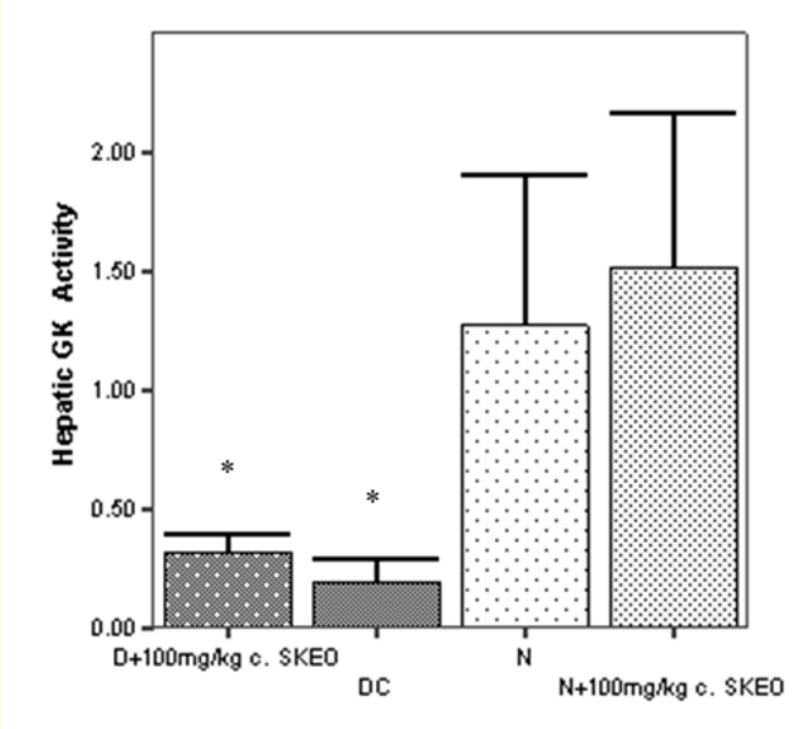

Figure 1: *Effect of oral administration of the cultivated SKEO on hepatic GK activity of the for 21 days. N, Normal; DC: Diabetic Control; D: Diabetic. Values (means ± S.E.M) were obtained each group of six rats. *p < 0.05 compare to normal control and normal + 100 mg/kg SKEO. Enzyme activity is expressed as nmoles/mg protein/min.*

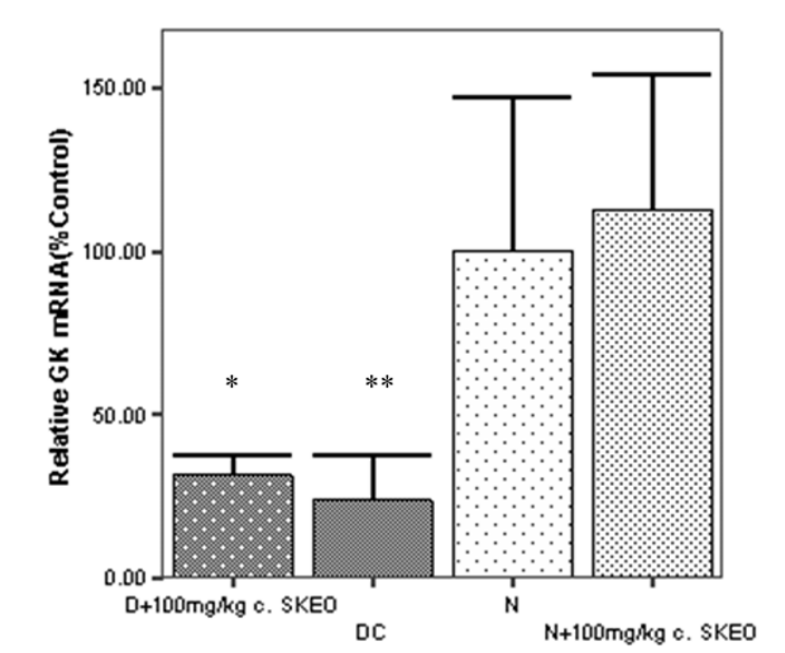

Figure 2: *Effect of oral administration of the cultivated SKEO on hepatic GK mRNA levels for 21 days. NC: Normal Control; DC: Diabetic Control; N: Normal; D: Diabetic. Values (means ± S.E.M) were obtained from each group of six rats. *p < 0.001 and **p < 0.05 compare to normal control and normal + 100 mg/kg SKEO.*

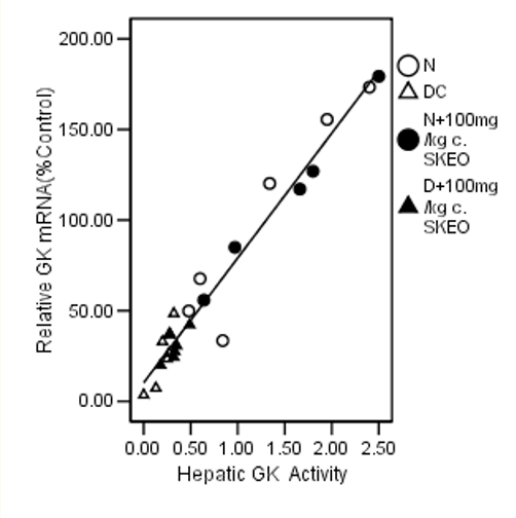

Figure 3: *Correlation between the GK mRNA levels and GK activity of rat livers. (a): Correlations are significant at p < 0.01 with r = 0.977.*

compared with diabetic control rats. Hepatic GP activity and its mRNA levels in normal rats treated with SKEO were associated with a 25% reduction in GP activity and a 20% reduction in its mRNA levels compared with those in the normal group of rats, although these differences are not significant ($p > 0.05$). As shown in figure 6, there was a significant correlation between the hepatic GP activity and the hepatic GP mRNA levels in all experimental groups of rats ($r = 0.931$ at $p < 0.01$).

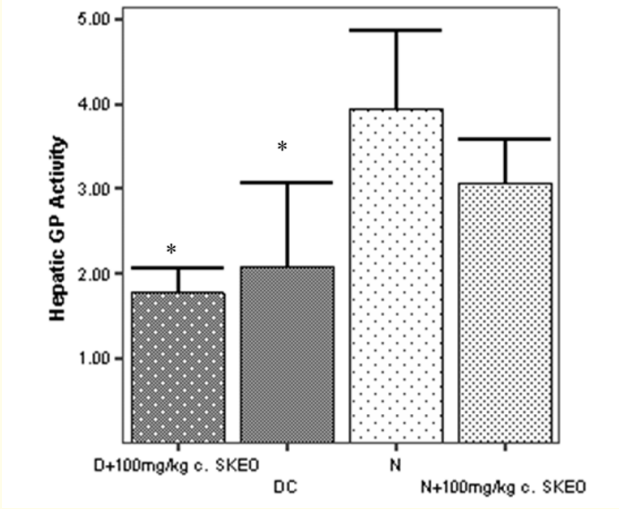

Figure 4: *Effect of oral administration of the cultivated SKEO on hepatic GP activity for 21 days. NC: Normal Control; DC: Diabetic Control; N: Normal; D: Diabetic. Values (means ± S.E.M) were obtained each group of six rats. *p < 0.05 compare to normal control and normal + 100 mg/kg SKEO. Enzyme activity is expressed as nmoles/mg protein/min.*

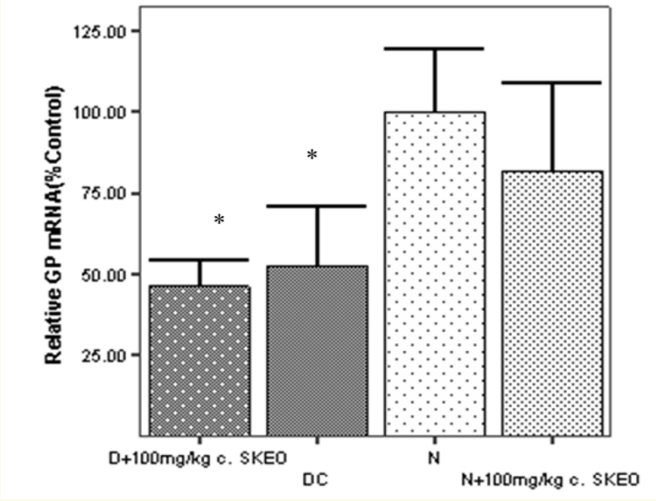

Figure 5: *Effect of oral administration of the cultivated SKEO on hepatic GP mRNA levels for 21 days. NC: Normal Control; DC: Diabetic Control; N: Normal; D: Diabetic. Values (means ± S.E.M) were obtained from each group of six rats. *p < 0.05 compare to normal control and normal + 100 mg/kg SKEO.*

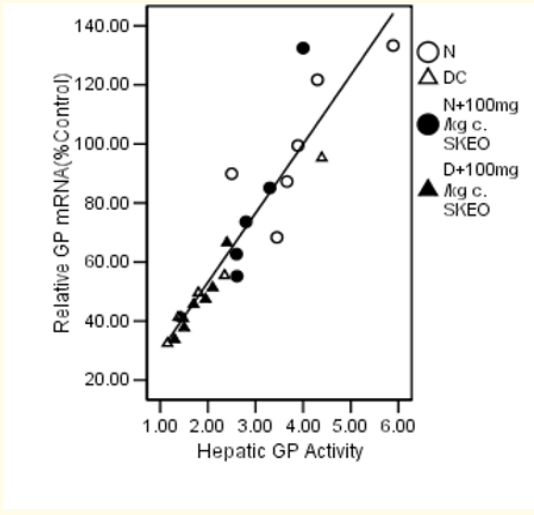

Figure 6: *Correlation between the GP mRNA levels and GP activity of rat livers; Correlations are significant at p ≤ 0.01 with r = 0.931.*

Effect of cultivated SKEO on hepatic PEPCK activity and its mRNA levels

Effect of oral administration of SKEO on hepatic PEPCK activity and its mRNA levels is shown in figure 7 and 8 respectively. Activity of the hepatic PEPCK and its mRNA levels of diabetic rats was significantly increased compared with those of normal control rats ($p < 0.001$). Normal and diabetic rats treated with SKEO were associated with a mean of 21% reduction in PEPCK activity and its mRNA levels compared with normal and diabetic controls, respectively ($p > 0.05$). As shown in figure 9, there was a high correlation between rates of activity and mRNA levels of PEPCK levels in all experimental groups of rats ($r = 0.948$ at $p < 0.01$).

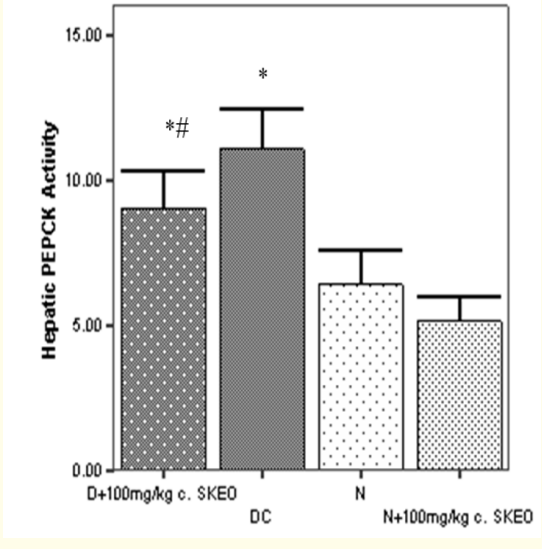

Figure 7: *Effect of oral administration of the cultivated SKEO on hepatic PEPCK activity for 21 days. NC: Normal Control; DC: Diabetic Control; N: Normal; D: Diabetic. Values (means ± S.E.M) were obtained each group of six rats. *p < 0.05 compare to normal control and normal + 100 mg/kg SKEO. #p < 0.05 compare to diabetic control. Enzyme activity is expressed as nmoles/mg protein/min.*

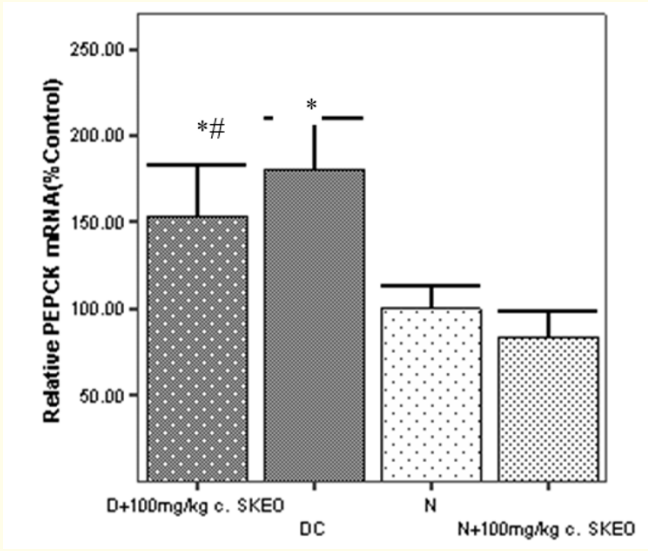

Figure 8: *Effect of oral administration of the cultivated SKEO on hepatic PEPCK mRNA levels for 21 days. NC: Normal Control; DC: Diabetic Control; N: Normal; D: Diabetic. Values (means ± S.E.M) were obtained from each group of six rats. *p < 0.05 compare to normal control and normal + 100 mg/kg SKEO. #p < 0.05 compare to diabetic control.*

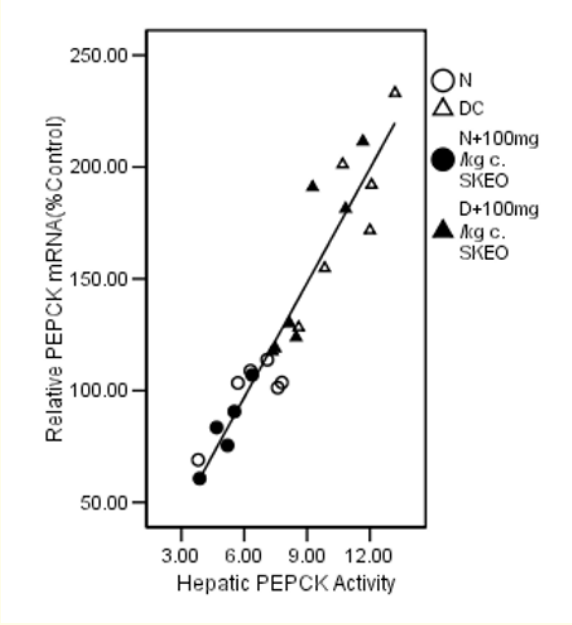

Figure 9: *Correlation between the PEPCK; mRNA levels and PEPCK, Activity of rat livers; Correlations are significant at p < 0.01 with r = 0.948 for PEPCK.*

Discussion

Diabetes type I significantly increased serum levels of FBS and decreased level of insulin in comparison with the control group. The level of FBS of diabetic rats receiving SKEO for 21 days were significantly decreased compared with those of diabetic control rats. The level of serum insulin of diabetic rats receiving SKEO for 21 days were not significantly decreased compared with those of diabetic control rats. The levels of serum insulin of normal rats treated with SKEO were not significantly altered compared with the levels in the normal control group. Hence, serum glucose lowering action of the cultivated SKEO was only observed in diabetic rats as a hyperglycemia state. The influence of cultivated SKEO on activities and gene expression of hepatic GK, hepatic GP and hepatic PEPCK in normal and STZ-induced diabetic rats was explored.

Hepatic GK activity and its mRNA levels decreased in diabetic untreated groups compared with control groups. The activity of GK and its mRNA levels of diabetic rats receiving SKEO for 21 days were not significantly increased compared with those of diabetic untreated rats. These data support the view that the slightly increase in the hepatic GK activity in diabetic rats treated with the cultivated is parallel to the GK mRNA levels that were moderately elevated ($r = 0.977$ at $p < 0.01$). These findings suggested that moderately enhanced GK activity and the GK mRNA levels in diabetic rats treated with SKEO are probably due to a slight increase in serum insulin levels.

Streptozotocin acts as a diabetogenic in the experimental animal models in pancreatic β-cells by increasing mitochondrial reactive oxygen species (ROS) formation. ROS plays a role in β-cell damaging and accelerates the process of β-cell destruction [17]. The main components of essential oil are rich in phenolic compounds (such as carvacrol, p-cymene, Linalool and Myrcene) and terpenoid compounds (such as γ-terpinene and terpinene-4-ol) which were isolated as antioxidant components from the cultivated *Satureja kuzestanica* Jamzed [18]. It has been reported that the flavonoids protect the islet β-cells in diabetic animal models which acts synergistically as an antioxidant [19]. This slightly increased level of insulin indicates that SKEO causes a short stimulation in insulin secretion from remnant β-cells and is possibly related to its antioxidant properties. A number of other plants have also been reported to have an antihyperglycemic effect and a stimulatory effect on insulin release [6,20,21]. The present study, hepatic GP activity and its mRNA levels were decreased in diabetic untreated groups compared with control group and slightly diminished after treated with SKEO at 21 days. In contrast to a previous report by Saadat., *et al.* [10] that reports increased GP activity in normal treated rats with SKEO compared with normal control rats, we observed reduced GP activity and mRNA levels (25% and 20%, respectively), although these differences are not significant ($p > 0.05$). On the other hand, the levels of insulin negligibly increased in diabetic rats treated with SKEO compared with levels in the diabetic control group. Hence, the chronic effect of insulin on the activation of the GP activity that is mediated through the stabilization of its mRNA levels was not observed [22].

The decrease in GP mRNA levels in normal and diabetic treated rat liver occurred parallel to the decrease in GP activity ($r = 0.931$ at $p < 0.01$). These data support the view that a decrease in GP activity and GP mRNA levels in normal and diabetic treated rat liver is probably due to a decrease in GP synthesis via the modulation of the factors involved in transcription rate or post-transcriptional steps.

We found that hepatic PEPCK activity and its mRNA levels significantly increased in diabetic untreated group compared with control group. Previous reports indicate that hyperglycemia results in generation of reactive oxygen species (ROS) ultimately leading to increased oxidative stress. This elevation of oxidative stress is involved in the up-regulation of gene expression of PEPCK via activating the stress-activated signaling pathways [23]. PEPCK activity and its mRNA levels were significantly decreased in normal and diabetic treated rats with SKEO compared with control and diabetic untreated groups, respectively, in agreement with the previously obtained results of Malardé., *et al* [23]. The high correlation between rates of activity and mRNA levels of PEPCK ($r = 0.948$ at $p < 0.01$) supports a fundamental role for SKEO in promoting inactivation of PEPCK synthesis through suppression of the factors involving the stress–activated signaling pathways that are mediated ROS. It this context, several antidiabetic plants have been reported to have the ability to decrease PEPCK activity through their antioxidant properties [24-26].

Our results demonstrated that SKEO significantly decreased PEPCK and slightly decreased GP activity as well as slightly increased GK activity may increase the efficiency of the serum glucose lowering action of SKEO.

Conclusion

This study showed that SKEO exerts beneficial effects on GP, GK and PEPCK activities and gene expression of GP, GK and PEPCK in the liver of diabetic rats treated with cultivated SKEO. SKEO has hypoglycemic effect may contribute through reduction of glycogenolysis and gluconeogenesis that association with elevation of hepatic uptake of glucose in diabetic treated rats. Therefore, hypoglycemic effects of SKEO seem to be related to beneficial effects of SKEO on activities and genes expression of hepatoglycoregulatory enzymes in diabetic treated rats. Also, this study showed that SKEO is a source easily accessible of natural good antioxidants such as Carvacrol (63.1%) and it may be suitable for use in food and pharmaceutical applications.

Conflict of Interest

We declare that we have no conflict of interest.

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