

ORIGINAL ARTICLE

EFFECTS OF *TEUCRIUM POLIUM* ON ORAL GLUCOSE TOLERANCE TEST, REGENERATION OF PANCREATIC ISLETS AND ACTIVITY OF HEPATIC GLUCOKINASE IN DIABETIC RATS

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Background and Objective – Hepatotoxicity associated with the hypoglycemic effects of an aqueous extract of *Teucrium polium* was previously described. In this investigation, the effects of the extract on oral glucose tolerance test, regeneration of pancreatic islets and hepatic glucokinase activity of streptozocin-induced diabetic rats were studied.

Methods – An aqueous extract of *T. polium* was fed by gavage tube to healthy and streptozocin-induced diabetic rats for several days. Oral glucose tolerance, number of pancreatic islets and hepatic glucokinase activity were measured using standard methods and compared between diabetic and healthy rats.

Results – In diabetic animals, the aqueous extract caused a significant reduction ($p < 0.001$) in the level of serum glucose during oral glucose tolerance tests. The number of pancreatic islets per unit area significantly increased ($p < 0.01$) and glucokinase activity was significantly elevated ($p < 0.01$) in diabetic animals treated with the extract.

Conclusion – Although aqueous extract of *T. polium* contains some hepatotoxic compounds, it also contains components that are beneficial in the treatment of streptozocin-induced diabetes.

Keywords diabetes mellitus glucose tolerance test islet of Langerhans rats *Teucrium polium*

Introduction

In a previous communication, the hepatotoxicity associated with the hypoglycemic effects of *Teucrium polium* or polygermander was reported and the literature on this herb was extensively reviewed.¹ In this study, attempts were made to elucidate the mechanisms of the hypoglycemic effects of the aqueous extract of the dried aerial parts of *T. polium* in bloom. Experiments were designed to study the effects of the extract on oral glucose tolerance test (OGTT), regeneration of pancreatic islets and hepatic glucokinase activity in streptozocin-induced

diabetic rats.

Materials and Methods

Reagents

Glucose, fatty acid-free bovine serum albumin, *Leuconostoc mesentroides* glucose-6-phosphate dehydrogenase (G6PD) and adenosine triphosphate (ATP, disodium salt) were obtained from Roche Chemical Company (Mannheim, Germany). Nicotinamide adenine dinucleotide (NAD⁺, disodium salt) from Fluka Chemical Company (Buchs, Switzerland), 4-(2-hydroxyethyl)-1-piperazineethane sulfanic acid (HEPES) buffer and dithioerythritol (DTE) from Sigma Chemical Company (St. Louis, MO, USA), streptozocin from Upjohn Co (Kalamazoo, MI, USA) and the enzymatic kit for glucose determination from Pars Azemoon Co (Tehran, Iran). All other reagents

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were of analytical grade and were purchased from other commercial sources.

Preparation of *T. polium* extract

T. polium aerial parts were purchased from herbalists in Kerman, Iran. After authentication, extracts were prepared as described previously.¹ The final aqueous extract, which corresponded to 4.5 g of the original dried aerial parts per mL, was used as the hypoglycemic agent throughout this study.

Animal experiments

Four groups of adult male Sprague-Dawley rats (10 rats per group) with weights ranging from 200 to 220 g were used. Housing conditions and feeding protocols were as described previously.¹ Two groups of rats were injected with streptozocin to induce diabetes.¹ After 2 weeks, the serum glucose level in diabetic rats reached 24 – 25 mM. One group of diabetic rats (diabetic experimental rats) received 1-mL aliquots of the aqueous extract twice a day through a gavage tube for a period of 12 days, while the other group of diabetic rats (diabetic control rats) received only water and rat chow *ad libitum*. The other two groups of rats with an average serum glucose level of 6.7 mM were used as healthy animals. The normoglycemic control rats received only water and rat chow *ad libitum* while normoglycemic experimental animals received 1 mL of the aqueous extract per day for 10 days in addition to *ad libitum* feeding. Serum glucose levels were determined at specified intervals in both normoglycemic and diabetic animals, as reported previously.¹ At the end of the gavage feeding of the extract, half of the rats in each of the four groups were fasted for 24 hours and underwent OGTT, while the other half were immediately sacrificed by decapitation. The liver was frozen in liquid nitrogen and kept at -70°C until hexokinase and glucokinase measurements were taken while the pancreas was placed in buffered 10% formalin and used for histological studies.

Oral glucose tolerance test

OGTT in fasted animals was performed using the procedure of Young et al.² Duplicate blood samples from animal tails were collected into heparinized microhematocrit tubes after 24 hours of fasting and the baseline serum glucose level was measured. At this time, animals received 1 mL of a glucose solution containing 0.6 g glucose (3 g/kg

body weight) by gavage and duplicate blood samples from their tails were collected after 45, 90 and 135 minutes. The glucose concentration data were used to compare glucose tolerance in the various groups.

Preparation of liver extract

Frozen liver tissue (1 g) was cut into small pieces and homogenized at 4°C in 9 mL of cold buffer (pH 7.4) containing Na-HEPES 50 mM, KCl 100 mM, EDTA 1 mM, MgCl₂ 5 mM and DTE 2.5 mM using a glass-Teflon Potter-Elvehjem homogenizer with 20 up-and-down motions at half maximum speed. The suspension was centrifuged at 12,000 g for 1 hour in a Sorval centrifuge at 4°C. The clear supernatant was immediately used to measure hexokinase and glucokinase activities.

Measurement of hexokinase and glucokinase activities

Liver hexokinase and glucokinase activities were measured using the coupled enzyme assay procedures of Davidson and Arion³ and Ferre et al.⁴ The incubation mixtures contained the following ingredients in a final volume of 1 mL: incubation buffer (pH 7.4) containing HEPES 50 μmol, KCl 100 μmol, MgCl₂ 7.5 μmol and DTE 2.5 μmol; fatty acid-free bovine serum albumin 10 mg; NAD⁺ 0.5 μmol; G6PD 4 units; liver extract 100 μL (hexokinase assay) or 10 μL (total hexokinase and glucokinase assays); and D-glucose 0.5 μmol (hexokinase) and 10 μmol (total enzyme activities). Both control and test tubes were pre-incubated at 25°C for 5 minutes. Water (0.2 mL) was added to the control tubes and, to start the reaction in the test tubes, 0.2 mL of a solution containing 0.5 μmol of ATP was added. Control tubes were adjusted to zero absorbance in a Shimadzu UV 160 spectrophotometer at 340 nm and the increase in absorbance in test tubes at this wavelength was plotted against time for a period of 15 minutes; the reaction was linear with time. Total enzyme activities (glucokinase + hexokinase) and hexokinase activities (mU/mL) were calculated. To obtain glucokinase activity, the hexokinase activity was subtracted from the total enzyme activity. Protein concentration in liver extracts was measured using the Biuret reagent⁵ and specific activities were expressed as mU/mg protein.

Histological studies

The pancreas was fixed in buffered 10% formalin.

Table 1. Effects of aqueous extract of *T. polium* on oral glucose tolerance tests of normoglycemic and diabetic rats.

Experimental groups	Zero time [*]	45 min	90 min	135 min
Normoglycemic control	3.4 ± 1.0 [†]	8.5 ± 0.6	5.8 ± 0.9	5.0 ± 0.5
Normoglycemic experimental [‡]	1.3 ± 1.4	7.12 ± 0.8	6.6 ± 0.5	5.3 ± 0.4
Diabetic control	1.5 ± 0.3	25.2 ± 0.6 [‡]	23.1 ± 1.4 [‡]	21.6 ± 1.9 [‡]
Diabetic experimental [§]	0.9 ± 0.2	11.7 ± 1.4 [‡]	10.1 ± 1.1 [‡]	8.7 ± 1.0 [‡]

* = zero glucose concentration refers to serum glucose concentration of 24 hr fasted animals at the end of each experiment prior to glucose feeding (see text); [†] = data are expressed as mean ± SEM of 5 rats in each group; [‡] = normoglycemic animals receiving 1 mL of the aqueous extract per day for 10 days; [§] = streptozocin-induced diabetic animals receiving 2 × 1 mL of the aqueous extract per day for 12 days; [‡] Student's *t*-test and Mann-Whitney U nonparametric test only showed a significant difference (*p* < 0.001) between the serum glucose level of diabetic control and diabetic experimental groups during glucose tolerance.

Two to three 5 μm thick sections were prepared and stained with hematoxylin and eosin. A pathologist who was not aware of the experimental treatments counted the islets in each section, expressed per cm².

Results

The effects of the aqueous extract of the aerial parts of *T. polium* on oral glucose tolerance tests of normoglycemic and streptozocin-induced diabetic rats are shown in Table 1. Intraesophageal administration of 1 mL of the extract for 10 days had no significant effect on glucose tolerance in normoglycemic animals. However, twice-daily treatment of diabetic rats with 1 mL aliquots of the extract significantly reduced serum glucose levels during the glucose tolerance test (*p* < 0.001) and in 135 minutes brought the level close to that of normoglycemic animals (8.7 ± 1.0 vs 5.0 ± 0.5 mM). The Figure, part A, shows a typical pancreatic section from a streptozocin-induced diabetic animal and Figure B shows a pancreatic section from a diabetic rat treated with the extract. The number of pancreatic islets per unit area was higher in extract-treated diabetic rats. Table 2 shows a comparison of the number of pancreatic

islets between normoglycemic control, diabetic control and diabetic experimental animals. The number of pancreatic islets per unit area significantly decreased in diabetic animals and extract treatment resulted in the regeneration of these islets to the normal range.

Table 3 shows the effect of the extract on hepatic hexokinase and glucokinase activities. Both hexokinase and glucokinase activities significantly decreased in diabetic animals and extract treatment brought the level of these enzymes to that reported in normoglycemic controls.

Discussion

According to one of the criteria devised by the National Diabetes Data Group (NDDG) for diagnosing diabetes mellitus,⁶ a plasma glucose greater than 11.1 mM at two time points (2 hours and one earlier time point) during an OGTT is indicative of diabetic glucose tolerance. As shown in Table 1, our streptozocin-induced diabetic animals satisfy this criterion. An NDDG criterion for the diagnosis of impaired glucose tolerance is a plasma glucose that exceeds 11.1 mM at least once between 0 and 2 hours during OGTT and that falls to 7.8 – 11.0 mM by 2 hours. As shown in Table 1, our diabetic animals treated with the extract satisfy this criterion. Thus, although this group is no longer classified as diabetic, it still shows impaired glucose tolerance. Rats with a blood glucose that never exceeds 7.1 mM during OGTT are truly normoglycemic (Table 1). As shown in the Figure part A and B and in Table 2, streptozocin-induced diabetes resulted in a significant decrease in the number of pancreatic islets per unit area and *T. polium* extract treatment apparently regenerated these islets to the normal level. Several flavonoids

Table 2. Effects of aqueous extract of *T. polium* on the number of pancreatic islets.

Experimental groups [*]	No. of islets of Langerhans (cm ²)
Normoglycemic control	20.5 ± 4.0 [†]
Diabetic control	5.0 ± 1.0 [†]
Diabetic experimental	14.9 ± 1.7 [†]

* = for the detail of experimental conditions see Table 1 and text; [†] = data are expressed as mean ± SEM of five rats in each group. The data were analyzed by one way ANOVA and Duncan test. The figures with different letter superscripts were significantly different at *p* < 0.01.

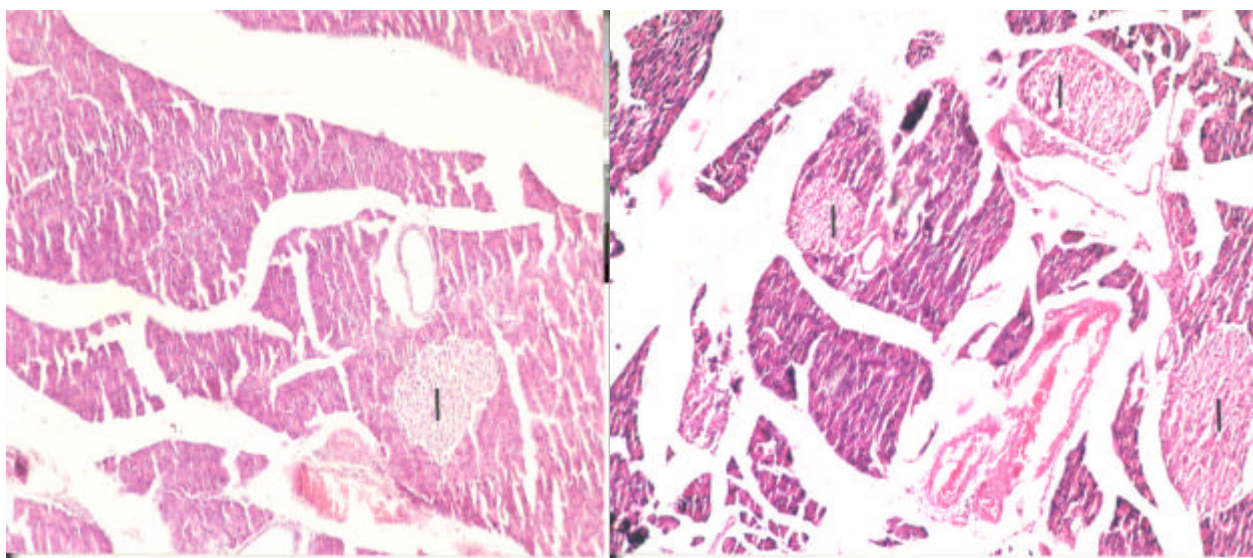


Figure. (left) A section of diabetic pancreas showing one islet of Langerhans (I). (right) A section of pancreas from diabetic animals treated with the extract showing three islets of Langerhans (I). Original magnification X 200.

are present in *T. polium*.⁷⁻⁹ One such flavonoid with hypoglycemic effects in diabetic animals is quercetin.¹⁰ Hii and Howell reported that quercetin may, at least in part, exert its effects on insulin release from rat islets of Langerhans via changes in Ca^{+2} metabolism.¹¹ Flavonoids with insulin-triggering and/or insulin-like properties have been extracted from other plants.¹² It is possible that the flavonoids present in the aerial parts of *T. polium* may be responsible for islet regeneration and possibly for β -cell regeneration and insulin release and/or they may have insulin-like properties.

As shown in Table 3, hepatic hexokinase activity in diabetic animals decreased to approximately 50% of that in normoglycemic animals and the level returned to normal after extract treatment. The specific activity of hepatic glucokinase, an insulin-inducible enzyme¹³ and the major glucose phosphorylating enzyme in the liver, decreased 7-fold in diabetic compared to normoglycemic animals; *T. polium* extract treatment increased the activity almost 9-fold (Table 3). This supports the

conclusion that flavonoids with insulin-releasing and/or insulin-like activities, such as quercetin, will promote induction of hepatic glucokinase and result in significantly increased activity in streptozocin-induced diabetic rats treated with the aqueous extract of the aerial parts of *T. polium*.

Although a significant increase in hepatic glucokinase indirectly demonstrates increased insulin release from β -cells, direct measurement of plasma insulin is necessary to confirm the insulin-releasing properties of this herbal extract.

In our previous communication,¹ we reported the adverse effects of *T. polium* extract on liver histology, but this extract also has some beneficial effects such as improvement of oral glucose tolerance, regeneration of the islets of Langerhans and normalization of hepatic glucokinase activity in streptozocin-induced diabetic rats.

Future experiments with quercetin, which is found in *T. polium*, will determine whether this flavonoid has these beneficial effects in the pancreas and the liver without the injurious effects

Table 3. Effect of aqueous extract of *T. polium* on hepatic hexokinase and glucokinase activities.

Experimental groups *	Hexokinase activity (mU/mg protein)	Glucokinase activity (mU/mg protein)
Normoglycemic control	2.0 \pm 0.2 [†]	4.4 \pm 0.98
Normoglycemic experimental	2.1 \pm 0.2	5.4 \pm 0.60
Diabetic control	0.8 \pm 0.2 [‡]	0.6 \pm 0.3 [§]
Diabetic experimental	1.9 \pm 0.2 [‡]	5.4 \pm 0.55 [§]

* = for the details of experimental conditions see Table 1 and text; [†] = data are expressed as mean \pm SEM of five rats in each group; [‡] $p < 0.05$; [§] $p < 0.01$ using Student's *t*-test and Mann-Whitney U nonparametric test.

on hepatocytes that are believed to be due to the presence of neoclerodane diterpenoids found in *T. polium*.¹

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References

- 1 Zal F, Vasei M, Rasti M, et al. Hepatotoxicity associated with hypoglycemic effects of *Teucrium polium* in diabetic rats. *Arch Iranian Med*. 2001; **4**: 188–92.
- 2 Young PW, Cawthorne MA, Coyle PJ, et al. Repeat treatment of obese mice with BRL 49653, a new and potent insulin sensitizer, enhances insulin action in white adipocytes. Association with increased insulin binding and cell-surface GLUT 4 as measured by photoaffinity labeling. *Diabetes*. 1995; **44**: 1087–92.
- 3 Davidson A, Arion WJ. Factors underlying significant underestimations of glucokinase activity in crude liver extracts: physiological implications of higher cellular activity. *Arch Biochem Biophys*. 1987; **253**: 156–67.
- 4 Ferre T, Pujol A, Riu E, et al. Correction of diabetic alterations by glucokinase. *Proc Natl Acad Sci USA*. 1996; **93**: 223–30.
- 5 Gornall AG, Bardwill CJ, David MM. Determination of serum proteins by means of the biuret reaction. *J Biol Chem*. 1949; **177**: 751–66.
- 6 National Diabetes Data Group. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. *Diabetes*. 1979; **28**: 1039–57.
- 7 Oganessian GB, Minatsakanyam VA. Flavonoids of cat thyme (*Teucrium polium* L). *Arm Khim Zh*. 1985; **38**: 57–61.
- 8 Rizk AM, Hammouda FM, Rimpler H, et al. Iridoids and flavonoids of *Teucrium polium* herb. *Planta Medica*. 1986; **2**: 87–8.
- 9 Verykokidov-Vitsaropoulou E, Vajias C. Methylated flavones from *Teucrium polium*. *Planta Medica*. 1986; **5**: 401–2.
- 10 Nuraliev IN, Avezov GA. The efficacy of quercetin in alloxan diabetes [in Russian]. *Eksp Klin Farmakol*. 1992; **55**: 42–4.
- 11 Hii CS, Howell SL. Effects of flavonoids on insulin secretion and 45Ca^{+2} handling in rat islets of Langerhans. *J Endocrinol*. 1985; **107**: 1–8.
- 12 Ahmad M, Akhtar MS, Malik T, et al. Hypoglycemic action of the flavonoid fraction of *Cuminum nigrum* seeds. *Phytother Res*. 2000; **14**: 103–6.
- 13 Nospikel T, Lynedjian PB. Insulin signaling and regulation of glucokinase gene expression in cultured hepatocytes. *Eur J Biochem*. 1992; **210**: 365–73.