Teucrium polium Extract Effects Pancreatic Function of Streptozotocin Diabetic Rats: A Histopathological Examination

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ABSTRACT

In an effort to evaluate the hypoglycemic activity of *T. polium*, the crude extract was administered orally to a group of Streptozotocin induced diabetic rats for 6 consecutive weeks. Significant decrease in blood glucose by 64%, total bilirubin by 35%, glutamate oxaloacetate transferase by 48% and glutamate pyruvate transferase by 30% was observed compared to untreated diabetic rats. However, the blood insulin level was enhanced by almost 160%. The insulinotropic property of the *T. polium* crude extract was further assessed by an *in vitro* investigation using isolated rat islets. Our data indicated that *T. polium* crude extract is capable of enhancing insulin secretion by almost 135% after one dose of treatment at high glucose concentration. Meanwhile, without affecting the time pattern of insulin secretion by the islets, the plant extract seems to be capable of regenerating the islets of langerhans in the treated compared to the untreated diabetic rats. These data clearly provide a mechanistic view concerning the hypoglycemic effect of *T. polium* extract through its significant effect on the pancreas. *Iran. Biomed. J. 9 (2): 81-85, 2005*

INTRODUCTION

It is known that diabetes mellitus, characterized by hypoglycemia, is a genetically and clinically heterogeneous group of disorders with common feature of glucose intolerance [1]. Based on WHO recommendation, diabetes mellitus is classified into three major subtypes: type I (insulin dependent diabetes mellitus, IDDM), type II (non-insulin dependent diabetes mellitus, NIDDM) and malnutrition-related diabetes mellitus. IDDM or Juvenile-onset diabetes results from a cellular mediated autoimmune destruction of the β-cells of the pancreas [2, 3]. However, NIDDM or adult-onset diabetes results from the development of insulin resistance and the affected individuals usually have insulin deficiency [4].

The use of medicinal plants has been among the earliest treatments of diabetes mellitus [5-7]. The Egyptian papyrus Eberm (1550 B.C.) recommended 3554 years ago, the use of high fiber diet of wheat germ and Aretaeus recommended the consumption of water boiled with autumn fruit(s), milk and gruels of different whole grains for treatment of diabetes mellitus. Nowadays, more than 1200 species of the organisms (from 725 genera belonging to 183 families) are used to treat symptoms of diabetes mellitus. Half of these species have been used traditionally for curing diabetes mellitus. The hypoglycemic property of almost fifty percent of these traditionally consumed medicines has been experimentally tested [8]. One of these traditional hypoglycemic herbs is *Teucrium polium* [9, 10], which belongs to Labiataea family. The aqueous extract of the dried aerial parts of *T. polium* is used traditionally to treat diabetes in Southern Iran.

In order to validate the local claimed hypoglycemic effect of this medicinal plant and also to evaluate its effect(s) on pancreas function, the crude extract was administered to a group of Streptozotocin (STZ)-induced diabetes rats for 6 consecutive weeks and the effects on blood glucose and insulin levels were evaluated. In addition, the insulinotropic property of *T. polium* crude extract was further evaluated using isolated rat islets.

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MATERIALS AND METHODS

Plant material. Arial parts of *T. polium L.* were collected from Fars province (Iran) during spring 1999. A voucher herbarium specimen (No. 570) was deposited in the Herbarium of the School of Pharmacy, Shaheed Beheshti University of Medical Sciences, Tehran, Iran. The plant arial parts were air-dried protected from direct sunlight and then powdered. The powder was kept in a closed container at 10°C.

Extraction. The powdered plant material (250 g) was extracted three times with ethanol-water (7:3, v/v) at room temperature. The combined extracts were concentrated under reduced pressure and the volume was adjusted to 500 ml (equivalent to 0.5 g plant powder per ml). The concentrated extract was divided into 25 ml aliquots and kept at -20°C for further investigation.

Animals. Male Wistar Albino rats (n = 30), 5-7 months old with a weight of 200-250 g, were purchased from the Pasteur Institute of Iran (Tehran). They were housed under conventional conditions and were allowed free access to food and water ad libitum.

Induction of experimental diabetes. STZ (Sigma, U.S.A; 40mg/kg body weight) was dissolved in 0.1 M sodium citrate buffer at pH 4.5 just before use [11], and injected intraperitoneally to 22 rats. Control animals (n = 8) received an equivalent volume of citrate buffer. One week after STZ administration, the diabetic rats with blood glucose concentration between 20-25 mmol/L were selected and divided into two groups (II and III).

Oral administration of the plant extract. The plant extract was administrated by gavage (i.g.) to 12 rats of group III daily in a dose of 1 ml/rat (equivalent to 0.5 g plant powder/kg body weight) for 6 consecutive weeks [9]. The control healthy rats (group I, n = 8) and the control diabetic rats (group II, n = 8) received the same volume of distilled water (i.g.). The blood glucose levels of the rats in each group were determined weekly using a Glucomen kit (A. Menarini, Diagnostics, Germany).

Insulin assay. The insulin level of each blood sample was measured by an enzyme-linked immunosorbent assay using a commercial kit (rat insulin ELISA; DRG Instruments GmbH, Germany), based on direct sandwich technique in which two mouse monoclonal antibodies are directed against separate antigenic determinants on the insulin molecules (according to manufacturer instructions).

Pancreatic islet isolation. The islet isolation was carried out according to Shewade's method [12]. Briefly, six healthy pancreases were isolated under sodium pentobarbital anesthesia (50 mg/kg body weight, i.p.). Each pancreas was cut into small pieces and was subjected to enzymatic (colla-genase) digestion at 37°C for 20 min on a magnetic stirrer. The medium consisted of DMEM supplemented with collagenase type V (1 mg/ml), soybean trypsin inhibitor (2 mg/ml), and BSA fraction V (2%). The digested tissue was then centrifuged at 235 ×g for 10 min, washed twice with phosphate buffer saline (pH 7.2) and seeded in culture flasks containing RPMI-1640 supplemented with 10% FCS and incubated at 37°C in an incubator with 5% CO_2. After 24 h of incubation, islets were separated by hand picking under an inverted microscope.

Insulin secretion: static incubation. Batches of 13 rat islets were placed into tubes containing 0.6 ml of the modified Krebs-bicarbonate medium with 20 mmol/L Hepes pH 7.4 and 2 mg/ml albumin [13] along with various plant extract concen-trations. After 2 hours of incubation in a CO_2 incubator at 37°C, samples were centrifuged at 2240 ×g to precipitate the islets. The supernatant was collected and stored at -20°C for insulin measurment.

Insulin secretion: time dependent. The triplicate culture flasks containing islets were transferred to tissue culture dishes and incubated at 37°C for 1 h in basal medium containing the modified Krebs-bicarbonate buffer with 2 mmol/L glucose. Doublicate samples were collected for insulin measurement every 5 min up to 50 min. Then, the culture media were changed with the modified Krebs-bicarbonate buffer containing 16 mmol/L glucose along with the plant extract (0.1 mg /ml). At 5 min intervals, doublicate aliquots were taken for insulin measurements up to 90 min. The medium was then changed back to basal medium containing 2 mmol/L glucose. Doublicate samples were again collected for insulin determination at 5 min intervals up to 120 mins [13].

Histopathology evaluation. Tissue samples from the pancreas were fixed in 10% buffered neutral formalin, embadded in parafin, sectioned at 5 μm and stained with hematoxylin-eosin and periodic acid-schiff [14].

Statistics. The significance of differences between the control and the test groups was established using the Student's *t*-test. significance set at $p<0.05$.

RESULTS AND DISCUSSION

The effect of T. polium extract on blood glucose level. One week after STZ injection, the diabetic rats with fasting blood glucose level in the range of 20-25 mmol/L (360-450 mg/dl), were selected for further studies. Figure 1 shows that prior to the extract administration, there was no significant difference between the blood glucose levels of the two diabetic groups of animals (II and III). However, after 6 weeks, the blood glucose levels of the treated rats were significantly lower than the controls (6.2 ± 1.64 and 27.7 ± 5.1 mmol/L, respectively). In contrast, the blood glucose level of the untreated diabetic rat remained elevated throughout the experimental period. The blood glucose level of the healthy (group I) remained unchanged during the course of the investigation. In addition, the blood insulin levels of the treated diabetic rats, after six consecutive weeks of treatments, were significantly enhanced (by 161%) compared to the untreated diabetic rats (Fig. 2).
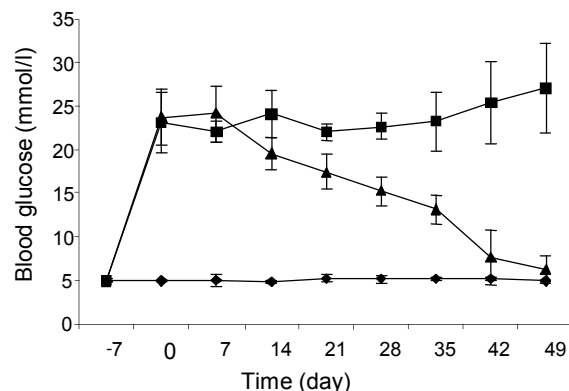

Fig. 2. Effect of EtOH-H_2O extract of *T. polium* (equivalent to 0.5 g plant powder per kg body weight) on the blood insulin level of treated diabetic rats compared with untreated diabetic and healthy control rats. Data are expressed as means ± SEM.

Figure 3 shows that, in contrast to normal rats, there was a significant ($p<0.05$) weight loss among group II and III rats, two weeks after crude extract administration. However, *T. polium*-treated rats showed sign of recovery in body weight gains three weeks after treatments (224 ± 30.2 g) compared to the diabetic control rats (173 ± 8.6 g).
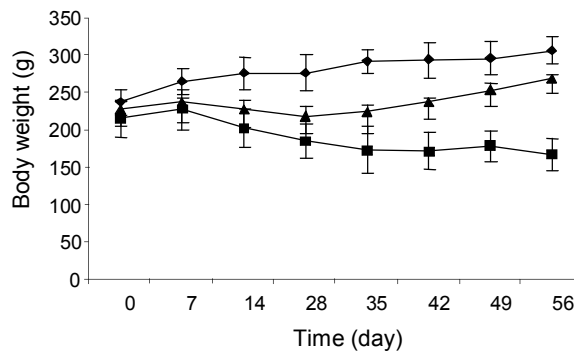
Fig. 3. The effect of EtOH-H_2O extract of *T. polium* (equivalent to 0.5 g plant powder per kg body weight) on the body weight of treated diabetic rats (▲), compared with untreated (■) and healthy control rats (◆). Data are expressed as means ± SEM.

The effect of T. polium extract on insulin release by the rat islets. Figure 4 shows that *T. polium* extract, in a dose range equivalent to 0.001-0.100 mg of the plant powder/ml, is capable of enhancing basal insulin release (at 2 mmol/L glucose) by a factor of 1.5 compared to the untreated islets. However, the insulinotropic effect of the crude extract on the pancreatic islets is less at higher doses (e.g., 1 mg/l). This observation is probably due to cytotoxicity effect of the crude extract or other physiological back responces of the islet cells exposed to high drug concentration.

Fig. 1. Effect of *T. polium* extract (equivalent to 0.5 g plant powder per kg body weight) on the rat blood glucose level: treated diabetic (▲), untreated (■) and the healthy control rats (◆). Data are expressed as means ± SEM.
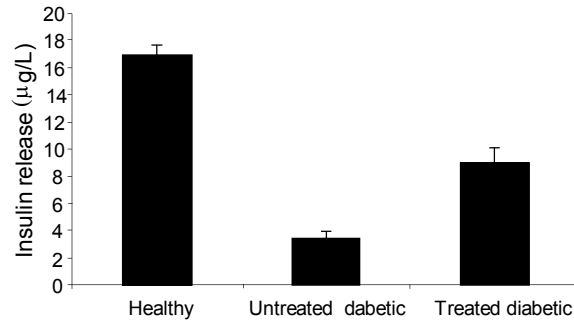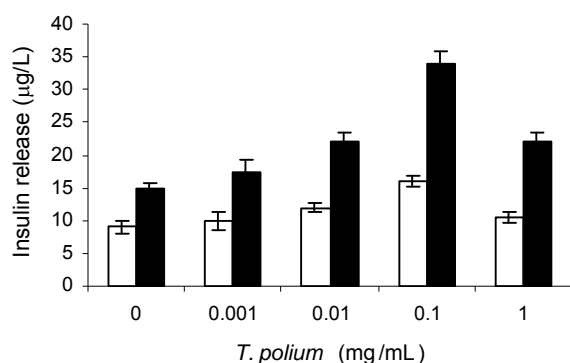
Fig. 4. Stimulation of rat islet insulin release by *T. Polium* extract. *T. Polium* extract induced a dose dependent initiation of basal (2 mmol/L glucose, □) and stimulated (16 mmol/ L glucose, ■) insulin secretion. Statistical significances of the observed differences in insulin release compared to the appropriate controls is/are: $p < 0.001$.

Exploration of this issue awaits further research and purification of the active component from the crude extract. The insulinotropic effect of *T. polium* was better evident at higher glucose concentration (16 mM, Fig. 5). Incubation of islets with 16 mmol/L glucose induced stimulation of basal insulin release, the rate of which fell rapidly when the high glucose medium was replaced by 2 mmol/L glucose. The addition of *T. polium* extract (0.1 mg/ml) to the cells cultured in the high glucose medium, resulted in an essentially unchanged pattern of insulin release with respect to the control cells. However, it was characterized by a more marked augmentation of secretion during stimulation period.
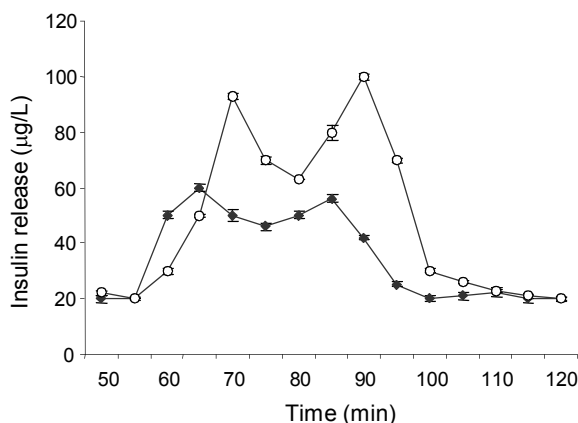

Fig. 5. The effect of *T. Polium* on the insulin secretion by the rat islets with respect to time. Islets were incubated in basal (2 mmol/L glucose) medium for 1 h, the medium was then changed with either 16 mmol/L glucose (■) or 16 mmol/L glucose plus 0.1 mg/ml *T. Polium* extract (○) for 30 min, followed by incubation under basal condition for another 30 min, the data present the mean ± SEM of three individual preparations of rat islets.

Histopathological examination of pancreas. Figures 6 and 7 represent two islets of langerhans from a normal and a STZ-induced diabetic rats, respectively. Comparison of these two Figures clearly indicates the reduction in the number of pancreatic islets as well as their number of β-cells in the diabetic rats. As it is evident from Figure 7, the islets are irregularly shaped and relatively small and atrophic. Most cells of the islets are small, degeranulated and dark with scanty cytoplasm.
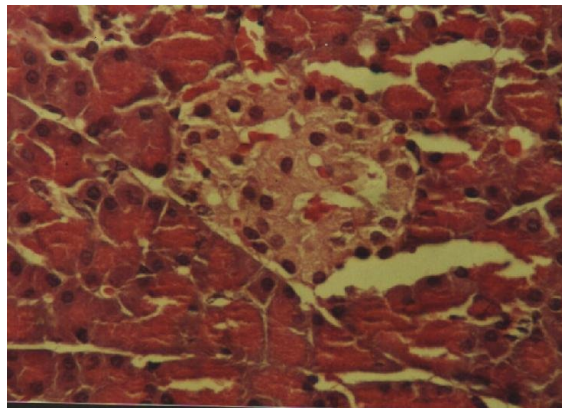

Fig. 6. An islet of langerhans from normal rat pancreas occupies the center of the field (hematoxylin-eosin, 640 ×).
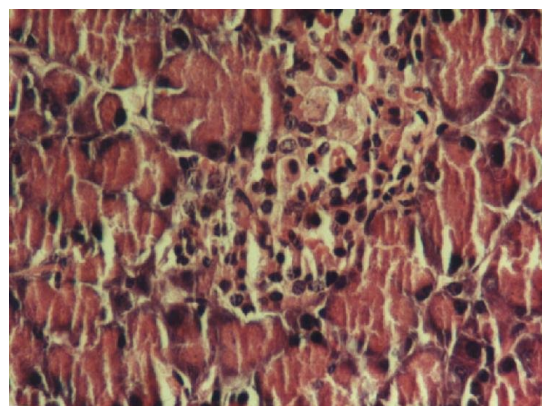

Fig. 7. An islet of langerhans from STZ-induced diabetic pancreas rat, occupies the center of the field which is infiltrated by numerous lymphocytes (hematoxylin-eosin, 640 ×).

Severe vaculation and degranulation are present in the β-cells of a number of islets. Insulitis (inflammation of the islets) is also detectable in the islets (Fig. 7). A exudate predominantly of lymphocytes, with a few macrophages and neutrophils is evident within and around the affected islets. However, compared to the untreated diabetic rats, histophatological examination of the plant extract treated diabetic rats revealed an
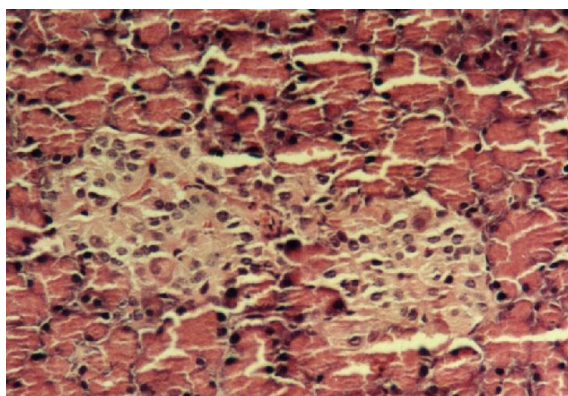
Fig. 8. Two islets of langerhans from treated diabetic pancreas rat. As it is evident, the islet cells are regenerated and the inflammatory infiltration has disappeared (hematoxylin-eosin, 640×).

increase in the number of pancreatic islets and the number of β-cells, along with a reduction in the number of initiated lymphocytes and macrophages (Fig. 8). In other words, the plant extract treated diabetic samples histopathologically approach the corresponding healthy pancreatic samples. The regeneration of the β-cells of the STZ-destructed islets is probably due to the fact that pancreas contains stable (Quiescent) cells which have the capacity of regeneration. Therefore, the surviving cells can proliferate to replace the lost cells [15,16].

In conclusion, our histopathological investigation along with the biochemical evaluations suggests the possibility of the islets regeneration upon plant extract treatment. Further work is required to explore exactly the mechanism of islet regeneration by the plant extract.

ACKNOWLEDGEMENTS

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