Antidiabetic effect of *Teucrium polium* aqueous extract in multiple low-dose streptozotocin-induced model of type 1 diabetes in rat

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A B S T R A C T

Background and Objective: *Teucrium polium* (TP) has shown hypoglycemic effect in type 1 diabetes induced by single high dose of the cytotoxic agent streptozotocin (STZ) in rats. This study was conducted to evaluate whether its aqueous extract could have such an effect in multiple low-dose STZ-induced model of type 1 diabetes in rats.

Materials and Methods: Male Wistar rats were divided into control, TP-treated control, diabetic, TP-treated diabetic groups. For induction of autoimmune model of type-1 diabetes, streptozotcin (STZ) was administered at a dose of 20 mg/kg/day for 5 days (multiple low-dose; MLD). Aqueous extract of TP was administered at a dose of 100 mg/kg for 3 weeks, started on 4th day post-STZ injection. Serum glucose level was determined before the study and at 2nd and 4th weeks after the study.

Results: TP extract-treated rats had a significantly higher weight versus diabetic rats at 4th week ($p<0.01$). In addition, serum glucose was significantly lower in TP-treated diabetic rats at 2nd and 4th weeks as compared to untreated diabetics ($p<0.005$). Meanwhile, treatment of control rats with TP extract did not significantly change serum glucose level.

Conclusion: Subchronic TP aqueous extract treatment of rats with autoimmune model of diabetes could attenuate abnormal changes in serm glucose and this may be of potential benefit in patients with type 1 diabetes.

1. Introduction

Diabetes mellitus is known as a heterogeneous complex of metabolic disorders characterized by the common phenotype hyperglycemia due to disturbances in insulin secretion, action or both (1). The development of chronic hyperglycemia in diabetes leads to severe damage in bodily tissues, organ dysfunctions and finally the irreversible failure of some critical organs of the body, especially the eyes, kidneys, nerves, and cardiovascular system (2). In addition to hyperglycemia, diabetes is itself followed by dyslipidemia and hyperlipidemia in affected patients with ensuing development of cardiovascular disorders, which are the major causes of morbidity and mortality (3). Deranged functioning of antioxidant system in diabetes leads to

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enhanced lipid peroxidation, inactivation of proteins, and protein glycation (4).

Type 1 diabetes is regarded as an autoimmune disease characterized by the infiltration of T-cells and macrophages in and around the islets of Langerhans (that is referred to as insulitis) with simultaneous and selective demolition of insulin-producing beta cells. These mononuclear cells may cause this event either directly and/or through the production and secretion of pro-inflammatory cytokines (5). This model is usually induced in rodents like rat via administering STZ at a low dose for five consecutive days (6).

Several approaches are presently used to lower the hyperglycemia in diabetes mellitus including insulin therapy which suppresses glucose production and increases glucose utilization, treatment by agents like sulfonylureas, which stimulates insulin secretion from pancreatic islet cells, agents like metformin with ability to reduce hepatic gluconeogenesis; inhibitors of α-glucosidase, which interfere with glucose absorption. Unfortunately, all of these therapies have limited efficacy and various side effects and thus searching for new classes of compounds is essential to overcome these problems (7). Recent interests have focused on the use of medicinal plants with antidiabetic and antioxidant potential in lowering the ensuing complications in diabetic patients (8). Plant-based pharmaceuticals have been employed in the management of various mankind diseases (8). They are as essential part of human diet and are present in plant extracts that have been used for centuries in oriental medicine. Antioxidant properties, ROS scavenging and cell function modulation of medicinal plants and their effective substances could mainly account for their pharmacological activity (8).

Teucrium polium L. is one of the species of the genus Teucrium from Lamiaceae family. T. polium is a perennial shrub, 20-50 cm in height, distributed widely in the dry and stony places of the hills and deserts of Mediterranean countries, South Western Asia, Europe and North Africa. T. polium (locally called as Kalpooreh) is abundantly and widely found in Iran (9). Phytochemical investigations have shown that T. polium contains various beneficial compounds including terpenoids and flavonoids. T. polium has been used in Iranian traditional medicine to treat many diseases such as abdominal pain, indigestion, common cold and urogenital diseases. The aqueous extract of the aerial parts of T. polium has been used by many type 2 diabetic patients, especially in the Southern Iran as an antidiabetic drug. In the Mediterranean countries, T. polium has been routinely used for various types of pathological conditions, such as gastrointestinal disorders, inflammations, diabetes and rheumatism (9-11). The aim of this study was to assess the hypoglycemic effect of subchronic administration of Teucrium polium aqueous extract in multiple low-dose streptozotocin-induced model of type 1 diabetes in rats.

2. Materials and Methods

2.1. Animals

Male albino Wistar rats (Pasteur's institute, Tehran, Iran) weighing 190-240 g were housed in an air-conditioned colony room at 21 ± 2 °C and supplied with standard pellet diet and tap water ad libitum. Procedures involving animals and their care were conducted in conformity with NIH guidelines for the care and use of laboratory animals.

2.2. Preparation of *Teucrium polium* aqueous extract

Fresh leaves of *Teucrium polium*, known by the local name Kalpooreh in Persian language, were collected from Alborz province in 2012. The leaves were botanically identified by the taxonomist of the Department of Botany, Shahid Beheshti University. A voucher specimen of the plant was deposited at the University's Botany Departmental herbarium. Leaves were air-dried at room temperature under shade. One hundred g of the air-dried leaves of the plant was milled into fine powder in a commercial blender. The powdered leaves were macerated and boiled in 1000 ml of distilled water for 10 min, extracted, and filtered three times. The combined aqueous extract was concentrated to waxy extract under reduced pressure in a rotary evaporator. The resulting crude aqueous extract was waxy in nature with a yield of 23% (w/w). The extract stock was kept in a 20 °C freezer until being used. Aliquot portions of the crude extract were weighed and dissolved in normal saline for use on each day of our experiment.

2.3. Experimental protocol

Male Wistar rats (n=32) were divided into equal-sized control, TP-treated control, diabetic, and TP-treated diabetic groups. Autoimmune model of type 1 diabetes mellitus was induced in rats by multiple low dose intraperitoneal injections of STZ (20 mg/kg body weight), freshly dissolved in normal saline, daily for five consecutive days. Age-matched normal animals that received an injection of an equivalent volume of normal saline comprised a non-diabetic control group. Diabetes was confirmed by the presence of hyperglycemia, polyphagia, polydipsia, polyuria and weight loss. Four days after the first STZ injection, TP aqueous extract was administered at a dose of 100 mg/kg for three weeks. Body weight and serum glucose level were recorded during the experimental period before the study (baseline) and at weeks 2 and 4.

2.4. Data and statistical analysis

All values were given as means ± SEM. Statistical analysis was carried out using repeated measure and one-way ANOVA followed by Tukey post hoc test. A statistical p value less than 0.05 considered significant.

3. Results

Body weight and serum glucose measurements (Figures 1 and 2) indicated that before diabetes induction, there were no significant differences among experimental groups. At 4th week, the weight of the vehicle-treated diabetic rats was found to be significantly decreased as compared to control rats ($p<0.05$) and TP-treated diabetic rats showed no decrease in body weight as compared to vehicle-treated diabetics and its weight was significantly higher versus diabetic rats in the same week ($p<0.01$).

Untreated diabetic rats had also an elevated serum glucose level over those of control rats ($p<0.001$-0.0005) and treatment of diabetic rats with TP extract caused a significant decrease in the serum glucose level at 2nd and 4th weeks ($p<0.005$) relative to vehicle-treated diabetics. In addition, TP extract treatment of control rats did not produce any significant change regarding serum glucose level.

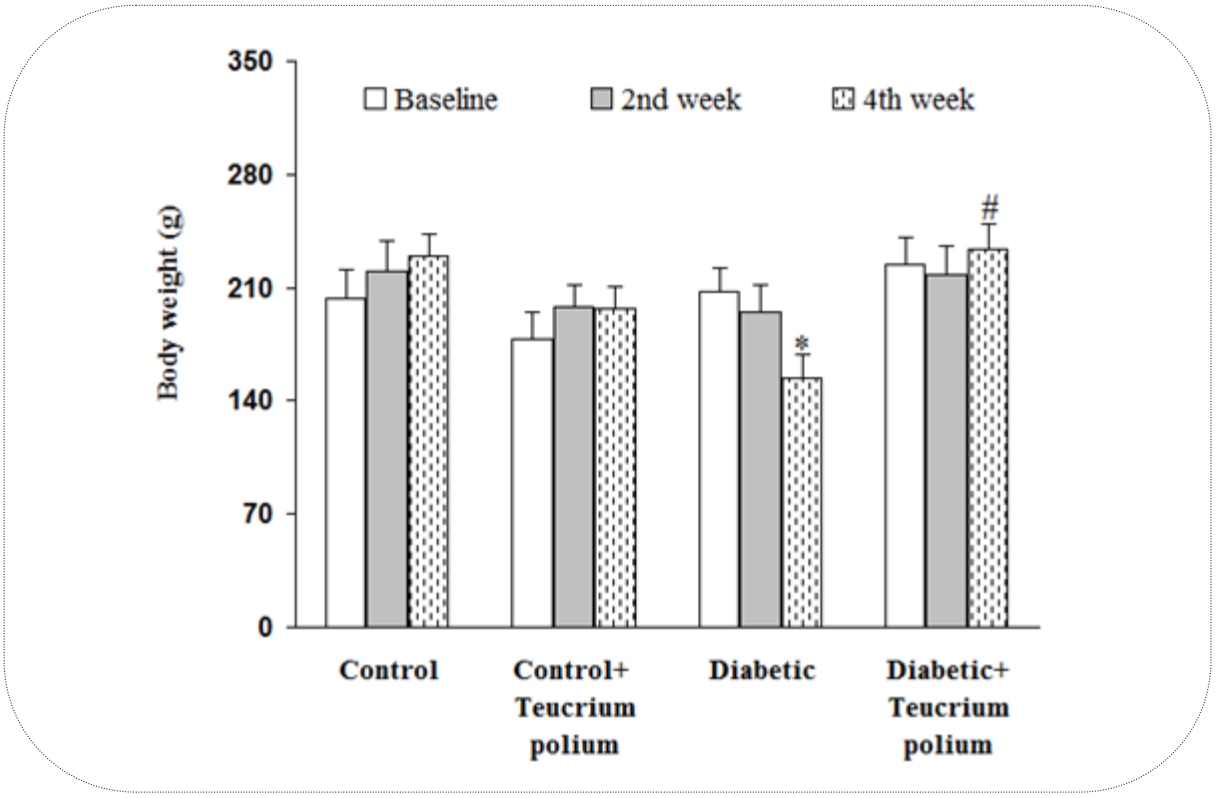

Figure 1. Body weight in different weeks (means ± S.E.M) * $p<0.05$ (as compared to baseline in the same group); # $p<0.01$ (as compared to diabetic in the same week)

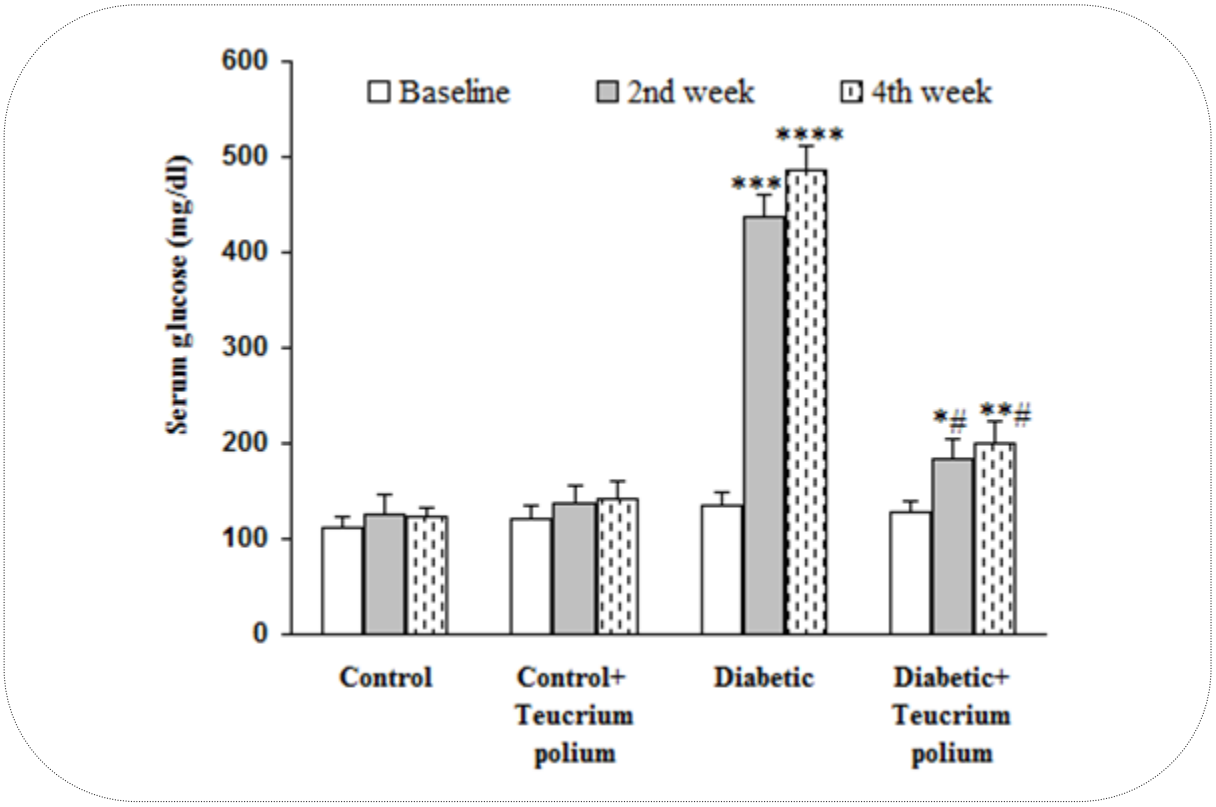

Figure 2. Serum glucose level in different weeks (means ± S.E.M) * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0005 (as compared to baseline in the same group); # p<0.005 (as compared to diabetic in the same week)

4. Discussion

In this study, TP extract-treated rats had a significantly higher weight versus diabetic rats at 4th week, serum glucose level was significantly lower in TP-treated diabetic rats at 2nd and 4th weeks as compared to untreated diabetics, and treatment of control rats with TP extract did not significantly change serum glucose level.

Although glucose-lowering effect of Teucrium polium aqueous extract was not significantly observed for control group in this study, but subchronic Teucrium polium treatment showed a marked hypoglycemic and antihyperglycemic effect in diabetic rats, indicating hypoglycemic mechanism of this medicinal plant to be different and specific in diabetic condition. The results of the previous studies have shown that TP administration to single dose STZ diabetic rats could protect and in part restore secretory function of beta cells in pancreatic tissue, in this way exerting its antihyperglycemic and antidiabetic effect (12). In addition, some flavonoids of the plant could have anti-diabetic and hypoglycemic potential (13). Such compounds have been suggested to inhibit hepatic gluconeogenesis through a ROS-dependent pathway (11). In addition, these flavonoids could exert an insulinomimetic effect and produce the cellular effects of insulin such as reducing gene expression of rate-limiting gluconeogenic enzymes (14). Furthermore, these flavonoids like the hormone insulin could increase tyrosine phosphorylation of the insulin receptor and insulin receptor substrate-1 and it reduces phosphoenolpyruvate carboxykinase gene expression in a phosphoinositide 3-kinase-dependent manner (14).

Since oxidative stress due to an increased production of ROS plays an important role in pathophysiology of diabetes, TP extract has the ability to attenuate oxidative stress and lipid peroxidation (11), and in this way maye have affected carbohydrate metabolism in this study.

In conclusion, subchronic TP aqueous extract treatment of rats with autoimmune model of diabetes could attenuate abnormal changes in serm glucose and this may be of potential benefit in patients with type 1 diabetes. More studies are

required to evaluate whether such therapy can be administered as an auxiliary beneficial therapeutic regimen in diabetic population.

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