



EFFECT OF DIFFERENT CHROMATOGRAPHIC FRACTION AQUEOUS AND ALCOHOLIC EXTRACTS OF TEUCRIUM POLIUM AERIAL PARTS ON THE INSULIN AND GLUCOSE CONTENT IN HYPERGLYCEMIC RATS

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ABSTRACT

In recent studies Teucrium polium (T. polium) was known as a hypoglycemic plant. But further research is needed to better understand the effect of Teucrium polium and biological active part of it. The purpose of this investigation is to examine the effect of different chromatographic fractions of aqueous and alcoholic extract of this plant on the level of insulin secretion and glucose content in hyperglycemic rat model. Also, our aim is determination of biological active fraction of aqueous and alcoholic extract of this plant. This study was carried out on the 36 rats. Hyperglycemia induced by administering of 50 mg/kg alloxan intraperitoneally and glucose level was monitored for hyperglycemic status. Hyperglycemia was confirmed by blood glucose measurement. In each experiment 100 grams of Teucrium polium aerial parts powder were boiled with 2 Litter of distilled water for 36 h. The decoction preparation was then filtered through a gauz cloth followed by filtration through filter paper. The extract was evaporated to one-fifth of its original volume and kept at 4°C until its use. Determination of different fraction aqueous extract effect of Teucrium polium on glucose level and insulin secretion was carried out. Blood was collected from the tail of the rats. Then glucose and insulin level was evaluated. The hyperglycemic animals showed significant decrease in the blood glucose level in rats administered with fourth fraction compared with other fractions. Administration of fourth fraction Teucrium polium aerial parts extract cause increase in insulin levels in alloxan-treated rats. Results suggest that treatment of fourth fraction Teucrium polium aerial parts extract may be useful in preventing the increase of glucose level in hyperglycemic rats. The interesting phenomenon of our results has shown that fourth fraction given parenterally possesses a hypoglycemic effect in alloxan hyperglycemic rats. Fourth fraction was found biological active and to be responsive to glucose challenge as evidenced by increase in insulin secretion.

Keywords: Aerial parts, Chromatography, Biological active, Glucose, Insulin, Teucrium polium



INTRODUCTION

With growing research interest in the use of plant materials to treatment of diabetic complication. Conflicting data have been reported regarding the role Teucrium polium in diabetic subjects. In this respect, some of investigators reported that T. polium extract did not show effect on blood glucose content in patients [1]. But, this function of T. polium extract in glucose level was challenged by other studies. In contrast to mentioned study, as reported by many other investigators T. polium extract caused reduction in blood glucose level administration [2]. Despite this apparently

conflicting effect several experimental studies reported that T. polium has antioxidant activity and hypoglycemic effects significantly improved survival of animals [3]. Previous studies have revealed that there is not any hypoglycemic effect of T. polium. A number of recent studies have reported that the bioactive metabolite of the effective substance of Teucrium has hepatotoxicity effects [4]. Previous studies have been reported that Teucrium polium play an important role in hyperglycemic status [5-7]. There is evidence that Teucrium polium extract effects on pancreatic function in diabetic rats [8]. Several experimental studies have investigated the Teucrium polium

effects on oral glucose tolerance test, regeneration of pancreatic islets and activity of hepatic glycokinase in diabetic rats[9]. Much evidence has accumulated in recent years, indicated that hepatotoxicity associated with hypoglycemic effects of *Teucrium polium* in diabetic rats was reported [10,11]. As described previously it may therefore be interesting possible that *Teucrium polium* has antioxidant activity[11-13]. The main objective of this investigation is first, to examine the effect of different chromatographic fraction of aqueous and alcoholic extract of this plant on the level of insulin secretion and glucose content in hyperglycemic rats. Second, determination of biological active part of this plant.

MATERIALS AND METHODS

Plant material: Plants were prepared from local store Mazandaran area in IRAN. Ethanol, p-anisaldehyde, sulfuric acid, methanol and were purchased from local chemical agency. All other chemicals were of the highest grade available.

Preparation of plant materials: Aerial parts of plant samples were dried at room temperature in laboratory. Then 100 g of the powdered plant materials were soaked in distilled water or 95% ethanol (100 ml per 10g) for 36 h. The crude aqueous and ethanol extracts were obtained after the solvent was evaporated at 50°C. Then aqueous and ethanol extract was fractionated by using column chromatography procedure.

Fraction collect: Fractionation by using column chromatography was carried out for aqueous and ethanol extracts that showed main six fractions. Aliquots (500 µl) of the aqueous and ethanol extract were applied 50 cm over the Column (30 mm, gel). Solutions of aqueous and methanol (25%, 75%) were used as eluents. Then fractions were collected manually and then 0.2 ml volume of each fraction was mixed with p-anisaldehyde and sulfuric (1/1) acid reagent and heated at 95°C for optimal color development. The volume of each fraction was 0.2 mL and the temperature was set to 25 °C. The absorption of each fraction was monitored at 360-380 nm. Then, total plant content in dried aerial parts of the plants was determined by the spectrometric method. For measurement of plant materials each fraction were mixed with p-anisaldehyde and sulfuric acid reagent and carefully heated at 95°C for optimal color development according previously reported[12,13].

Animal study: A total of 36 rats were used for the study, with a weight of 190-220 g were purchased from Animal center. Rats were housed under

conventional conditions and were fed laboratory chow and water *ad libitum* and used for experiments. The environment temperature and light was controlled (12 hr light/12 hr dark). The study carried out according to the principles of the animal care. The experimental protocols were approved by Babol University of Medical Sciences. Hyperglycemic was induced after an overnight fast with a single intraperitoneal injection of alloxan (50 mg/kg), insulin and serum glucose levels were measured every time point, and rats with serum glucose over 250 mg/kg were used as the hyperglycemic rats.

In this experiment rats were divided into twelve groups of three animals each. The aqueous and alcoholic extract of *T. polium* at different dose levels: 0.0, 0.1, 0.2, 0.3, 0.4 and 0.5 g/kg body weight, were administered weekly for 8 consecutive weeks. All groups were followed for 8 weeks. Rats were anesthetized and were sacrificed by decapitation under anesthesia 24 hr after the last treatment. Blood samples were taken from the tail vein of animals. Blood samples were collected and serum samples were obtained by centrifugation. Also, pancreas was obtained, weighed and placed in formalin for histological evaluation.

Histology study of pancreas tissues: Pancreas were fixed in buffered formalin. Paraffin sections were made and stained. Microscopic evaluation was carried out in the laboratory of Histology Department. The light microscopic examination of pancreas tissues of rats treated acutely as control or with fraction no 4 exhibited several pathological changes in the pancreas. Such changes were not seen in control rat's pancreas tissue.

Determination of glucose and insulin content: Glucose level was measured by spectrophotometric method (Jenway uv/vis, 6505 model, Dunmow, UK) using glucose kit of Pars Azmoon Co., Tehran, Iran. The insulin test was performed following ELISA assay using insulin kit of rat made by Mercodia Co., Sweden (Stat Fax -2100, Awareness Technology Inc, Plant city, USA, FL 34990). Experiments were performed in triplicate in at least three separate experiments.

Statistical analysis: All data were presented as Mean ± SD. t-test was used for comparing means. Statistical analysis was done using SPSS, version 18.0, and P values <0.01 were considered to be statistically significant.

RESULTS

One biological active fraction of *Teucrium polium* aerial parts extract, termed 4TP, was separated by column chromatography. We observed administration of the fourth fraction of *Teucrium polium* aerial parts extract decreased glucose level in hyperglycemic rat. Also, it is interesting that we observed administration of this biological active *Teucrium polium* aerial parts extract increase insulin content. This fraction of extracts showed a potential anti-glycemic activity and induces increasing in the number of pancreas islets. While no significant histological changes in the pancreas were seen in the 1st, 2nd, 3rd, 5th and 6th fractions. Also 1st, 2nd, 3rd and 5th and 6th fractions did not stimulate insulin secretion. It is interesting that we observed that fourth fraction of the *T. polium* aerial parts extracts caused a significant ($P < 0.05$) increase in insulin release during a eight weeks, from the basal level of $5.87 \pm 0.23 \mu\text{g/l}$ at 1st day to a peak value of $9.04 \pm 0.38 \mu\text{g/l}$ (figure 1). Also, fourth fraction of the *T. polium* aerial parts extracts powder/mL caused a significant ($P < 0.05$) decrease in glucose level during a eight weeks, from the basal level of $251.3 \pm 17.4 \text{ mg/dl}$ to a peak value of $117.5 \pm 19.6 \text{ mg/dl}$ (figure 2). Characteristic of fraction extracts of *teucrium polium* aerial Parts on column chromatography was shown (figure 3).

DISCUSSION

One of the major findings of this study is that one of fraction of *Teucrium polium* aerial parts extract is biological active and demonstrated the possibility of the antiglycemic function. The biological active of this plant was fourth fraction in column chromatography. A significantly lower level of blood glucose was recorded in alloxan-induced rats treated with fourth fraction of *Teucrium polium* aerial parts extract. Fourth fraction of *Teucrium polium* aerial parts extract compared with the control rats ($P < 0.05$), whereas no significant difference was observed in level of blood glucose between alloxan-induced rats treated with 1st, 2nd, 3rd and 5th and 6th fractions and the control rats ($P > 0.05$). Also, our findings indicate that the fourth fraction *Teucrium polium* aerial parts extract functions as stimulator insulin secretion and biological active. Our present findings, taken together with previous results [14-16] we suggest that fourth fraction of *Teucrium polium* aerial parts extract has potency to effects on pancreatic function of alloxan hyperglycemic rats by stimulate pancreas repair. This function might be due to partial regeneration in the b-cells or may be attributed to its constituents. Although it has been known that hyperglycemia may exhibit most of the diabetic complications through a decrease in the activity of carbohydrate metabolism enzymes.

The exact mechanism involved in the carbohydrate metabolism in diabetes is not been fully elucidated.

Our results were in agreement with those reported previously [16-18]. We hypothesized that the reduction in blood glucose level following the administration of fourth fraction can be attributed to increase secretion of insulin. The precise mechanism of fourth fraction of *Teucrium polium* aerial parts extract in glucose-lowering action has not been fully understood. Further systematic investigations and much research effort into the chemical constituents, pharmacological actions, and regeneration activity of the fourth fraction materials will be needed to better understand the function. In addition, the cellular and molecular mechanisms of the *Teucrium polium* still need to be determined in animal models and detailed information on their usage, duration and dosage must be more investigated. Given the benefits fourth fraction of *Teucrium polium* in lowering blood glucose, we speculate that this fraction is biological active.

CONCLUSION

The experimental results presented in this study demonstrated that administration fourth fraction of *Teucrium polium* aerial parts extract might be useful in preventing hyperglycemia by having insulin stimulator action. In future studies, further work on the role of fourth fraction of *Teucrium polium* aerial parts in diabetic patients is merited.

Limiting the findings: Limiting the clinical usefulness of this plant material beyond all these positive results and suggestion, some limitations and methodological flaws of our study should be mentioned. This study had a number of limitations; first we did not have data on molecular level. Second, our sample was small. Third number of fraction was small.

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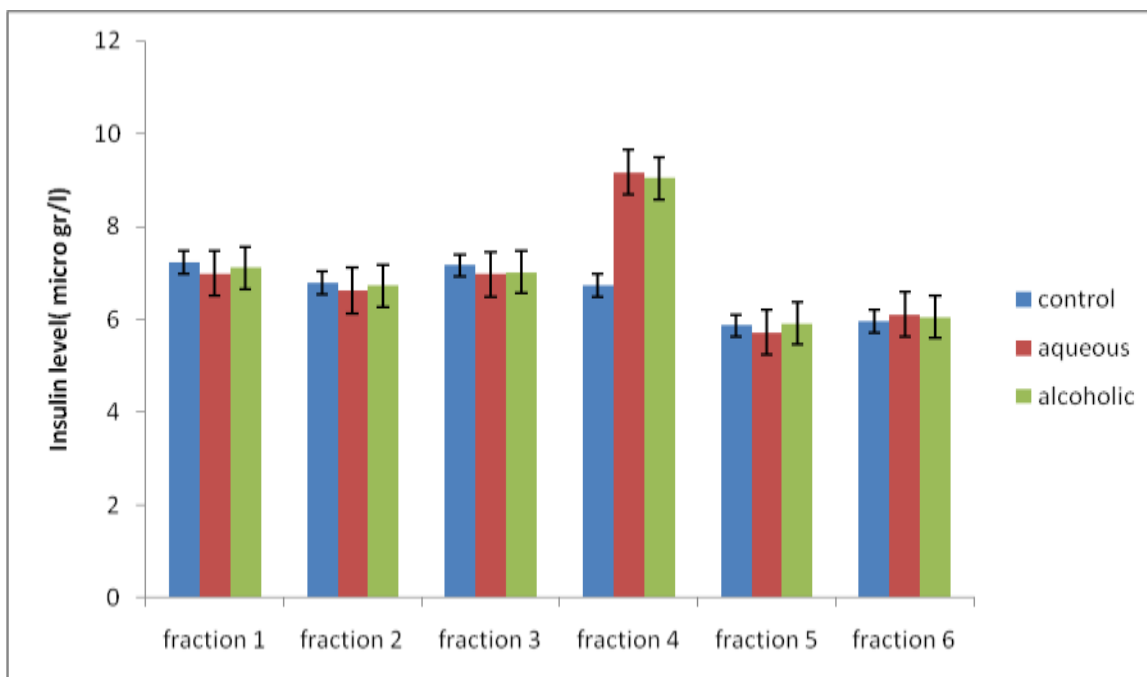


Figure 1. Insulin content ($\mu\text{g/l}$). Each value is the mean \pm SD of 6 experiments. (Column 1; control without administration, column 2; with aqueous extract administration and column 3; with ethanol extracts administration).

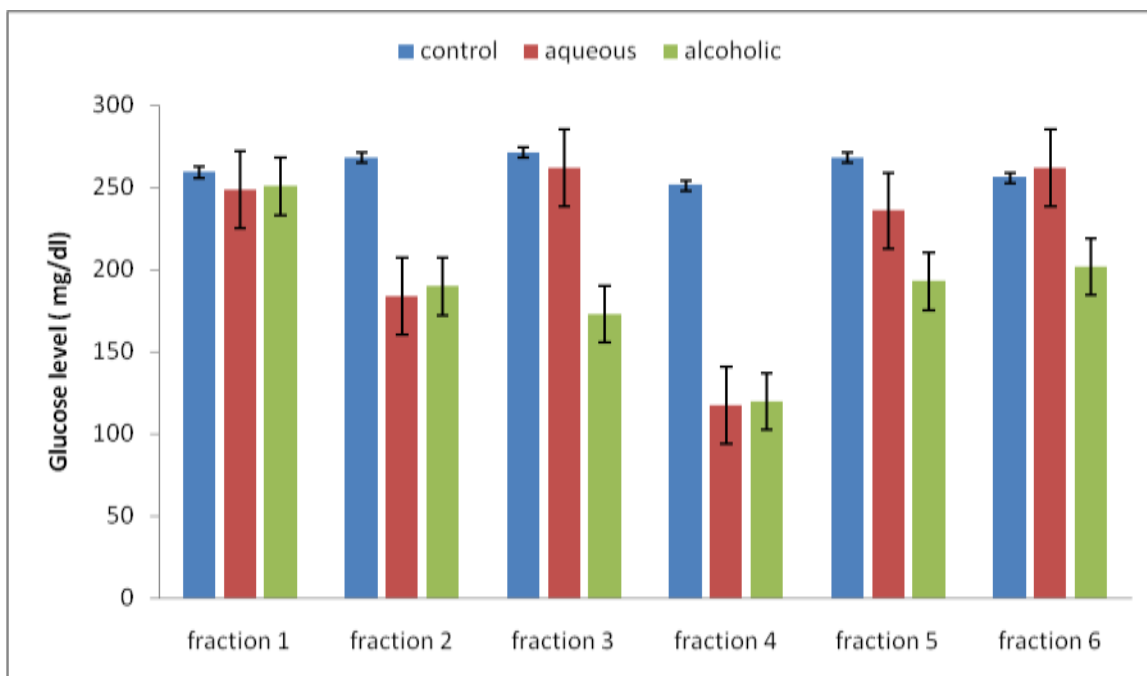


Figure 2. Glucose level (mg/dl). Each value is the mean \pm SD of 6 experiments. (Column 1; control without administration, column 2; with aqueous extract administration and column 3; with ethanol extracts administration).

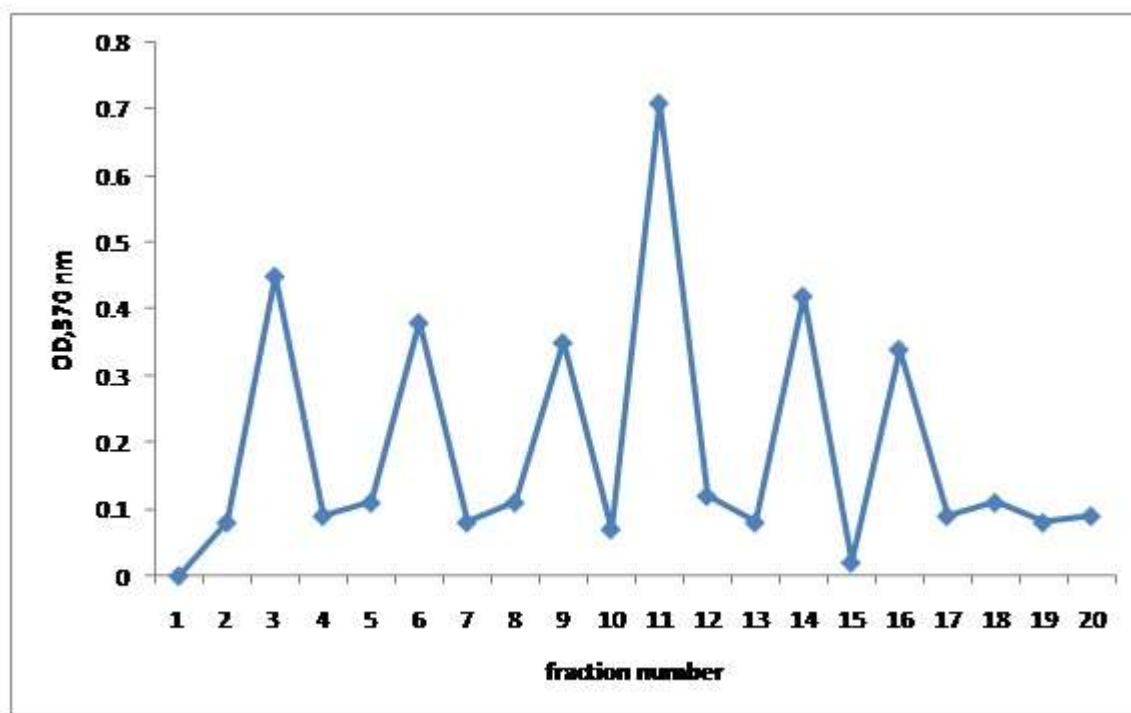


Figure 3.Characteristic of fraction extracts of *Teucrium polium* aerial Parts on column chromatography.

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