Full Length Research Paper

Effect of *Teucrium polium* flower extract on the activities of nucleoside diphosphate kinase and acetyl-CoA carboxylase in normal and diabetic rats

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Accepted 29 March, 2012

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Abbreviations: TP, *Teucrium polium* L.; NDPK, nucleoside diphosphate kinase; ACC, acetyl-CoA carboxylase; i.p., intraperitoneal; Mg, milligram; kg, kilogram; ml, milliliter; mM, millimolar; μl, microliter.

*Teucrium polium* L. (TP) has been used for the treatment of diabetes, gastric inflammation and convulsion in traditional medicine. The aim of this study was to assess in vivo effects of aqueous and ethanolic extracts of TP flower on activities of acetyl-CoA carboxylase (ACC), nucleoside diphosphate kinase (NDPK), insulin and serum glucose levels in normal and diabetic rats. The experimental animals were randomly distributed among 6 groups of 7 or 8 rats. The treatment groups received 150 mg/kg of alloxan. The rats were fed aqueous and ethanolic extracts of *T. polium* L. (TP) flower at 500 mg/kg/day doses for two weeks period. Subsequently, glucose, insulin levels, ACC and NDPK activities were measured. The present study showed significantly lower levels of glucose and elevated levels of insulin in the group of rats that was treated with ethanol and aqueous extracts of TP flowers, when compared with alloxan group (p<0.01). The results showed that the TP flower extracts increased the ACC and NDPK activities (p<0.05). The findings suggest that aqueous and ethanolic extracts of TP flower have hypoglycemic effect in alloxan diabetic rats and may be hypoglycemic activity of TP extract attributed increased activity of ACC as glucose sensor for insulin secretion.

Key words: *Teucrium polium*, nucleoside diphosphate kinase, acetyl-CoA carboxylase, diabetes, ethanolic extract, aqueous extract.

INTRODUCTION

Metabolic syndrome and especially diabetes mellitus (DM) is the most common endocrine disorder and by the year 2010, it is estimated that more than 200 million people worldwide will have DM and 300 million will subsequently have the disease by 2025 (Mahjoub and Masrour Roudsari, 2012b; Amos et al., 1997). The metabolic syndrome (MS) which is highly significant as a major cause of DM and cardiovascular diseases, has become one of the major public health challenges worldwide (Mahjoub et al., 2012a; Mahjoub Masrour Roudsari, 2012b).

Several studies currently focused on effects of herbal plants in controlling DM (Büyükbalci and Sedef, 2008; Rahimi et al., 2011; Wadkar et al., 2008). The author and colleagues investigated on in vivo and in vitro effects of aqueous and alcoholic extracts of some herbal plants in DM. Our latest study indicated that the alcoholic extract of *Urtica dioica* leaves can decrease glucose level and increase insulin secretion, acetyl coenzyme A carboxylase (ACC), and nucleoside diphosphate kinase (NDPK) activities in the alloxan diabetic animals (Qujeq et al., 2011).

Herbal plants serve as excellent resources for new
medications, and today, an increasing interest is observed in their usage (Hasani-Ranjbar et al., 2008; Rafiq et al., 2009; Focho et al., 2009; Singh et al., 2011). Medicinal herbs grow vastly in Iran and are widely used for natural treatment of diseases. One of these medicinal plants, which have been recommended in Iranian folk medicine for treatment of DM since centuries ago, is “Teucrium polium L.” (TP).

TP (family Lamiaceae) is one of the 300 species of the genus Teucrium and is found mainly in the Mediterranean and Western Iranian-Turanian sphere (Afifi et al., 2005). In Iran, this medicinal plant, also named as Calpoureh, is widely distributed and traditionally used by native inhabitants as herbal tea, a spice or a hypoglycemic agent that is recommended by herbalists (Hasani-Ranjbar et al., 2008). Some biological and therapeutic effects have been reported for TP such as anti-inflammatory, anti-oxidant, anti-microbial, anti-nociceptive, anti-pyretic, anti-gastric ulcer, hepatoprotective, hypolipidemic and hypoglycemic effects (Qujeq et al., 2011; Hasani-Ranjbar et al., 2008; López et al., 2007; Mirmazandari et al., 2010; Hasani-Ranjbar et al., 2010; Abdollahi et al., 2003; Rasekh et al., 2001; Zal et al., 2001). As literature review shows, there is no study to assess the effects of aqueous and ethanolic extracts of TP flower on activities of acetyl-CoA carboxylase (EC 6.4.1.2) and nucleoside diphosphate kinase (E.C. 2.7.4.6). Acetyl-CoA carboxylase (ACC), convert acetyl-CoA to malonyl-CoA. Phosphorylation and dephosphorylation of ACC cause the enzyme’s inactivation and activation, respectively, and serve as the enzyme’s short-term regulatory mechanism (Kim, 1997). Insulin activates ACC. This activation involves dephosphorylation by a protein phosphatase. ACC activity could be enhanced in primary cultures of hepatocytes from adult rats by insulin as well as by glucose (Katz and Ick, 1981). Also ACC is equipped as glucose sensor for insulin secretion. ACC, the unique enzyme to catalyze the production of malonyl-CoA, suggest a significant defect in NDPK expression and function under glucoolipotoxic conditions (Veluthakal et al., 2009). NDPK is an enzyme that catalyzes the transfer of the terminal phosphate from nucleoside triphosphates to nucleoside diphosphate (Muimo et al., 2006). NDPK is tightly linked to the energy metabolism of the cell (Hammargren et al., 2008). There is not found any reports about the effects of aqueous and ethanolic extracts of TP flower on activities of ACC and NDPK. TP is widely distributed and its use by people implies a relative lack of toxicity. However, further studies are needed to elucidate the mechanisms of its hypoglycemic action. In our ongoing research project on the medicinal plants used for the treatment of hyperglycemia, we undertook the present study in order to clarify the possible role of TP in treatment of hyperglycemia. In addition, the purpose of this study was to assess in vivo effect of aqueous and ethanolic extracts of T. polium flower on activities of ACC, NDPK activities, insulin level and serum glucose concentration.

MATERIALS AND METHODS

Plant materials

The plant was collected in June 2008 from the hills of Ferdows, Southern Khorassan province. It was authenticated by the Agricultural Research Center and Natural Resources of Mazandaran province. The fresh flower of TP was separated, cleaned and dried at room temperature while kept from direct sunlight; the flowered part was separated and powdered.

Extractions

Thereafter, 100 g of dried flowers was ground and its powder was mixed with 2000 ml of distilled boiling water for 60 min under continuous stirring. The obtained mixture was filtered twice through a mesh and then two times through a whatman paper No. 2 and the obtained liquid was dried using a magnet stirrer and finally, evaporated under the vacuum, at 70°C to reduce the solution volume to 1/8 of initial value. This aqueous extract was maintained at -20°C until used. To prepare ethanolic solution, 100 g of dried flower powder was added to 2 L of 70% ethanol and mixed using a shaker for 24 h at room temperature. Again, the nonsoluble part was separated using mesh and the solution was then passed two times through whatman paper No. 2. The solution was evaporated at 60°C under the vacuum to reduce the solution volume to 1/8 of initial value. Both aqueous and ethanolic solutions were kept four days in sterilized plates under the hood to let the solvents evaporate. This extract was maintained at -20°C until used.

Experimental animals

Rats (totally 45 Vistar rats) of 8 to 12 weeks old with approximate weight of 200 to 240 g were used in this study. The rats were provided by the animal center of Babol University. Age-matched rats were used as control animals. The rats were placed in suspended bracket cages in an air conditioned room with temperature of 22 ± 5°C and photoperiod of 12L: 12D. All animals were carefully maintained under standard animal house conditions with free access to food, water and ad libitum. The rats were fasted 14 h before injection. The approval of the Ethics Committee of Babol University was also obtained (No. 1825).

Animals’ treatments

The treatments in this study included: (1) Non treated animals (control group), which received orally 0.7 ml normal saline; (2) Hyperglycemic group that were intraperitoneally injected by 150 mg/kg of fresh solution of alloxan (alloxan monohydrate from Sigma Co.) dissolved in distilled water; (3) Animals which received aqueous extract; (4) Animals that received ethanolic extract; (5) Hyperglycemic animals (received 150 mg/kg alloxan intraperitoneal) and treated with aqueous extract. (6) Hyperglycemic animals (received 150 mg/kg alloxan intraperitoneal) and treated with ethanolic extract. After 72 h from treatments, blood samples of rats were obtained from conjunctiva and glucose plus insulin of blood serum were measured. At 24 h after blood sampling, the rats were treated orally with concentration of 250 mg/kg/dose of TP flower extract and distilled water (control) respectively, for 14 days. The blood samples were taken 24 h after completion of this period to measure glucose and insulin levels. Hyperglycemic was developed gradually as assessed by blood glucose level, which reached approximately 250 mg within 2 days. In each experiment, 0.5 g of rat liver was thawed and suspended in PBS, after stirring for 60 min at 35°C insoluble materials removed by centrifugation at 13000 x g.
Table 1 Glucose (Mean ± S.E.M) level three days after alloxan injection and before treatment with aqueous and ethanolic extracts of *T. polium* flower for normal and diabetic rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>87.4 ± 3.0</td>
</tr>
<tr>
<td>Alloxan</td>
<td>7</td>
<td>418.1 ± 34.9 *</td>
</tr>
<tr>
<td>Control and aqueous extract</td>
<td>8</td>
<td>84.5 ± 2.1</td>
</tr>
<tr>
<td>Control and ethanolic extract</td>
<td>7</td>
<td>86.3 ± 5.3</td>
</tr>
<tr>
<td>Alloxan and aqueous extract</td>
<td>7</td>
<td>387.3 ± 31.7 *</td>
</tr>
<tr>
<td>Alloxan and ethanolic extract</td>
<td>7</td>
<td>413.1 ± 41.7 *</td>
</tr>
</tbody>
</table>

*p<0.001 with respect to control group

Determination of glucose and insulin levels

Glucose level was determined by spectrophotometric method (Jenway uv/vis, 6505 model, Dunmow, UK) using glucose kit of Pars Azmoon Co., Tehran, Iran. The insulin test was carried out using insulin kit of rat made by Mercodia Co., Sweden by ELISA assay (Stat Fax -2100, Awareness Technology Inc Plam city, USA, FL 34990).

Protein assay

Protein concentration in the rat liver homogenized samples was determined by dye-binding method of Bradford using BSA as the standard (Bradford, 1976).

Acetyl-CoA carboxylase activity assay

ACC activity was determined by spectrophotometry as described previously with modification (Bijleveld and Geelen, 1987). The assay was conducted in a mixture, containing 50 mM Tris-HCl (pH 7.6), 10 mM potassium citrate, 10 mM MgCl2, 3.75 mM Glutamate, 0.75 mg bovine serum albumin in 1 ml and 0.125 mM acetyl-CoA, 20 units of pyruvate kinase and 20 units of lactate dehydrogenase, adjusted with distilled water to 1 ml. Subsequently, 10 μl rat liver homogenized and incubated (for) 10 min at 37°C was added. Monitoring of absorbance at 340 nm follows the oxidation of NADH. The specific activity of 1 unit of enzyme is defined as the turnover of 1 μmol of substrate in 1 min per milligram of protein (Qujeq et al., 2011).

NADP kinase activity assay

The activity of NADP kinase was investigated by spectrophotometry using a coupled pyruvate kinase lactate dehydrogenase enzyme system as described previously with modification (Johansson et al., 2008). The assay was carried out in a 1 ml reaction mixture, containing 50 mM Tris-HCl (pH 7.6), 5 mM MgCl2, 0.05 mM KCl, 0.1 mM phosphoenoxypruvate, 0.5 mM ATP, 0.1 mM TDP, NADH (0.1 mg/ml), 2 units of pyruvate kinase, and 2.5 units of lactate dehydrogenase. The reaction was initiated by addition of 0.5 to 5 μg of NDP kinase at 25°C adjusted with distilled water to 1 ml. Then 10 μl rat liver homogenized and incubated (for) 10 mins at 37°C was added. Monitoring of the decrease in absorbance at 340 nm follows the oxidation of NADH, which reflect ADP formation by NDP kinase. The specific activity of 1 unit of enzyme is defined as the turnover of 1 μmol of substrate in 1 min per milligram of protein.

Statistical analysis

All values were presented as Mean ± S.E. Statistical analysis was done using statistical package for social sciences (SPSS), One-way analysis of variance (ANOVA) test. Turkey's test was used for all comparisons of ANOVA and p values<0.05 were considered to be statistically significant.

RESULTS

In the present study, the hypoglycemic effect of the TP, as used in folk medicine, was tested. Administration of aqueous and ethanolic extracts of TP flower produced a significant reduction in blood glucose concentration in alloxan-induced diabetic rats, as compared to the controls which received saline only. The aqueous and ethanolic concentrate yields were 20.29 and 9.1 g from the 100 g dry powders, respectively. Table 1 shows the effect of alloxan on glucose level of blood in all 6 groups. The effect of aqueous and ethanolic extracts of TP flowers on insulin and glucose concentrations in normal and hyperglycemic rats is also shown in Table 2. Our results revealed that serum glucose value significantly decreased after TP aqueous and ethanolic extracts administration (272.1 ± 22.2 and 296.4 ± 23.3 mg/dl) respectively, when compared with alloxan group (393.6 ± 24.9 mg/dl), p<0.01. Table 3 shows activities of ACC and NDPK enzymes after treatment with aqueous and ethanolic extracts in normal and diabetic rats. Our results showed that the TP flower extracts elevated the ACC and NDPK activities, p<0.05.

DISCUSSION

The main goal of this study was to assess in vivo effect of aqueous and ethanolic extracts of *T. polium* flower on ACC, NDPK activities, insulin level and serum glucose concentration.

In the present study, treated with aqueous and ethanolic extracts of TP in alloxan diabetic rats caused a
rise in ACC and NDPK activities compared to control group. Also, our results revealed that serum glucose value was significantly decreased after TP aqueous and ethanolic extracts administration, when compared with alloxan group. These results are in accordance with other investigators (Wadkar et al., 2008; Esmaeili and Yazdanparast, 2004) which showed that TP decreased the serum glucose level of diabetic animals. In addition, our results revealed that serum insulin level was significantly increased after TP aqueous and ethanolic extracts administration, when compared with alloxan group. These findings are consistent with other studies (Wadkar et al., 2008; Esmaeili and Yazdanparast, 2004). Some differences in our results compare to other reports may be due to the difference in method of TP administration. Oral method was used, whereas the other may have used other methods administration (Afifi et al., 2005). In most researches, its significant effect on glucose has been proved (Wadkar et al., 2008; Zal et al., 2001; Esmaeili and Yazdanparast, 2004).

One of most important mechanism which is assumed for hypoglycemic effects of TP is being an antioxidant (Hasani-Ranjbar et al., 2010; Abdollahi et al., 2003). Antioxidants can combine with free radicals. This prevents destruction of β-cells of pancreas. Plants secondary metabolites such as flavonoids and polyphenols compounds exhibited important commercial and biological role due to their antioxidants activity (Mahdi et al., 2011). The hypoglycemic effect of TP due to its antioxidant effect proves its protection effect in diabetic rats (Hasani-Ranjbar et al., 2010).

To explain the hypoglycemic action of T. polium extract in alloxan diabetic rat, we hypothesized that the reduction in blood glucose level following the administration of TP may be attributed to the increasing circulating insulin levels or independent of insulin and/or due to the possibility that TP increases the peripheral utilization of glucose. Also, it is possible that the flavonoids present in the TP may be responsible for islet regeneration. T. polium is widely distributed and its use by people implies a relative lack of toxicity. However, further studies are needed to elucidate the mechanisms of its hypoglycemic action. No report was found about the effects of aqueous and ethanolic extracts of TP flower on activities of ACC and NDPK. Our results showed that the TP flower extracts elevated the ACC and NDPK activities in alloxan-induced diabetic rats. This effect of TP flower extract is the same with the effect of U. dioica leaves extract reported in our latest study (Qujeq et al., 2011). This may be as a result of the fact that hypoglycemic activity of TP extract is attributed to the increased activity of ACC as a glucose sensor for insulin secretion. However, NDPK might be probably used as an energy metabolism of the cell. Anyway, the action mechanism remains unclear and further studies are needed to clarify this mechanism.

**Conclusion**

The findings suggest that aqueous and ethanolic extracts
of TP flower have hypoglycemic effect in alloxan diabetic rats and may be hypoglycemic activity of TP extract attributed increased activity of ACC as glucose sensor for insulin secretion. Also, for the first time we showed that aqueous and ethanolic extracts of this plant increase the ACC and NDPK activity.

ACKNOWLEDGMENTS

This project was supported by Grant No. 78215 from the office of the Vice Chancellor for Research, Babol University of Medical Sciences. The authors wish to express their gratitude to Dr. Jila Masrour Roudsari for her excellent assistance.

REFERENCES


