HEPATOTOXICITY ASSOCIATED WITH HYPOGLYCEMIC EFFECTS OF *TEUCRIUM POLIUM* IN DIABETIC RATS

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Abstract

Background-Aqueous extract of *Teucrium polium* has been used traditionally as an antidiabetic agent in many Iranian provinces. The goal of this study was to investigate the hypoglycemic effect and histopathological changes in the liver following the ingestion of an extract of *T. polium* in streptozocin-induced diabetic rats.

Methods-An aqueous extract of *Teucrium polium* was fed intra-esophageal to healthy and streptozocin-induced diabetic rats for several days. Serum glucose levels of the case group were measured daily and compared to those of the healthy control. At the end of the extract ingestion period, parts of the animal liver were excised, fixed in formalin and studied histologically.

Results-Treatment of diabetic animals with the aqueous extract resulted in a significant decrease (p<0.001) in the serum glucose level after 24h, which reached those of the normoglycemic animals in 8 days. Liver sections from *T. polium*-treated rats showed marked cytoplasmic hydropic changes in 1/3 to 2/3 of the liver lobule in perivenular and midzonal areas. Apoptopic bodies were also noted in the perivenular zone and Kupffer cells increased in number. Nuclear enlargement with anisonucleosis and prominence of 1 or 2 nucleoli suggestive of regenerative changes were also observed.

Conclusion-Although the aqueous extract of *T. polium* has strong hypoglycemic properties in experimental animals, human application should be discouraged.

Keywords • *Teucrium polium* • diabetes • liver • toxicity • rats

Introduction

Polygonermander or *Teucrium polium*, L., (Kalporeh or Arpeh in Farsi) is a member of the Labiatae family and its flowering aerial parts have proven hypoglycemic effects in both normoglycemic and streptozocin-induced hyperglycemic rats.1,2

The blossoms of a related species, *Teucrium chamaedrys* or germander, have also been used in France for the preparation of herbal teas and capsules and were employed in folk medicine for the treatment of obesity. Several cases of hepatotoxicity have been reported as a result of *T. chamaedrys* consumption in the form of tea or capsules.3,4 Loeper, et al4 showed that an intragastric administration of the lyophilisate (1.25 g/kg) of *T. chamaedrys* to mice would produce midzonal liver necrosis after 24 h. They concluded that a cytochrome P4503A metabolite of one of the constituents of this plant (furano neoclerodane diterpenoids) probably acts as the major hepatotoxic factor. The aqueous extract of the dried aerial parts of *T. polium* in bloom is being used in many Iranian provinces, particularly in Kerman, as an antidiabetic agent. In 1995, Mattei, et al5 reported a case of severe liver failure caused by a herbal medicine containing *T. polium*, for whom liver transplantation was performed. Several neoclerodane diterpenoids, the probable hepatotoxic precursors of this herb have been reported to...
be present in the aerial parts of the plant. This study, which reports the histopathological changes in the liver induced by *T. polium* in streptozocin-induced diabetic rats, is the first report of a series of investigations performed to evaluate the antidiabetic potential of this herbal medicine.

### Materials and Methods

#### Reagents

Reagents used in this study included streptozocin (Upjohn Co., Kalamazoo, MI, USA) and the enzymatic kit for glucose determination (Pars Azemoon Co., Tehran, Iran). All other reagents were of analytical grade and obtained through other commercial sources.

#### Preparation of *T. polium* extract

The dried aerial parts of *T. polium* were purchased from herbalists in Kerman and were authenticated by the Center for Research on Natural Resources and Livestock (Ministry of Agricultural Jihad, Isfahan, Iran) as *Teucrium polium* L.

The cleaned aerial parts (90 g) were suspended in one liter of water for an hour prior to simmering on a boiling water bath for another hour. The suspension was cleared upon passing through several layers of cheese cloth and freeze-dried (Heto Dry Winner, Model DW3). Approximately 20 g of dried powder was obtained from every 90 g of the original plant. The dried powder was dissolved in distilled water at a concentration of 1 g/mL. This preparation which is referred to as the aqueous extract and corresponds to 4.5 g of the original aerial parts per mL is employed as the hypoglycemic agent throughout this study (see below).

#### Animal experiments

Four groups of adult male Sprague-Dawley derived rats (5 rats per group) with a weight ranging from 200 to 220 g were used as experimental animals. Each group was separately housed in a cage and fed a rat chow diet (Pars Dam Co, Tehran, Iran) and water *ad libitum* throughout this study. Two groups of the rats were injected with streptozocin (40 mg/kg body weight) two weeks prior to the initiation of treatments as mentioned previously. After obtaining blood from the tails using heparinized microhematocrit tubes, serum glucose of all groups was measured weekly prior to the beginning of the aqueous-extract treatment and on specified days during the treatment. Two weeks after streptozocin treatment, the serum glucose level of the diabetic rats reached 24-25 mM. One group of the streptozocin-induced hyperglycemic rats (experimental diabetic rats) received 1 mL aliquots of the aqueous extract twice a day (8:30 AM and 1:30 PM) through a gavage for a period of 12 days. The other diabetic group (diabetic control rats) had only the *ad libitum* feeding schedule. A group of normoglycemic animals (normal experimental rats) received 1 mL aliquots of the aqueous extract once a day (8:30 AM) for a period of 10 days and the second normoglycemic group (normal control rats) followed only the *ad libitum* feeding protocol. Since normoglycemic rats which received 1 mL aliquots of the extract twice daily became extremely hypoglycemic and died, it was decided to treat normoglycemic animals with only 1 mL aliquot of the extract once daily.

Serum glucose determination was performed on days 0 (just prior to aqueous-extract treatment), 1, 4, 8 and 12 in diabetic rats and on days 0, 2, 6 and 10 in normoglycemic animals after the intragastric feeding of the extract to the experimental groups in the morning.

#### Histological studies

After 10 days of the aqueous extract treatment of the normoglycemic animals and 12 days after the extract treatment of the streptozocin-induced hyperglycemic rats, the animals were sacrificed and a portion of the livers were immediately placed in buffered 10% formalin. Two to three 5-micron-thick sections of the liver were prepared and stained with hematoxylin and eosin.

### Table 1. Effects of aqueous extract of *Teucrium polium* on serum glucose levels of normoglycemic rats.

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Serum glucose (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day zero</td>
</tr>
<tr>
<td>Normoglycemic control</td>
<td>6.7±0.35&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Normoglycemic experimental&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.7±0.25</td>
</tr>
</tbody>
</table>

<sup>a</sup>Results are expressed as mean±SEM of 5 rats in each group. <sup>b</sup>Normoglycemic animals receiving 1 mL of the aqueous extract per day for 10 days. Student’s *t*-test and Mann-Whitney *U* non-parametric test showed no significant difference between the control and the experimental groups.
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Table 2. Effects of aqueous extract of Teucrium polium on serum glucose levels of streptozocin-induced hyperglycemic rats.

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Serum glucose (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day zero</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>24.6±1.15a</td>
</tr>
<tr>
<td>Diabetic experimentalb</td>
<td>25.6±0.89</td>
</tr>
</tbody>
</table>

* Results are expressed as mean±SEM of 5 rats in each group. Streptozocin-induced diabetic animals receiving 2 x 1 mL of the aqueous extract per day for 12 days.* Student’s t-test and Mann-Whitney U non-parametric test showed a significant difference (p <0.001) between the respective control and the experimental groups.

Results

The effects of the aqueous extract of the aerial parts of T. polium on serum glucose levels of normoglycemic and streptozocin-induced diabetic rats are shown in Tables 1 and 2, respectively. As shown in Table 1, intra-esophageal administration of 1 mL of the extract for 10 days had no significant effect on serum glucose level of the normoglycemic animals. However, twice daily treatment of the diabetic rats with 1 mL aliquots of the extract resulted in a highly significant reduction (p<0.001) in serum glucose level after 24h; the level reached those of the normoglycemic animals after 8 days of extract treatment (Table 2).

Figure 1 represents a typical normal hepatic lobule with a central vein and a portal tract. Liver sections from T. polium-treated rats showed marked hydropic change of the cytoplasm in one-third to two-third of the areas of the liver lobules (Figure 2). This type of change was mostly seen in the perivenular and midzonal areas. Isolated hepatocyte necrosis (apoptotic bodies) was also easily observed especially in the perivenular zone (Figure 3). There was no significant inflammatory cell infiltrate in the portal as well as the injured areas, but Kupffer cells increased in number. Fatty changes were absent. The individual hepatocytes showed vesicular nuclei, nuclear enlargement with anisonucleosis and prominence of 1 or 2 nucleoli, suggestive of regenerative changes (Figure 3).

Discussion

As noted in Table 2, intra-esophageal feeding of 2x1 mL aliquots of the aqueous extract of T. polium from Kerman province significantly decreased serum glucose level of the streptozocin-induced diabetic rats within 24 h and with the continual feeding of 2 x 1 mL aliquots of the extract per day, the blood glucose level of the diabetic rats decreased about 71% after 8 days and remained in the normal range thereafter. Several flavonoids17-19 and terpenoids7,9,15 have been demonstrated to be present in T. polium. One of such flavonoids is quercetin20 which has hypoglycemic effects only in diabetic rats21. Two different terpenoids with hypoglycemic effects were also shown to be effective in diabetic animals only.22 It is, therefore, possible to conclude that the hypoglycemic effects of the aerial parts of T. polium may be due to its content of flavonoids and/or terpenoids. However, in our preliminary experiments (unpublished data), we were unable to observe any hypoglycemic effect of T. polium from Shiraz, Iran. It is, therefore, probable that the hypoglycemic effects of T. polium from Kerman area is due to its contents of K, Zn, Ca, and Cr which have been shown to have some hypoglycemic properties23. It is suggested that the hypoglycemic property of this plant may depend on the type of soil, in which it is grown.

The histopathological changes of the liver
Figure 2. Liver section from a diabetic rat treated with *T. polium* extract. Hydropic change of the cytoplasm and focal cell necrosis in 2/3 of the hepatic lobules and absence of inflammatory cells. C, central vein; P, portal vein. Original magnification x 200.

(Figures 2 and 3) agree with the study of Mattei, et al, who reported massive hepatocyte necrosis predominantly in the centrilobular areas of the liver in a patient with severe acute liver failure after *T. polium* consumption. Loeper, et al have also shown mid-zonal and centrilobular necrosis after 24 h in mice fed with 2.5 g of *T. chamaedrys* lyophilisate per kilogram body weight. Several cases of hepatitis due to *T. chamaedrys* have also been reported in France. In this study, we observed midzonal and perivenular necrosis and hydropic changes in these two areas were also observed. Absence of inflammatory cells and the presence of scattered individual hepatocyte necrosis, suggests that cell injury had occurred by the apoptopic process following direct and rapid hepatotoxic damage passing through cellular swelling. Presence of cell activity i.e. nuclear enlargement and prominent nucleoli may demonstrate regenerative capacity and reversibility of the injurious process.

In conclusion, we should emphasize that although the aqueous extract of *T. polium* aerial parts has potent hypoglycemic effects in experimental animals, it is strongly recommended that its use will be legislated against due to its proven hepatotoxic effects in both animals and humans.

Attempts should be made to evaluate the hypoglycemic effects of the organic and the inorganic constituents of this plant with the hope of separating the hypoglycemic factor(s) from the hepatotoxic ones.

**Acknowledgment**

This project was partially supported by Grant No. 78-639 from the Office of Vice-Chancellor for Research, Shiraz University of Medical Sciences.

**References**

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