Effects of two Turkish medicinal plants *Artemisia herba-alba* and *Teucrium polium* on blood glucose levels and other biochemical parameters in rabbits

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Abstract

*Artemisia herba-alba* (Asteraceae) and *Teucrium polium* (Lamiaceae) are traditional plants that are used for the conventional therapy of several diseases such as diabetes mellitus and gastrointestinal disorders in Turkey. Our aim in the present study is to investigate the effects of these two plants on blood glucose levels and some other biochemical parameters to demonstrate their possible therapeutic effects on diabetes. The extracts of aerial parts of *Artemisia herba-alba* (85 mg/kg) and *Teucrium polium* (82 mg/kg) were administered orally to control and Streptozotocin (STZ)-induced diabetic rabbits. Applied doses did not cause any acute toxicity or behavioural changes. Blood glucose levels were estimated before and 2, 4, 6 and 8 hours after administration of the extracts. The *Artemisia herba-alba* produced a significant (p<0.05) hypoglycaemic effect in normal and diabetic rabbits while *Teucrium polium* had insignificant (p >0.05) effect.

Keywords: *Artemisia herba-alba*, *Teucrium polium*, hypoglycaemia, streptozotocin, diabetes.

Türkiye’de yetişen iki tibbi bitki *Artemisia herba-alba* ve *Teucrium polium’un tavşanlarda kan glukoz düzeyi ve diğer biyokimyasal parametreler üzerinde etkileri

Özet


Anahtar Sözcükler: *Artemisia herba-alba*, *Teucrium polium*, hipoglizemi, streptozotosin, diyabet
Introduction

Since ancient time phytotherapy has been used as folk medicine to treat various diseases including diabetes mellitus. More than 400 traditional plants treatments for diabetes mellitus have been recorded, but only small numbers of these have received scientific and medical evaluation to assess their efficacy (Bailey and Day, 1989, Satyavati et al., 1987).

Diabetes mellitus is characterised by elevated plasma glucose concentration resulting from insulin insufficiency and insulin resistance, or both (ADA, 1989).

*Artemisia* species (Fam: Asteraceae), (Mossa 1985; Al-Shamaony et al., 1994; Subramoniam et al., 1996) and *Teucrium* species (Fam: Lamiaceae) (Suleiman et al., 1988; Gharabeh et al., 1988; Tanira et al., 1996; Konuklugil et al., 1997) are reported to possess antidiabetic effects and have been used in many countries of Middle East and Turkey as a herbal medicine for treatment of diabetes, high blood pressure and gastrointestinal ailments.

French Ministry of Health and Humanitarian Action has suspended marketing authorization for medical products containing extracts from germander (*Teucrium chamaedrys* Fam: Lamiaceae). Several cases of hepatitis have been reported following administration of germander (WHO 1992, Larrey et al., 1992). These findings were marked by jaundice and increased aminotransferase levels in blood serum. A focal loss in purkinje cells in the cerebellum in rats treated with *T. stocksianum* was reported (Tanira et al., 1996).

These symptoms appeared 3-18 weeks following after administration. However, there is no detailed study demonstrating therapeutic effects, acute and chronic toxicity of these plants.

In our present work, by using experimental diabetic animal model we investigated the effects of these plants on diabetes and the possibility of using them as alternative therapeutic drugs.

Materials and methods

Animal

Adult New Zealand rabbits of both sex weighing 1.350-2.050 kg and 5-6 months old were used. They were housed in-groups in large cages and fed on green vegetables and chaw pellets, and allowed tap water ad libitum. The animals were kept in laboratory for two months, before starting the experiments for acclimation.

Plant material and the extract preparation

Aerial part of *A. herba alba* and *T. polium* were obtained from a herbal and folk medicine market in Sanliurfa Turkey. The plants were botanically authenticated and the samples were stored in Herbarium of Science and Art Faculty, Harran University, Sanliurfa/Turkey.

The aerial parts of *A. herba-alba* and *T. polium* were coarsely powdered and macerated with distilled water for 16 hours with occasional stirring. The 8.5 mg/ml *A. herba-alba* and *T. polium* 8.2 mg/ml have been prepared from the filtered extract.

Experimental induction of diabetes in rabbits

Diabetes was induced by 65 mg/kg of streptozotocin (STZ) Sigma® administered intraperitoneal (i.p.) in physiologic saline with pH adjusted to 4.5 using citric acid as described (Palanichamy et al., 1988). After 8 days of STZ administration and 18 h. of fasting, blood glucose was measured by collecting 3-5 ml of blood from the marginal ear vein. The glucose levels that were above 250 mg/ml were included in the experiments.

Grouping of animals

Diabetic rabbits were divided into 3 groups of 10 animals each. Normal rabbits were divided randomly into 3 groups of 10 rabbits each and treated as follows:

A- Groups I-III including normal rabbits were treated as follows.
   Group I- received 10 ml/kg tap water and served as control.
   Group II- received 85 mg/kg *Artemisia herba-alba* aqueous extract.
   Group III- received 82 mg/kg *Teucrium polium* aqueous extract.

B- Group IV-VI including diabetic rabbits were treated as follows.
   Group IV- received 10 ml/kg tap water and served as control.
   Group V- received 85 mg/kg *Artemisia herba-alba* aqueous extract.
   Group VI- received 82 mg/kg *Teucrium polium* aqueous extract.
Drug administration

The amounts of Artemisia herba-alba and Teucrium polium extract were calculated for each rabbit on body weight basis 85 mg/kg and 82 mg/kg respectively, this was equivalent to 2g/kg of dried plant (Tanira et al., 1996) and prepared by dissolved in 10 ml of water (Gharaibeh et al., 1988; Tanira et al., 1996). The water and extracts were administered to each rabbit using a stomach tube attached to 20-ml standard syringe. The tube was inserted into stomach through the oesophagus, and the plunger of syringe was pressed slowly and steadily. Drugs were administered at doses 85 and 82 mg/kg body weight to evaluate the chronic toxicity.

Collection of blood and biochemical determination

Blood was collected from the marginal vein of the rabbits using 22 gauge sterile syringe from the marginal vein of the ear, prior and 2, 4, 6, 8 hours after drug administration. Plasma glucose levels were then measured by commercial kits (Boehringer Manheim, Germany) by using a glucose oxidize method with a HITACHI 917 autoanalyser.

Blood plasma was separated by centrifugation at 3000 rpm for 3 minutes. Blood urea, alanin transaminase (ALT), aspartate transaminase (AST), creatinine, cholesterol, and trigliserides were measured using colorimetric and ultraviolet methods as recommended by the German Society for Clinical Chemistry. Reagents were obtained from Sigma-Aldrich.

Pathological estimation

All animals were sacrificed by dislocation at the end of the experimental period. Liver, kidney, brain and medulla spinalis tissues from control and diabetic treated groups were fixed in Bouin fixative. After routine tissue processing they were embedded in paraffin and sectioned 5 μm thick from each block and stained with haematoxylin-eosin for ordinary light microscopic studies.

Statistical analysis

The data were subjected to analysis of variance (Duzgunes et al., 1987) using SPSS 10.0 for Windows program. The data were analysed with ANOVA in SPSS. Duncan’s test was used in all data where appropriate.

Results

There was no mortality in any experimental group throughout the investigation period. The effect of aqueous extract of aerial parts of A. herba-alba and T. polium on blood sugar values on hours 2, 4, 6, and 8 of euglycemic and STZ-induced diabetic rabbits are shown in Figure 1 and 2 respectively. 82 mg/kg body weight of T. polium aqueous extract was insignificant (p>0.5) neither euglycemic nor STZ-induced diabetic rabbits when compared with control group.

However 85 mg/kg body weight of A. herba-alba aqueous extract attenuated the hyperglycaemic effect of STZ-induced diabetic rabbits. The blood sugar values of STZ-induced rabbit group on 2, 4, 6 and 8 hours were 269.7±4.8, 246.5±5.2, 253.9±9.5 and 271.5±4.4 respectively and the hypoglycaemic effect of A. herba-alba extracts was estimated to be 36 % of initial value.

As shown in Table 1 administration of A. herba-alba aqueous extract resulted in large reductions (p<0.05) in alanine aminotransferase, aspartate aminotransferase and urea levels of STZ-induced diabetic rabbits group and changes in any biochemical parameters except increasing in urea levels.

Histopathological results

No significant histopathological changes were noted in the studied organs (liver, heart, kidney, brain and medulla spinalis. In A. herba alba group after chronic and acute treatment. However animals treated with T. polium showed normal histology of liver, heart and brain, and simple apoptosis in the kidney sections of treated groups.

Discussion

The aim of this paper is to study the hypoglycemic effect and the toxicity of A. herba-alba and T. polium and to approve its traditional usage for controlling diabetes mellitus or other diseases.

This present study reveals that oral administration of A. herba-alba aqueous extract showed significant (p<0.05) hypoglycemic effect in both normal and STZ
treated rabbits, while the effect of *T. polium* aqueous extract was insignificant (p>0.05). These results are similar with those of Twaij and Al-Badr, 1988), Al-Shamaony et al., (1994) and Marrif et al.,(1995) who reported that *A. herba alba* produced hypoglycemia in both normal and hypoglycemic rabbits. Gharaibeh et al., (1988) reported that *T. polium* extract caused significant reduction in blood glucose concentration 4 hours after intravenous (i.v.) administration and 24 hours after i.p. administration. Ansari et al., (2000) reported that *T. polium* did not show any effect on blood sugar levels in patients.

<table>
<thead>
<tr>
<th>Table 1. The effects of <em>A. herba alba</em> and <em>T. polium</em> on biochemical and haematological parameters (n=10 for 6 groups).</th>
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<tr>
<td><strong>Biochemical parameters</strong></td>
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<tr>
<td>Alanine Aminotransferase (U/L-1)</td>
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<td>Aspartate Aminotransferase (U/L-1)</td>
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<td>Cholesterol (mol/L-1)</td>
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<td>Creatinine (mg/dL-1)</td>
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<td>Tryglycerides (mg/L-1)</td>
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<td>Urea (mg/dL-1)</td>
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<td>Glutamyl transferase (Unit/ L-1)</td>
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<td>Total protein (mg/dL-1)</td>
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Values are means ± s.d.(n=10)

* p<0.05 significantly different from STZ-induced hyperglycaemic rabbit control group.

** p>0.05 insignificantly different from control group

Figure 1. Effects of *A. herba alba* and *T. polium* on blood glucose level in nondiabetic rabbits

Figure 2. Effects of *A. herba alba* and *T. polium* on glucose levels in STZ-induced hyperglycaemic rabbits
Our results are at variance with those of Gharaiibeh et al. (1988), but similar with Ansari et al. (2000). It is well known that sulphonylurea drugs like tolbutamide lower blood glucose level by stimulating pancreatic β-cells to release insulin in to the blood stream, which have been reported to produce hypoglycaemia, thus increasing glycogen deposition in the liver causing reduction of glucose levels which possibly increase the insulin receptors. STZ-induced diabetes by destroying β-cells and impairing renal function. However in this study tolbutamide exhibited only anti hyperglycaemic animals induced by STZ-diabetic rabbits (Wadood et al., 1992). However in this study aqueous extract of A. herba alba (85 mg/kg) showed significant (p<0.05) effect of both normoglycemic and hyperglycemic rabbit.

This suggest that the main mechanism action of the extract not only due to the potentiation of insulin action released from pancreatic β-cells but may be the drug effect on insulin independent diabetes also. A similar mechanism has also been offered to explain the effect of Momordica caranta (Akhtar et al., 1981) Cuminum nigrum (Akhtar and Ali, 1985) Tinospora cordifolia (Wadood et al., 1992). The blood glucose and cholesterol levels were estimated before and after administration of A. herba alba extract. The treatment with A. herba alba extract effort a significant (p<0.05) hypoglycaemic effect in normal as well as in STZ-treated diabetic rabbits and did not show any toxicity limits.

Also administration of 82 mg/kg Teucrium polium did not cause any reduction in blood glucose levels in both STZ-treated and untreated rabbits. Results suggest that treatment of this plant extract may not be useful in preventing the increase of glucose level in diabetic rabbits. However Gharaiibeh et al., (1988) reported that intraperitoneally and i.v. injection of T. polium extract resulted in a significant decrease of insulin secretion rather than an increase insulin secretion. Ansari et al. (2000) and Yaniv et al. (1987) reported non significant antidiabetic effects of T. polium was observed, Kouzi et al. (1994) reported that in mice bioactive metabolite of teucrine A (diterpence constituent of T. chamaedrys) is the responsible compound for T. chamaedrys hepatotoxicity. Larrey et al. (1992) reported several cases of hepatitis characterized by jaundice and severe hepatocellular injury and a marked increase in serum aminotranferase levels occurring within 4-8 weeks of treatment with germander (T. chamaedrys). Tanira et al. (1996) reported that treatment with T. stocksianum did not cause a major hepatotoxic effect but a neurotoxic effect in treated animals. Histological studies of sections obtained from kidneys of rabbit groups, showed congestion of the renal tubules and glamorous along with loss of cells cytoplasm. Larrey et al. (1992) and Ansari et al. (2000) did not mention any symptoms, however Tanira et al. (1992) reported that brain sections of both group of rats showed neurocytotoxicological effect of T. stocksianum. T. polium contains flavonoids which has antioxidant activity (Gulcin et al., 2003) and hypoglycemic effects. However we were unable to observe any hypoglycemic effect of T. polium. These parameters were considered according to findings of Kouzi et al. (1994) who reported that the bioactive metabolite of the effective substance of Teucrium was responsible for hepatotoxicity. No change was furthermore noticed in ALT, AST. Altogether these observations indicate minimal hepatotoxicity potential of T. polium in rabbits. It is well known that sulphonylurea drugs like tolbutamide can lower glucose level by stimulating pancreatic β-cells to release insulin. STZ-induces diabetes by destroying β-cells and injury renal function, in the present study tolbutamide exhibited only marginal antihyperglycemic effect in the STZ-diabetic animals. However, in these rabbits the aqueous extract of A. herba alba (85 mg/kg) showed marked antidiabetic effect. This suggest revealed that treatment with A. herba alba acts as hypoglycemic by stimulating pancreatic β-cells to release more insulin into blood stream (Wadood et al., 1992). Thus increasing glycogen deposition in the liver causing a reduction of glucose levels or may by increasing the number of insulin receptors (Kouzi et al., 1994).

From our results we concluded that administration A. herba alba might be useful in preventing hyperglycemia by having insulin-like action and can significantly reduce the blood glucose in normoglycemic and hyperglycemic rabbits but do not shows any action on creatinine and cholesterol in both normal and STZ-induced diabetic rabbits in acute diabetic experiment while T. polium did not preventing or delaying, or at least retarding hyperglycemia in diabetic rabbits in acute experiment.
Acknowledgements

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References


