Effects of Parsley (Petroselinum crispum) on the Liver of Diabetic Rats: a Morphological and Biochemical Study

S. Bolkent1,6, R. Yanardag2, O. Ozsoy-Sacan2 and O. Karabulut-Bulan1

1Department of Biology, Faculty of Science, Istanbul University, 34459-Vezneciler, Istanbul, Turkey
2Department of Chemistry, Faculty of Engineering, Istanbul University, 34850-Avcılar, Istanbul, Turkey

Parsley is used by diabetics in Turkey to reduce blood glucose. The present study aims to investigate both the morphological and biochemical effects of parsley on liver tissue. Rat hepatocytes were examined by light and electron microscopy. Degenerative changes were observed in the hepatocytes of diabetic rats. These degenerative changes were significantly reduced or absent in the hepatocytes of diabetic rats treated with parsley. Blood glucose levels, alanine transaminase and alkaline phosphatase were observed to be raised in diabetic rats. Diabetic rats treated with parsley demonstrated significantly lower levels of blood glucose, alanine transaminase and alkaline phosphatase. The present study suggests that parsley demonstrates a significant hepatoprotective effect in diabetic rats. Copyright © 2004 John Wiley & Sons, Ltd.

Keywords: parsley; Petroselinum crispum; diabetes mellitus; liver; rat.

INTRODUCTION

The use of herbal folk remedies to control diabetes recently has gained widespread popularity (Neef et al., 1995; Lemus et al., 1999). Parsley from the family Umbellifera, is widely used in the food, pharmaceutical, perfume and cosmetic industries (Lopez et al., 1999) and is an established antidiabetic agent in Turkish folk medicine. According to folk medicine parsley has antimicrobial, anticoagulant, antihyperlipidaemic and antihypertensive effects (Oztürk et al., 1991), membrane protective effects (Fejes et al., 2000) and is an antioxidant (Nielsen et al., 1999). Parsley seeds are used as a diuretic (Marczal et al., 1997). Phytochemical analysis shows the presence of flavonoids, carotenoids, ascorbic acid, myristicin, apiole, terpenoids and coumarins (Tunalı et al., 1997). The reported antidiabetic properties could be due to terpenoids (Pino et al., 1997), flavonoid glucosides (Tomas et al., 1972), coumarins (Anand et al., 1981) or ascorbic acid (Davey et al., 1996). The present study aims to evaluate the hepatoprotective effect of an extract of parsley in diabetic rats.

MATERIALS AND METHODS

Animals and treatments. Male Swiss albino rats, 6–6.5 months old were used in light and electron microscope investigations (n = 24) and in biochemical investigation (n = 75). The animals were divided into four groups as two controls and two diabetics. Group I: Control (untreated, non-diabetic) animals. Group II: Control animals given parsley extract. Group III: Diabetic animals. The rats in diabetic groups were fasted for 18 h and rendered diabetic on day 0 by an intraperitoneal injection of 65 mg/kg streptozotocin (STZ) in a freshly prepared citrate buffer (pH 4.5). Group IV: Diabetic animals given parsley extract. The plant extract was administered by gavage technique to rats at a dose of 2 g/kg every day for 28 days, 14 days after experimental animals were made diabetic.

Preparation of aqueous parsley extract. The parsley leaves were collected from Büyükçekmece-Istanbul. They were carefully washed with tap water and left to dry in the shade at room temperature. Dried parsley leaves (100 g) were extracted by adding 1000 mL distilled water and boiling for 30 min. Then, the extract was filtered and the filtrate was evaporated to dryness under reduced pressure using a rotary evaporator. The extract was dissolved in distilled water before administration to normal and diabetic rats.

Morphological investigations. On day 42, the liver tissues were taken from animals, which were fasted overnight, under ether anaesthesia. The paraffin sections were stained by Masson’s tri-dye and examined under a Carl Zeiss Ultraphot II microscope. The pieces of liver tissues were prefixed with 2% glutaraldehyde and postfixed with 1% osmium tetroxide. The ultrathin sections stained with uranyl acetate and lead citrate were examined using a Carl Zeiss EM 9 S-2 microscope.

Biochemical investigations. Blood samples from rats were collected from the tail vein at 0, 7, 14, 21, 28, 35 and 42 days after STZ injection. Fasting blood glucose

* Correspondence to: Dr S. Bolkent, Department of Biology, Faculty of Science, Istanbul University, 34459-Vezneciler, Istanbul, Turkey.
E-mail: sbolkent@superonline.com
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by the o-toluidine method (Relander and Raiha, 1963), alanine transaminase (ALT) by Reitman and Frankel (1957) and ALP activities by the two point method (Walter and Schütt, 1974) were determined.

Statistical analysis. The results were evaluated using an unpaired t-test and Anova variance analysis using the NCSS statistical computer package (Hintze, 1986).

RESULTS AND DISCUSSION

Degenerative changes in the hepatocytes of the diabetic rats were observed. Observation by light microscopy showed dilation and hyperemia in the sinusoids, picnotic nuclei, large granules in the cytoplasm and rupturing in the epithelium of the central veins (Fig. 1). In the diabetic rats treated with parsley extract, dilation and hyperemia in the sinusoids were absent and there were fewer picnotic nuclei. The rupturing of the epithelium of some central veins remained (Fig. 2).

Compared with the control group, the diabetic rat hepatocytes showed swelling in the cisternae of the granular endoplasmic reticulum and mitochondrial cristae, dilation in the perinuclear space, picnotic nuclei including invaginations, and an increase in smooth endoplasmic reticulum and lipid accumulation (Fig. 3). In the diabetic rats treated with parsley extract the swelling in the cisternae of the granular endoplasmic reticulum and mitochondrial cristae and dilation in the perinuclear space were either absent or significantly reduced. Few picnotic nuclei were observed and there was a marked reduction of smooth endoplasmic reticulum. Lipid accumulation was present, an increase in glycogen accumulation and parallel granular endoplasmic reticulum cisternae containing many ribosomes were observed (Fig. 4).
All animals had similar body weights when the study began ($P_{\text{ANOVA}} = 0.754$). The nondiabetic animals increased in body weight over days 0–42 (Table 1). The diabetic animals showed a gradual decrease in body weight over days 0–42. No effect on body weight was observed in the nondiabetic group treated with parsley extract during days 14–42 ($p_{\text{ANOVA}} = 0.965$). The diabetic rats treated with parsley had a body weight during days 14–42 similar to the untreated diabetic group.

Initial blood glucose levels were similar for all animals ($P_{\text{ANOVA}} = 0.586$) (Table 2). During the experiment blood glucose levels remained stable among the nondiabetic untreated (control) group and nondiabetic treated (control + parsley) group. On day 7 blood glucose levels for the diabetic groups were three times the initial levels. The blood glucose levels for the untreated diabetic rats remained high during the experiment and decreased on day 42. In the diabetic treated group blood glucose levels gradually reduced over days 14–42. A maximum reduction of about 50% was recorded on day 42 (Table 2).

Initial serum ALT levels were similar for all animals ($P_{\text{ANOVA}} = 0.307$) (Table 3). There was no significant difference observed in the ALT levels during the experimental period in both treated and untreated nondiabetic groups. A significant increase in ALT levels was observed in both diabetic groups over 7–42 days ($P_{\text{ANOVA}} = 0.0001$). The ALT levels were not reduced in the rats treated with parsley over days 14–42.

Changes in serum ALP values are given in Table 4. There was a significantly increase in ALP in both diabetic groups ($P_{\text{ANOVA}} = 0.0001$). The ALP values of the diabetic rats treated with parsley extract decreased compared with the diabetic untreated group during the experimental period.

As is well known, alterations in insulin levels affect liver function. In this investigation, histology of the liver during diabetes showed that there were structural alterations in the liver due to the absence of insulin. After parsley extract treatment, the hepatic lobuli structure of the liver was almost similar to that of the control and this extract prevented the damage to the liver tissue of diabetic rats. The ultrastructural findings underline the light microscopy findings that diabetes causes the liver cells to degenerate. In this study, the increase in the amount of liver glycogen in the hepatocyes of the diabetic group given parsley extract can be explained as stored glycogen or peripheral usage of glucose. Insulin is associated with carbohydrate, protein and lipid metabolisms. In the diabetic group given parsley extract, the increase in GER cisternae containing many ribosomes also demonstrated an increase in protein synthesis. The increase in lipid accumulation in the diabetic group and the diabetic group given parsley can be explained by an increase in the amount of triglyceride stores in the liver.

In this study, a decrease in body weight and an increase in blood glucose levels of the diabetic rats were
observed. After giving the parsley extract, decreases in blood glucose and body weights of the diabetic rats were observed. The reduction in body weight of the diabetic rats given parsley may be explained partly by the diuretic effect of parsley (Marczal et al., 1997). In other studies on parsley, it was observed by Tunali et al. (1999) that the administration of parsley caused a decrease in blood glucose levels of diabetic rats. In another study in which the pancreas was examined by light and electron microscopies, it was noted that parsley extract did not increase insulin release from B cells of the pancreas but decreased blood glucose levels by causing usage of glucose via extrapancreatic ways (Yanardag et al., 2003). In the current study, increased activities of serum ALP and ALT were observed in diabetic rats. It has been demonstrated that tissue antioxidant status is an important factor in the development of diabetic complications (Wohiaieb and Godin, 1987). The increase in the levels of these enzymes in diabetes may be as a result of leakage from the tissues and migration into the bloodstream (Chaudry et al., 1993; Stanely et al., 2000). Administration of parsley extract brought about a reduction in the activity of ALP and ALT. Flavonoids, ascorbic acid, tocopherol (Larson, 1988) and essential oils (Lagouri et al., 1993) are antioxidant compounds. Parsley contains large amounts of flavonoids (apin, luteolin, apigenin-glycosides), ascorbic acid, tocopherol and essential oils (apiole, myristicin) with antioxidant properties and these may prevent oxidative damage (Nielsen et al., 1999; Fejes et al., 2000). The protective and antidiabetic effects of parsley observed in this study might be related to its ascorbic acid, tocopherol, flavonoids and essential oil content.

As a result, it is suggested that the extracts of parsley showed a significant protective effect on the liver injury of diabetic rats in the present study.

Acknowledgements

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REFERENCES


Table 4. Mean levels of serum ALP for all groups (U/L)*

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Day 42</th>
<th>p (t-test)</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>15</td>
<td>77 ± 28.3</td>
<td>0.004</td>
</tr>
<tr>
<td>Control + parsley</td>
<td>20</td>
<td>50 ± 16.4</td>
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<tr>
<td>Diabetic</td>
<td>20</td>
<td>283 ± 74.4</td>
<td>0.0001</td>
</tr>
<tr>
<td>Diabetic + parsley</td>
<td>20</td>
<td>172 ± 50.2</td>
<td></td>
</tr>
<tr>
<td>PANOVA</td>
<td></td>
<td>0.0001</td>
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</tbody>
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*Mean ± SD.

n = Number of animals.