PHYTOTHERAPY RESEARCH
Phytother. Res. **18**, 996–999 (2004)
Published online in Wiley InterScience (www.interscience.wiley.com). **DOI**: 10.1002/ptr.1598

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

S. Bolkent[1,*], R. Yanardag[2], O. Ozsoy-Sacan[2] and O. Karabulut-Bulan[1]

[1]Department of Biology, Faculty of Science, Istanbul University, 34459-Vezneciler, Istanbul, Turkey
[2]Department 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 (Manderfeld *et al.*, 1997), antihypertensive, anticoagulant, antihyperlipidaemic and antihepatotoxic 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.*, 1999). 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 evalute 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 tetraoxide. 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
Contract/grant sponsor: Research Fund of Istanbul University; Contract/grant number: 875/090896; B-225/070799.

Received 9 October 2000
Accepted 19 September 2001

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).

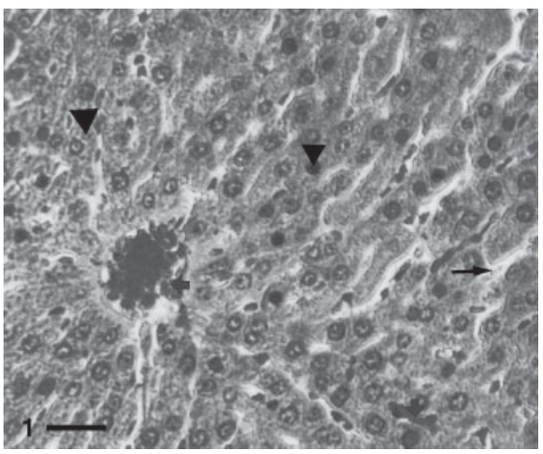

Figure 1. The liver of diabetic rat. The dilation in the sinusoids (→), picnotic nuclei (▼), hyperemia (➔), big cytoplasmic granules (▼). Masson's tri-dye. ×400. (1 cm = 1 μm).

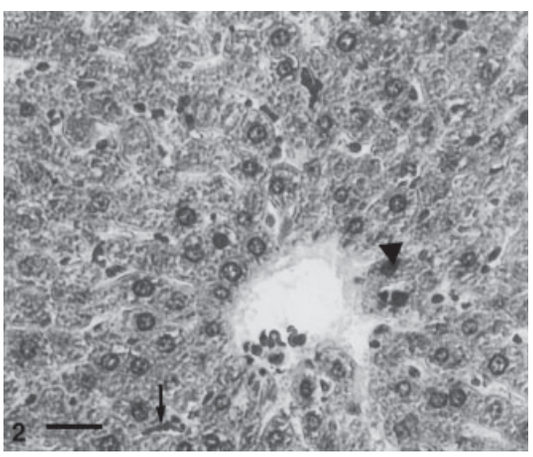

Figure 2. The liver of diabetic rat given parsley extract. An ordinary histological appearance except decreased hyperemia (→) and picnotic nuclei (▼). Masson's tri-dye. ×400. (1 cm = 1 μm).

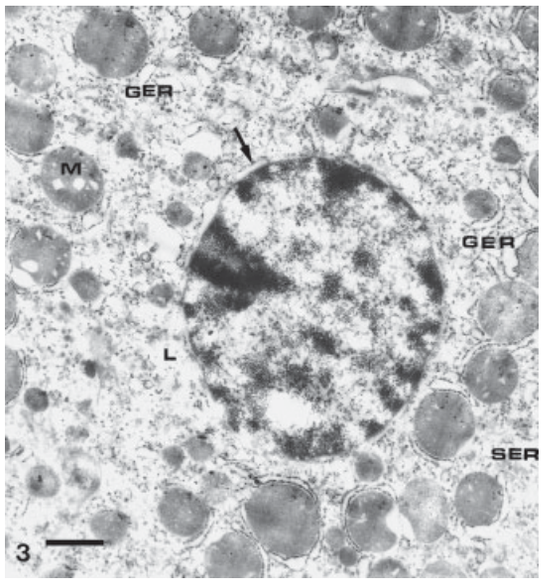

Figure 3. Electron micrographs of hepatocytes of the diabetic rats. Swelling in granular endoplasmic reticulum (GER) and mitochondrion (M), a dilation in perinuclear space (→), an increased smooth endoplasmic reticulum (SER) and lipid accumulation (L). ×13500. (1 cm = 6 μm).

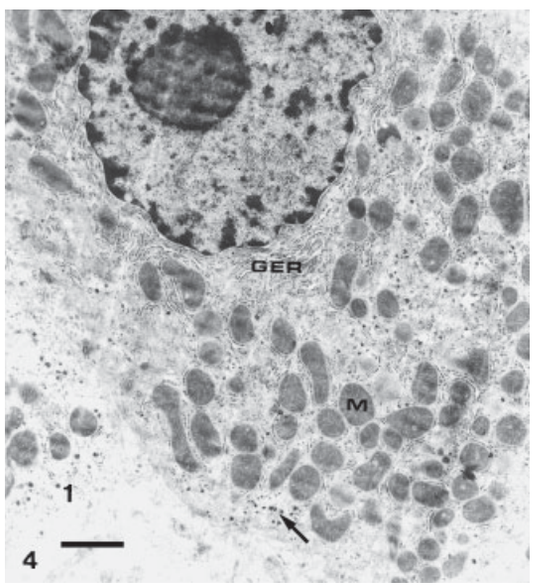

Figure 4. Electron micrographs of hepatocytes of the diabetic rats given parsley extract. An ordinary cytological appearance. Parallel granular endoplasmic reticulum (GER) and mitochondrion (M), glycogen accumulation (→). ×13500. (1 cm = 6 μm).

Table 1. Mean levels of body weight for all groups (g)[a]

Group	n[b]	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42	p (*t*-test)
Control	15	248 ± 26.8	257 ± 24.0	251 ± 32.3	253 ± 22.0	257 ± 22.5	260 + 24.3	259 ± 22.5	0.856
Control + parsley	20	260 ± 37.1	250 ± 31.1	257 ± 28.6	251 ± 30.7	251 ± 29.6	253 ± 30.7	258 ± 35.3	0.965
Diabetic	20	256 ± 30.8	223 ± 29.5	207 ± 28.0	207 ± 37.0	197 ± 22.4	194 ± 25.7	190 ± 31.9	0.0001
Diabetic + parsley	20	257 ± 20.3	220 ± 27.1	207 ± 20.1	193 ± 15.2	188 ± 21.7	192 ± 31.2	190 ± 31.2	0.0001
p_{ANOVA}		0.754	0.001	0.0001	0.0001	0.0001	0.0001	0.0001	

[a] Mean ± SD.
[b] n = Number of animals.

Table 2. Mean levels of blood glucose for all groups (mg %)[a]

Group	n[b]	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42	p (*t*-test)
Control	15	71 ± 17.7	79 ± 14.1	77 ± 15.3	80 ± 15.1	68 ± 7.6	84 ± 16.8	102 ± 12.0	0.495
Control + parsley	20	66 ± 23.0	73 ± 14.7	75 ± 17.3	71 ± 15.0	77 ± 18.3	82 ± 15.3	67 ± 10.2	0.117
Diabetic	20	71 ± 21.1	225 ± 65.9	181 ± 57.7	191 ± 70.7	198 ± 11.9	190 ± 81.2	158 ± 85.1	0.0001
Diabetic + parsley	20	63 ± 14.3	221 ± 65.2	67 ± 49.3	168 ± 57.6	139 ± 59.3	116 ± 48.2	111 ± 45.1	0.0001
p_{ANOVA}		0.586	0.0001	0.0001	0.0001	0.0001	0.0001	0.018	

[a] Mean ± SD.
[b] n = Number of animals.

Table 3. Mean levels of serum ALT for all groups (U/L)[a]

Group	n[b]	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42	p (*t*-test)
Control	15	18 ± 7.32	13 ± 2.93	13 ± 3.20	20 ± 6.40	22 ± 9.96	14 ± 2.24	20 ± 11.26	0.033
Control + parsley	20	14 ± 6.77	11 ± 5.23	11 ± 8.36	13 ± 9.34	15 ± 8.97	10 ± 7.20	14 ± 8.73	0.668
Diabetic	20	15 ± 8.29	100 ± 14.79	59 ± 13.08	61 ± 21.34	96 ± 31.98	101 ± 18.09	122 ± 20.16	0.0001
Diabetic + parsley	20	12 ± 6.23	64 ± 24.59	27 ± 5.49	62 ± 10.89	125 ± 31.47	67 ± 12.49	103 ± 16.17	0.0001
p_{ANOVA}		0.307	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	

[a] Mean ± SD.
[b] n = Number of animals.

All animals had similar body weights when the study began (p_{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 ($p_{t\text{-test}}$ = 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_{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_{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 ($p_{t\text{-test}}$ = 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_{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 hepatocytes 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

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

Group	n[b]	Day 42	p (t-test)
Control	15	77 ± 28.3	0.004
Control + parsley	20	50 ± 16.4	
Diabetic	20	283 ± 74.4	0.0001
Diabetic + parsley	20	172 ± 50.2	
p_{ANOVA}		0.0001	

[a] Mean ± SD.
[b] n = Number of animals.

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 Tunalı *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 (Wohaieb 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 (apiin, 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

This study was supported by the Research Fund of Istanbul University, project numbers 875/090896 and B-225/070799.

REFERENCES

Anand NK, Sharma ND, Gupta SR. 1981. Coumarins from *Apium petroselinum* seeds. *Natl Acad Sci Lett* **4:** 249–251.

Chaudry AR, Alam M, Ahmad M *et al.* 1993. Studies on medicinal herbs. II. Effect of *Colchicum luteum* on biochemical parameters of rabbit serum. *Fitoterapia* **64**: 510–515.

Davey MW, Bauw G, Montagu MV. 1996. Analysis of ascorbate in plant tissue by high performance capillary zone electrophoresis. *Anal Biochem* **239:** 8–19.

Fejes SZ, Blazovics A, Lemberkovics E *et al.* 2000. Free radical scavenging and membrane protective effects of methanol extracts from *Anthriscus cerefolium* L. (hoffm.) and *Petroselinum crispum* (Mill.) Nym. Ex A.W. Hill. *Phytother Res* **14:** 362–365.

Hintze JL. 1986. Number Cruncher Statistical System Copyright C, 865, east 400, North Kaysville, Utah, 84037 (801) 546-0445.

Lagouri V, Blekas G, Tsimidou M *et al.* 1993. Composition and antioxidant activity of essential oils from Oregano plants grown wild in Greece. *Z Lebensm Unters Forsch* **197:** 20–23.

Larson RA. 1988. The antioxidants of higher plants. *Phytochemistry* **27:** 969–978.

Lemus I, Garcia R, Delvillar E, Knop G. 1999. Hypoglycaemic activity of four plants used in Chilean popular medicine. *Phytother Res* **13**: 91–94.

Lopez MG, Sanchez-Mendoza IR, Alejo O. 1999. Comparative study of volatile components and fatty acids of plants and *in vitro* cultures of parsley (*Petroselinum crispum* (Mill) Nym ex Hill). *J Agric Food Chem* **47:** 3292–3296.

Manderfeld MM, Schafer HW, Davidson PM, Zottola EA. 1997. Isolation and identification of antimicrobial furanocoumarins from parsley. *J Food Prot* **60**: 72–77.

Marczal G, Balogh M, Verzar-Petri G. 1997. Phenol-ether components of diuretic effect in parsley I. *Acta Agron Acad Sci Hung* **26**: 7–13.

Neef H, Declercq P, Laekeman G. 1995. Hypoglycemic activity of selected European plants. *Phytother Res* **9:** 45–48.

Nielsen SE, Young JF, Daneshvar B *et al.* 1999. Effect of parsley (*Petroselinum crispum*) intake on urinary apigenin excretion, blood antioxidant enzymes and biomarkers for oxidative stress in human subjects. *Br J Nutr* **81:** 447–455.

Oztürk Y, Başer CHK, Aydın S. 1991. Hepatoprotective (antihepatotoxic) plants in Turkey *Proceedings of the 9th Symposium on Plant Drugs. Baser KHC (ed) 16–19 May, Eskişehir, Turkey*. Baser KHC (ed) 40–50.

Pino JA, Rosada A, Fuentes V. 1997. Herb oil of parsley (*Petroselinum crispum* Mill.) from Cuba. *J Essent Oil Res* **9**: 241–242.

Reitman S, Frankel SA. 1957. Colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am J Clin Path* **28**: 56–63.

Relander A, Raiha CE. 1963. Differences between the enzymatic and o-toluidine methods of blood glucose determination. *Scand J Clin Lab Invest* **15**: 221–224.

Stanely P, Prince M, Menon VP. 2000. Hypoglycaemic and other related actions of *Tinospora cordifolia* roots in alloxan-induced diabetic rats. *J Ethnopharmacol* **70:** 9–15.

Tomas F, Mataix JJ, Corpena, O. 1972. Flavonoid glucosides in *Petroselinum sativum. Rev Agroquim Technol Aliment* **12**: 263–268.

Tunalı T, Yarat A, Yanardağ R *et al.* 1999. Effect of parsley (*Petroselinum crispum*) on the skin of STZ induced diabetic rats. *Phytother Res* **13**: 138–141.

Walter K, Schütt C. 1974. Acid and alkaline phosphatase in serum (two point method). *Methods of Enzymatic Analysis*, **vol 2**, Bergmeyer (ed.) Verlag chemie GmbH: Florida, 856–860.

Wohaieb SA, Godin DY. 1987. Alterations in free radical tissue defense mechanisms in streptozotocin-induced diabetes in rat. Effects of insulin treatment. *Diabetes* **3:** 1014–1018.

Yanardağ R, Bolkent S, Tabakoğlu-Oğuz A, Ozsoy-Saçan O. 2003. Effects of *Petroselinum crispum* extract on pancreatic B cells and blood glucose of streptozotocin-induced diabetic rats. *Biol Pharm Bull* **26**: 1206–1210.