Effects of *Petroselinum crispum* Extract on Pancreatic B Cells and Blood Glucose of Streptozotocin-Induced Diabetic Rats

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This study investigated both morphologically and biochemically whether parsley (*Petroselinum crispum*), which is used as a folk remedy to decrease blood glucose, has any antidiabetic effect on pancreatic B cells of rats. Parsley extract was given to male diabetic rats. In the diabetic group given parsley extract, it was detected that the number of secretory granules and cells in islets and other morphologic changes were not different from the control diabetic group, while the blood glucose levels in the diabetic group given the plant extract were reduced in comparison to the diabetic group. In addition, a decrease was observed in the weight of the control diabetic group and the diabetic group given the plant extract. It is suggested that the plant therapy can provide blood glucose homeostasis and cannot regenerate B cells of the endocrine pancreas.

Key words B cell; diabetes mellitus; endocrine pancreas; hypoglycemic plant; *Petroselinum crispum*

Diabetes mellitus is a metabolic disorder affecting carbohydrate, fat, and protein metabolism. A worldwide survey showed that diabetes mellitus affects nearly 10% of the population.[1)] The treatment of diabetes mellitus is based on oral antihyperglycemic agents and insulin. The oral antihyperglycemic agents currently used in clinical practice have characteristic profiles of serious side effects. This leads to increasing demand for herbal products with antidiabetic activity and fewer side effects.[1,2)]

For centuries, more than 800 plants worldwide have been documented as beneficial in the treatment of diabetes.[3,4)] It is known that diet plays a major role in the management of diabetes mellitus and that many traditional medicines are from plant sources that do not form the constituents of our normal diet. The plants used as traditional medicines are herbs and spices, vegetables, and fruits.[5)] The World Health Organization has recommended accordingly that assessment of traditional plant treatments for diabetes mellitus merits further investigation.[6)]

Parsley (*Petroselinum crispum*) is a member of the Umbelliferae family that has been employed in the food, pharmaceutical, perfume, and cosmetics industries.[7)] Parsley is widely distributed in Turkey, and grown in gardens and fields. Parsley has been reported to have a number of possible medicinal attributes including, antimicrobial,[8)] antianemic, menorrhagic,[9)] anticoagulant, antihyperlipidemic,[10)] antihepatotoxic,[11)] antioxidative,[12)] and laxative.[13)] It has been used to treat lumbago, as a blood pressure regulator, to treat eczema, knee ache, impotence,[10)] and nose bleed.[14)] Parsley seeds are also used as a diuretic.[15)] The hypoglycemic activity of parsley has been shown by Yanardağ and Özsoy.[16)] The constituents of parsley which include ascorbic acid, carotenoids, flavonoids, coumarins, myristicin, apiole, various terpenoic compounds, phenyl propanoids, phthalides, furano coumarins,[17)] and tocopherol,[18)] have been chemically investigated.

The aim of this study was to determine whether *P. crispum* extract, which is used as a folk remedy to decrease blood glucose, has any antidiabetic effects on endocrine pancreatic B cells and blood glucose homeostasis of streptozotocin-induced diabetic rats.

MATERIALS AND METHODS

Preparation of Aqueous Parsley Extract Parsley leaves were collected from Büyükçekmece-Istanbul. They were carefully washed with tap water and left to dry for 1 week in the shade at room temperature. Then they were stored in well-sealed cellophane bags. Dried parsley leaves (100 g) were extracted by adding 1000 ml of distilled water and boiling for 30 min. Then the extract was filtered and the filtrate evaporated to dryness under reduced pressure using a rotary evaporator. The extract yield was 31.57% w/w. It was dissolved in distilled water before administration to normal and diabetic rats.

Experimental Animals Swiss albino male 6- to 6.5-month-old rats were used. They were clinically healthy, fed with chow laboratory pellets and given water *ad libitum*. Every experimental animal group was randomly divided into four subgroups, two controls and two diabetics: group I, intact group (untreated control animals); group II, control group given parsley extract; group III, diabetic group; and group IV, diabetic group given parsley extract. Diabetes in rats was induced by a single intraperitoneal injection of streptozotosin 65 mg/kg body weight in freshly prepared citrate buffer (pH 4.5).[19)] After day 14 from the induction of diabetes, rats in every experimental group were administered, by intragastric intubation 2 g/kg of parsley extract containing water prepared from dried plants daily for 28 d.[16,17)] Twenty-four animals were used for light and electron microscopic analysis and 75 for biochemical analysis.

Tissue Morphological Analysis On day 42, pancreatic tissue samples were obtained after the rats had been fasted overnight ether anesthesia. The samples of pancreatic tissue were fixed in Bouin's fixative for light microscopy and subsequently processed using traditional paraffin-embedding medium. The tissue sections were stained with aldehyde fuchsin-fast green and also treated with an immunohistochemical kit to show insulin amounts in all experimental and control B cells of pancreatic islets. In addition, samples of pancreatic tissue were prefixed in 2% glutaraldehyde and postfixed in 1% osmium tetraoxide for electron microscopy. Then the tissue fragments were dehydrated in ethanol, infil-

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trated with propylene oxide, and embedded in Epon 812.[20] Electron micrographs were taken with a Carl Zeiss EM 9 S-2.

Immunohistochemical Investigation Four micron-thick sections were mounted on poly-L-lysine-coated glass slides for immunocytochemical studies. An indirect immunocytochemical method, using the streptavidin-biotin-peroxidase kit (Zymed, 95-9943, San Francisco, CA, U.S.A.), was applied to paraffin sections. Endogen peroxidases were blocked by exposure to 0.3% hydrogen peroxide. Then the sections were washed with phosphate buffered saline (PBS) and incubated with nonimmune serum. Biotinylated secondary antibody was applied for 10 min after washing with PBS following incubation with insulin primer antibody (guinea pig antiserum against insulin: Zymed, 08-0067) for 60 min. The sections were washed again with PBS and incubated with streptavidin-peroxidase for 10 min. After washing, the sections were incubated with AEC (3-amino-9-ethyl-carbazole) for 5—10 min. Hematoxylin was used as a counterstain.[21]

Biochemical Assays Blood samples from the male rats were collected from the tail vein at 0, 7, 14, 21, 28, 35, and 42 d. Fasting blood glucose levels (after 18-h fasting) were determined using the *o*-toluidine method.[22] Blood was treated with 3% trichloroacetic acid, mixed, and centrifuged. The supernatant was treated with 6% *ortho* toluidine reagent and placed in a boiling water bath for 8 min. The color developed was read using a UV-120 Shimadzu spectrophotometer at an absorbance of 625 nm.

Statistical Analysis All results are shown as mean±standard deviation. The results were evaluated using an unpaired *t*-test and analysis of variance (ANOVA) using the NCSS statistical computer package.[23]

RESULTS AND DISCUSSION

The control group given parsley extract was not different from the intact control group considering the insulin amounts in B cells (Figs. 1, 2). Secretory material in B cells of the diabetic group giving a positive reaction with both aldehyde fuchsin and immunohistochemical kit was decreased except in a few cells (Fig. 3). There was no difference between the amount of insulin in B cells in the diabetic group and diabetic group given parsley extract with respect to both aldehyde fuchsin and immunohistochemical reaction (Fig. 4). In B cells of diabetic groups, a general decrease in secretory granules, an increase in clear vesicles, picnotic and lobular nuclei, and widened intercellular areas were observed. Dilated granular endoplasmic reticulum cisternae with the exception of a few full secretory granules in some B cells in some islets were observed (Figs. 5, 6). B cells of the diabetic group given parsley extract showed the same characteristics as those in the diabetic group (Figs. 7, 8).

The mean body weight of the four groups is given in Table 1. Before inducing diabetes, there was no difference in the body weight between the groups (Table 1) ($p_{ANOVA}=0.754$). On day 7, body weight was significantly lower than on day 0 for the diabetic and diabetic+parsley groups ($p_{ANOVA}=0.001$). A significant decrease was observed in the weight of diabetic and diabetic+parsley group from 7—42 d ($p=0.0001$) compared with 0 d. The administration of the parsley extract for 28 d caused a significant decrease in body weight in diabetic groups. Administration of parsley extract had no effect on body weight over days 14—42 in control+parsley rats ($p=0.965$).

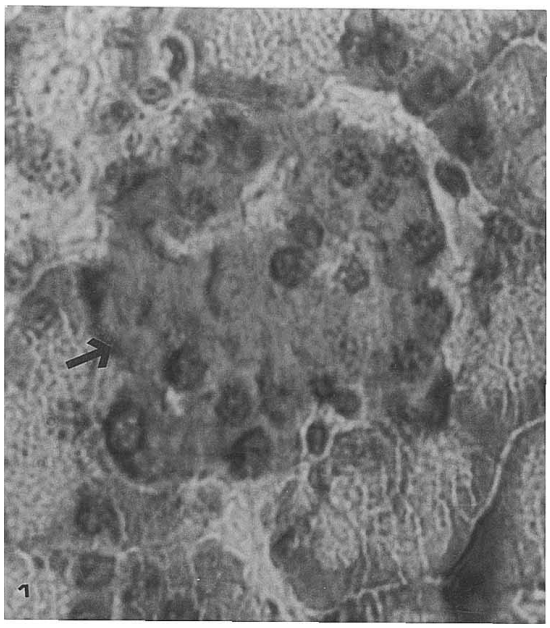


Fig. 1. Insulin Immunoreactivity (→) in B Cells of Langerhans Islet of Intact Rats

Streptavidin-biotin-peroxidase technique. Counterstained with hematoxylin. ×400.

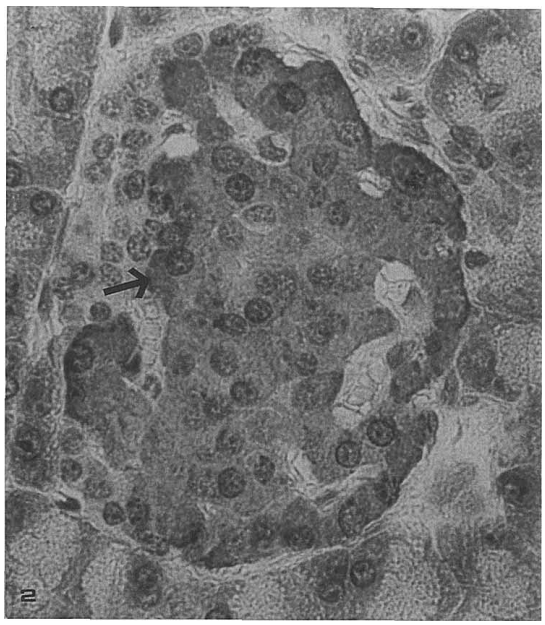


Fig. 2. Insulin Immunoreactivity (→) in B Cells of Langerhans Islet of the Control Rats Given Parsley Extract

Streptavidin-biotin-peroxidase technique. Counterstained with hematoxylin. ×400.

The mean blood glucose levels are shown in Table 2. Prior to inducing diabetes there was no difference in blood glucose between the groups ($p_{ANOVA}=0.586$). During the course of the experiment, blood glucose levels of control (nondiabetic) rats were similar to the initial values. On day 7, a significant increase was observed in the blood glucose levels of diabetic rats which continued to day 42 compared to day 0 ($p=0.0001$). In control+parsley rats, the blood glucose lev-

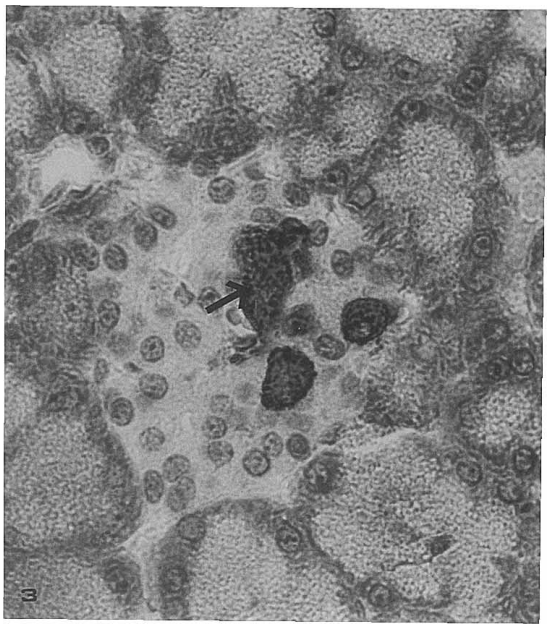


Fig. 3. Dark-Stained Insulin Immunoreactivity (→) in B Cells of Langerhans Islet of Diabetic Rats

Streptavidin-biotin-peroxidase technique. Counterstained with hematoxylin. ×400.

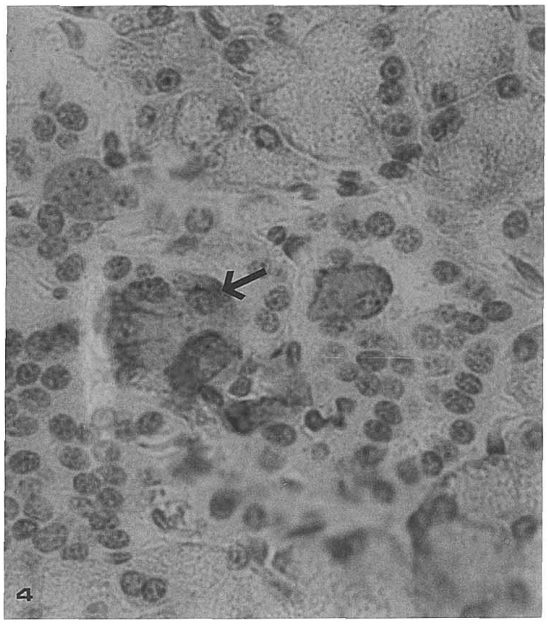
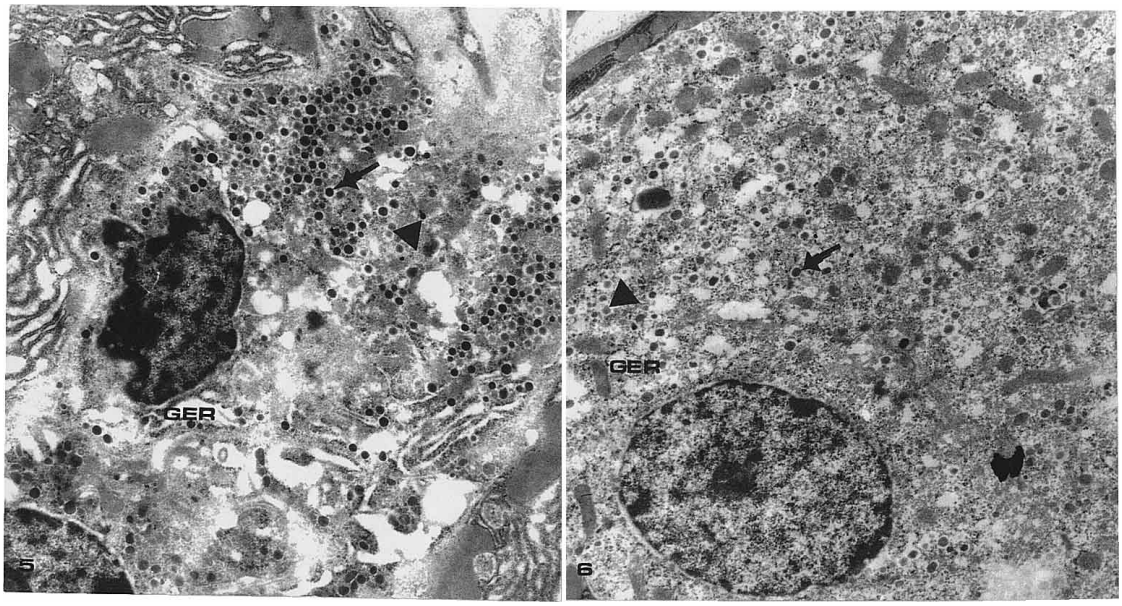


Fig. 4. Insulin Immunoreactivity (→) in B Cells of Langerhans Islet of Diabetic Rats Given Parsley Extract

Streptavidin-biotin-peroxidase technique. Counterstained with hematoxylin. ×400.



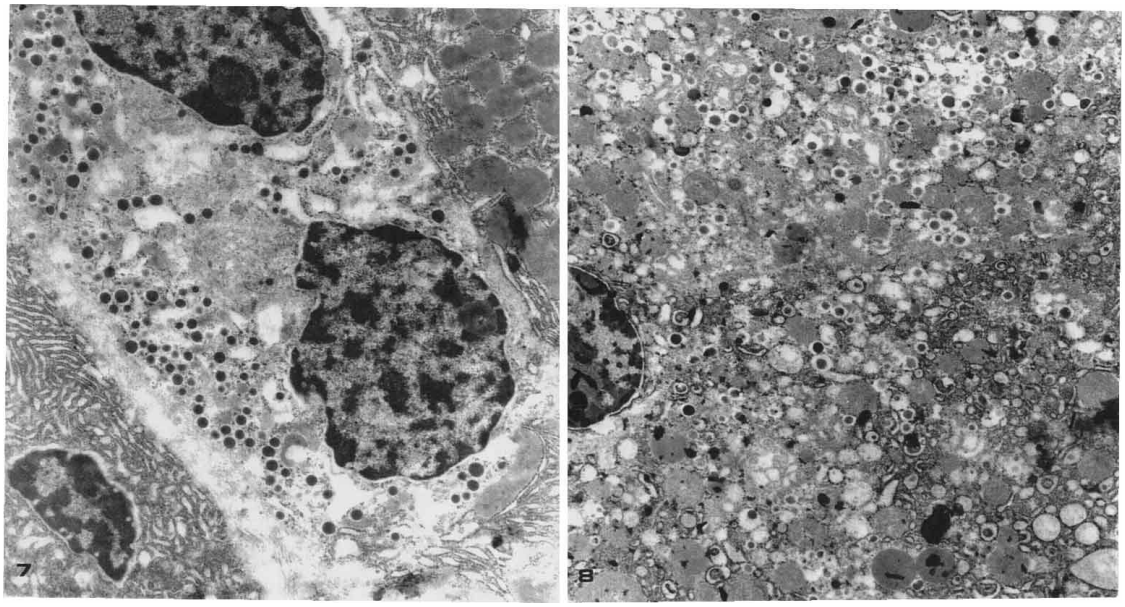
Figs. 5, 6. Electronmicrographs of Diabetic Rats

Decreased secretory granules (→), clear vesicles (▲), and dilated granular endoplasmic reticulum (GER) cisternae are seen. ×13500.

els did not change significantly during the experimental period (Table 2). Diabetic+parsley rats showed a gradual reduce in blood glucose levels over days 14—42. Maximum reduction in the blood glucose levels was observed on the day 42, and the reduction was about 50%.

In recent years, various plant extracts have been investigated for their antidiabetic effect. *P. crispum* is one of those plants. This study was carried out morphologically by light and electron microscopy and biochemically to detect whether this plant used in folk remedies for decreasing blood glucose had an effect on pancreatic B cells. It is known that there are structural changes in the liver and kidney as a result of the lack of insulin in diabetes. Both the morphologic and biochemical results in the diabetic group were in good agreement with the results of other investigators.[24—26] After parsley extract treatment, the number of secretory granules and B cells giving a positive reaction in islets and the ultrastructural changes in the pancreatic B cells were almost similar to those in the diabetic group.

Yanardağ and Özsoy reported that 1—4 g/kg doses of aqueous extracts of dried parsley leaves the blood glucose of normal rabbits.[16] In this study, 2 g/kg of parsley extract was given to rats by gavage once a day. It was found that the parsley extract inhibited the increase in blood glucose levels in



Figs. 7, 8. Electronmicrographs of Diabetic Rats Given Parsley Extract
The appearance is the same as that in the control diabetic group. ×13500.

Table 1. Body Weight in All Groups (g)[a)]

Group	*n*	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Control	15	248.1±26.8	256.9±24.0	250.6±32.3	252.8±22.0	257.2±22.5	260.4±24.3	259.0±22.5
Control+parsley	20	259.9±37.1	250.1±31.1	257.3±28.6	251.0±30.7	251.4±29.6	253.4±30.7	257.6±35.3
Diabetic	20	255.8±30.8	222.6±29.5	206.6±28.0	207.1±37.0	197.4±22.4	194.2±25.7	190.1±31.9*
Diabetic+parsley	20	256.8±20.4	219.9±27.1	207.4±20.1	193.2±15.2	188.3±1.7	191.8±31.2	189.5±31.2**
p_{ANOVA}		0.754	0.001	0.0001	0.0001	0.0001	0.0001	0.0001

a) Mean±S.D. *n*=Numbers of animals. *,** Significant differences (Student's *t*-test), $p<0.0001$ with respect to day 0.

Table 2. Blood Glucose Levels in All Groups (mg/dl)[a)]

Group	*n*	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Control	15	71.5±17.7	78.6±14.1	77.2±15.3	80.2±15.1	68.1±7.6	84.0±16.8	102.3±12.0
Control+parsley	20	66.1±22.9	73.2±14.7	75.1±17.3	71.4±15.0	76.6±18.3	81.6±15.3	66.7±10.2
Diabetic	20	71.1±21.1	225.0±65.9	181.0±57.7	190.6±70.7	198.3±1.9	189.7±81.2	158.1±85.1*
Diabetic+parsley	20	63.2±14.3	220.7±5.2	167.3±49.3	168.2±57.6	138.7±59.3	115.7±48.2	110.9±45.1**
p_{ANOVA}		0.586	0.0001	0.0001	0.0001	0.0001	0.0001	0.018

a) Mean±S.D. *n*=Numbers of animals. *,** Significant differences (Student's *t*-test), $p<0.0001$ with respect to day 0.

the diabetic group. The antidiabetic effect of some oral hypoglycemics has been attributed to the reduced hepatic uptake of endogenous insulin or the altered effectiveness of cyclic AMP.[27)] A significant decrease observed in the blood glucose in our investigation may be explained by usage of glucose *via* extrapancreatic path ways. Hypoglycemic effects have been reported for some plants that contain flavonoids.[28)] Phytochemical results showed that the extracts were rich in flavone apigenin[29)] and other flavonoids.[12)] It is possible that the antihyperglycemic effect is related to this component. In addition, a decrease was observed in the weight of the diabetic group and the diabetic group given plant extract. The reduction in the body weight of diabetic rats given parsley extract may be a result of the diuretic effect of parsley.[13)]

This study clearly demonstrates that parsley extract significantly reduces blood glucose in streptozotocin-induced diabetic rats. The extract has no insulin like activity. Inhibition of gluconeogenesis and direct stimulation of glycolysis may be involved in the mechanism of action of this plant, but further detailed studies are needed to establish the mode of action of *P. crispum* and to establish its use in the treatmant of diabetes mellitus.

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