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Effects of Parsley (*Petroselinum crispum*) Extract and Glibornuride on the Kidney of Streptozotocin-Induced Diabetic Rats

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The purpose of this study was to investigate the effect of parsley (*Petroselinum crispum*) extract and compare the effects with a hypoglycemic agent glibornuride on the kidney as histological and biochemical in normal and streptozotocin-induced diabetic rats. The parsley extract was administered by gavage technique to rats a dose of 2 g/kg daily for 28 d; 14 d after experimental animals were made diabetic. 5 mg/kg glibornuride were given by same method, 14 d after the experimental animals were made diabetic, to one of the diabetic group and also one of the control group, daily for 28 d. The kidney tissues were examined histologically, blood glucose, serum urea and creatinine levels were determined, spectrophotometrically. The distinct degenerative changes were observed in the kidney tissue of streptozotocin-induced rats. On the other hand, the injury to kidney tissue was minimal or absent in diabetic group given parsley extract. The damage of kidney tissue was minimal in streptozotocin-induced group given glibornuride. Blood glucose, serum urea and creatinine levels significantly increased in diabetic groups. Administration of parsley extracts and glibornuride significantly reduced blood glucose, serum urea and creatinine levels in diabetic groups. According to these results, it is concluded that parsley extract is more effective in comparison to glibornuride in the protection of kidney tissue from the damage of streptozotocin-induced diabetic rats.

Key Words: Diabetes mellitus, Glibornuride, Kidney, *Petroselinum crispum*, Rat.

INTRODUCTION

Diabetes mellitus is a heterogeneous metabolic disease characterized by altered carbohydrate, protein and fat metabolism[1] and is responsible for well-recognized renal functional and morphological changes in man and

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experimental animals[2]. The treatment of diabetes mellitus is based on oral hypoglycemic agents, including sulphonylureas and biguanides and insulin. In recent years, various plant extracts have been claimed to be useful for the therapy of diabetic hyperglycemia and widely investigated[3-5].

Parsley is used as a hypoglycemic agent by diabetic patients in Turkey. Parsley (*Petroselinum crispum*) is a member of the Umbelliferae family that has been employed in the food, pharmaceutical, perfume and cosmetic industries[6]. Parsley is widely distributed in Turkey and this plant is grown in garden and field. Parsley has been used in Turkey[7] and world[8] as a traditional medicine for diabetes. In folk medicine, parsley is used as antimicrobial[9], antianemic, menorrhagic[10], against lumbago, as blood pressure regulator, anticoagulant, antihyperlipidemic, against eczema, kneeache, impotence[7], nose bleeding[11] and as antihepatotoxic[12], membrane protective effects[13] and antioxidant activity[14]. Parsley seeds have a strong diuretic activity due to its high essential oil content[15]. The hypoglycemic activity of parsley has been shown by many researchers[7,16-18]. Phytochemical screening of parsley have revealed the presence of flavonoids (apiin, luteolin, apigenin-glycosides)[13], carotenoids[19], ascorbic acid[20], tocopherol[21], volatile compounds (myristicin, apiole), cumarines (bergapten, imperatorin)[13] phthalides, furanocoumarins and sesquiterpenes[22].

The mechanisms by which oral agents and the plant extracts help to control hyperglycemia are only partially understood[23,24]. Glibornuride is a sulphonylurea derivative which has been used as an oral hypoglycemic agent[25]. Glibornuride treatment reduces the incidence of renal complications and the blood glucose[26]. On the other hand, both the liver and kidney participate to the clearance of hypoglycemic sulphonylurea from the bloodstream[27].

This study was carried out as morphological and biochemical in order to detect whether this plant extract had a protective effect on kidney tissue, the blood glucose, serum urea and creatinine levels of the streptozotocin induced diabetic rats or not and the results were compared with a hypoglycemic agent glibornuride.

EXPERIMENTAL

Preparation of aqueous parsley extract: Parsley leaves were collected from Buyukcekmece Istanbul in Turkey. Parsley leaves were carefully washed with tap water and left to dry in the shade at room temperature. They were stored in well scaled cellophane bags.

The air-dried leaves (100 g) were extracted with the addition of 1000 mL distilled water and boiled for 0.5 h. The extract was then filtered and the filtrate was evaporated to dryness under reduced pressure using a rotary evaporator. The extract was dissolved in distilled water before administered to normal and diabetic rats.

Administration of parsley extract and glibornuride: The parsley extract was given by gavage technique to rats at a dose of 2 g/kg daily for 28 d; after 14 d experimental animals were made diabetic, to one of the diabetic groups and also one of the control groups, daily for 28 d. 5 mg/kg body weight glibornuride (Roche) dissolved in distilled water were given by gavage method, after 14 d the experimental animals were made diabetic.

Experimental animals: Male 6 to 6.5 month old Swiss albino rats were used. The experiments were reviewed and approved by Local Institute's Animal Care and Use Committees. They were maintained in standard environmental conditions and fed a standard laboratory diet and water *ad libitium*. Fasted (deprived of food for at least 18 h) animals had free access to water. The animals were randomly divided into 4 groups. **Group I**: Control (untreated, non diabetic) animals. **Group II**: Control animals given parsley extract, **Group III**: Control animals given glibornuride, **Group IV**: Diabetic animals, **Group V**: Diabetic animals given parsley extract, **Group VI**: Diabetic animals given glibornuride.

Preparation of diabetic rats: Diabetes was induced by a single intraperitoneal injection of 65 mg/kg body weight of streptozotocin (STZ) to overnight fasted rats. STZ was dissolved in freshly prepared 0.01 M citrate buffer (pH: 4.5)[28].

Histological assays: On the 42nd day, kidney tissues were taken from animals for histological evaluation, which were fasted overnight, under ether anesthesia. The tissues which were fixed in Bouin's solution and subsequently processed using traditional paraffin embedding techniques for preparation of paraffin sections were stained with haematoxylin and eosin (H.E.), Masson's triple dyes (Masson) and Periodic-Acid Schiff (PAS).

Biochemical assays: After STZ injection blood samples from the male rats were collected from the tail vein at 0, 14 and 42 d. Fasting blood glucose levels (after 18 h period of fasting) were determined by *o*-toluidine methods[29]. On the 42nd day, blood samples were taken from control and diabetics animals. Serum urea and creatinine levels were determined by diacetylmonooxime method[30] and Jaffe reaction[31], respectively.

Statistical analysis: The results were evaluated using an unpaired t-test and Anova variance analysis using the NCSS statistical computer package[32]. Values were considered statistically significant when $p < 0.05$.

RESULTS AND DISCUSSION

Normal histological appearance was observed in the kidney tissue of the control (untreated) rats and control group given parsley extract and glibornuride (Fig. 1A). In STZ-induced rats, the distinct degenerative changes were observed especially in the proximal tubular cells and the renal corpuscles when compared with the controls. It is observed

occasional ruptures at the brush border, cytoplasmic debris and desquamated nuclei in the lumens and the picnotic nuclei in the cytoplasm of proximal tubular cells. It is also noticed ruptures at the epithelium and picnotic nuclei in the cytoplasm of distal tubular cells. The excessive edema is established in the cytoplasm of some proximal tubules and especially collecting tubules of this group. Hemorrhage is detected, a loss of cells and an expansion in capsular spaces of renal corpuscles in this group. In addition, PAS positive reaction in the glomeruli was increased (Fig. 1B). Although the individual differences in STZ-induced group given the plant extract the damage to kidney tissue was minimal or absent (Fig. 1C). On the other hand, the damage to kidney tissue was minimal in diabetic group given glibornuride (Fig. 1D). In the groups given plant extract and glibornuride, PAS positive reaction that is almost similar to that of the control individuals was observed.

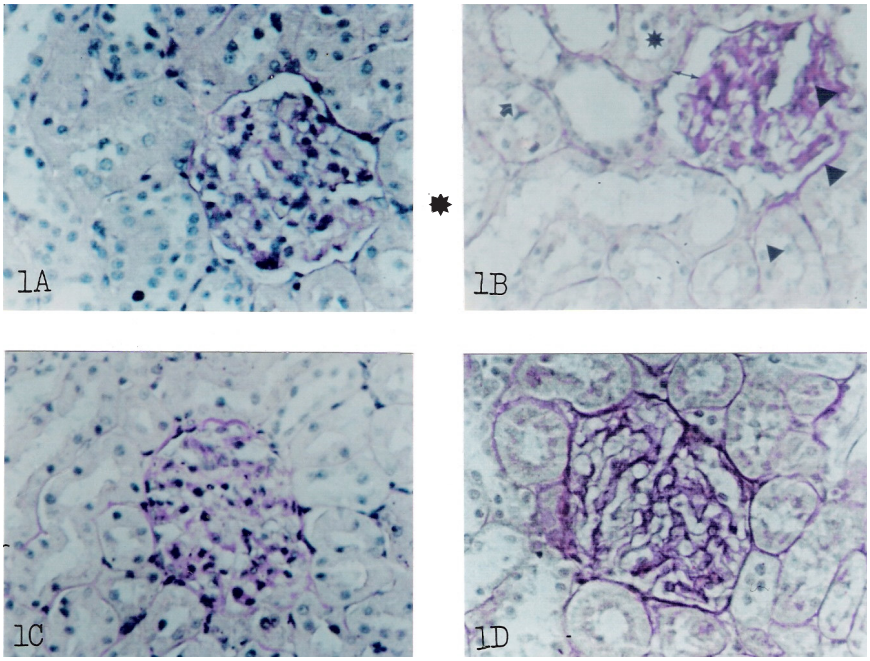

Fig. 1. Normal histological appearance of kidney tissue of control rats, (**A**). The histological appearance of kidney tissue of a diabetic rat. Ruptures at the brush border of proximal tubules(➡), cytoplasmic debris ( ), desquamated nuclei ( ) in the lumen, picnotic nuclei in the cytoplasm of proximal and distal tubules (**X**), an expansion in capsular spaces of renal corpuscles (⟷), increased PAS positive reaction (▶) in the glomeruli, (**B**). Structure of kidney tissue of diabetic rat given parsley extract, (**C**). and glibornuride, (**D**). The morphology of kidney tissue was noticed to be nearly same as those of the controls with administration of parsley extract and glibornuride. PAS. X400

Table-1 demonstrates the level of blood glucose levels in normal and experimental animals at 0, 14 and 42 d. There was a significant increase in blood glucose in STZ-induced diabetic rats. The administration of parsley extract to diabetic rats resulted in a significant decrease in the level of blood glucose. Parsley did not change the concentration of fasting blood glucose levels after 28 d of treatment in the control group (Table-1). The administration of glibornuride to STZ-induced rats resulted in a significant decrease in the level of blood glucose. Administration of parsley extract and glibornuride tends to bring the values to near normal. Parsley extract was found more effective than glibornuride to control of blood glucose.

TABLE-1
MEAN LEVELS OF BLOOD GLUCOSE (mg/dL) FOR ALL GROUPS*

Groups	n	Day 0	Day 14	Day 42
Control	15	71.49 ± 17.71	77.20 ± 15.29	73.85 ± 11.96
Control + Parsley	20	66.13 ± 22.94	75.08 ± 17.30	66.66 ± 10.16
Control + Glibornuride	13	70.10 ± 6.39	87.41 ± 15.37	70.58 ± 24.93
Diabetic	20	71.05 ± 21.06	181.02 ± 57.67[a]	158.08 ± 85.11[b]
Diabetic + Parsley	20	63.24 ± 14.30	167.29 ± 49.25	110.89 ± 45.15[c]
Diabetic+ Glibornuride	14	64.72 ± 14.11	178.93 ± 45.16	125.95 ± 37.46[d]
P_{Anova}		0.528	0.00001	0.0001

*Mean ± SD; n = Number of animals; [a]p = 0.0001 compared to control group; [c]p = 0.055 compared to diabetic group; [b]p = 0.001 compared to control group; [d]p = 0.295 compared to diabetic group.

The mean serum urea levels of the 6 groups are given in Table-2. Serum urea levels were significantly increased in STZ-induced rats as compared the control groups ($P_{t\text{-test}}$ = 0.009). Administration of parsley extract and glibornuride caused reduction in serum urea levels in diabetic and control groups ($P_{t\text{-test}}$ = 0.0001). Administration of parsley extract and glibornuride tends to bring the values to near normal. However, glibornuride was more effective than parsley extract in control of serum urea levels. Table-2 shows the content of serum creatinine levels of normal and experimental animals. In 42 d, the serum creatinine levels in STZ-induced group were significantly increased as compared to the control group ($P_{t\text{-test}}$ = 0.002). Administration of parsley extract and glibornuride tends to bring the values to near normal but in comparison parsley extract was found more effective than glibornuride in control of serum creatinine levels.

TABLE-2
MEAN LEVELS OF SERUM CREATININE AND UREA FOR ALL GROUPS*

Groups	n	Urea (mg/dL)	Creatinine (mg/dL)
Control	15	29.96 ± 6.15	0.51 ± 0.08
Control + Parsley	20	26.46 ± 6.62	0.46 ± 0.09
Control + Glibornuride	13	15.58 ± 5.24	0.36 ± 0.07
Diabetic	20	73.82 ± 11.92[a]	0.66 ± 0.10[d]
Diabetic + Parsley	20	52.34 ± 11.35[b]	0.47 ± 0.09[e]
Diabetic+ Glibornuride	14	34.20 ± 3.04[c]	0.50 ± 0.21[f]
P_{Anova}		0.0001	0.0001

*Mean ± SD n= Number of animals; [a]p = 0.0001 compared to control group; [b,c]p= 0.0001 compared to diabetic group; [d]p = 0.002 compared to control group; [e]p = 0.0001 compared to diabetic group; [f]p= 0.046 compared to diabetic group.

Many medicinal plants have been found to possess active principles useful for treating disease. Plant drugs are frequently considered to be less toxic and free from side effects than synthetic chemicals. The synthetic hypoglycemic agents can produce serious side effects including hematological, cutaneous and gastrointestinal reactions, hypoglycemic coma and disturbances of liver and kidney. In addition, they are not found suitable for use during pregnancy[33]. Therefore, the search for more effective and safer hypoglycemic agents has continued to be an area of active research. The treatment of diabetes mellitus is based on oral hypoglycemic agents, including sulphonylureas and biguanides and insulin. Glibornuride of sulphonylurea group exerts its action through pancreas by stimulating insulin secretion of B cells of the islets[34,35]. The other mechanism of action is extrapancreatic and its effect is on target organs[34]. Its mechanism of action is a still controversial and sulphonylureas can be considered to exert antidiabetic effect by increasing insulin sensitivity of target organs[1]. The aim of this work was to investigate the effects of parsley extract and compare the effects with glibornuride which is a hypoglycemic agent on the kidney morphology of STZ-induced diabetic rats. Blood glucose, serum urea and creatinine levels have been chosen as biochemical parameters.

Kidney is a major organ involved in diabetic complications[36]. Diabetic kidney exhibits characteristic changes leading to renal insufficiency or complete kidney failure[37]. The light microscopic results indicate that damage in proximal, distal and collecting tubules and glomerulus exists. Ruptures at the brush border of proximal tubular cells show disruption of the structural integrity of the membrane. It leads to functional disorders in membrane-dependent functions especially in the proximal tubules.

Increased PAS positive reaction in the glomeruli due to the thickenings of capillaries of the glomeruli shows an increase in glycoprotein of the basal lamina. In our study, histological findings in STZ-induced group were in agreement with the findings of other studies[38,39]. The partial decrease of the degeneration in STZ-induced group given parsley extract and glibornuride indicates that they prevent the damage in the kidney tissue of STZ-induced rats. However, parsley extract is more effective in comparison to glibornuride in the protection of kidney tissue from the damage in STZ-induced diabetic rats.

The observed significant increase in the level of blood glucose in STZ-induced diabetic rats could be due to the destruction of pancreatic B cells by streptozotocin. In this study, the pancreas was examined by light and electron microscopes, it is noted that parsley extract didn't increase insulin release from B cells of pancreas but it decreased blood glucose levels by causing usage of glucose *via* extrapancreatic ways[18]. In present study, creatinine, a marker of renal function was significantly increased in diabetic rats. Serum urea levels also increased significantly in diabetic rats. Administration of parsley extract and glibornuride caused significant reduction in serum urea and creatinine in diabetic rats. It was reported a similar effect with the aqueous extract of *Aegle marmelose*, β-*vulgaris* L. var cicla[38], *Aleo vera* L.[39] and *Aegle marmelose*[40] in diabetic rats. It is a known fact that the kidney functioning is disturbed during diabetes. In this study, treatment with plant extract may have normalized the kidney function as indicated by reversal of blood urea and creatinine levels. The significant decrease in the serum urea levels following administration of parsley extract may also be attributed to impairment of the urea cycle leading to reduced production of urea.

Diabetic kidney complications are one of the major problems occurring in diabetic patients, which caused by many factors such as increased serum urea and creatinine levels and defects in antioxidant defense system. There is evidence that diabetes alters free radical metabolism in tissue. Parsley contains large amounts of flavonoids (apigenin, luteolin)[13], ascorbic acid[20], tocopherol[21] and essential oils[15] as antioxidant compounds. These compounds may prevent oxidative damage in kidney. For this reason, parsley extract could be used for the treatment of diabetic kidney complication. There was a significant regeneration of tubular epithelium of the kidney tissue and a decrease in serum urea and creatinine levels after the administration of glibenclamide[39]. In present study, it was also observed that glibornuride has a similar effect with glibenclamide.

As a result, it may be concluded that parsley extract is more effective in the protection of kidney tissue from the damage of STZ-induced diabetic rats in comparison to glibornuride which has been used as an oral

hypoglycemic agent commonly. Finally, the present study indicates parsley extract might have therapeutic potential as hypoglycemic agent.

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