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THE EFFECT OF PARSLEY LEAVES AND SEED EXTRACTS ON BLOOD GLUCOSE LEVELS IN RABBITS[1]

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SUMMARY

Parsley *(Petroselinum crispum)* is one of the plants used in Turkey and World folk medicine for the treatment of diabetes mellitus. In this study, oral administration of parsley leaves and seed aqueous extracts (2g/kg) and methanolic extracts (200mg/kg and 400mg/kg), to normal rabbits produced significant hypoglycemic activity, which was consistent and time-dependent.

ÖZET

Maydanoz, Türkiye'de ve dünyada şeker hastalığına karşı halk arasında kullanılan bitkilerden biridir. Çalışmada, maydanoz yaprak ve tohumlarından elde edilen sulu (2 g/kg) ve metanollü (200 mg/kg ve 400 mg/kg) ekstrelerin normal tavşanlarda zamana bağlı olarak anlamlı bir hipoglisemik etki gösterdiği bulunmuştur.

Key words: Parsley, *Petroselinum crispum*, Diabetes mellitus, Antidiabetic effect.

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INTRODUCTION

Many plant species are known to be medically used for the treatment of diabetes due to their hypoglycemic activities (1-6). Scientifically documented plants with an hypoglycemic activity have been reviewed recently (7-11). *Petroselinum crispum* (parsley) is widely disributed in Turkey and this plant is grown in garden and field. In folk medicine, the plant is used as antimicrobial (12), antianemic, menorrhagic (13), against lumbago, as blood pressure regulator, anticoagulant, antihyperlipidemic, against eczema, kneeache, impotence (14), nose bleeding (15) and as antihepatotoxic (16). Parsley seeds are also used as diuretic (13,17) and for rheumatism treatment (13). Parsley have been used in Turkey (14) and world (10,18) as a traditional medicine for diabetes. The constituents of parsley which include some phenolic acid (hydroxycinnamic and p-hydroxy benzoic acid) (19), rosmarinic acids (20), carotenoids (21), flavonoid glucosides (13,22), tocopherol (Vit E) (23), ascorbic acid (24), mono and sesquiterpenes, phenylpropanoids, phthalides and furanocoumarins (25), flavonoids (26), myristin, apiole and various terpenoic compounds (27) and coumarins (28) have been chemically investigated.

There is no experimental evidence for the hypoglycemic action of this plant. Therefore, the present work has been undertaken with the aim to study the effect of seed and leaf extracts of parsley on the blood glucose levels of normal and glucose loaded rabbits.

RESULTS AND DISCUSSION

In this study, the possible hypoglycemic effect of the parsley leaves and seeds on normal rabbits was investigated. The effect of oral administration of parsley leaves aqueous and methanolic extracts on blood glucose level of normal as well as glucose loaded rabbits is presented in Table 1 and hypoglycemic activity of different samples prepared from parsley leaves in Table 2. The administration of NaCl (0.9 %) did not change the blood glucose levels of rabbits (Tables 1,2).

The mean percent decrease in blood glucose levels produced by 1g/kg of parsley leaves extract at 1, 2, 3 and 4 hours were 14.38, 11.30, 5.26 and 5.26, respectively. Maximum reduction was observed at the first hour. The doses of 2 g/kg parsley leaves aqueous extract induced a significant decrease in glycemia after oral administration. Maximum reduction was observed at 3h, at which time the percentage variation in blood glucose was about 19.95 for the aqueous extract. When methanolic extracts in doses of

Table 1: Effect of parsley leaves aqueous and methanolic extracts on blood glucose levels of normal rabbits and action of the aqueous extract on glucose level of glucose loaded rabbits

Groups	Dose	Blood Glucose Level (mg %)*				
		0h	1h	2h	3h	4h
1 Control (0.9%NaCl)	1ml/kg	61.07 ± 4.97	69.14 ± 12.96	66.64 ± 14.89	68.59 ± 13.62	67.65 ± 11.89
2 (Aqueous extract)	1g/kg	74.00 ± 18.53	63.36 ± 12.23	65.64 ± 12.00	70.11 ± 11.57	70.11 ± 11.63
3 (Aqueous extract)	2g/kg	71.92 ± 25.29	66.26 ± 23.79	71.39 ± 29.44	57.57 ± 14. 51	58.68 ± 14.17a
4 (Aqueous extract)	4g/kg	76.62 ± 13.45	79.92 ± 17.55	84.87 ± 14.93	73.08 ± 11.07	76.05 ± 14.58
5 Control (70%Glucose)	1g/kg	59.46 ± 10.62	87.62 ± 18.33	60.67 ± 11.66	56.03 ± 17.53	55.93 ± 12.55
6 (Aqueous extract	2g/kg	57.98 ± 16.12	82.76 ± 34.20	65.59 ± 24.00	58.03 ± 17.02	57.71 ± 14.99
+70%Glucose)	+1g/kg					
7 (Methanolic extract)	200mg/kg	63.79 ± 5.40	60.71 ± 5.31	58.39 ± 5.40	57.46 ± 9.63	56.22 ± 10.03b
8 (Methanolic extract)	400mg/kg	68.86 ± 19.47	67.07 ± 16.69	64.00 ± 20.40	60.89 ± 17.82	59.39 ± 17.17c

* Mean ± SD
a) $0.01 < p < 0.02$ b) $p < 0.001$ c) $0.001 < p < 0.01$

Table 2: Hypoglycemic activity of different samples prepared from parsley leaves.

Group	Dose	% Change of Blood Glucose Level			
		1h	2h	3h	4h
1 Control (0.9% NaCl)	1 ml/kg	+13.21	+9.12	+12.31	+10.77
2 (Aqueous extract)	1 g/kg	-14.38	-11.30	-5.26	-5.26
3 (Aqueous extract)	2 g/kg	-7.86	-0.74	-19.95	-18.41
4 (Aqueous extract)	4 g/kg	+4.31	+10.77	-4.62	-0.74
5 Control (70% Glucose)	1g/kg	+47.36	+2.03	-5.77	-5.94
6 (Aqueous extract + 70% Glucose)	2g/kg+1g/kg	+42.74	+13.13	+0.08	-0.47
7 (Methanolic extract)	200 mg/kg	-4.83	-8.47	-9.92	-11.87
8 (Methanolic extract)	400 mg/kg	-2.60	-7.06	-11.57	-13.75

200 mg/kg and 400 mg/kg were given, maximum reduction was observed in 200 mg/kg and 400 mg/kg at 4h, at which time percentage variation on blood glucose was about 11.87 and 13.75 respectively. Doses of 4 g/kg and glucose loaded (2 g/kg (aqueous) + 70% glucose) had no effect on glycemia.

Table 3 summarizes the effect of parsley seed extracts on the blood glucose levels of normal rabbits. It was found that 1 g/kg aqueous, 400 mg/kg methanolic and 200 mg/kg (methanolic + 70 % glucose) did not statistically decrease the blood glucose level of normal rabbits but 2 g/kg of aqueous extracts and 200 mg/kg methanolic extracts produced a significant decrease in blood glucose levels of normal rabbits. Maximum decrease was observed at 3h, for the methanolic extract, at which time the percentage variation in blood glucose was about 21.54. When the aqueous extract was given, maximum reduction was observed in 2 g/kg at 4h, which time the percentage variation on blood glucose was about 15.09 % (Table 4).

In the results obtained from the experiments, it was observed that aqueous extracts of parsley leaves reduce the blood glucose level at 2 g/kg and the same effect was seen of the methanolic extracts at 200 mg/kg doses in normal rabbits. Also, aqueous extracts of parsley seeds reduce the blood glucose level at 2 g/kg doses, methanolic extract at 200 mg/kg and 400 mg/kg doses, in normal rabbits.

In several studies, active substances in different chemical structures were isolated from the plants which showed hypoglycemic activity. It was reported that the active substances were alkaloids (29), pyridine derivates (30), indole derivates (30), purine derivates (30), flavonoids (31,32), isoflavones (33), coumarins (34), furacoumarins (30), steroids (35), terpenoids (36), polypeptide (37, 38), glycoprotein (39) and polysaccharide (40, 41). However the chemical screening has revealed the presence of flavonoid glucosides (13, 22), furano coumarins (25), terpenoic compounds (27), and coumarins (28) in parsley. One or more of these compounds may be responsible for the antidiabetic activity of parsley leaves and seeds, more experiments are needed to determine the exact nature of the active principles and the mechanisms of action.

EXPERIMENTAL

Plant Material: Parsley leaves were collected from Büyükçekmece, İstanbul during June and July. The leaves were carefully washed with tap water to remove dust and any other foreign materials. Then, they were dried under shade (at room temperature). Parsley seeds were purchased from local market of Çemberlitaş, İstanbul.

Table 3: Effect of parsley seed aqueous and methanolic extracts on blood glucose levels of normal rabbits and action of the aqueous extract on glucose level of glucose loaded rabbits.

Groups	Dose	Blood Glucose Level (mg %)*				
		0h	1h	2h	3h	4h
1 Control (0.9%NaCl)	1ml/kg	61.07 ± 4.97	69.14 ± 12.96	66.64 ± 14.89	68.59 ± 13.62	67.65 ± 11.89
2 (Aqueous extract)	1g/kg	74.93 ± 6.46	81.50 ± 7.23	83.22 ± 7.62	70.52 ± 7.46	71.21 ± 8.85
3 (Aqueous extract)	2g/kg	80.71 ± 2.37	79.60 ± 10.73	79.11 ± 6.22	70.49 ± 4.64	68.53 ± 4.77a
4 (Methanolic extract)	200mg/kg	88.05 ± 17.62	72.16 ± 8.02	72.27 ± 12.29	69.08 ± 5.20	70.53 ± 5.09b
5 (Methanolic extract)	400mg/kg	65.57 ± 12.53	62.36 ± 5.98	65.36 ± 8.33	60.00 ± 9.32	63.89 ± 9.78c
6 (Control+70%Glucose)	1g/kg	59.46 ± 10.62	87.62 ± 18.33	60.67 ± 11.66	56.03 ± 17.53	55.93 ± 12.55
7 (Methanolic extract + 70% Glucose)	200mg/kg + 1g/kg	67.14 ± 6.37	116.00 ± 32.73	87.36 ± 28.75	77.97 ± 15.13	69.79 ± 9.97

* Mean ± SD

a) $0.02 < p < 0.05$ b) $p < 0.001$ c) $0.001 < p < 0.01$

Table 4: : Hypoglycemic activity of different samples prepared from parsley seeds.

Group	Dose	% Change of Blood Glucose Level			
		1h	2h	3h	4h
1 Control (0.9 % NaCl)	1 ml/kg	+13.21	+9.12	+12.31	+10.77
2 (Aqueous extract)	1 g/kg	+ 8.77	+11.06	-5.89	-4.96
3 (Aqueous extract)	2 g/kg	-1.38	-1.98	-12.66	-15.09
4 (Methanolic extract)	200 mg/kg	-18.05	-17.92	-21.54	-19.90
5 (Methanolic extract)	400 mg/kg	-4.90	-0.32	-8.45	-2.56
6 Control(70% Glucose)	1g/kg	+47.36	+2.03	-5.77	-5.94
7 (Methanolic extract +70% Glucose)	200mg/kg + 1g/kg	+72.77	+30.12	+16.13	+3.95

Preparations of Extracts

Aqueous Extract: 100g dried parsley leaves were extracted by boiling for 30 minutes in 1000ml distilled water. The extract was then filtered and the filtrate was evaporated under reduced pressure to dryness. Then, the extract was dissolved in distilled water and administered to normal rabbits in graded doses (1g/kg, 2g/kg and 4g/kg) orally. Parsley seeds were extracted with distilled water in the same manner then, the extract was given to normal rabbits orally in graded doses (1g/kg and 2g/kg).

Methanolic Extract: Dried parsley leaves were extracted with methanol in Soxhlet apparatus for 8 hours.Then, methanol was evaporated by a rotatory evaporator under reduced pressure.The extract was dissolved in distilled water and administered to normal rabbits orally.The methanolic extract was also made with parsley seeds.

Experimental Animals

In this study, female, adults, healthy rabbits weighing between 2-3 kg were used. The animals were fed with commercial feed with free access to tap water. The animals of all the groups were fasted overnight (approximately 16h) (water allowed ad libitum) prior to administration of extracts and control solution.

Grouping of the rabbits

The experimental animals were divided into various groups, each containing five rabbits. The rabbits were given orally the aqueous extract (1g/kg, 2g/kg and 4g/kg of original dry starting materials) and methanolic extract (200mg/kg and 400 mg/kg body weight) prepared from parsley leaves. They were also given orally the aqueous extract (1g/kg and 2 g/kg) and methanolic extract (200g/kg and 400 g/kg) prepared from parsley seeds. The control groups were given 1ml/kg saline orally. Glucose control was given as 70% glucose solution (1g/kg) and the other groups were given the aqueous extract (2g/kg) suspended in the same glucose solution.

Collection of Blood and Blood Glucose Estimation

The rabbits were held in a wooden rabbit holder and immediately before the administration of the drug, 0.1ml of blood for glucose estimation was collected from an ear vein. Similar blood samples were also collected at 1, 2, 3 and 4 hours after the drug administration.

Blood glucose estimation was done by spectrophotometry, according to the o-toluidine method (42) each determination was carried out in duplicate. In this study, the percentage change in glycemia was calculated by applying the following:

% Change of Glycemia: $G_x - G_o / G_o X100$

The blood glucose concentration was determined and noted as inital glycemia (G_0). Then, the sample was administered orally and glucose values were determined at 1, 2, 3 and 4 hours (G_x).

Statistical Analysis

Mean blood glucose levels were expressed as % mg ± SD in all the experiments and Student's t test was used to check their significance.

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