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Biochemical Studies on the Effect of Parsley (*Petroselinum Crispum*) in Experimentally Induced Diabetic Rats

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ABSTRACT

This study was conducted to evaluate the effect of Parsley (Petroselinum crispum) on some biochemical profiles in streptozotocin diabetic rats. For this purpose, 40 male albino rats were divided into 4 groups according to the program assigned 10 rats per group: Group 1: control group kept on basal ration, Group 2: Parsley group kept on basal ration + Parsley 2gm/kg body weight, Group 3: Diabetic group rats injected intra peritoneal with STZ 45mg/kg body weight and kept on basal diet, Group 4: Diabetic + Parsley rats injected intra peritoneal with STZ 45mg/kg body weight and kept on basal diet + Parsley 2gm/kg body weight. Results showed that: Treatment of the STZ induced diabetic rats with parsley resulted in significant decrease in fasting blood glucose and glycated haemoglobin compared to control group but there is no significant difference within all groups in fructose amine, Alanine aminotransferase, aspartate aminotransferase, Total protein, Albumin, Urea and Creatinine also in Total cholesterol, Triglyceride and HDL while STZ resulted in significant increase in liver antioxidants compared to Parsley treated

1. INTRODUCTION

Diabetes, often referred by doctors as diabetes mellitus, describes a group of metabolic diseases in which the person has high blood glucose (blood sugar), either because insulin production is inadequate, or because the body's cells do not respond properly to insulin, or both (Davis et al.,2005). Patients with high blood sugar will typically experience polyuria (frequent urination); they will become increasingly thirsty (polydipsia) and hungry (polyphagia) (Bell, 2003). The most common types are: Type 1 diabetes (IDDM) where the body's immune system attacks and destroys the cells that produce insulin and type 2 diabetes (NIDDM) where the body doesn't produce enough insulin, or the body's cells don't react to insulin. However type 2 diabetes is widely spread than type1 (WHO, 2013). Diabetes increases risk for many serious health problems over the long-term complications developed gradually due to hyperglycemia microenvironment in both types of diabetes damage, dysfunction and failure of multiple organs (Olsen et al., 2004). Parsley is a popular culinary and medicinal herb recognized as one of the functional food for its unique antioxidants, and disease preventing properties. This wonderful, fragrant rich biennial herb is native to the Mediterranean region belongs to the Apiaceae family, in the genus; Petroselinum. Its botanical name is Petroselinum crispum (Mozafarian, 2007). Also; its phytochemical constituents are flavonoids, carbohydrates, essential oil components, coumarin (Chaves et al., 2011). Wide range of pharmacological activity of Parsley including antioxidant, hepatoprotective, brain protective, anti-diabetic (Ziyyat, 1997), analgesic, spasmolytic, immunosuppressant, anti-platelet, gastro protective, cytoprotective, laxative, estrogenic, diuretic (Rehecho, et al 2011). Also hypoglycemic constituents in this plant which are used in folklore medicine to treat diabetes mellitus (El-Aboudi and Afifi, 2010). Therefore this study was undertaken to test the efficacy of parsley on improvement of blood glucose level in streptozotocin diabetic rats.

2. MATERIAL AND METHODS

2.1. Plant Material and Extraction Procedure

Parsley leaves were collected from Egypt local market 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. The air-dried leaves of the plant were milled into fine powder in a Waring commercial blender. 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 in this research as cited by (Refiye et al., 2003).

2.2. Rats

Forty albino male rats, weighting 150- 200 g and of average age 2 months, purchased from health institute were used. The experiment was conducted according to the ethical norm approved by Institutional Animal Ethics Committee (IAEC). The rats were left in clean and disinfectant cages (10 rate/ cage). Rats in all groups were provided with food (based diet) and water was available adlibtum. They were given basal diet before experiment for acclimatization and to ensure normal growth of rats at the end of experimental period 30 days.

2.3. Chemicals and reagents

1-Streptozotocine, (STZ) (2-deoxy-2-(3-methyle-3nitrosoureido)-D-glycopyranoside), as a diabetogenic agent, was purchased from sigma Aldrich Company., Citric acid and Sodium citrate was purchased from El-Nasr Pharmaceutical chemical company, Egypt, Kits for determination of diabetic profile, lipid profile AST, ALT, total protein and albumin were purchased from Spectrum Company (Egypt), Kits for determination of MDA, NO, GSH, total antioxidant capacity were purchased from Biodiagnostic company (Egypt).

2.4. Experimental design and sampling

Forty male rats were divided equally into four groups each group contains 10 rats as follow: Group 1: control group kept on basal diet for 30 days, Group 2: Parsley group kept on basal diet + Parsley 2gm/kg body weight by gavage (Tunalı et al., 1999) for 30 days, Group 3: Diabetic group rats injected intra peritoneal with STZ 45mg/kg body weight and kept on basal diet, Group 4: Diabetic + Parsley rats injected intra peritoneal with STZ 45mg/kg body weight and kept on basal diet + Parsley 2gm/kg body weight by gavage. The treatment was given for one month after that the rats were fasted overnight, anaesthetized using di-ethyl ether and the blood samples collected from canthus of eye using hematocrit tubes then immediately after euthanized, the rats were dissected and the livers were collected, washed with ice cold saline and stored at -18 Celsius for biochemical measurement.

2.5. Serum biochemical analysis

The serum concentrations of Fasting blood glucose (Kaplan 1984), fructosamine concentration (Roger et al., 1983), serum glycated hemoglobin concentration (Roberts et al., 2002), serum insulin concentration (Andrew et al., 2009), Calculation of HOMA-IR (Wallace et al.,2004), serum total cholesterol level (Deeg and Zeigenohrn, 1983), serum triglyceride level Fossati and Prencipe (1982), serum high density lipoprotein concentration (Burstein et al., 1970), serum low density lipoprotein (Friedewald, 1972), serum total protein (Witt and Trendeienburg, 1982), serum albumin (Doumas et al., 1971), serum alanine transaminase (Reitman and Frankel, 1957), serum aspartate transaminase (Reitman and Frankel, 1957), blood urea concentration (Tabacco et al., 1979), serum creatinine concentration (Henry, 1984), MDA concentration in liver homogenate (Placer et al., 1966), GSH concentration in liver homogenate (Sedlack and Lindsay, 1968), Nitric oxide concentration (Montgomery and Dymock 1961), Total antioxidant capacity concentration (Ahsan et al., 2004) were calorimetrically measured using diagnostic kits according to the manufacturer′s instructions.

3. RESULTS

The data presented in table (1,2) showed that there is no significant difference in fasting blood glucose, HOMA, HbA1c and fructose amine between the group given parsley extract and the intact control group. While the injection of STZ resulted in significant increase in fasting blood glucose, HbA1c, decrease insulin.

The data summarized in Table (3) revealed that, STZ injection caused non-significant increase in serum Ch, TG, vLDL-c, LDL-c and decreased HDL-c level. Furthermore, in table (7) and fig. (23 and 24) there is not a significant change in diabetic group compared to diabetic group treated with parsley in the following parameters Cardiac risk coefficient and atherogenic coefficient.

The data presented in table (4) Showed that there is no significantly differs in total protein, serum

albumin, globulin, GPT and GOT from parsley group compared to control one. Also, there is no significant change in serum albumin, globulin, GPT and GOT while the total protein significantly decrease in STZ injected group compared with control one. While treatment of STZ-induced diabetic rats with parsley significantly elevate the serum protein concentration and decreased GOT compared to control diabetic group. However serum albumin, globulin, GPT does not significantly changed in STZ injected group fed with parsley compared with diabetic control group.

The data summarized in Table (5) revealed that, the injection of STZ resulted in significant increase in serum urea level and no significant change on serum creatinine level.

The result in table (6) showed significant decrease in MDA and NO levels while the GSH and total lipid peroxide levels increased significant in parsley group compared to control group. Also administration of parsley to STZ-induced diabetic rats cause significant decrease in MDA level compared to control group. While the GSH and total lipid peroxide levels in table (6) showed significant increase in parsley group compared to control group. Furthermore administration of parsley to STZ-induced diabetic rats cause significant decrease in GSH and total lipid peroxide levels compared to control group.

Table 1 Effect of Parsley on Diabetic profile in in STZ-induced diabetic rats compared to control:

	Parameter		
Group	FBS (mg/dl)	Insulin (mIU/L)	HOMA-IR
Control	81.6 ± 1.91^{b}	5.56 ± 0.20^{b}	1.12 ± 0.05^{ab}
Parsley	87.0 ± 6.24^{b}	9.46 ± 0.32^{a}	2.02 ± 0.12^{a}
Diabetic	270.0 ± 23.14^{a}	2.11 ± 0.18^{c}	1.45 ± 0.23^{ab}
Diabetic + Parsley	87.4 ± 5.68^{b}	3.07 ± 0.54^{c}	0.69 0.14^{b}

Values are Means ± SEM. Means without a common superscript in a column differ significantly (P<0.05).
FBS: Fasting blood glucose HOMA-IR: Homeostatic model assessment-insulin resistance.
Means within the same column with different letters are significantly differed (P ≤ 0.05).

Table 2 Effect of Parsley on Fructosamine and HBA1C in STZ-induced diabetic rats compared to control:

	Parameter	
Group	Fructosamine (mmol/L)	HbA1C (%)
Control	1.67 ± 0.04^{a}	3.52 ± 0.07^{b}
Parsley	1.88 ± 0.09^{a}	3.67 ± 0.06^{b}
Diabetic	2.11 ± 0.13^{a}	7.74 ± 0.60^{a}
Diabetic + Parsley	1.91 ± 0.19^{a}	5.67 ± 0.66^{ab}

Values are Means ± SEM.
Means without a common superscript in a column differ significantly (P<0.05).
Means within the same column with different letters are significantly differed (P ≤ 0.05).

Table 3 Effect of Parsley on lipid profile in STZ-induced diabetic rats compared to control:

	Parameter				
Group	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)
Control	118 ± 3.98^{a}	79 ± 5.88^{b}	35.2 ± 1.77^{a}	15.8 ± 1.18^{b}	67.4 ± 5.64^{a}
Parsley	104 ± 5.21^{a}	129 ± 13.6^{a}	34.9 ± 1.50^{a}	25.8 ± 2.71^{a}	43.4 ± 6.08^{b}
Diabetic	118 ± 4.83^{a}	105 ± 15.5^{ab}	36.4 ± 2.73^{a}	21.0 ± 3.11^{ab}	60.5 ± 5.11^{ab}
Diabetic + Parsley	124 ± 10.73^{a}	111 ± 11.6^{ab}	40.4 ± 2.84^{a}	22.2 ± 2.33^{ab}	61.2 ± 10.0^{ab}

Values are Means ± SEM. Means without a common superscript in a column differ significantly (P<0.05).
HDL: High density lipoprotein cholesterol; VLDL: Very low density lipoprotein cholesterol; LDL: Low density lipoprotein cholesterol. Means within the same column with different letters are significantly differed (P ≤ 0.05).

Table 4 Effect of Parsley on liver function test in STZ-induced diabetic rats compared to control:

	Parameter				
Group	Total protein(gm/dl)	Albumin (gm/dl)	Globulin(gm/dl)	GPT(U/L)	GOT(U/L)
Control	7.36 ± 0.05[a]	3.98 ± 0.10[a]	3.38 ± 0.13[a]	73.8 ± 8.87[a]	22.2 ± 2.63[a]
Parsley	7.32 ± 0.05[a]	3.98 ± 0.07[a]	3.33 ± 0.10[a]	74.2 ± 3.88[a]	18.1 ±2.34[ab]
Diabetic	6.68 ± 0.07[c]	3.66 ± 0.10[ab]	3.02 ± 0.15[a]	86.5 ± 9.08[a]	24.4 ± 2.60[a]
Diabetic + Parsley	7.10 ± 0.08[b]	3.63 ± 0.13[b]	3.47 ± 0.16[a]	67.1 ± 6.31[a]	13.9 ± 1.35[b]

Values are Means ± SEM. Means without a common superscript in a column differ significantly (P<0.05).
GPT: Glutamic pyruvic transaminase; GOT: Glutamic oxalacetic transaminase. Means within the same column with different letters are significantly differed (P ≤ 0.05). Means within the same column with different letters are significantly differed (P ≤ 0.05).

Table 5 Effect of Parsley on kidney function test in STZ-induced diabetic rats compared to control:

	Parameter	
Group	Urea (mg/dl)	Creatinine (mg/dl)
Control	28.4 ± 1.25[b]	0.83 ± 0.03[a]
Parsley	43.6 ± 2.27[a]	0.76 ± 0.02[a]
Diabetic	54.9 ± 7.40[a]	0.80 ± 0.02[a]
Diabetic + Parsley	49.3 ± 3.50[a]	0.91 ± 0.05[a]

Values are Means ± SEM.nMeans without a common superscript in a column differ significantly (P<0.05).
Means within the same column with different letters are significantly differed (P ≤ 0.05).

Table 6 Effect of Parsley on liver antioxidants in STZ-induced diabetic rats compared to control:

	Parameter			
Group	MDA (nmol/g wet tissue)	GSH (µmol/g wet tissue)	Nitric oxide (µmol / g)	Total antioxidant capacity (nmol/g)
Control	15.0 ± 0.57[b]	6.33 ± 0.43[c]	4.81 ± 1.14[a]	4.76 ± 0.78[a]
Parsley	11.4 ± 0.60[c]	9.29 ± 0.72[b]	2.64 ± 0.22[b]	5.08 ± 0.30[a]
Diabetic	20.9 ± 1.25[a]	9.10 ± 0.45[b]	3.66 ± 0.69[ab]	1.57 ± 0.58[b]
Diabetic + Parsley	15.1 ± 0.63[b]	13.66 ± 0.38[a]	2.58 ± 0.22[b]	4.78 ± 0.53[a]

Values are Means ± SEM. Means without a common superscript in a column differ significantly (P<0.05).
MDA: Malondialdehyde: GSH: Reduced glutathione. Means within the same column with different letters are significantly differed (P ≤ 0.05).

Table 7 Effect of Parsley on atherogenic indices

	Parameter		
Group	Atherogenic index of plasma	Cardiac risk coefficient	Atherogenic coefficient
Control	0.34 ± 0.05[b]	3.55 ± 0.30[a]	2.56 ± 0.30[a]
Parsley	0.55 ± 0.04[a]	3.02 ± 0.28[a]	2.02 ± 0.28[a]
Diabetic	0.41 ± 0.08[ab]	3.32 ± 0.21[a]	2.33 ± 0.21[a]
Diabetic + Parsley	0.42 ± 0.04[ab]	3.13 ± 0.39[a]	2.13 ± 0.39[a]

Values are Means ± SEM. Means without a common superscript in a column differ significantly (P<0.05).
Means within the same column with different letters are significantly differed (P ≤ 0.05).

3. DISCUSSION

Diabetes mellitus (DM) is one of the metabolic diseases in which there are high blood sugar levels over a prolonged period (WHO, 2013). DM is characterized by abnormal insulin secretion, action or both with derangement in carbohydrate, lipid and protein metabolism and is diagnosed by the presence of hyperglycemia (Bell, 2003). Also, DM is a major worldwide health problem if neglected over long periods of time; the metabolic abnormalities are capable of contributing towards the development of complications such as nephropathy, retinopathy, neuropathy, and cardiovascular diseases (Bate and Jerums, 2003).

STZ is a compound commonly used for the induction of diabetes in experimental rats; STZ causes diabetes by rapid depletion of b-cells, which leads to a reduction of insulin release. It is well established that parsley produces hypoglycemia, these alternative treatments are supposed to help control blood sugar levels, reduce resistance to insulin, and prevent diabetes-related complications (Ziyyat, 1997).

The data presented in table (1,2) showed that there is insignificant difference in fasting blood glucose, HOMA, HbA1c and fructose amine between the group given parsley extract and the intact control group.

While the injection of STZ resulted in significant increase in fasting blood glucose, HbA1c, decrease insulin this come parallel to what cited by (Sajad et al.,2008) who reported that, significant increase in fasting blood glucose and decrease in insulin concentrations were recorded in STZ induced diabetic rabbits compared to control rabbits , the same authors cited that the elevation in fasting blood glucose and the reduction in insulin concentrations reflect abnormalities in beta cell function induced by STZ .

The data summarized in Table (3) revealed that, STZ injection caused insignificant increase in serum Ch, TG, vLDL-c, LDL-c and decreased HDL-c level.

These results come in agreement with those obtained by (Cathrine et al., 1991) who reported that, a significant increase in serum Ch, TG and as well as liver Ch were increase in alloxan induced diabetic rabbits compared to control rabbits.

Furthermore, in table (7) there is insignificant change in diabetic group compared to diabetic group treated with parsley in the following parameters Cardiac risk coefficient and atherogenic coefficient.

The data presented in table (4) Showed that there is insignificant different in total protein, serum albumin, globulin, GPT and GOT from parsley group compared to control one. Also, there is insignificant change in serum albumin, globulin, GPT and GOT while the total protein significantly decrease in STZ injected group compared with control one. While treatment of STZ-induced diabetic rats with parsley significantly elevate the serum protein concentration and decreased GOT compared to control diabetic group. However serum albumin, globulin, GPT does not significantly changed in STZ injected group fed with parsley compared with diabetic control group.

The data summarized in Table (5) revealed that, the injection of STZ resulted in significant increase in serum urea level and no significant change on serum creatinine level. These results come in agreement with those obtained by (Sajad et al., 2008) Who showed that, a significant increase in blood urea and serum creatinine were recorded in STZ induced diabetic rabbits compared to control rabbits.

The result in table (6) showed significant decrease in MDA and NO levels while the GSH and total lipid peroxide levels increased significant in parsley group compared to control group. Also administration of parsley to STZ-induced diabetic rats cause significant decrease in MDA level compared to control group. While the GSH and total lipid peroxide levels in table (6) showed significant increase in parsley group compared to control group. Furthermore administration of parsley to STZ-induced diabetic rats cause significant decrease in GSH and total antioxidant capacity compared to control group. These results come in agreement with those obtained by (Lei et al., 2008) who concluded that, rats treated with STZ showed a significant increase in total antioxidant capacity in livers compared with controls.

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