Ameliorative Effect of Parsley Extract on Some Diabetes Complications in the Pregnant Rats

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Abstract: One of the important types of diabetes is the gestational diabetes mellitus which leads to many obstetrical and postnatal disorders. This study aimed to investigate the antioxidant effects of the parsley aqueous extract (PAE) on streptozotocin-induced diabetes in pregnant rats (40 mg/kg b.wt.). Diabetic pregnant rats were treated with PAE (1ml/150 g b.wt.) on the1st and 7th to 19th day of gestation one hour post-injection with STZ. The results exhibited that STZ - induced diabetes in pregnant rats showed increased levels of serum glucose, alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), alkaline phosphatase activities (ALP) and malondialdhyde (MDA). While, insulin hormone, catalase (CAT) activity, reduced glutathione (GSH) levels were decreased. On the other hand, PAE improved STZ - induced abnormalities in the biochemical and antioxidant parameters.
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Keyword: Diabetes, streptozotocin, Parsley, antioxidants

1. Introduction:

Diabetes mellitus is considered a major worldwide health problem. Because antidiabetic drugs have many undesirable effects, there is a great demand of researches on antidiabetic medicinal plants that produce minimum side effects [1]. DM is characterized by abnormal regulation of glucose [2], results from impaired beta-cell functions, leading to insulin deficiency which leads to hyperglycemia [1].

Diabetes mellitus is a group of chronic metabolic disorders; it has 3 types. Type1: insulin dependent diabetes mellitus (IDDM) which is an autoimmune β-cell destruction, type 2: non insulin dependent diabetes mellitus (NIDDM) that is a combination of β-cell failure and peripheral tissues resistance to insulin action. The third type is the gestational diabetes [3] resulting from failure of beta cells of pancreas to produce insulin and defects in insulin secretion or action during pregnancy [4]. During pregnancy diabetes leads to disturbances in the metabolic states in both the mother and fetuses [5].

Streptozotocin (STZ) is considered the agent of choice for diabetes induction in experimental animals; its diabetogenic effect is due to its highly destructive effects on beta cells of pancreas by increasing production of oxygen-free radicals which result in oxidative stress [6].

Parsley (*Petroselinum crispum*) is an annual herb and an important dietary source that contains zinc, vitamins C, B, calcium, iron, phosphorous, and luteolin. This accounts for its hepatoprotective effect [7]. The presence of some compounds such as flavonoids, carotenoids, tocopherol in parsley can scavenge free radicals and protect membrane oxidation [8]. Freshly prepared parsley leaf extract can inhibit lipid oxidation [9]. It has been used as antioxidant, antidiabetic, cytoprotective and hepatoprotective [7]. Liver is a very important organ for a healthy life of mammals. It plays a major role in metabolism and detoxification [10]. Herbal extracts were used to treat various diseases. Natural antioxidants enhance the endogenous antioxidants defenses against reactive oxygen species and keep the optimal equilibrium by neutralizing reactive species [7]. Therefore, the objective of this study was to assess antidiabetic, hepatoprotective, and antioxidant effects of parsley on STZ - induced diabetes in pregnant albino rats.

2. Materials and Methods

Animals

Adult Albino rats of 50 female and 25 males, weight 200-220 g were selected. Rats were kept on a standard diet and water. Before the experiment, all rats were kept for 2 weeks in metal cages under standard laboratory conditions of light (12 h light/dark cycle), temperature 25±5 and good ventilation.

Pregnancy induction

Vaginal smears were collected daily for determination of estrous cycle and then the estrous rats were kept with healthy male for mating in the ratio of 2:1. In the following morning, mating is confirmed by detection of sperms in the vaginal smears and vaginal plug. It was considered as day zero of pregnancy [3]. The pregnant rats were labeled and separated, while non-pregnant rats were excluded.

Generation of gestational diabetes mellitus

Induction of diabetes in rats was done by a single intraperitoneal injection of freshly prepared STZ (40 mg/kg body weight) on day one of gestation [3]. This

preparation dissolved in citrate buffer (0.1 mol/l, pH = 4.5). After 24 hrs of STZ injection if blood glucose levels ranged between 120-250 mg/dl, the rats were selected and considered as gestational diabetes mellitus.

Aqueous extract of parsley:

Fresh parsley leaves were selected from local markets and carefully washed with tap water, chopped into small pieces and left to dry in dark room. They were kept in well-closed cellophane. The dried leaves (100 g) were extracted through adding one liter of boiled distilled water for 30 min. The extract was then filtered and the filtrates were given 1 ml/150 g orally by stomach tube to pregnant rats [3].

Experimental groups:

About 50 pregnant Albino rats were used and categorized into seven groups after mating: **group 1**: **control** untreated group **(C), groups 2,3**: received 1ml/150 g b.wt. of parsley (*Petroselinum crispum*) extract on the 1st and 7th day of gestation **(PAE1, PAE7**) **group**s **4,5**: received single intraperitoneal streptozotocin (STZ) injection (40 mg kg^{-1} b.wt.) on the 1st and 7th day of gestation **(D1, D**7) **groups 6,7**: pregnant diabetic rats treated with parsley extract (1ml/150 g b.wt.) on the1st and 7th to 19th day of gestation post injection with STZ **(D1+PAE), (D7+PAE)** respectively. The biochemical parameters, such as serum glucose, insulin hormone, liver function tests, some antioxidants such as CAT, GSH and lipid peroxide indicator (MDA) were estimated in the blood samples collected on the 19th day of pregnancy.

Methods

The serum glucose concentration was determined according to the method described in Ref. [11]. Insulin concentration was carried out by ELISA assay according to the method of described in Ref. [12], alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT) enzyme activites were determined according to the method of described in Ref. [13] and alkaline phosphatase (ALP) according to the method of described in Ref. [14]. Glutathione (GSH) content was estimated according to the method described in Ref. [15], catalase activity (CAT) according to the method of described in Ref. [16] and contents of malondialdhyde (MDA) according to the method described in Ref [17].

Statistical Analysis

The data obtained were expressed as the mean ± standard deviation (M±SD) and analyzed by one-way analysis of variance (ANOVA) and t-student test (t-test). Statistical analysis was carried out using the statistical package for social science (SPSS 11.0 software). Values at $P < 0.05$ were considered significant, less than 0.01 were considered highly significant (P<0.01), less than 0.001 were considered very highly significant (P<0.001). While levels more than 0.05 were considered insignificant (P>0.05) [18].

3. Results

Data presented in table (1) and illustrated in figures (1A & B).

Showed that induction of diabetes caused a significant increase in serum glucose and decrease in insulin hormone levels in **D1** and **D7** pregnant rats when compared to the untreated control pregnant rat group (**C**). Meanwhile, oral administration of PAE caused a significant improvement in glucose and insulin levels in **D1+PAE** and **D7 +PAE** groups.

Table (1): Serum glucose and insulin hormone levels in the control and different treated groups of pregnant female rats.

Groups / Parameters	Control	PAE1	PAE7	D1	D7	D1+PAE	D7+PAE
Glucose (mg/dl)	86.5± 4.58	85.52± 4.73+	85.19± 4.31+	178.35± 7.13*	181.35± 8.17*	95.82± 3.85#	86.8± 86.81#
Insulin (µIU/ml)	2.63± 0.16	2.64± 0.13+	2.61± 0.12+	1.51± 0.11*	1.62± 0.15*	2.54± 0.16#	2.61± 0.13#

Data were represented as Mean ±S.D. *Highly statistically significant (P< 0.01), +statistically insignificant (P> 0.05) with respect to the pregnant control. # Highly statistically significant (P< 0.01) with respect to STZ-treated groups (D1 & D7) (n=6 in each group).

Results found in table (2) and illustrated in figures (2A, B & C)

Indicated that the subcutaneous injection of STZ exerted a significant increase in ALAT, ASAT and ALP activities in the diabetic pregnant groups (D1 and **D7**) in comparison with the control group. After treatment of the diabetic pregnant rats with parsley extract (**D1+ PAE and D7 + PAE)** enzyme activities reached somewhat near to normal enzyme activities as in the control group.

Results observed in table (3) and illustrated in figures (**3A, B & C**)

Showed that injection of STZ exerted a significant decline in CAT enzyme activities and GSH levels. While, increase in MDA levels were detected in the diabetic groups **(D1+D7**) with respect to the

control. Meanwhile, administration of PAE caused an improvement in CAT enzyme activities, GSH and MDA levels in **(D1+PAE and D7+PAE)** when compared to the diabetic groups **(D1+D7)** respectively.

Table (2): Serum liver enzymes activities (ALAT, ASAT and ALP) in the control and different treated groups of pregnant female rats.

Groups / Parameters	Control	PAE1	PAE7	D1	D7	D1+PAE	D7+PAE
ALAT (U/L)	42.76 ± 3.93	41.76 ± 4.25+	41.19 ± 3.68+	69.19 ± 6.08*	64.55 ± 3.03*	44.36 ± 3.53#	43.02 ± 3.71#
ASAT (U/L)	66.01± 4.39	64.94± 4.54+	64.59± 4.77+	92.52± 3.51*	87.02± 5.72*	67.07± 5.42#	65.94± 5.32#
ALp (U/L)	65.11± 2.83	64.94± 3.21+	64.56± 1.99+	118.42± 6.27*	116.62± 7.87*	67.48± 4.12#	66.91± 3.43#

Data were represented as Mean ±S.D. * highly statistically significant (*P*< 0.01), +statistically insignificant (*P*> 0.05) with respect to the pregnant control. # Highly statistically significant (*P*< 0.01) with respect to STZ-treated groups (D1 & D7) (n=6 in each group).

Table (3): Serum CAT activity, GSH, MDA levels in the control and different treated groups of pregnant female rats.

Groups / Parameters	Control	PAE1	PAE7	D1	D7	D1+PAE	D7+PAE
CAT (U/L)	67.87 4.46	68.71 3.44+	64.56 1.99+	49.81 3.01*****	47.94 2.07*	69.38 5.34#	68.01 5.29#
GSH (mmol/L)	16.06 1.11	16.49 1.44+	16.52 1.23+	8.26 0.59*	9.52 0.55*	15.09 0.71#	15.54 0.41#
MDA (nmol/ml)	28.64 2.75	27.85 3.45+	27.48 4.07+	39.4 4.35*	37.21 4.41*	29.88 3.73#	28.95 3.59#

Data were represented as Mean ±S.D. * Highly statistically significant (*P*< 0.01), +statistically insignificant (*P*> 0.05) with respect to the pregnant control. # Highly statistically significant (*P*< 0.01) with respect to STZ-treated groups (D1 & D7) (n=6 in each group).

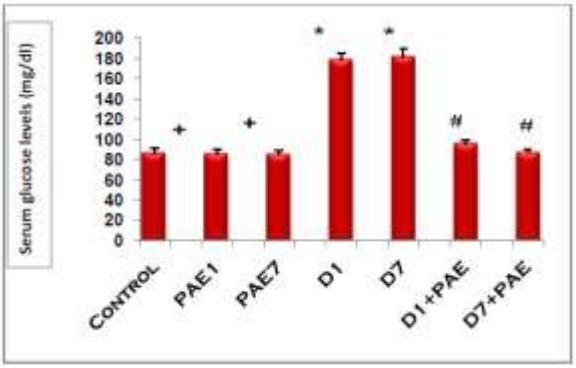

Figure 1A: Serum glucose (mg/dl) levels in the control and different treated groups. Each bar with vertical line represents the mean± S.D. (n = 6 in each group). *Highly statistically significant (*P*< 0.01), +statistically insignificant (*P*> 0.05) with respect to the pregnant control. # Highly statistically significant (*P*< 0.01) with respect to STZ-treated groups.

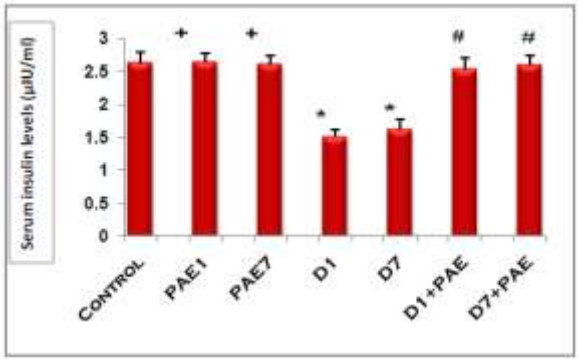

Figure 1B: Serum insulin (µIU/ml) levels in the control and different treated groups. Each bar with vertical line represents the mean± S.D. (n = 6 in each group). * Highly statistically significant in insulin levels (*P*< 0.01), +statistically insignificant (*P*> 0.05) with respect to the pregnant untreated control. # Highly statistically significant (*P*< 0.01) in comparison with STZ-treated groups.

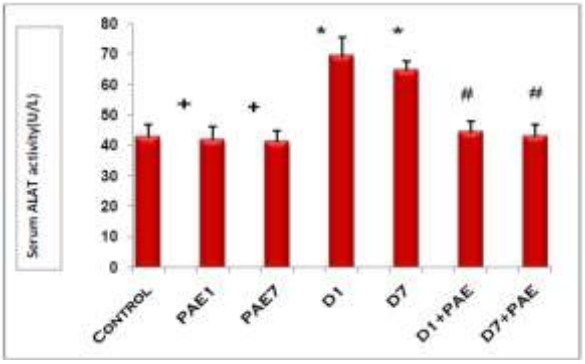

Figure 2A: Serum alanine amino transferase (U/L) activities in the control and different treated groups. Each bar with vertical line represents the mean± S.D. (n = 6 in each group). * Highly statistically significant *(P*< 0.01) +statistically insignificant (*P*> 0.05) with respect to the pregnant untreated control. # Highly statistically significant (*P*< 0.01) when compared to STZ-treated groups.

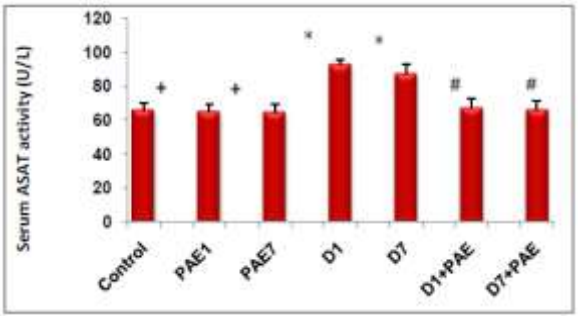

Figure 2B: Serum aspartate amino transferase (U/L) activities in the control and different treated groups. Each bar with vertical line represents the mean± S.D. (n = 6 in each group). * Highly statistically significant *(P*< 0.01)**,** +statistically insignificant (*P*> 0.05) when compared to the control. # Highly statistically significant (*P*< 0.01) when compared to STZ-treated groups.

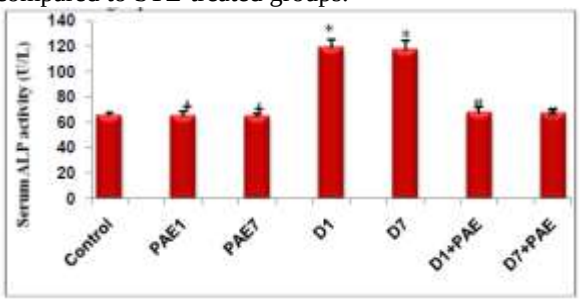

Figure 2C: Serum alkaline phosphatase (U/L) activities in the control and different treated groups. Each bar with vertical line represents the mean± S.D. (n = 6 in each group). *Statistically significant *(P*< 0.01), +statistically insignificant (*P*> 0.05) when compared to the control. # Statistically significant (*P*< 0.01) in comparison with STZ-treated group.

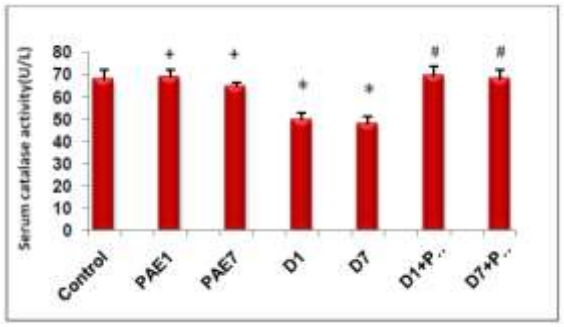

Figure 3A: Serum catalase (U/L) enzyme activities in the control and different treated groups. Each bar with vertical line represents the mean± S.D. (n = 6 in each group). * Highly statistically significant *(P*< 0.01), +statistically insignificant (*P*> 0.05) with respect to the pregnant control. # Highly statistically significant (*P*< 0.01) with respect to STZ-treated groups.

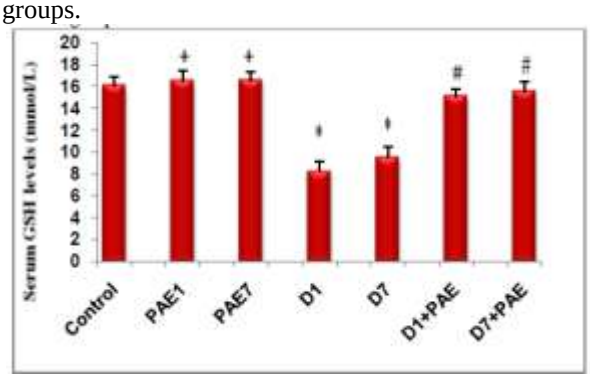

Figure 3B: Serum glutathione (mmol/L) levels in **the control and different treated groups**. Each bar with vertical line represents the mean± S.D. (n = 6 in each group). *Statistically significant *(P*< 0.01), +statistically insignificant (*P*> 0.05) with respect to the pregnant control. #Statistically significant (*P*< 0.01) with respect to the diabetic groups **(D1+D7)** respectively.

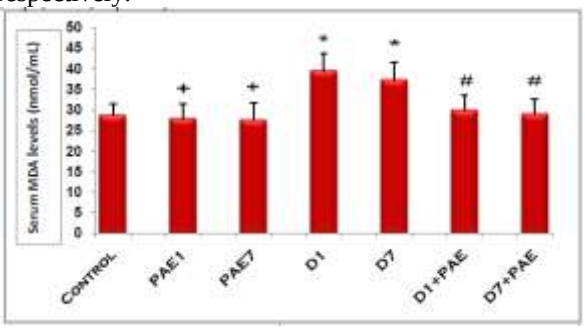

Figure 3C: Serum malondialdhyde (nmol/ml) levels in **the control and different treated groups**. Each bar with vertical line represents the mean± S.D. (n = 6 in each group). * Highly statistically significant *(P*< 0.01), +statistically insignificant (*P*> 0.05) with respect to the pregnant control. # Highly statistically significant (*P*< 0.01) with respect to the diabetic groups **(D1+D7)** respectively.

4. Discussion

The present study showed significant increase in glucose level and significant decrease in insulin level in diabetic pregnant rats when compared to the control group. This finding is in agreement with the results of [19]. Administration of parsley to diabetic rats improved the plasma glucose and insulin levels. This finding suggested that administration of PAE improved the destructed β-cells that led to release of insulin from the pancreas. Results are in agreement with the findings of [20] and support the role of PAE in protecting the pancreatic tissue against the destructive effect of STZ. The hypoglycemic effect of PAE may be due to the presence of flavonoids (apiin, luteolin, and apigenin-glycosides) [3]. The flavonoid is able to reduce β cell apoptosis in pancreatic islets [21], regenerates pancreatic β cells and protects the new β cells from autoimmunity. The hypoglycemic action of PAE may be via inhibition of gluconeogenesis and stimulation of glycolysis [20]. The present investigation also showed an increase in the serum activities of ALAT and ASAT in the diabetic pregnant groups with respect to the control. Results agree with the results of [21] who showed an increase in ALAT and ASAT activities in the diabetic rats and increase in ALP activity comes in agreement with the result of [22]. Parsley extract improved liver enzymes activities, indicating that parsley had the ability to regenerate liver cells because PAE contains flavonoids, (particularly, quercetin) [23]. Flavonoids have antioxidant activity; they can protect the liver cells against the oxidative damage. Also, parsley has highly nutritive values of vitamins such as riboflavin, niacin, A, C, minerals such as Ca, K, Fe, Mg, P, Na and Zn [24]. Decreased CAT activity in the diabetic group may be due to high production of reactive oxygen species (ROS) and their accumulation exceeding the detoxification capacity of antioxidant enzymes with subsequent severe damage to the liver cells [25]. Increased CAT activities after treatment with PAE correlates with the results of [26]. Parsley is a good source of iron because of iron demand in catalase composition. So, administration of parsley caused an increase in catalase enzyme activity [27].

In this study the induced diabetes caused alteration in GSH content in plasma. This finding was in consistent with the results of [28] who suggested that the GSH depletion in the diabetic rats referred to the enhanced utilization of GSH for detoxification of toxic products. Administration of PAE restored the GSH level. These results came in agreement with the results of [29] which indicated that parsley extract is able to resist STZ- induced GSH depletion. This finding reflects the ability of PAE to scavenge free radicals and regulate the synthesis of GSH [30]. Elevated level of MDA indicated that the formation of free radicals led to generation of oxidative stress. This was in agreement with the results of [6]. This finding reflected the destruction in beta cells of pancreas and the mobilization of free fatty acids from fat depots that caused destabilization in the membrane fluidity and permeability finally leds to cell lysis [8]. Generation of free radicals, which can exhaust antioxidant defense led to the alteration of cellular function and oxidative damage to the cell membranes and enhanced the susceptibility to lipid peroxidation [31].

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