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Research Article

ANTIOXIDANT AND ANTIDIABETIC ROLE OF *PETROSELINUM CRISPUM* AGAINST STZ-INDUCED DIABETES IN RATS

Nadia A Eltablawy[1], Hanan A. Soliman[2], Mona S Hamed[1]

[1]Biochemistry Division, National Organization for Drug Control and Research (NODCAR), Cairo, Giza, Egypt.

[2]Department of Chemistry, Faculty of science, Beni Suef University, Beni Suef city, Egypt.

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ABSTRACT

Petroselinum crispum (Parsley) has been reported to have a number of possible medicinal attributes**.** The aim of this work is to evaluate the ameliorative effect of parsley aqueous extract (PAE) against STZ-induced diabetic rats. Gliclazide was used as hypoglycemic standard drug. Total phenolic content (TPC), total flavenoid content (TFC) and ferric reducing power were determined in PAE. STZ was injected subcutaneously in four doses (27.25mg/kg b.wt. as initial dose followed by three low doses, 11.25 mg/kg b.wt.) within two weeks. Sixty diabetic rats were equally divided into four groups as the following: control diabetic rats, diabetic rats treated with PAE, gliclazide and a combination of both. In addition, sixty normal rats were equally divided into four control groups as the following: control non-treated rats, rats treated with PAE, gliclazide and a combination of both. The treatment of normal and diabetic rats lasted for 45 days. The obtained results revealed a relatively high TPC and TFC with considerable antioxidant capacity as evaluated by FRAP assay. STZ injection induced a significant increase in blood glucose and plasma MDA levels in association with a significant decrease of serum insulin level, erythrocyte SOD activity. Pancreatic β-cell and expression of caspase-3 were affected by STZ injection as evaluated by immunohistochemical examination. Administration of PAE to diabetic rats significantly attenuated the undue effects of STZ and preserving pancreatic β-cells integrity. In conclusion, PAE is a potent hypoglycemic agent that preserves and protects β-cells of pancreas against oxidative damage.

Keywords: Hyperglycemia, *Petroselinum crispum*, Gliclazide, Caspase-3, Insulin antibody and *In- vitro* antioxidant studies.

INTRODUCTION

Diabetes mellitus (DM) represents a major public health concern, and its prevalence continuous to escalate worldwide **[1].** DM has become a major cause of mortality and morbidity in the world, mainly contributed by developing countries **[2].** DM is an endocrine-metabolic disorder of multiple etiologies characterized by chronic hyperglycemia that leads via several mechanisms (glucose auto oxidation, stimulation of polyol pathway, activation of reduced form of nicotinamide adenine dinucleotide phosphate oxidase and production of advanced glycation end products) to an increased production of reactive oxygen species. The resulting oxidative stress can play a key role in diabetes pathogenesis **[3,4].**

It has been shown that, oxidative stress starts at early onset of diabetes. The consequence of oxidative stress can be involved in the structural tissue damage (liver, kidney and pancreas) and in the development of diabetic complications **[5,6].** Nowadays, the use of complementary and/or alternative medicine and especially the consumption of botanicals have been increasing worldwide, because of supposedly less frequent side effects when compared to therapeutic agents **[7].**

Parsley [*Petroselinum crispum* (Mill.) Nym.ex A.W. Hill] family Umbelliferae has been used medicinally for many centuries in European, Mediterranean and Asian countries **[8].** Parsley has been reported to have a number of possible medicinal attributes including, antimicrobial, anticoagulant and antihepatotoxic **[9].**

Phytochemical screenings of parsley have revealed the presence of polyphenolic compounds that includes flavonoids such as kaempferol, quercetin apigenin and luteolin, which occur as glycosidic form in nature. These compounds represent the major flavonoids found in parsley and other apiaceous vegetables **[10].** Flavonoids possess a wide range of biochemical and pharmacological effects and have been recommended as chemopreventive agents or nutritional supplements. The predominant mechanism of their biological actions is

**Corresponding author: Mona S Hamed | E-mail: monasaber1282011@yahoo.com*

thought to result from antioxidant activity, enzyme inhibition and the capacity to scavenge free radicals **[11,12].** It has been reported that parsley is a powerhouse of nutrition, rich in tocopherol and vitamin A **[13,14].** It contains starch, Vitamins B and C, β-carotene and zinc **[10].**

The Consumption of dietary antioxidant inhibits oxidative stress **[15].** Thus we envisage that parsley aqueous extract may indicate protective activity which might have promising therapeutic potential against oxidative damage caused by STZ- induced diabetic rats. For this purpose, the hypoglycemic and antioxidant effects of parsley extract were analyzed with the glucose, insulin, plasma MDA levels and erythrocytes SOD activity were measured. Further, the destructive effect of STZ on pancreatic β-cells and the protective effects of PAE were examined by immunohistochemistry. Gliclazide was used in the present study as a reference hypoglycemic drug.

Materials and Methods

Preparation of parsley aqueous extract (PAE):

Parsley was purchased from Egyptian local market, the dried leaves (100 g) were extracted by adding 1000 ml distilled water and boiled for 30 minutes. The extract was then filtered through a cotton piece and the filtrates were evaporated using rotary evaporator under reduced pressure to dryness**.** The dried matter was dissolved in distilled water for using in the experimental studies. The daily oral dose of PAE is (2g/kg b.wt.) [11].

Preparation of gliclazide suspension:

Gliclazide was obtained from Miscellaneous lab., National Organization of Drug Control and Research (NODCAR). Gliclazide tablets were grounded in a glass mortar; suspended in 100 ml redistilled H_2O with few drops of Tween 80. The concentration of the gliclazide suspension was 0.84 g %. The recommended rat dose calculated according to Paget and Barnes [16].

Preparation of streptozotocin (STZ):

Streptozotocin (STZ) purchased from Sigma Aldrich chemical Co (St Louis, MO, USA)**.** STZ dissolved in 0.1M citrate buffer pH 4.5, freshly prepared and injected within five minutes.

Animals and experimental design

A total number of 120 male albino rats (160 ± 30 g) from the laboratory stock colony of National Organization of drug control and research (NODCAR) were used in the present study. The animals were kept under normal environmental conditions for two weeks before the initiation of the experiment. The animals were allowed free access to water and fed on a standard diet. The local ethics committee of NODCAR approved study protocols.

Sixty male albino rats were injected subcutaneously within freshly prepared STZ preparation with an initial dose of 27.5 mg/kg b.wt, booster injection of three successive doses (11.25 mg/kg b.wt.) were given within two weeks according to **Said *et al.* [17].** Blood samples were withdrawn 48 hours after each injection to assess the induction of diabetes. Only rats confirmed with permanent glucose level around 250 mg/dl were used in this study. Hyperglycemic rats were equally divided into four groups as follows:

STZ group: Received saline by stomach tube and served as diabetic control group.
STZ+ PAE group: Rats treated with a daily oral dose of PAE (2g/kg b.wt.) for 45 days.
STZ + Gliclazide group: Rats were treated with a daily oral dose of gliclazide (8.4 mg/Kg b.wt.) for 45 days.
STZ + Gliclazide + PAE group: Rats treated with a daily oral dose of gliclazide in a recommended dose (8.4 mg / Kg b.wt) followed by another oral dose of PAE (2 g/kg b.wt.) for 45 days.
Control groups: Normal 60 rats equally divided into the following four subgroups:
Normal control group: Received saline by stomach tube for 45 days.
PAE group: Healthy rats treated with an oral dose of PAE (2 g/kg b.wt.) for 45 days.
Gliclazide group: Healthy rats orally treated with gliclazide suspension (8.4 mg /Kg b.wt.) for 45 days.
Gliclazide + PAE group: Healthy rats treated with PAE and gliclazide with a daily oral dose of gliclazide (8.4 mg/Kg b.wt) followed by another oral dose of PAE (2 g/kg b.wt.) for 45 days.

Some phytochemical studies on parsley aqueous extract

Determination of Total phenolic content:

Total phenolic content of parsley aqueous extract (PAE) was determined according to the method of **Singleton *et al.* [18]**. Total phenolic content of PAE determined by Folin-Ciocalteau (F-C) assay using gallic acid as a standard phenolic compound. F-C method based on the oxidation of phenolic compounds by a molybdotungstate in F-C reagent to yield a colored product that measured at 750 nm. The standard gallic acid curve was prepared and total phenol values were expressed in in the term of mg Gallic acid equivalent/g of plant material. Total content of phenolic compound was calculated by the following equation:

$C = (c*m)/V$

Where, C= total content of phenolic compound in Gallic acid equivalent, c= concentration of Gallic acid

established from the standard curve (µg/ml), m=weight of crude plant extract, V=volume of plant extract.

Determination of total flavonoid content:

The total flavonoid content was determined according to **Meda *et al.* [19].** The flavonoid content was expressed as quercetin equivalents (mg QU/g extract). Total content of flavonoid compound was calculated by the following equation:

C= (c*m)/V

Where, C=total content of flavonoid compound in quercetin equivalent, c= concentration of quercetin established from the standard curve (µg/ml), m=weight of crude plant extract, V=volume of plant extract.

Ferric reducing antioxidant power (FRAP):

The FRAP assay was carried out according to the method of **Al-Farsi *et al.* [20].** FRAP assay was done to assess the total antioxidant capacity of PAE that is based on the stability of the extract to reduce the ferric 2, 4, 6-tripyridyl-*s*-triazine complex (Fe^{3+}-TPTZ complex) to its blue ferrous form (Fe^{2+}-TPTZ complex). The absorbance values were plotted against the concentration of $FeSO_4$.7H2O and the results were expressed in mM Fe (II) /100 g of extract.

Biochemical Analysis

At the end of the experimental period, blood samples were collected as described by **Schermer [21].** Each blood sample was collected in two tubes; the first one contains heparin and the second one to maintain serum. The whole blood was used for the determination of hemoglobin [22]. and then the tubes were centrifuged at 4000 rpm for 15 minutes at cooling centrifuge to separate plasma for the determination of glucose colorimetrically [23] and plasma lipid peroxidation index in the term of MDA [24]. After plasma separation the red blood cells were washed and haemolysed for the determination of superoxide dismutase (SOD) activity [25]. Serum was separated to measure insulin level, serum insulin level was determined by ELISA technique [26].

Histopathological Examination

At the end of the experimental period rats were decapitated, the pancreas was taken washed with cold saline. Autopsy samples were taken from the pancreas of different groups of rats fixed in 10% formalin for twenty-four hours for histopathological examination [27]. The obtained tissue sections were stained by hematoxylin and eosin stain for histopathological examination through the electric light microscope**.**

Immunohistochemistry Examination

A-Anti-caspase 3 polyclonal antibody:

The immunohistochemical staining for caspase-3 was performed on paraffin embedded pancreatic tissues using specific anti-caspase-3 primary antibody (Gennova Kit, Spain) according to the manufacture's instructions **[28]**.

B-Insulin Antibody (polyclonal):

Immunolocalization technique for anti-insulin was performed on 5–6 m thickness sections and stained with the streptavidin–biotin–peroxidase staining method **[29].** Using anti-sera containing primary antibodies for rat insulin (polyclonal antibody) supplied by Bio Genex Cat. No. AR. 295-R.

Immunohistochemical Evaluation

The ordinary light microscope was used to detect and localize the immunostain. All the sections were examined by an image analyzer computer system using the software Leica Qwin 500. Six random fields in each specimen were captured using a magnification (X400) to determine the area percentage of the positive cells. The data obtained as mean area % and standard error (mean of Area % ± SE).

Statistical analysis

The results were expressed as mean ± standard deviation of the mean (M±SD) for seven animals in each group. In the evaluation of Area % of insulin anti-body and caspas-3, the results were expressed as mean ± standard error (M±SE), Differences between groups were assessed by one-way analysis of variance (ANOVA). Subsequent multiple comparisons between the different groups were analyzed by Duncan's multiple comparisons test. Data were statistically analyzed using the statistical package for social science (SPSS 11.0 software) values at $P < 0.05$ were considered significant.

Results

Total phenolic content:

In the current work the determination of total phenolic content of PAE by Folin Ciocalteu reagent and Gallic acid as a standard revealed that, parsley extract contains a measurable amount of phenolic compounds with respect to Gallic acid, approximately 369.33±15.5 mg GAE/g extract (Table 1).

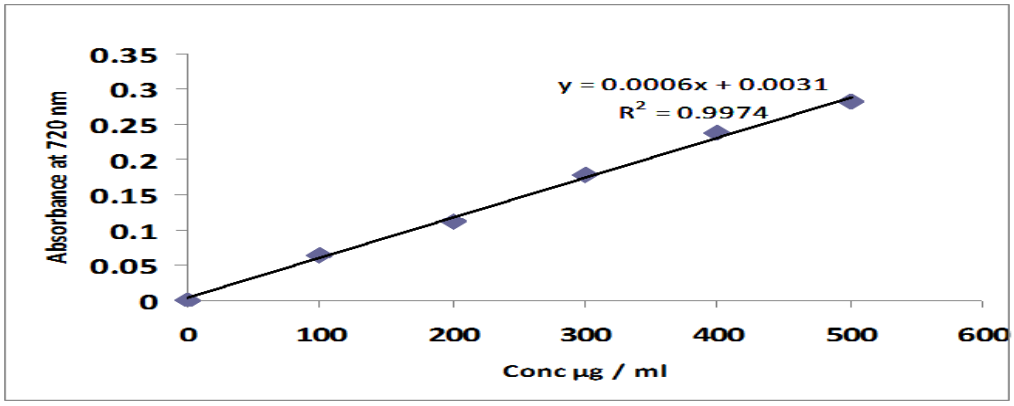


Figure 1: Standard curve of Gallic acid

Total flavonoid content:

In the current study the determination of flavonoids content of PAE by using quercetin as standard revealed that, parsley extract contains a relatively high amount of flavonoids content, approximately 407.7±10.35 mg QU/g extract (Table 1).

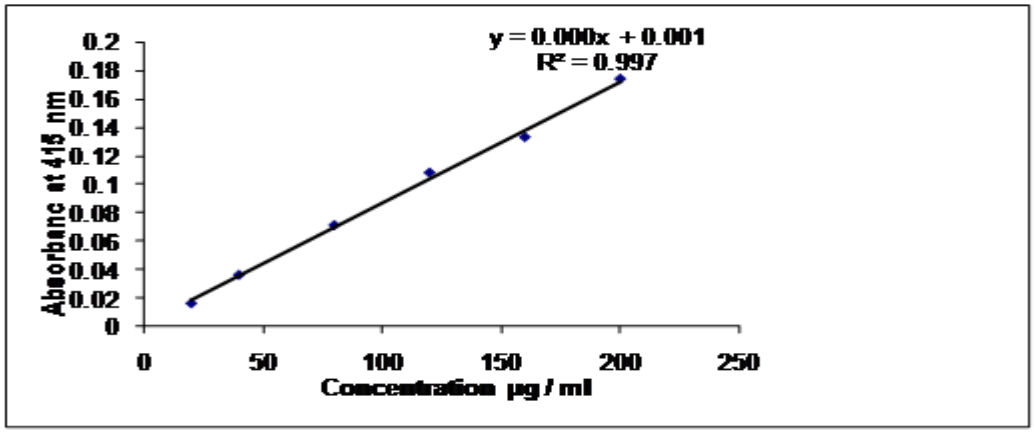


Figure 2: Standard curve of Quercetin

Ferric reducing antioxidant power (FRAP):

The obtained data revealed that parsley extract elicited a ferric reducing power equivalent to 195.35±5.03 mM Fe (II)/100 g extract. This value reflects the redox properties of the polyphenolic contents of PAE that play an important role in adsorbing and neutralizing free radicals (Table 1).

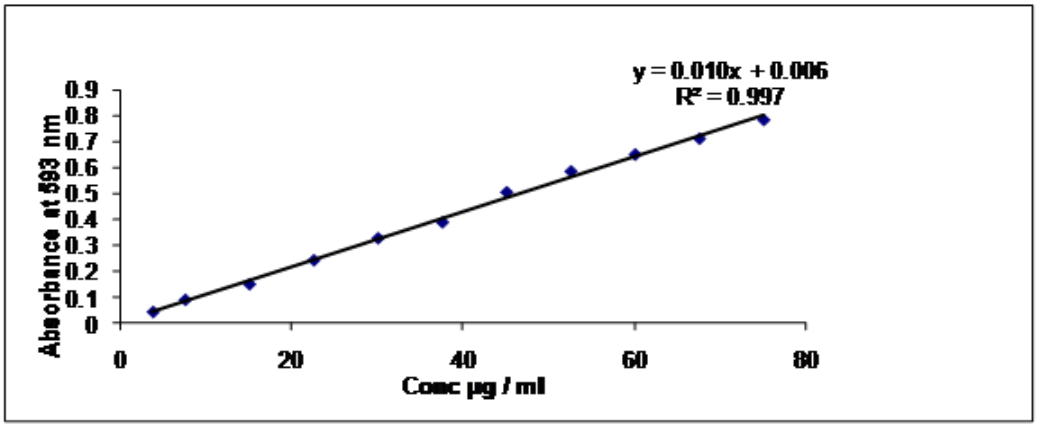


Figure 3: Standard curve of ferrous sulfate

Table (: The total phenolic content, total flavonoid content and Ferric reducing antioxidant power of parsley aqueous extract:

Assay	Result
Total phenolic content (mg GAE acid/g extract)	369.33±15.5
Total flavenoid content (mg QU /g extract)	407.7±10.35
FRAP mM Fe (II)/100 g extract	195.35±5.03

- Values are mean of 3 determinations for each test ± SD

Effect of PAE or/and gliclazide on blood glucose and insulin level in normal and diabetic rats

Assessment of the hypoglycemic effect of parsley extract, gliclazide or the combined administration of both via the measurement of blood glucose level after 45 days of daily oral administration revealed that, the administration of parsley extract caused a significant reduction in glucose level (Table 2). This reduction of glucose levels is associated with a significant increase in insulin levels. The obtained result also revealed that the hypoglycemic effect of parsley extract and its ability to increase insulin level is equivalent to that induced by gliclazide that used as a reference drug in the current study when statistically compared with STZ-treated group (Table 2). However, the combined administration of parsley extract and gliclazide caused a significant decrease in glucose level and increase of insulin, but this effect is less than that induced by the sole administration of parsley extract or gliclazide to diabetic rats (Table 2). The sole administration of PAE or/and gliclazide to normal rats elicited no change in glucose and insulin levels with respect to control non-treated group (Table 2).

Table 2: Effect of oral administration of PAE, gliclazide and the combination of both on the levels of blood glucose and insulin in normal and diabetic rats.

Groups	Glucose (mg/dl)	Insulin (ng/ml)
Control	111.51 ± 1.46^{A}	1.62 ± 0.122^{E}
Parsley only	111.54 ± 2.93^{A}	$1.51\pm0.136^{D,E}$
Gliclazide only	100.43 ± 4.11^{A}	1.58 ± 0.164^{E}
Gliclazide+Parsley	109.20 ± 2.06^{A}	1.55 ± 0.143^{E}
STZ	240.93 ± 8.62^{D}	0.64 ± 0.094^{A}
STZ +Parsley	138.34 ± 11.6^{B}	1.36 ± 0.114^{C}
STZ+Gliclazide	133.90 ± 4.10^{B}	$1.39\pm0.100^{C,D}$
STZ+Gliclazide+Parsley	146.84 ± 9.81^{C}	1.15 ± 0.104^{B}

- Values are mean ± SD (n=7 for glucose and n=6 for insulin).
- The presence of different capital letters means significant differences between groups in the same columns. ANOVA test followed by Duncan's multiple comparisons between groups at $P < 0.05$ were employed.

Effect of PAE or/and gliclazide on plasma MDA level and erythrocyte SOD enzymatic activity in normal and diabetic rats

Table 3 proves that, one of the most important diabetic complications is the production of free radicals as evaluated by the significant increase of MDA level in STZ-treated group when statistically compared with control non-treated group. Also, the gained result revealed that the oral administration of PAE, gliclazide or the combined administration of both exerted a significant decreasing effect on MDA level when compared with STZ-treated group.

Data in Table 3 show that, the induced hyperglycemia caused a significant reduction in erythrocytes SOD activity. Meanwhile, the oral administration of the examined materials to diabetic rats displayed a significant increment in SOD activity and the most pronounced effect was induced by PAE with respect to STZ-treated rats. The sole administration of parsley extract or/and gliclazide to normal rats exerted no effect on plasma MDA level and erythrocytes SOD activity with respect to normal rats (Table 3).

Table 3: Effect of oral administration of PAE, gliclazide or the combination of both on plasma MDA level and erythrocyte SOD activity in normal and diabetic rats.

Groups	MDA (nM/ml)	SOD (U/g Hb /ml)
Control	3.31 ± 0.25^{A}	$330.14\pm21.9^{C,D}$
Parsley only	3.33 ± 0.13^{A}	$340.83\pm14.69^{C,D}$
Gliclazide only	3.63 ± 0.25^{A}	341.86 ± 6.79^{D}
Gliclazide+Parsley	3.70 ± 0.12^{A}	$332.14\pm36.0^{C,D}$
STZ	6.10 ± 0.40^{E}	262.90 ± 13.4^{A}
STZ +Parsley	3.67 ± 0.34^{B}	$331.47\pm8.53^{C,D}$
STZ+ Gliclazide	4.16 ± 0.19^{C}	$318.57\pm5.9^{B,C}$
STZ+ Gliclazide+Parsley	4.54 ± 0.33^{D}	305.43 ± 22.0^{B}

- Values are mean ± SD (n=7).
- The presence of different capital letters means significant differences between groups in the same columns. ANOVA test followed by Duncan's multiple comparisons between groups at $P < 0.05$ were employed.

Effect of PAE or/and gliclazide on caspase-3 expression and insulin antibody expression in normal and diabetic rats

As a marker of apoptosis, the expression level of caspase-3 was increased in STZ injected group. Parsley extract or/and gliclazide showed a significant decrease in % area of caspase-3 expression in pancreatic tissues of STZ injected rats, indicating their promising effect on STZ-induced apoptosis. The sole administration of parsley extract or/and gliclazide to normal rats exerted no change in area % of caspase-3 expression in pancreatic tissues with respect to normal rats (Fig 4 and Fig 5).

The obtained data show that, the area % of insulin antibody is markedly decreased in pancreatic tissues of STZ injected rats. Diabetic rats treated with parsley extract or/and gliclazide showed a significant increase in % area of insulin antibody. Meanwhile, the administration of parsley extract or/and gliclazide to normal rats exerted no change in area % of insulin antibody expression in pancreatic tissues with respect to normal rats (Fig 6 and Fig 7).

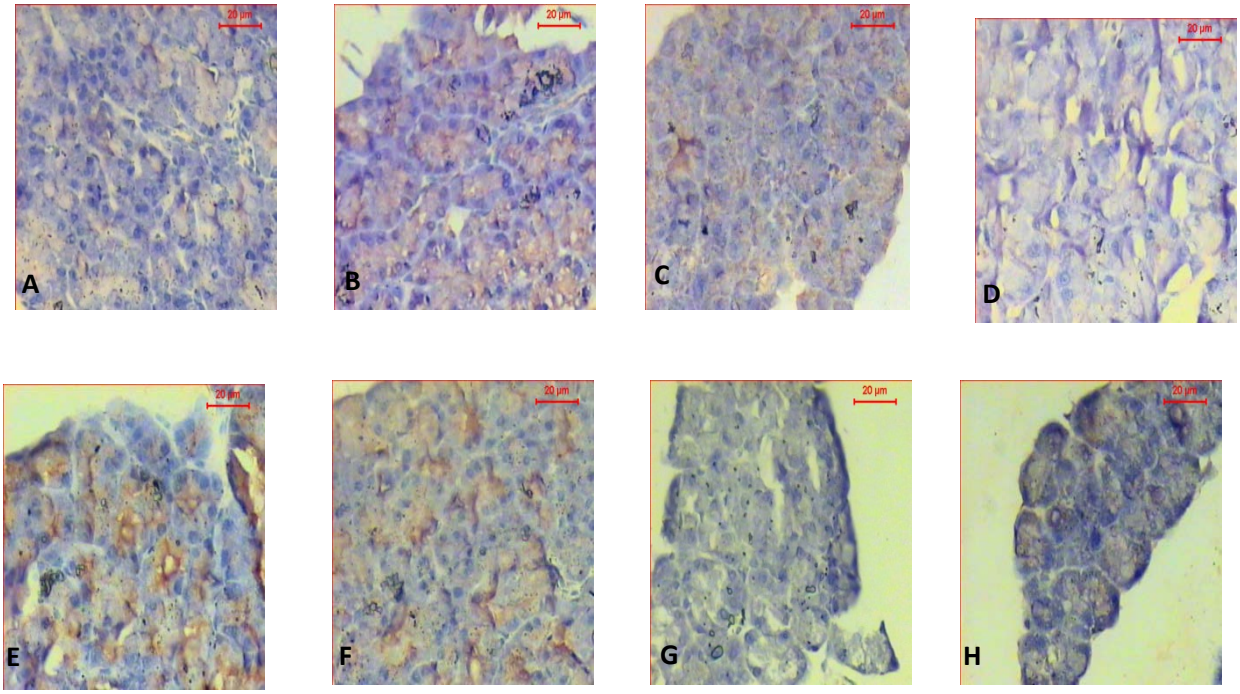


Figure 4: Immunohistochemical detection of caspase-3 expression in pancreas of tested groups: A) Control non-treated group showing weak staining, B) PAE group showing weak staining, C) Gliclazide group showing weak staining, D) A combination group showing weak staining, E) Diabetic group showing strong staining, F) Diabetic treated with PAE group showing moderate staining, G) Diabetic treated with gliclazide group showing moderate staining, H) Diabetic treated with a combination of PAE and gliclazide group showing moderate staining.

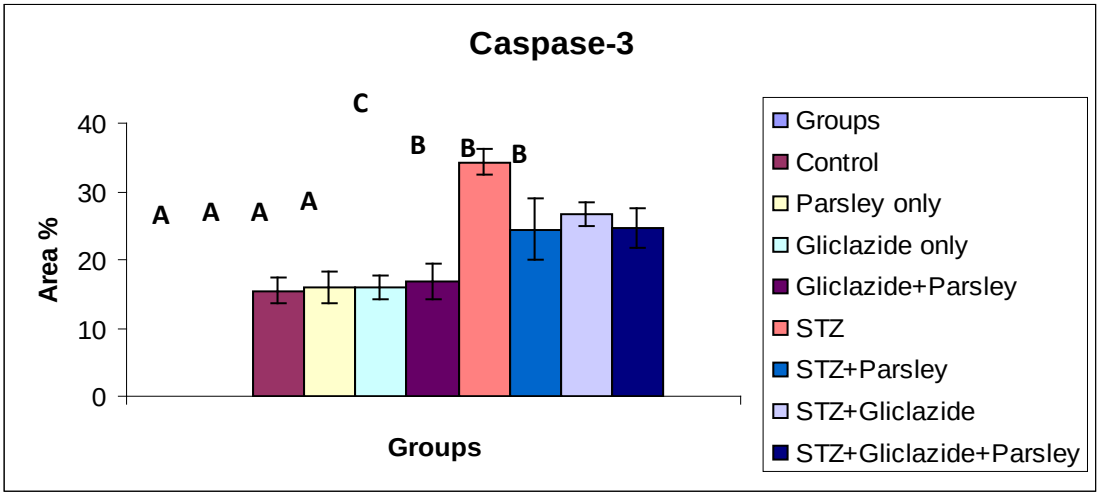


Figure 5: Each bar represents area % of caspase-3 immunopositivity/field (mean of 6 fields ± SE). The presence of different capital letters means significant differences between groups. ANOVA test followed by Duncan's multiple comparisons between groups at $P < 0.05$ were employed.

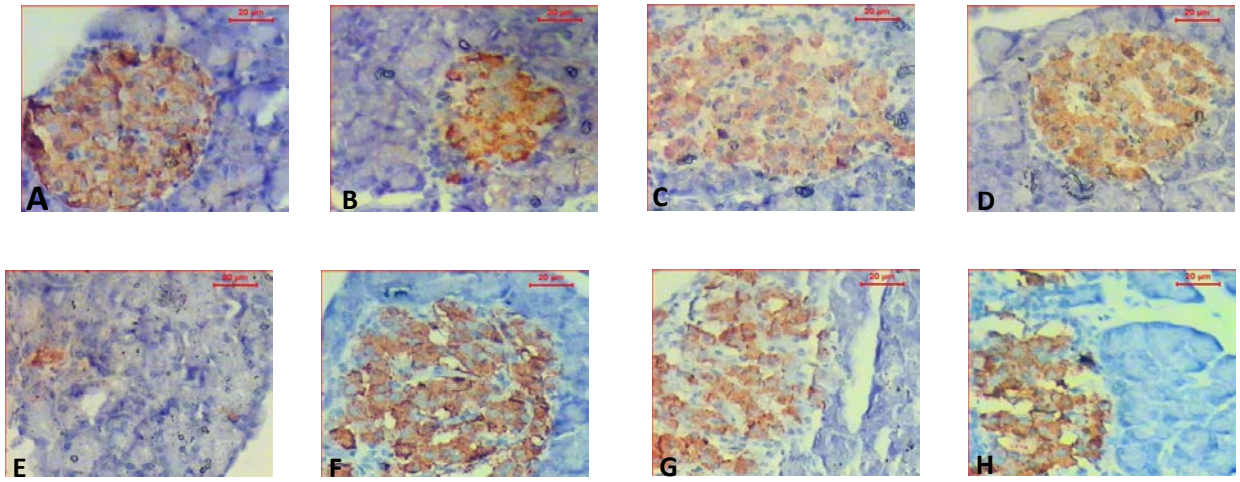


Figure 6: Immunohistochemical detection of anti-insulin antibody in β-cells in the islet of Langerhans of tested groups: A) Control non-treated group showing strong staining, B) PAE group showing strong staining, C) Gliclazide group showing strong staining, D) A combination group showing strong staining, E) Diabetic group showing weak staining, F) Diabetic treated with PAE group showing moderate staining, G) Diabetic treated with gliclazide group showing moderate staining, H) Diabetic treated with a combination of PAE and gliclazide group showing moderate staining.

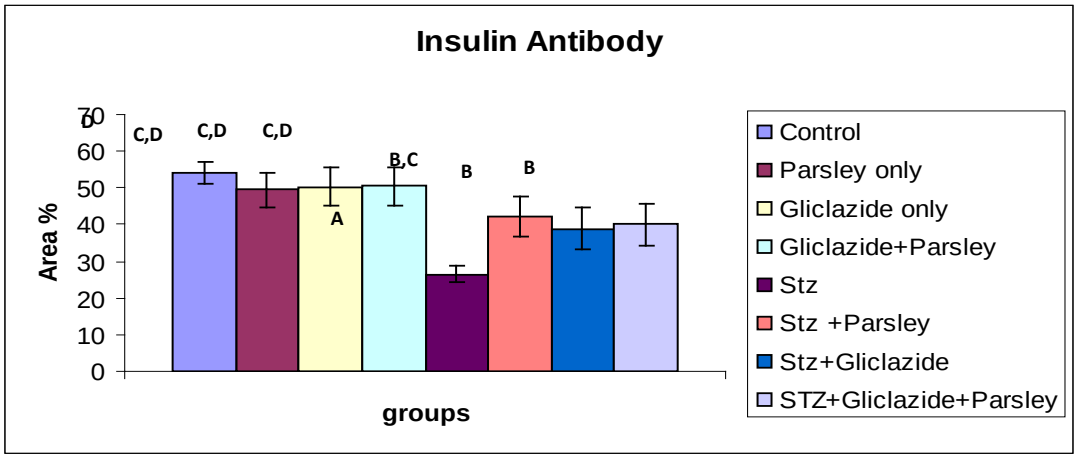
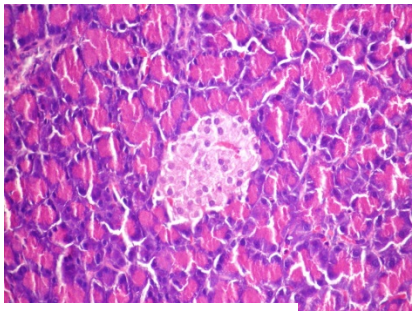
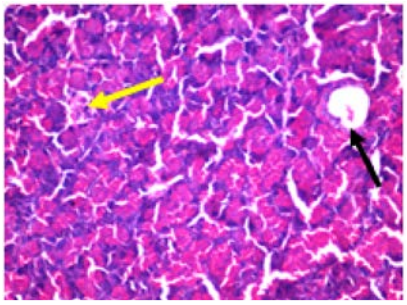


Figure 7: Each bar represents area % of anti-insulin antibody immunopositivity/field (mean of 6 fields ± SE). The presence of different capital letters means significant differences between groups. ANOVA test followed by Duncan's multiple comparisons between groups at $P < 0.05$ were employed.

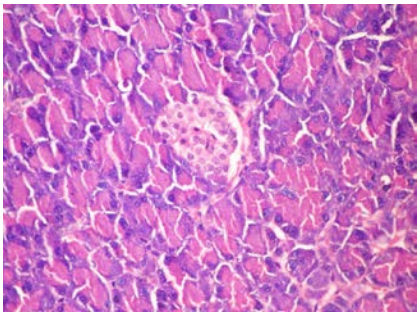
Histopathological Examination

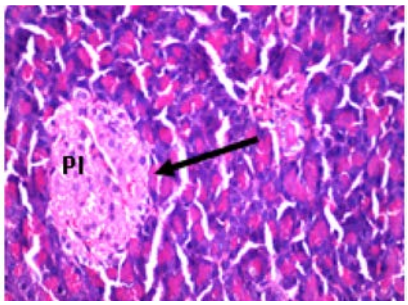
Histological examination of pancreatic tissues of control non-treated group showing intact pancreatic tissues with normal architecture (Fig. 6A). Pancreatic tissues of STZ-induced diabetic rats revealed many pathological alterations in form of dilation and congestion of most of pancreatic vasculature accompanied with perivascular edema and inflammatory reaction. Perifibrous tissues together with proliferated interstitial connective tissue and most of pancreatic islets display different pathological changes where many appeared with reduction in number, size and cellularity (Fig. 6B). Histological examination of pancreatic tissues of diabetic rats treated with PAE revealed moderate curative impact, where many pancreatic islets restore normal size and cellularity (Fig. 6D). Diabetic rats treated with gliclazide revealed a significant curative effect, in form of presence of many intact pancreatic islets, with moderate cellularity accompanied with normal pancreatic acini (Fig. 6F). Diabetic rats treated with gliclazide and PAE revealed good impact, where many pancreatic islets display normal size and cellularity (Fig. 6H). In normal rats treated with PAE, gliclazide and administration of both display intact pancreatic tissues with normal architecture of pancreatic tissues (Figs. 6 C, E and G).



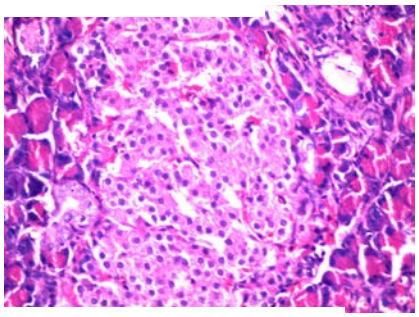


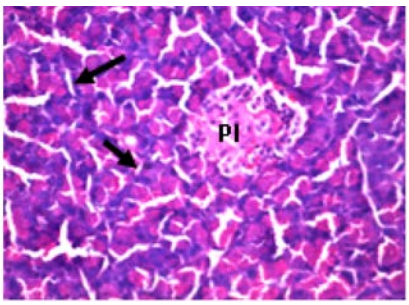
B) Photomicrograph of STZ diabetic group showing hypocellularity and sever reduction in size (yellow arrow) pancreatic duct with degenerative changes in their lining cells (arrow) (H&E) (X:400).



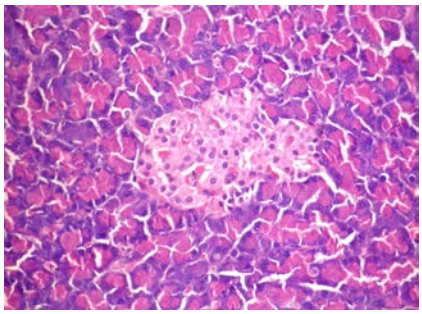


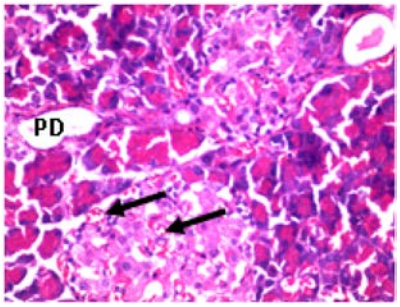
D) Photomicrograph of diabetic group treated with PAE showing moderate cellularity in pancreatic islets (PI) vaculations in and between islets cells (arrow) (H&E)(X:400)





F) Photomicrograph of diabetic group treated with gliclazide showing moderate cellularity in pancreatic islets (PI), intact pancreatic acini (arrow) (H&E) (X:400).





H) Photomicrograph of diabetic group treated with a combined PAE and gliclazide showing moderate cellularity in pancreatic islets, dilated blood capillary (arrow) intact pancreatic duct (PD) (H&E) (X:400).

Figure 6: Histopathological examination of pancreatic tissue

Discussion

Data of the present study revealed a considerable amount of total phenolic and flavenoid contents in the examined PAE (Table 1). Phenolics are the well-known group of secondary metabolites and comprise a large group of biologically active compounds. Polyphenols are phenolics that contain at least two phenolic rings **[30].** It has been reported that, the antioxidant activity of plant materials is well correlated with the content of their phenolic compounds **[31].** Phenolic compounds contribute to the overall antioxidants activities of plants mainly due to their redox properties. Generally, the mechanisms of phenolic compounds for antioxidant activity are neutralizing lipid free radicals and preventing decomposition of hydroxides into free radicals **[32].**

Flavonoids are a family of phenolic compounds that characterized by a benzo-γ-pyrone structure, it is ubiquitous in fruits and vegetables and make a great contribution to their antioxidant activity [33]. In this study the determination of flavonoids content in PAE revealed a relatively high amount of flavonoids in corresponding to quercetin. The obtained findings in the current study are in consistent with that of **Chaves *et al.,*** and **Al-Daraji *et al.* [34,9]** who screened PAE through phytochemical examination and revealed the presence of relatively high phenolic content and flavonoids, (kaempferol and quercetin) and flavones (apigenin and luteolin), coumarins, terpenes**,** apiol, monoterpenes (α-pinene), myristicin and furanocoumarins. It has also been reported that in addition to flavenoids and phenolic compounds the fresh leaves of parsley are rich source of manganese, phosphorus, vitamins (as vitamin C, carotenoids and tochopherol) and calcium **[35,8,10].** According to **Bahnas *et al.,* ; Vora *et al.,*** and **Mahmood *et al.* [35,8,10]** and our results, these compounds make PAE one of the most potent natural antioxidants.

In the present study, free radical scavenging activity of PAE was determined by ferric reducing ability power (FRAP). In FRAP assay the ability of the sample to reduce the ferric ion is used as a criterion on the antioxidant capacity. Ferric reduction based on FRAP is the only assay that directly measures antioxidants or reductants in the examined sample. FRAP assay measures the reducing ability of antioxidant that react with ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex and produced a colored ferrous tripyridyltriazine (Fe^{2+}-TPTZ) complex. In FRAP assay the results were expressed as the combined concentrations of all electron-donating reductants which occur in the sample **[36].**

It has been proposed that STZ acts as a diabetogenic agent owing to its ability to destroy pancreatic β-cells **[37].** In the present work, the subcutaneous administration of STZ at low three doses given through two weeks caused a significant elevation in blood glucose levels. This finding assessed the efficiency of STZ administration route in inducing a hyperglycemic state that sustained until the end of the experimental period. This finding is in agreement with the results of **Ozsoy-Sacan *et al.*,** and **Al-Bishri and Al-Attas [11,38]** who reported that, STZ administration at lower doses produces partial destruction of pancreatic β-cells with permanent diabetes condition.

In the present work, elevated level of MDA was observed in STZ-induced diabetes which indicates the formation and propagation of free radicals that lead to the generation of oxidative stress. This finding is in agreement with the results of **Budin *et al.,*** and **Okoro *et al.,* [39,40]** who reported that, the increase in plasma MDA level of diabetic rats reflects the destruction of pancreatic β-cells and the mobilization of free fatty acids from fat depots, that cause the destabilization, disintegration and alteration in membrane fluidity and

permeability, all events which increase the rate of protein degradation and eventually leads to cell lysis **[41,40].**

The impairment of the antioxidants defense system is a critical step in STZ- induced diabetes. Evidence has shown that the undue effect of STZ is characterized by change in circulating SOD antioxidant enzyme activity **[42,41,40].** In the present study, the obtained increase in plasma MDA and reduction in erythrocyte SOD activity indicates and signify the imbalance between the pro-oxidant and antioxidant states in the body that leads to an imbalance in systematic redox status. This finding is in agreement with the results of **Chandramohan *et al.,* ; Almeida *et al*.,** and **Baghdadi [43,44,45]** who reported that the decrease in erythrocytes SOD activity in STZ-treated rats might be resulted from the involvement of deleterious oxidative change due to the accumulation of toxic products.

The histopathological examinations of pancreatic islets in STZ-treated rats show hypocellularity and sever reduction in size, pancreatic duct with degenerative changes in their lining cells. In addition the immunoreactivity for anti-insulin antibody revealed a marked decrease in number of insulin positive cells after STZ-injection. This findings is in the harmony with the results of **Yanardağ *et al.*, ; Adewole and Ojewole** and **Abo Gazia and Hasan [46,47,48]** who reported similar results**.**

In the present study, the immunohistological examination of pancreatic tissue in STZ - treated rats shows a significant elevation of caspase-3 expression. Cell death is the last stage of cellular damage and it can occur by apoptosis **[49].** Caspases are cysteine-aspartyl specific proteases that play a key role in apoptosis **[50,51].** In this study the recorded increase in caspase-3 expression suggests that, apoptosis plays a significant role in pancreatic β-cells destruction. In agreement with the obtained results the findings of **Maedler *et al.*,** and **Haligur *et al.*, [52,53]** who reported that caspase-3 dependant apoptotic pathways are essential for pancreatic β-cells apoptosis.

Data of the current study revealed that, gliclazide significantly reduced the blood glucose levels of STZ diabetic rats after 45 days of daily oral dose administration. This finding is in accordance with the results of **Salman and Inamdar [54].** In addition, gliclazide significantly elevate serum insulin level in STZ-diabetic rats. This finding is in the harmony with the results of **Sarkar *et al.,*** and **Gadiko *et al.*, [55,56]** who reported that, gliclazide stimulates insulin release from rat pancreatic islets.

In this study the obtained increase of insulin level in diabetic rats treated with gliclazide is referred to its binding with sulphonylureas receptors on β-cells leading to blocking of K^{+}ATP channels, opening of voltage gated calcium channels and increase in Ca^{2+} influx leading to insulin release from pancreatic β-cells. This finding is supported by the marked increase in insulin positive cells as recorded by the immunohistological examination. This results is in accordance with that given by **Aquilante,** and **Sliwinska *et al.,* [57,58].**

Gliclazide, which is used as a reference hypoglycemic agent in the present study is a member of the 'second-generation' sulphonylureas. As a class, sulphonylureas enhance and increase the release of endogenous insulin from pancreatic β-cells. They also promote and facilitate peripheral tissue uptake and utilization of glucose **[59].** It has been proposed by **Jackson and Bressler (1981); Sarkar *et al.* (2011)**; **Gadiko *et al.* (2013)** that sulphonylureas produce their hypoglycemic effects via three main mechanisms, namely; (1) increased insulin release from pancreatic β-cells, (2) potentiation of insulin's action on target tissues and increased glucose removal from the blood, and (3) reduction of blood glucagon levels. Therefore, any plant, secondary metabolite or chemical constituent that is capable of affecting pancreatic β- or α-cell secretion in any of these three ways will be a good mimicker of sulphonylureas and will produce hypoglycemic effects in mammals via mechanisms similar to those of the sulphonylureas **[56].**

In the current study, the administration of parsley extract to diabetic rats induced a marked decreasing effect on blood glucose levels that was associated with a significant increase in insulin level. The current immunohisto chemical examination showed that pancreatic β-cells were destroyed by STZ whereas PAE administration prevented degeneration of β-cells. PAE administration increases the area of insulin immunoreactive β-cells significantly. This finding suggests that the administration of parsley extract displayed an improvement in the destructive β-cells that leads to the release of insulin from the pancreas. The obtained results are parallel to the findings of **Ozsoy-Sacan *et al.*, ; Ameho *et al.*, ; Abd El-Baky** and **Mahmood *et al.,* [11,61,62]** and support the role of PAE in protecting the pancreatic tissue against the destructive effect of STZ.

Depending on these aforementioned results it could be suggested that the hypoglycemic effect of parsley aqueous extract is must probably attributed to its constituents of flavonoids that exert a stimulatory effect on insulin secretion by changing Ca^{+2} concentration as described by **Elberry *et al.* [63],** who reported that the phytochemical investigation of PAE led to the characterization of several flavonoids that possess hypoglycemic activity, as well as antioxidant properties.

The obtained results clearly declare the efficient hypoglycemic activity of the examined parsley extract and suggest that phenolic compounds of parsley could mimic the hypoglycemic effect of gliclazide that used in the present study as a reference hypoglycemic drug.

In the present study, administration of gliclazide to diabetic rats significantly regulates the antioxidants status as manifested by the significant decrease in lipid peroxidation as well as a marked increase in SOD activity. These findings reflect the antioxidant properties of gliclazide that may be attributed to the presence of aminoazabicyclo-octane ring that possesses free radical scavenging property as described by **Salman and Inamdar [54].** It has been also reported that, this ring is absent in other sulphonylureas, that is distinct this drug from the others **[64,56].** Consequently, the obtained reduction of caspase-3 expression may be expected and this finding is in agreement with the results of **Li and Renier [65]** who reported that, the anti-apoptotic effect of gliclazide was associated with an increase in protein kinase B activity and a decrease in caspase-3 and -9 activities.

In this study administration of parsley aqueous extract to diabetic rats inhibited the formation of MDA in plasma. The ability of parsley extract to lower lipid peroxidation and modulate oxidative stress has been demonstrated in several studies [66,67]. Since parsley is a polyphenols - rich herb, its polyphenolic compounds may be able to bind the reactive oxygen species directly and scavenge them or act as sacrificial antioxidant to inhibit the lipid peroxidation cascade as seen in this study.

Results of this study further showed that, the administration of parsley extract to diabetic rats reversed the observed change in the activity of erythrocyte SOD. This modulation of SOD activity could be ascribed to the direct quenching property of PAE towards ROS generated by STZ-induced diabetes. This finding confirms the efficient role of parsley in detoxifying free radicals and reflects its antioxidant properties.

In the present study the administration of PEA to diabetic rats significantly decreased the expression of caspase-3 level in pancreatic tissues, indicating its promising effect on STZ-induced apoptosis. This finding supports the antioxidant role of parsley in suppressed the apoptosis in pancreatic cells and suggests that ROS generated by hyperglycemia likely play a critical triggering role in apoptotic cell death in STZ-induced diabetes.

The obtained results revealed that, however the combined administration of PAE and gliclazide to diabetic rats exhibited a significant reduction in blood glucose levels in association with a marked elevation of insulin level as well as a significant attenuation in the oxidative damage resulted from STZ injection, but this ameliorating effect is less than that obtained by the sole administration of parsley or gliclazide to diabetic rats. According to these findings, it could be stated that there is no significant interaction between PAE and gliclazide when used in combination on any of the aforementioned parameters. It follows that the two treatments can be taken together safely without fear of any serious reactions. The absence of additive action between the two drugs observed in this study may be attributed to the use of doses that give maximal response, thus no potentiating of action was observed. It has been reported that, when the drug is taken orally it travels through the digestive system in mostly the same way as food and herbs taken. Therefore, when it is mixed with herb, each can alter the others pharmacokinetic profile, that is, absorption, distribution, metabolism, and/or excretion. Some drugs interfere with the Body's ability to absorb herbs. Similarly, some herbs and food can lessen or increase the impact of a drug **[68,69].**

In conclusion, PAE is a potent antioxidant and hypoglycemic agent that preserves and protects pancreatic β-cells against oxidative damage with the enhancement of insulin secreation.

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