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Hanan A. Soliman
Department of Chemistry, Faculty of science, Beni Suef University, Beni Suef city, Egypt.

Nadia A Eltablawy
Biochemistry Division, National Organization for Drug Control and Research (NODCAR), Giza, Egypt.

Mona S Hamed
Biochemistry Division, National Organization for Drug Control and Research (NODCAR), Giza, Egypt.

Correspondence:
Mona S Hamed
Biochemistry Division, National Organization for Drug Control and Research (NODCAR), Giza, Egypt.

The ameliorative effect of *Petroselinum crispum* (parsley) on some diabetes complications

Hanan A. Soliman, Nadia A Eltablawy, Mona S Hamed

Abstract
This study is aimed to evaluate the hypolipidemic effect of parsley aqueous extract (PAE) and its effect on heart tissue of diabetic rats. Diabetes induced by subcutaneous injection of streptozotocin (STZ). Sixty diabetic rats were equally divided into four groups: control diabetic rats, diabetic rats treated with PAE and/or gliclazide. In addition, sixty normal rats were equally divided into four control groups: control non-treated rats, normal rats treated with PAE and/or gliclazide. The experiment lasted for 45 days. STZ injection induced a significant increase in plasma total cholesterol, triglycerides, low-density lipoprotein levels and glutathione transferase activity in association with a significant decrease in plasma high-density lipoprotein, whole blood glutathione and serum homocysteine levels. Expression of caspase-3 in cardiac tissue was affected by STZ injection. In conclusion, administration of PAE significantly attenuates the hyperlipidemia, overcomes the oxidative stress and improves heart tissue of diabetic rats.

Keywords: *Petroselinum crispum*, Hyperlipidemia, Oxidative stress, Homocysteine and Caspase-3 expression.

1. Introduction
Diabetes mellitus (DM) is one of the most severe, incurable metabolic disorders characterized by abnormal regulation of glucose and lipid metabolism [1]. Hyperlipidemia is a complication associated with DM [2] due to qualitative and quantitative abnormalities in lipoproteins. In a diabetic condition, increased serum lipids are due to the increased lipolysis of adipose tissue, and thereby cause abnormal lipoprotein concentration [3]. During diabetes, free radicals oxidize the lipoproteins and various irregularities of lipoprotein metabolism also occur in low-density lipoprotein (LDL-C) and high-density lipoprotein (HDL-C). Hyperglycemia, *per se* or by the promotion of lipid peroxidation of LDL-C can result in the production of free radicals. There is considerable evidence that oxidative stress plays a key role in diabetes complications such as micro/macro vascular damage [4]. The abnormal high concentration of serum lipids in STZ-induced diabetes is mainly due to the increase in the mobilization of free fatty acids (FFA) from fat depots [5].
Homocysteine (Hcy) is an amino acid not use in protein synthesis. Its role is to serve as intermediate in methionine metabolism. Hcy itself is located at a branch point of metabolic pathways either it is irreversibly degraded via the transsulfuration pathway to cysteine or it remethylated back to methionine [6]. There is a relationship between Hcy level and oxidative stress in DM [7]. Hcy contains a reactive sulfydryl group that can react with plasma constituents and this may promote oxidative damage [8]. Hcy levels in patients with DM have been reported as either low or elevated when compared to control non-diabetic group as reported by **Jacobs *et al.*** [9] **and Paul *et al.*** [10].
Apoptosis is termed as a programmed cell death, which is characterized by cell shrinkage, chromatin condensation, DNA fragmentation and the activation of specific cysteine proteases known as caspases. Caspase-3 is a critical component of the cell death machinery, being regarded as the most downstream enzyme in the apoptotic process due to its location in the protease cascade pathway [11].
For the past several years natural product have received scientific and medicinal status with their antioxidant property which represents a better option to cure various diseases [12]. Parsley, [*Petroselinum crispum* (Mill.) Nym.ex A.W. Hill] family Umbelliferae, locally known as Baqdunis, has been used medicinally for many centuries in European, Mediterranean and Asian countries [13]. Parsley is rich with an antioxidant arsenal that includes luteolin, flavonoid that searches out and eradicates free radicals in the body that cause oxidative stress in cells [14]. Parsley is a powerhouse of nutrition, rich in tocopherol and vitamin A [15, 16]. It contains starch,

vitamins B, C, β-carotene and zinc [17]. In popular medicine, parsley is used to treat various illnesses such as Alzheimer's disease, thrombosis and strokes. Parsley is widely employed against cardiovascular diseases [18]. Besides having significant nutritional value, parsley also exhibits antioxidant and neutralizing properties [19].

Therefore, the objective of this study is to evaluate the ameliorative effect of PAE on hyperlipidemia, oxidative stress and its effect on heart tissue of diabetic rats. We hypothesized that a combined treatment between the examined extract and gliclazide could give more pronounced effect, which will be ascertained.

Materials and Methods

Plant material and the 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 and the filtrates were evaporated using rotary evaporator under reduced pressure to dryness [15]. The dried matter was dissolved in distilled water for using in the experimental studies.

Gliclazide and the preparation of its 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** [20].

Preparation of streptozotocin (STZ)

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 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. This study was approved by the local ethics committee of NODCAR.

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.*** [21]. Blood samples were withdrawn 48 hours after each injection to assess the induction of diabetes. Diabetes was developed and stabilized within two weeks. Diabetes confirmed by the elevated blood glucose level after 48 hours (after each injection). Only rats confirmed with permanent glucose level around 250 mg /dl were used in this study. Diabetic 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 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.

Blood sampling

At the end of the experimental period blood samples were collected as described by **Schermer** [22]. Each blood sample was collected in two tubes; the first one is contain heparin and the second one to maintain serum. The whole blood was used for the determination of hemoglobin and reduced glutathione (GSH). The blood was centrifuged at 4000 rpm for 15 minutes at cooling centrifuge to separate plasma for the determination of Glutathione transferase (GST) activity, the levels of total cholesterol (TC), triglycerides (TG) and high-density lipoprotein (HDL-C). Serum was separated to measure Hcy level.

Biochemical analysis

Blood hemoglobin was determined according to the method of Van-Kampen and Zijlstra [23]. GSH content in whole blood was determined according to Beutler *et al.* [24] method**.** GST activity was carried out according to Habig *et al.* [25] method**.** TC was determined colorimetrical according to the method of **Richmond** [26]. HDL-C was determined according to **Warnick and Albers** [27] method. TG was determined according to **Scheletter *et al.*** [28] method. Low-density lipoprotein (LDL-C) was evaluated according to the formula of **Friedewald *et al.*** **[29]** as follow: LDL-C = TC – (TG / 5 + HDL -C). Hcy concentration was measured by the IMX system provided by ABBOTT laboratories diagnostic division-Germany [30].

Histopathological Examination

At the end of the experimental period rats were decapitated, the heart was taken and washed with cold saline. Autopsy samples were taken from the heart of different groups of rats fixed in 10% formalin for twenty-four hours for histopathological examination according to **Banchroft *et al.*** [31]. The obtained tissue sections were stained by hematoxylin and eosin stain for histopathological examination through the electric light microscope**.**

Immunohistochemistry Examination

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 instructions [32].

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 the mean ± standard deviation (M±SD) for seven animals in each group. 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 comparison tests. Data were statistically analyzed using the statistical package for social science (SPSS 11.0 software) values at $P < 0.05$ were considered significant**.**

Results

The obtained results revealed that, the sole administration of PAE or gliclazide to normal rats induced a significant decrease in TC and TG levels with no change in HDL-C when compared to normal control group (Table 1). The obtained data also show that administration of PAE to normal rats exhibited a more pronounced effect on TG and LDL-C levels. The combined administration of both to normal rats exerted no effect on TC and TG levels with significant changes in HDL-C and LDL-C levels when compared to control non-treated rats (Table 1). Data also revealed that, induction of diabetes caused a disturbance in lipid profile as manifested by the significant increase in the levels of TC, TG and LDL-C in association with a marked reduction of HDL-C level in STZ-induced diabetic rats. Meanwhile, the oral administration of PAE, gliclazide or the combination of both to diabetic rats caused a significant decrease in TC, TG and LDL-C levels in association with a significant improvement in HDL-C level (Table 1).

Table 1: Effect of oral administration of parsley extract, gliclazide and the combined administration of both on lipid profile in normal and diabetic rats

Groups	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL-C (mg/dl)	LDL- C
Control	98.5 ± 5.63^{C}	91.53 ± 2.50^{C}	50.23 ± 2.5^{C}	$27.5\pm2.13^{B,C}$
Parsley only	84.9 ± 8.5^{A}	$75.31\pm7.75^{A,B}$	49.2 ± 3.21^{C}	21.6 ± 3.08^{A}
Gliclazide only	$88.5\pm8.7^{A,B}$	$82.92\pm10.76^{B,C}$	49.72 ± 4.70^{C}	26.9 ± 4.03^{B}
Gliclazide+Parsley	$96.3\pm6.78^{B,C}$	85.10 ± 9.70^{C}	44.24 ± 2.76^{B}	34.7 ± 1.19^{D}
STZ	150 ± 7.91^{D}	134.13 ± 5.14^{E}	29.60 ± 5.02^{A}	92.9 ± 7.4^{F}
STZ +Parsley	$88.4\pm7.59^{A,B}$	71.37 ± 3.80^{A}	49.43 ± 5.2^{C}	$28\pm3.72^{B,C}$
STZ+Gliclazide	101 ± 6.26^{C}	92.6 ± 12.14^{C}	49.20 ± 3.33^{C}	$31.8\pm3.1^{C,D}$
STZ+Gliclazide+Parsly	103 ± 3.09^{C}	105.8 ± 8.7^{D}	43.40 ± 3.74^{B}	38.8 ± 2.53^{E}

- Values are mean ± SD number of samples (n) (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.

Table (2) shows that, the sole administration of the examined materials to normal rats had no effect on whole blood GSH level, plasma GST activity and Hcy level. The obtained data revealed that, the subcutaneous injection of STZ exerted a significant decline in whole blood GSH and Hcy levels when compared with control non-treated group. Meanwhile, the activity of GST recorded a significant increase in STZ-treated rats. A significant increase in whole blood GSH level of diabetic rats was obtained after treatment with the examined materials when statistically compared with STZ-treated group. Administration of PAE and /or gliclazide to diabetic rats caused a significant reduction in plasma GST activity with respect to STZ-treated group (Table 2). The obtained results also revealed that, administration of the examined materials to diabetic rats exerted a significant improving effect on Hcy level, but the combined administration of parsley and gliclazide elicited a less improving effect than that produced by the administration of each one alone to diabetic rats when compared with STZ-treated rats (Table 2).

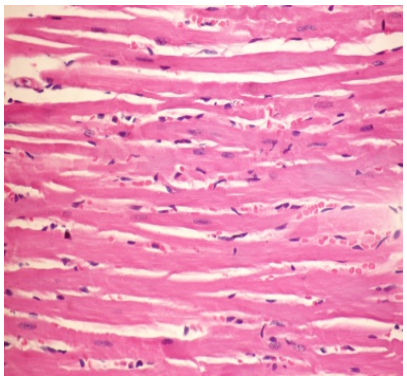
Table 2: Effect of oral administration of parsley extract, gliclazide and the combined administration of both on GSH level, GST activity and Hcy level in normal and diabetic rats

Groups	GSH (mg / g Hb)	GST (mg/ml)	Hcy (µmol /L)
Control	2.51 ± 0.143^{C}	$3.82\pm0.204^{A,B}$	11.51 ± 1.03^{C}
Parsley only	2.51 ± 0.157^{C}	$3.8\pm0.085^{A,B}$	12.10 ± 1.59^{C}
Gliclazide only	2.53 ± 0.189^{C}	3.66 ± 0.085^{A}	10.95 ± 2.09^{C}
Gliclazide+Parsley	2.57 ± 0.227^{C}	$3.80\pm0.35^{A,B}$	10.43 ± 1.14^{C}
STZ	1.7 ± 0.140^{A}	7.50 ± 0.43^{D}	5.19 ± 1.85^{A}
STZ +Parsley	2.03 ± 0.180^{B}	4.20 ± 0.57^{C}	8.30 ± 1.20^{B}
STZ+Gliclazide	2.12 ± 0.190^{B}	$3.96\pm0.17^{A,B,C}$	8.21 ± 1.80^{B}
STZ+Gliclazide+Parsley	2.12 ± 0.123^{B}	$4.10\pm0.25^{B,C}$	$6.80\pm1.31^{A,B}$

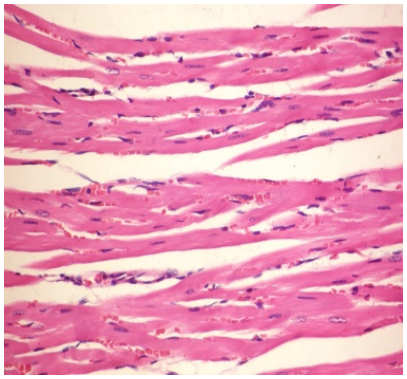
- Values are mean ± SD (n=7 for GSH, GST and n = 6 for Hcy).
- The presence of different capital letters means significant differences between groups in the same columns. ANOVA test followed by Duncan's multiple comparisons between groups at $P < 0.05$ were employed.

Histopathological examination

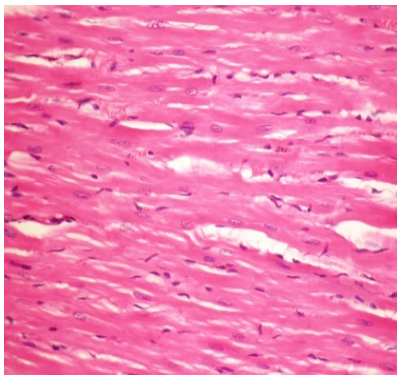
Cardiac tissue of control non-treated group, normal rats treated with PAE, gliclazide and the combination of both showed normal architecture of the heart (Fig. 1 A,B,C and D). Cardiac tissues of STZ-induced diabetic rats revealed many pathological alterations in form of focal area of disrupted architecture and cardiomyocytes with atrophied cardiomyocytes. This is blood vasculature display dilatation and congestion and inflammatory cells infiltrate (Fig. 1 E). Meanwhile the administration of PAE and/or gliclazide to diabetic rats showed a marked improvement in heart tissue with respect to STZ injected rats (Fig. 1 F, G and H).



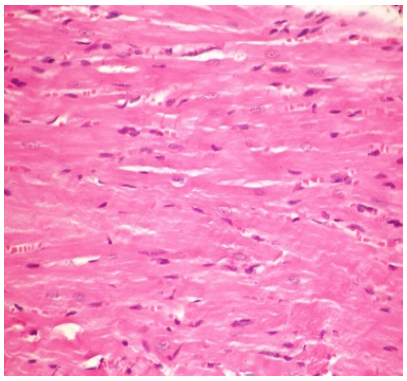
A) Photomicrograph of control group showing intact cardiomyocytes (H&E) (X:400)



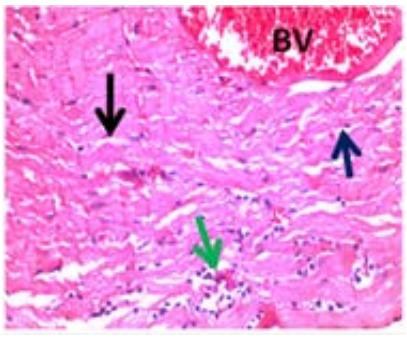
B) Photomicrograph of PAE group showing intact cardiomyocytes (H&E) (X:400)



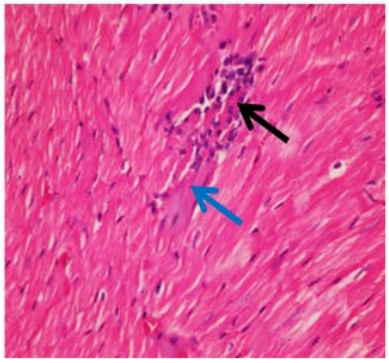
C) Photomicrograph of gliclazide group showing intact cardiomyocytes (H&E)(X:400)



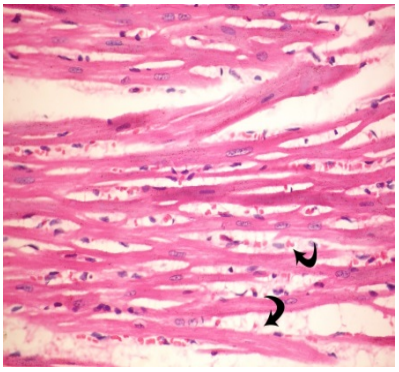
D) Photomicrograph of PAE and gliclazide combined administration group showing intact cardiomyocytes (H&E) (X:400)



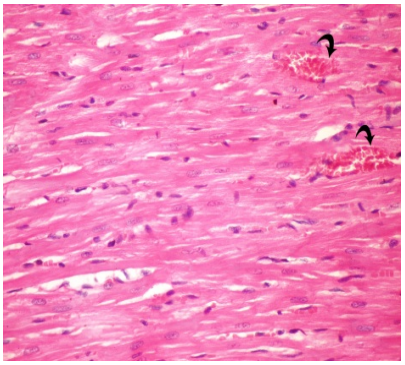
E) Photomicrograph of diabetic rat group showing focal area of disturbed architecture (arrow), inflammatory aggregates (green arrow), dilated congested blood vessels (BV) and atrophied cardiomyocytes (blue arrow), (H&E) (X:400)



G) Photomicrograph of diabetic rat treated with gliclazide showing cellular infiltrate (arrow), cardiomyocytes with hyalinized cytoplasm (blue arrow) (H&E) (X: 400)



F) Photomicrograph of diabetic rat treated with PAE showing slight intermuscular oedema (arrow), (H&E) (X:400)



H) Photomicrograph of diabetic rat treated with a combined administration of PAE and gliclazide showing mild congestion of blood vessels (H&E) (X: 400)

Fig 1: Histopathological examination of cardiac tissue

Immunohistochemical Examination

Figures 2 and 3 revealed that, the sole administration of the examined materials to normal rats had no effect on caspase-3 expression level in heart tissue when statistically compared with control non-treated group. The subcutaneous injection of STZ induced a significant increase in the expression of caspase-3 in heart tissue with respect to control group. Meanwhile, the administration of parsley extract, gliclazide and the combination of both to diabetic rats caused a significant reduction in caspase-3 expression in comparison with STZ-treated group.

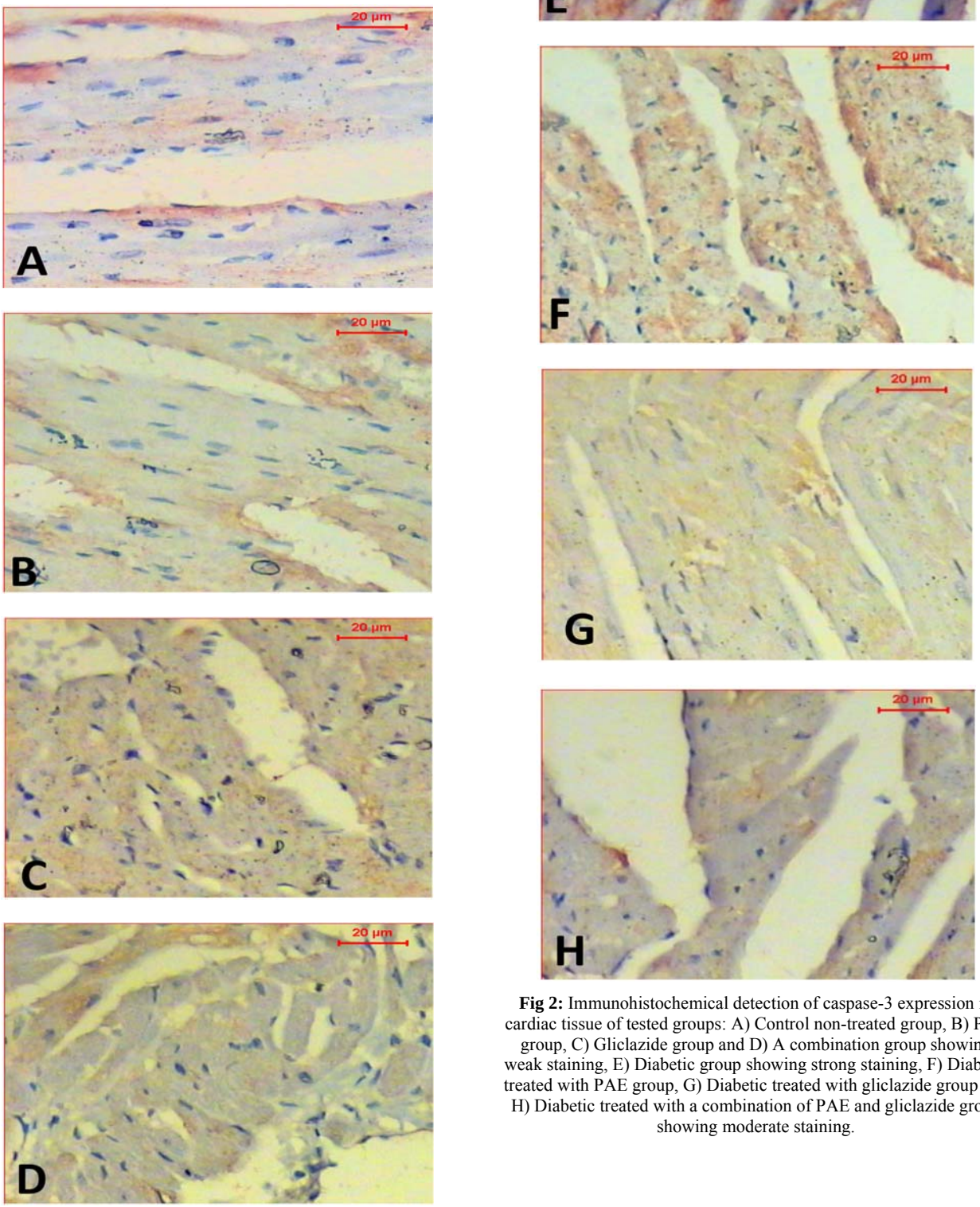


Fig 2: Immunohistochemical detection of caspase-3 expression in cardiac tissue of tested groups: A) Control non-treated group, B) PAE group, C) Gliclazide group and D) A combination group showing weak staining, E) Diabetic group showing strong staining, F) Diabetic treated with PAE group, G) Diabetic treated with gliclazide group and H) Diabetic treated with a combination of PAE and gliclazide group showing moderate staining.

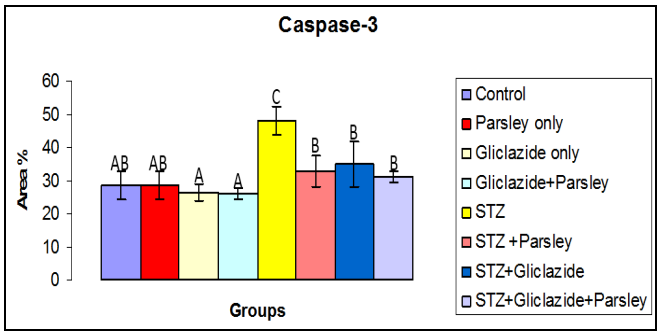


Fig 3: Immunohistochemical detection of caspase-3 expression in heart tissue of the tested groups**.** Each bar represents area % of caspase-3 immunopositivity/field (mean of 6 fields ± SE). The heart of different capital letters means significant differences between groups. ANOVA test followed by Duncan's multiple comparisons between groups at $P < 0.05$ were employed.

Discussion

In the present study, induction of diabetes caused a significant increase in the levels of plasma TC, TG and LDL-C levels in association with a marked decrease in HDL-C level that clearly proved the disturbance of lipid profile and the induction of hyperlipidemia in diabetic rats. This finding is in consistent with the results of Rajeswari and Rajagopalan [5] who attributed this disturbance in lipid profile to the increased mobilization of free fatty acids (FFA) from adipose tissue in diabetic rats. Moreover, the obtained hypercholesterolemia in the current study is in consistent with Subash Babu *et al.* [33] who attributed the increase of cholesterol to the increased intestinal absorption and increased cholesterol biosynthesis in STZ treated rats.

In the current study, hypertriglyceridemia in diabetic rats could be attributed to increase in the activity of hormone-sensitive lipase, which catalysis the mobilization of fatty acids from triacylglycerols stored in adipocytes as described by Almeida *et al*. [34]. Thus, the greater quantities of fatty acids returning to the liver are reassembled into triacylglycerols. It has also been reported that the activity of lipoprotein lipase (an enzyme bound to endothelial cells, which catalysis the hydrolysis of triacylglycerol) is reduced in diabetes and this reduction promotes diabetic hypertriglyceridemia [35].

In this study the obtained increase in plasma LDL-C level of diabetic rats could be resulted from glycosylation of the lysyl residues of apolipoprotein B, which leads to the decrease in LDL metabolism due to a decrease in the affinity of LDL for its receptors as explained by Rajeswari and Rajagopalan [5]. The recorded decrease of plasma HDL-C level in STZ-treated rats is in consistent with the results of Nwoneri-Chidozie *et al.* [36] who referred this decrease in plasma level of HDL-C of untreated diabetic rats to the diminished lecithin cholesterol transferase activity.

The derangements in lipid metabolism in DM are often important determinants of course and status of the disease. Some studies have linked hypertriglyceridemia to higher blood small dense LDL particles, atherothrombosis and impaired endothelial function, the hallmarks of several prevalent cardiovascular diseases as well as their complications [34]. Hypercholesterolemia and abnormalities in lipoprotein metabolism are considered other serious risk factors and important early events in the pathogenesis of atherosclerosis in both peripheral and coronary circulation [37] and clearly proved that DM is associated with profound alteration in blood lipid and lipid profile that may be a risk factor for coronary heart disease.

Treatment of diabetic rats with the examined parsley extract showed a significant decrease in total cholesterol, triglycerides and LDL-C concentrations and significantly increased HDL-C levels. This results are in agreement with the findings of Gohil *et al.* [38]. The lowering effect of parsley extract on total cholesterol, triglycerides and LDL-C levels could be mainly attributed to the presence of flavonoids, that play a role in decreasing the activity of both hepatic enzyme 3-hydroxy-3-methylglutaryl conenzyme A (HMG-CoA) reductase and Acyl Conzyme A: Cholestrole O-acyltransferase (ACAT), or may be due to the presence of some HMG-CoA reductase inhibitors as described by Marzouk *et al.* [39].

The treatment of diabetic rats with PAE induces a significant increase in HDL-C. This increase in plasma HDL-C could be referred to the stabilizing effect of polyphenols on plasma lipoproteins or due to the systemic effect of flavonoids to modulate various enzyme activities that can effect on lipoprotein leading to an augmentation of HDL-C. The positive effect of parsley extract on HDL-C seemed to be in agreement with the results of Abd El-Baky [40] and Baghdadi [41]. Young *et al.* [42] reported that the increase in HDL-C facilitates the transport of cholesterol from the serum to the liver where it is catabolized and extracted from the body**.**

Treatment of diabetic rats with gliclazide showed a significant decrease in TC, TG and LDL-C concentrations and significantly increased HDL-C levels. A higher content of HDL-C is very important because it is correlated with a reduced risk of coronary heart disease [45]. The improving effect of gliclazide is attributed to its stimulatory action on insulin release [43]. The released insulin activates lipoprotein lipase which hydrolyzes TG as described by Lee *et al.* [44] that lead to the significant improvement in lipid profile. The obtained results are in consistent with those of Salman and Inamdar [45]. Sena *et al.* [46] reported that, in diabetic animal models, gliclazide potentially protects the vasculature through improvements plasma lipids and platelet function. Mechanisms may include the ability of the drug to increase tissue plasminogen activator, and its properties as a free radical scavenger**.** Moreover, Salman and Inamdar [45] reported that gliclazide inhibited low-density lipoprotein oxidation and enhanced total plasma antioxidant capacity.

The obtained data revealed that, treatment of diabetic rats with gliclazide or parsley extract elicited equivalent effect in increasing the levels of HDL-C suggest that the mechanism whereby gliclazide and parsley may affect these elevations seemed to be through the same mechanism via the activation of various enzymes that can affect lipoprotein, cholesterol and triglycerides synthesis.

DM is associated with oxidative stress that is imbalance between free radicals production and antioxidant level [4]. In this study the induced alteration in GSH content in whole blood and plasma GST activity reflect the generation of oxidative stress in diabetic rats. This finding is in consistent with the results of Sudhakara *et al.* [47] who suggested that, the increase in GST activity and GSH depletion in diabetic rats referred to the enhanced utilization of GSH for detoxification of toxic products.

Administration of PAE to diabetic rats, restored the whole blood GSH level to value comparable to those found in control non-treated group, indicating that parsley extract is able to protect against STZ- induced GSH depletion. This finding reflects the ability of PAE to quench free radicals and up regulate the synthesis of GSH [48, 15]. This antioxidant property of parsley referred to its content of polyphenols, vitamins E and C [16].

In the present study, the administration of parsley extract to

diabetic rats tends to bring the GST activity to be near the control value. Under this condition of parsley administration, most of the test parameters namely; TC, TG and HDL-C displayed a great tendency to be improved, where marked reduction of oxidative stress occurs [49, 50]. Consequently the indicated improvement in the activity of GST may reflect the efficiency of the antioxidants component of PAE to regulate the activity of GST. The obtained decrease in GST activity is in consistent with the results of Kolarovic *et al.* [51].

In this study, administration of gliclazide to diabetic rats significantly regulates the antioxidants status as manifested by a significant increase in the content of whole blood GSH that associated with a significant reduction in plasma GST activity. These findings are in the harmony with the results of Salman and Inamdar [45] who reported that gliclazide possesses antioxidant properties that produce measurable clinical effects at therapeutic doses as well as gliclazide improves the oxidative status of patients due to its hemovascular properties independent of its hypoglycemic action, in addition gliclazide administration lead to improvements in hypercoagulability, endothelial function and platelet reactivity which have not been demonstrated by other sulphonylureas [45, 46]. The antioxidant properties of gliclazide attributed to the presence of aminoazabicyclo-octane ring that possesses free radical scavenging property and is distinct this drug from the others [45].

Hcy is considered a risk factor for cardiovascular diseases. Several studies have been reported a link between the increased Hcy level, increased lipid profile and cardiovascular diseases in DM [9, 52]. In the present study, a significant decrease in serum Hcy level was obtained in diabetic rats. This decrease in Hcy level could be attributed to the disturbance in transsulfuration pathway to cysteine or it remethylated back to methionine as reported by Blom and Smulders [6]. The recorded decrease in Hcy level is in the agreement with the results of Wijekoon *et al.* [53]. In addition Jacobs *et al.* [9] reported a 40 % lower Hcy level in STZ-induced diabetic rats than those of normal controls**.** This decrease in Hcy level was referred to the increased hepatic cystathionine beta synthase (CBS) activity in diabetics as described by Gursu *et al.* [52]. While the high serum Hcy is widely recognized as a cardiovascular disease risk factor, individuals with low Hcy may also be at risk. The risk of hypohomocysteinemia derives from the fact that Hcy is normal intermediate for conversion of methionine into cysteine and thus for production of glutathione, taurine and sulfate. Individuals with low Hcy have limited capacity for response to oxidative stress and certain kinds of toxin exposure [10]. The limited production ability exacerbated in conditions that cause increased demand for any of the sulfur compounds produced from Hcy.

The obtained depletion of whole blood GSH and increased GST activity could be the reason for decreased Hcy level, Lord and Fitzgerald [54] reported that oxidative stress draws Hcy into glutathione synthesis, potentially causing a drop of plasma Hcy to levels where total body glutathione status is critical.

Administration of PAE, gliclazide or the combination of both revealed a significant improvement in serum Hcy level of diabetic rats. This improvement in serum Hcy and blood GSH levels signify the improvement of redox state in the diabetic treated rats and proved the role of the examined materials as antioxidant for attenuating the undue effects of STZ-induced oxidative stress.

In the present study the histological examination of PAE, gliclazide or the combination of both elicited no change in heart tissue that reflects the safety use of these materials. Meanwhile the histological examination of STZ-induced diabetic rats showed focal area of disturbed architecture, inflammatory aggregates and dilated congested blood vessels as well as atrophied cardiomyocytes. These changes in heart of diabetic rats could be referred to the obtained disturbance in lipid profile and the generation of oxidative stress. The obtained changes in heart tissue of diabetic rats are in consistence with the results of Huang *et al.* [55]. The treatment of diabetic rats with the examined materials showed a significant improvement in heart tissues. That proved the ameliorative effect of the examined materials against diabetes induced changes in the heart.

Cell death is the last stage of cellular damage and it can occur by apoptosis [56]. In the present study, the immunohistological examination of heart tissue in STZ-treated rats shows a significant elevation of caspase-3 expression in heart tissue. Caspases are cysteine-aspartyl specific proteases that play a key role in apoptosis [57, 11]. In particular, caspase-3 is the most widely studied effectors caspases. In agreement with the present study Huang *et al.* [55] confirmed the role of ROS and caspase-3 in the production of apoptosis in diabetic rats.

In the present study the administration of PEA and/or gliclazide to diabetic rats significantly decreased the expression of caspase-3 in heart tissue. This finding support the antioxidant role of parsley and / or gliclazide in suppressed the apoptosis in myocardial cells and the obtained data suggest that ROS generated by diabetes likely play a critical triggering role in apoptotic cell death in the diabetic myocardium.

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 [58].

In conclusion, PAE is a potent hypolipidemic agent that could be administrated safely in case of diabetes mellitus.

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