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Research Article

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Pomegranate (*Punica granatum*) Peels Ameliorates the Glycemic Indices and Antioxidant Gene Transcripts in Diabetic Rabbits

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Abstract ***Context:*** Pomegranate peel (PP) characterized by high content of antioxidant activity. So, there is an increased interest in its potential role in the amelioration of diabetes mellitus (DM). ***Objective:*** This study focused on the ability of PP powder to restrain the detrimental effect of streptozotocin (STZ)-high fat diet (HFD) induced diabetes in rabbits. ***Material and Methods:*** A total of forty white male New Zealand rabbits were allocated tofour equal groups of ten animals each. Control group, received basal diet; diabetic group, injected with STZ (35 mg/ kg BW., i.p) and fed on HFD; PP I, diabetic rabbit were fed on PP powder 1.0% of the basal diet and PP II, diabetic rabbit was fed on PP powder 2.0% of the diet. ***Results:*** PP powder significantly increased ($P < 0.05$) insulin level, catalase (CAT) activity, superoxide dismutase (SOD) activity and gene expression, and total antioxidant capacity (TAC). Furthermore, PP significantly decreased blood glucose level, lipid peroxidation, kidney and liver damage biomarkers, and lipid profile in diabetic rabbits. ***Conclusion:*** PP powder has a therapeutic effect against STZ-HFD-induced biochemical alteration, increased insulin secretion and sensitivity, reduced glucose level, and has the potent antioxidative capacity and lipid lowering effects.

Keywords Diabetes mellitus, Antioxidant, Lipid peroxidation, Pomegranate peel, Gene expression

1. Introduction

Diabetes mellitus (DM) is a chronic disease characterized by either absolute or relative insulin deficiency. Hyperglycemia is a common consequence of uncontrolled diabetes and over time leads to serious damage to many of the body's systems [1]. DM caused marked liver and renal injuries reflected in decreased total protein, albumin and increased serum alanine aminotransferase (ALT, EC 2.6.1.2), aspartate aminotransferase (AST, EC 2.6.1.1), urea, and creatinine as well as increased blood glucose level and lipid profiles [2].

Pomegranate (*Punica granatum*) is a small tree, belonging to the Punicaceae family. Pomegranate juice has become increasingly popular because of its important biological actions such as antioxidant and hepatoprotection(Schubert et al., 2002). Anti-diabetic has been reported for extracts from different parts of Pomegranate [3]. Pomegranate peel (PP) is a rich source of antioxidants, especially polyphenols, such as ellagic acid, quercetin, anthocyanidins, punicalagin, and many plant polyphenols [4].

Therefore, the aim of present study was evaluating the anti-diabetic and antioxidant potential effect of PP against STZ-HFD induced diabetic rabbits.

2. Material and methods

2.1. Chemicals

Streptozotocin was purchased from Sigma-Aldrich (Sigma Chemical Co., St. Louis, MO, USA.). Kits for determination of all liver functions, kidney functions, lipid profile, and antioxidant parameter were purchased from the Biodiagnostic Company, Cairo, Egypt. All chemicals used were of analytical grade.

2.2. Induction of Diabetes Mellitus

Streptozotocin was prepared by dissolving in sodium citrate buffer (pH 4.5) and injected immediately to avoid degradation [5] then rabbits were fed with HFD till the end of the experiment.

Rabbits were fed with high-fat diet, prepared using 20% fat (20% sheep tallow) for weeks two as recommended by Samah (2014) [6]. After which rabbits were injected intraperitoneally with STZ by 35 mg/kg BW. Ten days after STZ injection, rabbits were screened for measuring blood glucose levels. Overnight fasted (8 hr) animals blood samples were taken from ear vein and kept without anticoagulant at room temperature for one hour then centrifuged and serum glucose level was measured. Rabbits having serum glucose more than 200 mg/dl were considered as diabetic and included in the experiment.

2.3. Pomegranate Peel Preparation

Pomegranate peels were obtained from Fathalla Market, Alexandria Governorate, Egypt. The peels were washed with distilled water, dried by sunlight exposure then ground to make a powder.

2.4. Gas chromatography–mass spectrometry (GC-MS) analysis of PP

The chemical composition of PP sample was performed using Trace GC Ultra-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness). The column oven temperature was initially held at 60°C and then increased by 5°C /min to 160°C withhold 2 min then increased to 250 with 5 °C/min. The injector and detector (MS transfer line) temperatures were kept at 250°C. Helium was used as a carrier gas at a constant flow rate of 1 ml/min. The solvent delay was 2 min and diluted samples of 1 µl were injected automatically using Autosampler AS3000 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 40–650 in full scan mode. The ion source and transfer line temperatures were set at 200 and 250°C respectively. The components were identified by comparison of their retention times and mass spectra with those of WILEY 09 and NIST 11 mass spectral database.

2.5. Animals

Forty white male New Zealand rabbits weighing 1.0 - 1.1 kg. They were obtained from the Faculty of Agriculture, Alexandria University, Egypt and acclimated to the laboratory conditions for two weeks prior to the initiation of the experiment. Rabbits were maintained on a stock diet (Table 1). Rabbits were accessed water and *ad libitum*.

Table 1: Ingredients of basal diet

Ingredient	Amount (gm)
Yellow corn	7.5
Wheat bran	24
Barley	20
Clover hay	22
Soybean meal (44% CP)	23.5
Limestone	1.15
Di-calcium phosphate	0.5
DI-Methionine	0.2
Anti-aflatoxin+Anti-coccidian	0.5
Vitamin and minerals premix	0.30
NaCl	0.35
Total	100

1Each kg of vitamin and mineral mixture contained: Vit A 2 000 000 IU; E 10 mg; B1 400 mg; B2 1200 mg; B6 400 mg; B12 10 mg; D3 180000 IU; Colin chloride 240 mg; Pantothenic acid 400 mg; Niacin 1000 mg; Folic acid

1000 mg; Biotin 40 mg; Mn 1700 mg; Zn 1400 mg; Fe 15 mg; Cu 600 mg; Se 20 mg; I 40 mg and Mg 8000 mg. GAE: Gallic acid equivalents

2.6. Experimental Design

A total of forty white male New Zealand rabbits were allocated into four groups; Group I: the control group received basal normal diet; Group II: Diabetic rabbits (Diabetes was induced by a single intraperitoneal injection of STZ (35 mg/kg body weight) and HFD 20%; Group III: Diabetic rabbit were fed on PP powder 1.0% of the diet; Group IV: Diabetic rabbit were fed on PP powder 2.0% of the diet. Blood glucose level was measured ten days after STZ injection using a one-touch glucometer to confirm diabetes.

2.7. Sampling and biochemical analysis

Blood samples were collected from the ear vein of 8 hours fasted rabbit two weeks, 4 weeks and 8 weeks from starting of the experiment. The blood was collected in the dried test tubes and then centrifuged at 3000 rpm for 10 min. The collected sera were used for determination of serum glucose level according to the method of Trinder (1969) [7], insulin hormone level by the solid phase radioimmunoassay (RIA) according to the method of Sapin et al. (1998) [8], insulin resistance was evaluated by homeostasis model assessment of insulin resistance (HOMA-IR) according toMatthews et al., (1985) [9]. Also, AST and ALT were determined according to the method of Morgenstern et al. (1966) [10], total protein evaluated by Biuret reagent [11], albumin according to the protocol of Doumas et al. (1971) [12], and globulin levels were calculated according to Soltan (2009) [13].Urea was determined according to Fawcett and Scott, (1960) [14]. Serum total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) were determined according to Stein and Myers, (1987) [15].

At the end of the 2ndmonth, rabbits were sacrificed and eviscerated and the livers were harvested from the carcass and washed by phosphate buffered saline (PBS) solution, pH 7.4 containing 0.16 mg /ml heparin to remove any red blood clots. The tissue was homogenized in 5 ml cold PBS per gram tissue (1:5 dilution). All samples were centrifuged at 4000 rpm for 15 min at 4°C. The supernatants were collected and stored at -20°C until the biochemical analysis of catalase activity, malondialdehyde (MDA), and Total antioxidant capacity (TAC) levels. Total antioxidant capacity was determined by the colorimetric method described by (Koracevic et al., 2001), catalase enzyme was determined according to (Aebi, 1984), MDA was determined according to Ohkawa et al., (1979) [16]. SOD was determined according to Nishikimi et al., 1972 [17].

Liver samples of 30mg were placed and ground thoroughly with a mortar and pestle. Then the tissue powder was transferred immediately into a 1.5 ml microcentrifuge tube containing 300 ul of lysis buffer supplemented with β-mercaptoethanol and thoroughly mixed by vortex for 10 sec then the homogenate was submitted to the RNA purification protocol, cDNA synthesis and amplification protocol of catalase gene in presence of housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Table 2). The primer sequences of selected genes were designed with Primer3 (http://primer3.ut.ee/) and BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch).

Table 2: Nucleotide sequences of primers used in RT-PCR

Gene symbol	Gene description	Accession # GenBank	Sequences (5'->3')
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	NM_001082253	F: ATCTCGCTCCTGGAAGATGG R: CAAAGTGGATGTTGTCGCCA
SOD	Superoxide dismutase	NM_001082627	F: CCCGGTCTTTGTACTCTCGT R: AAGGATGAAGAGAGGCACGT

Purification of total mRNA was done by the kit of Thermo-Scientific Company [18]. Further, cDNA samples were synthesized by the kit of Thermo-Scientific Company. Amplification of the reverse transcription reaction product occurred by the kit manufactured by Thermo-Scientific Company.

2.8. Statistical analysis

Data was analyzed by one-way analysis of variance (ANOVA), with Duncan's multiple range tests for significant between means by SPSS software package v.20. A p value of less than 0.05 was considered statically significant.

3. Results

The data presented in Figure (1) and illustrated in Table (3) revealed the chemical composition of one sample of pomegranate peel (PP) was carried out using the GC–MS analysis led to the identification of twelve different components; phenol (33.86 %), oleic acid (14.07%), eugenol (9.55%), octadecenoic acid (8.08%), hexadecanoic acid (6.37%), caryophyllene (4.84%), dotriacontane(4.43), Phenol (5.06%), octadecenoic acid (4.41%), heptadecyne (2.45%), heptacosane (3.18%), pentadecanoic (1.82%), isochiapn (1.04), l.arginine (0.81%) and heptatriacotanole (0.65%).

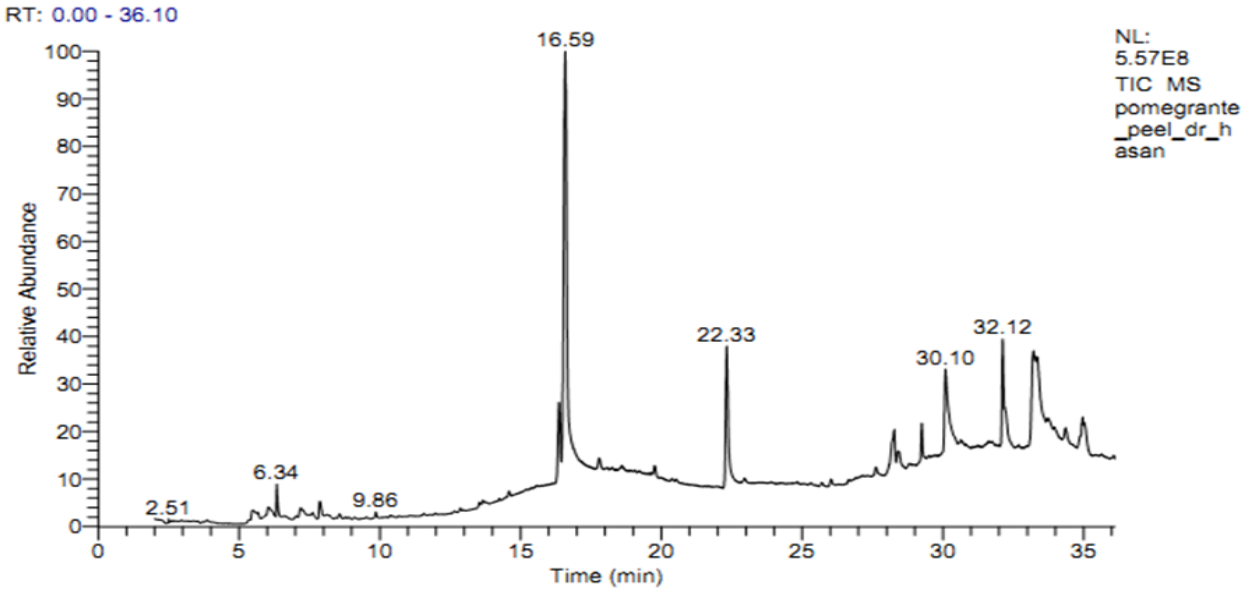

Figure 1: GC-MS chromatogram of PP methanolic extract

Table 3: Result of Gas chromatography–mass spectrometry (GC-MS) analysis of PP

No.	Compound name	RT (Minutes)	Area %	Molecular Formula
1	Phenol	16.58	33.68	$C_{10}H_{12}O_2$
2	Oleic acid	32.12	14.07	$C_{18}H_{34}O_2$
3	Eugenol	22.32	9.55	$C_{10}H_{12}O_3$
4	Octadecenoic acid	32.11	8.08	$C_{19}H_{36}O_2$
5	Hexadecanoic acid	30.09	6.37	$C_{16}H_{32}O_2$
6	Caryophyllene	16.37	4.84	$C_{15}H_{24}$
7	Dotriacontane	34.46	4.43	$C_{32}H_{66}$
8	Heptacosane	28.27	3.18	$C_{27}H_{56}$
9	Pentadecanoic	29.72	1.82	$C_{17}H_{34}O_2$
10	Isochiapn	34.36	1.04	$C_{19}H_{22}O_6$
11	6-Methyl-3-nitro-4-trifluoro methyl pyridone-2	17.8	0.81	$C_6H_{14}N_4O_2$
12	Heptatriacotanole	33.75	0.65	$C_{37}H_{76}O$

Injection of STZ resulted in significant increase in serum glucose level and HOMA-IR, while a significant decrease in insulin level in blood. Administration of diabetic rabbits with PP extract 1 and 2 % significantly decrease serum

glucose level and HOMA-IR level, while insulin level there is a significant increase. Administration of PP extract 2% significantly decrease the level of glucose and HOMA-IR and increase the level of insulin in blood than treated with PP extract 1% but did not reach to the control one Table (4).

Table 4: Effect of DM and PP 1 and 2% on serum glucose, insulin, and HOMA-IR between control and Diabetic group's blood of rabbits

Weeks	Group	Glucose (mg/dl)	Insulin (µU/mL)	HOMA-IR
		Mean±SE	Mean±SE	Mean±SE
2nd week	**Control**	94.33±4.81^{d}	12.25±0.03^{a}	2.84±0.14^{c}
	Diabetic	203.00±4.62^{a}	10.13±0.05^{d}	5.07±0.08^{a}
	PP 1%	168.67±8.09^{b}	11.14±0.04^{c}	4.63±0.21^{a}
	PP 2%	141.00±6.35^{c}	11.29±0.03^{b}	3.93±0.16^{b}
4th week	**Control**	93.67±5.46^{c}	12.20±0.02^{a}	2.82±0.16^{c}
	Diabetic	210.67±8.88^{a}	9.12±0.05^{d}	4.73±0.18^{a}
	PP 1%	149.00±6.43^{b}	10.84±0.14^{c}	4.01±0.13^{b}
	PP 2%	144.33±3.76^{b}	11.13±0.03^{b}	3.95±0.11^{b}
8th week	**Control**	93.67±5.21^{c}	12.28±0.02^{a}	2.84±0.15^{d}
	Diabetic	303.33±4.48^{a}	7.67±0.16^{d}	5.73±0.04^{a}
	PP 1%	193.00±5.29^{b}	10.30±0.08^{b}	4.89±0.10^{b}
	PP 2%	207.33±4.63^{b}	8.77±0.11^{c}	4.47±0.04^{c}

Means bearing different letters within the same column within each period are significant at (P<0.05)

Table (5) revealed the effect of PP 1 and 2% administration on kidney function and liver enzymes in serum of diabetic rabbit. Serum urea, creatinine, ALT, and AST activities were significantly increased (P<0.05) in diabetic control rabbits compared to normal control group. Moreover, treatment of diabetic animals with both PP 1 and 2.0% induced potential improvement (P<0.05) of these altered parameters. PP 2% seemed to be more effective than PP 1.0% in improving serum AST and ALT activities.

Table 5: Effect of PP 1 and 2% on kidney function (urea, creatinine) and liver enzyme of rabbit

Weeks	Group	Urea (mg/dl	Creatinine (mg/dl	ALT (U/L)	AST (U/L)
		Mean±SE	Mean±SE	Mean±SE	Mean±SE
2nd week	**Control**	21.00±1.15^{c}	0.84±0.04^{b}	25.00±2.08^{a}	27.67±2.40^{a}
	Diabetic	27.67±2.40^{ab}	1.10±0.11^{a}	30.33±0.88^{a}	33.33±2.33^{a}
	PP 1%	29.00±1.73^{a}	0.93±0.05^{ab}	27.00±2.31^{a}	30.67±1.20^{a}
	PP 2%	22.00±2.08^{bc}	0.84±0.05^{b}	27.67±1.45^{a}	29.00±1.15^{a}
4th week	**Control**	21.00±1.73^{c}	0.95±0.08^{b}	19.67±2.03^{c}	20.67±2.03^{b}
	Diabetic	36.67±2.03^{a}	1.69±0.08^{a}	50.33±2.03^{a}	66.33±5.46^{a}
	PP 1%	29.00±1.15^{b}	1.12±0.14^{b}	36.33±2.60^{b}	25.67±2.91^{b}
	PP 2%	23.67±2.40^{bc}	0.96±0.07^{b}	21.67±1.76^{c}	26.67±2.33^{b}
8th week	**Control**	24.33±3.53^{c}	0.83±0.05^{c}	23.33±3.18^{c}	26.67±2.33^{c}
	Diabetic	57.00±3.21^{a}	2.89±0.12^{a}	52.00±2.08^{a}	61.00±1.15^{a}
	PP 1%	47.67±2.40^{b}	2.74±0.04^{ab}	36.00±3.21^{b}	40.33±0.88^{b}
	PP 2%	39.00±1.73^{b}	2.54±0.06^{b}	32.00±1.73^{b}	37.00±3.06^{b}

Means bearing different letters within the same column within each period are significant at (P<0.05)

The levels of total protein and albumin were significantly decreased (P<0.05) in diabetic rabbits as compared with normal control animals. Administration of PP extract to diabetic rabbits significantly (P<0.05) improved the altered

levels of total protein and albumin; PP extract 2 % seemed to be more effective than PP extract 1 % in improving the levels of total protein and albumin. similarly, globulin levels and A/G ratio were significantly lower in experimentally diabetic rabbits compared to healthy control group all over the experimental period. Treatment of diabetic rabbits by PP extract significantly (P<0.05) elevated the altered levels of globulin and A/G ratio. PP extract 2 % seemed to be more effective than PP extract 1 % in improving the levels of levels of globulin and A/G ratio (Table 6).

Table 6: Effect of PP 1 and 2% on total protein, albumen, Globulin and Albumen/globulin ratio of rabbit liver

Weeks	Group	Protein(g/dl)	Albumen(g/dl)	Globulin (g/dl)	A/G ratio
		Mean±SE	Mean±SE	Mean±SE	Mean±SE
2nd week	Control	7.21±0.05^{a}	4.12±0.03^{a}	3.09±0.02^{a}	1.33±0.01^{b}
	Diabetic	5.8±0.06^{d}	3.32±0.03^{c}	2.57±0.05^{bc}	1.29±0.02^{b}
	PP 1%	6.26±0.05^{c}	3.87±0.03^{b}	2.39±0.08^{c}	1.62±0.06^{a}
	PP 2%	6.76±0.06^{b}	4.15±0.08^{a}	2.67±0.07^{b}	1.53±0.04^{a}
4th week	Control	7.21±0.04^{a}	4.17±0.03^{a}	3.04±0.02^{a}	1.37±0.01^{a}
	Diabetic	5.93±0.03^{d}	3.29±0.03^{d}	2.64±0.01^{d}	1.25±0.01^{c}
	PP 1%	6.34±0.02^{c}	3.64±0.03^{c}	2.71±0.01^{c}	1.34±0.02^{ab}
	PP 2%	6.62±0.04^{b}	3.77±0.03^{b}	2.85±0.01^{b}	1.32±0.01^{b}
8th week	Control	7.31±0.02^{a}	4.21±0.01^{a}	3.09±0.02^{a}	1.36±0.01^{c}
	Diabetic	5.53±0.03^{d}	3.21±0.01^{d}	2.65±0.34^{ab}	1.39±0.00^{c}
	PP 1%	5.83±0.03^{c}	3.69±0.01^{c}	2.14±0.02^{b}	1.73±0.01^{a}
	PP 2%	6.06±0.03^{b}	3.79±0.03^{b}	2.28±0.01^{b}	1.66±0.02^{b}

Means bearing different letters within the same column within each period are significant at (P<0.05).

The data summarized in Table (7) showed that injection of STZ resulted in significant increase in Ch, TG, LDL-C and VLDL-C compared with the control group, while administration of PP extract with 1 % resulted in decreasing in Ch , TG, LDL-C, and VLDL-C and increased in HDL-C compared with the diabetic group. In addition, administration of PP extract 2 % resulted in a decrease in Ch, TG, LDL-C and VLDL-C , increased in HDL-C compared with the diabetic group and group 1%.

Table 7: Effect of PP 1 and 2% on serum cholesterol level, Triglyceride level, HDL, LDL and VLDL level of rabbit

Weeks	Group	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL(mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
		Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE
2nd week	Control	115.67±4.37^{c}	97.67±5.81^{c}	40.08±0.09^{a}	56.02±3.28^{c}	19.53±1.16^{c}
	Diabetic	167.00±6.43^{a}	145.00±3.46^{a}	25.87±0.16^{d}	112.10±6.37^{a}	29.03±0.69^{a}
	PP 1%	143.00±4.36^{b}	126.00±2.52^{b}	31.78±0.27^{c}	89.67±2.84^{b}	24.53±0.55^{b}
	PP 2%	142.33±2.40^{b}	122.33±4.63^{b}	38.85±0.18^{b}	79.67±2.28^{b}	24.47±0.93^{b}
4th week	Control	140.00±2.31^{b}	116.67±3.38^{ab}	40.42±0.05^{a}	76.20±2.82^{c}	23.37±0.64^{ab}
	Diabetic	215.67±10.33^{a}	128.67±1.45^{a}	21.48±0.11^{d}	168.40±10.07^{a}	25.47±0.37^{a}
	PP 1%	154.67±2.73^{b}	105.00±6.11^{b}	30.00±0.23^{c}	103.70±4.15^{b}	21.00±1.22^{b}
	PP 2%	145.00±3.46^{b}	85.00±3.46^{c}	33.04±0.27^{b}	95.00±3.72^{bc}	17.00±0.69^{c}
8th week	Control	155.00±6.43^{c}	107.33±2.91^{d}	40.31±0.09^{a}	93.20±7.06^{c}	21.47±0.58^{c}
	Diabetic	253.00±5.29^{a}	205.33±5.90^{a}	19.98±0.08^{d}	192.00±4.11^{a}	40.53±1.57^{a}
	PP 1%	213.33±6.49^{b}	163.67±3.28^{b}	28.54±0.29^{c}	151.05±6.54^{b}	32.73±0.66^{b}
	PP 2%	205.33±6.49^{b}	149.67±3.76^{c}	31.04±0.04^{b}	144.63±6.23^{b}	29.97±0.75^{b}

Means bearing different letters within the same column within each period are significant at (P<0.05)

Lipid peroxidation in liver tissue homogenate of diabetic rabbits showed a highly significant (P<0.05) elevation in fasting state as compared with normal animals. The treatment of diabetic animals with PP-induced a potential improvement (P<0.05) of elevated values. The higher dose of PP 2.0% was more effective than the 1.0% (Table 8).

Table (8) and Fig (2) revealed a significant decrease (P<0.05) in catalase activities in STZ diabetic rabbits than the healthy control group. The STZ diabetic rabbits treated with PP 1.0 and 2.0% showed a significant increase (P<0.05) in catalase activity compared to the non-treated diabetic group. The higher dose of PP 2.0 % was more effective than 1.0%.

Table 8: Effect of PP 1 and 2% on antioxidant (Catalase, TAC, MDA and SOD) of rabbit blood

Group	Catalase (U/mg protein)	TAC (mM/g tissue)	MDA nM/g tissue)	SOD (U/mg protein)
	Mean±SE	**Mean±SE**	**Mean±SE**	**Mean±SE**
Control	4.29±0.08a	2.59±0.05a	64.33±2.03d	297.33±6.74a
Diabetic	0.44±0.04d	1.18±0.10b	124.67±2.03a	110.67±3.48c
PP 1%	1.81±0.09c	1.57±0.22b	86.33±3.18b	138.00±5.51b
PP 2%	2.91±0.19b	2.43±0.13a	77.67±2.60c	151.33±1.45b

Means bearing different letters within the same column are significant at (P<0.05)

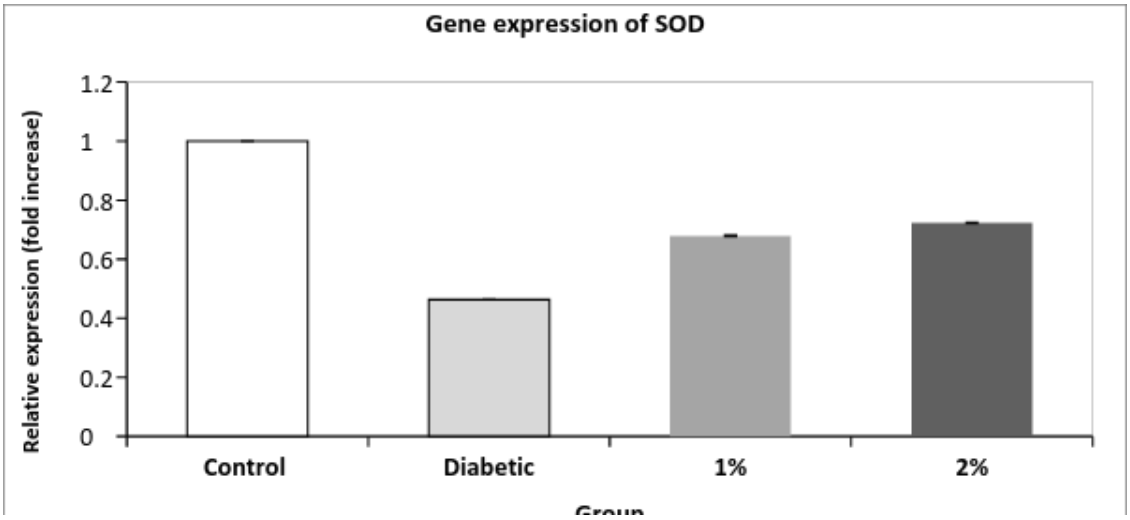

Figure 2: Mean values of gene expression of catalase between diabetic groups treated with PP 1 and 2% and control groups

Antioxidant capacity in liver tissue homogenate of diabetic rabbits showed a highly significant (P<0.05) decrease as compared with normal animals. The treatment of diabetic animals with PP extract induced significant increase (P<0.05) of total antioxidant capacity. The higher dose of PP 2.0% was more effective than 1.0%.

Lipid peroxidation (MDA) in diabetic rabbits showed a highly significant (P<0.05) increase as compared with normal animals. The treatment of diabetic rabbits with PP extract induced significant decrease (P<0.05) of MDA. The higher dose of pomegranate peel 2.0% was more effective than 1% in decreasing lipid peroxidation (Table 8).

Catalase and SOD activity in tissue homogenate of diabetic rabbits showed a highly significant (P<0.05) decrease as compared with normal animals (Table 8). The treatment of diabetic animals with PP extract induced significant increase (P<0.05) of SOD. The higher dose of PP 2.0% was more effective than 1.0%.

The obtained data in Figure (2) showed a significant decrease (P<0.05) of SOD gene expression levels in diabetic group than healthy control group. While diabetic group treated with PP extract especially 2.0% showed significant increase (P<0.05) of SOD gene expression levels as compared with non-treated diabetic group.

4. Discussion

Punica granatum is widely used a plant that has high nutritional value. This study assessed the effect of (PP) extract 1 and 2% administration on rabbits treated with STZ-induced DM.

The chemical composition of one sample of PP was carried out using the GC–MS analysis led to theidentification of different components; phenol, oleic acid, eugenol, octadecenoic acid, hexadecanoic acid, caryophyllene, dotriacontane, heptacosane, pentadecanoic , isochiapn, 6-Methyl-3-nitro-4-trifluoro methyl pyridone-2, and heptatriacotanole, respectively most of which had antioxidant activity.

After PP administration, glucose level was significantly decreased compared to diabetic rabbits; also, HOMA-IR was significantly decreased compared to diabetic rabbits. While insulin level decreased significantly in diabetic group II compared to control. PP extract 1 and 2% administration increased the level of insulin significantly ($P \leq 0.05$) in groups (III & IV) compared to the diabetic group. The obtained results were agreed with (Khalil, 2004)who reported that administration of PP extracts 0.43 g/kg BW associated with a reduction of serum glucose level in alloxan-induced diabetic rats. These results reflected that the potential antidiabetic effect of PP might occur through increased insulin secretion and decreased insulin resistance. A comparable studies of [19-23] revealed that olive leaves extract; green tea and vitamin D, respectively had glucose lowering effect in STZ-induced diabetic rats.

Urea, creatinine, ALT and AST revealed a significant increase in diabetic rabbits compared to control. Moreover, treatment of diabetic animals with both PP 1.0 and 2.0% induced potential improvement of these altered parameters. Our results in agreement with Ahmed and Ali (2010) [24] which reported that PP extract treatment reduced the elevated levels of serum urea and creatinine that are marker parameters of kidney toxicity. Al-Hussaini (2014) [25] found that animals treated with PP ethanolic extract at a dose of 200 mg/kg/day orally for 15 days showed a significant reduction in urea, creatinine, and ALT that were resulted from hepatocytes damage caused by ethanol.

The present results partially agreed with the results obtained by Osman et al., (2012) [26] who examined the antioxidant effect of PP and juice on DM-induced by alloxan in female Rats. The results showed that AST and ALT were significantly increased in the diabetic group, but after treatment with PP and juice, AST and ALT levels decreased and become near to the control level especially ALT value. This effect is due to the antioxidant content of PP and juice.

The injection of STZ resulted insevere liver damage reflected in decreased total protein, albumin, globulin and A/G ratio in diabetic rabbits as compared with normal control animals. These obtained data indicated the hepatocellular damage induced by STZ. The result comes in agreement with those obtained by Ramesh et al., (2007) [27]. Administration of PP extract to diabetic rabbits significantly ($P<0.05$) improved the altered levels of total protein, albumin, and globulin. these come in agreement with those obtained by Khan et al. (2015) who found that supplementation of lambs with PP in the diet at the level of (1, 2 and 4%) had a significant increase total protein, serum albumin, and serum globulin.

STZ significantly increased serum Triglyceride, cholesterol, LDL-C, and VLDL-c level and decreased HDL-c level in diabetic group compared to normal control one. The present findings come in accordance with those obtained by Kumar et al., (2013) [28] who reported that STZ induced diabetic rats had hyperlipidemia with significant elevation of TC, TGs, LDL-c, VLDL-c and AI and significantly decreased HDL-C when compared to normal control rats. The same authors reported that the hypercholesterolemia and hypertriglyceridemia are mostly found in DM due to lipid abnormalities. The level of TGs increased due to insulin deficiency result in failure to activate lipoprotein lipase causing hypertriglyceridemia.

The obtained results revealed that a significant decrease ($P<0.05$) in CAT, TAC, and SOD in STZ diabetic rabbits than the healthy control group. The STZ diabetic rabbits treated with PP 1.0 and 2.0 % showed a significant increase ($P<0.05$) in CAT, TAC, and SOD compared to the non-treated diabetic group. The treatment of diabetic rabbits with PP extract 2.0% was more effective in induced significant decrease ($P<0.05$) of MDA than PP extract 1.0 %. Our results come in agreement with those obtained by Moneim et al. (2011)that investigated the antioxidant properties of Pomegranate in hepatic and renal tissues of rats. They found that administration of Pomegranate juice and methanol extract of PP as 200 mg/kg for twenty-one days reduces MDA, also, authors found a significant increase in SOD, CAT, and TAC of rats received Pomegranate was observed. These findings demonstrated that pomegranate has a potent anti-oxidative effect.

The obtained results agreed with Osman et al., (2012) [26] who investigated the antioxidant effect of MEPP against oxidative damage in STZ-induced diabetic rats and revealed that using the peel extract for 4 weeks significantly

enhanced the activities of antioxidant enzymes in liver and kidney tissues and elevated the total serum of TAC. A comparable study of Taha, N. et al. (2013) [22-23] reported that lipoic acid impedes oxidative markers in liver of STZ-induced diabetes in rats.

The obtained data showed a significant decrease ($P<0.05$) of SOD gene expression levels in diabetic group than healthy control group, while the diabetic group treated with pomegranate peel extract especially 2.0% showed a significant increase ($P<0.05$) of SOD gene expression levels as compared with the non-treated diabetic group.

5. Conclusion

This study demonstrates that PP powder 1.0 and 2.0 % has anti-diabetic and antioxidant activity reflected in normalization of glycemic indices and antioxidant status due to the presence of biologically active antioxidant compounds. We recommend the introduction of PP in the regime of diabetic management.

Conflicts of interest

The authors declare no conflicts of interest.

References

1. Rahimi, R., Nikfar, S., Larijani, B., Abdollahi, M., 2005. A review on the role of antioxidants in the management of diabetes and its complications. Biomedicine & Pharmacotherapy 59(7), 365-373.
2. Sadek, K., Taha, N., Korshom, M., Mandour, A., 2013. THIAMINE AMELIORATE HEPATIC, RENAL DYSFUNCTION AND DYSLIPIDAEMIA IN DIABETIC RATS. Journal of current research in science 1(1), 35.
3. Belal, S.K., Abdel-Rahman, A.H., Mohamed, D.S., Osman, H.E.H., Hassan, N.A., 2009. Protective effect of pomegranate fruit juice against aeromonas hydrophila-induced intestinal histopathological changes in mice. World Appl Sci J 7(2), 245-254.
4. Guo, S., Deng, Q., Xiao, J., Xie, B., Sun, Z., 2007. Evaluation of antioxidant activity and preventing DNA damage effect of pomegranate extracts by chemiluminescence method. Journal of agricultural and food chemistry 55(8), 3134-3140.
5. Atanasovska, E., Tasic, V., Slaninka-Miceska, M., Alabakovska, S., Zafirov, D., Kostova, E., Pavlovska, K., Filipce, V., Labacevski, N., 2014. Six week followup of metabolic effects induced by a high-fat diet and streptozotocin in a rodent model of type 2 diabetes mellitus. Pril (Makedon Akad Nauk Umet Odd Med Nauki) 35, 169-179.
6. Samah, A., 2014. Effect of Ficus sycomorus L. Leaves on High Fat Diet-Fed RatsPossible Mechanisms behind the Prevention of Obesity and Its Related Disorders. . IOSR Journal of Environmental Science, Toxicology and Food Technolog 8(12), 7-16.
7. Trinder, P., 1969. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Annals of clinical Biochemistry 6(1), 24-27.
8. Sapin, R., Ongagna, J.-C., Gasser, F., Grucker, D., 1998. Insulin measurements in haemolysed serum: influence of insulinase inhibitors. Clinica chimica acta 274(1), 111-117.
9. Matthews, D., Hosker, J., Rudenski, A., Naylor, B., Treacher, D., Turner, R., 1985. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28(7), 412-419.
10. Morgenstern, S., Oklander, M., Auerbach, J., Kaufman, J., Klein, B., 1966. Automated determination of serum glutamic oxaloacetic transaminase. Clinical chemistry 12(2), 95-111.
11. Abdelbaset, A.E., Ellah, M.A., ElGhaffar, S.K.A., Sadiek, A.H., 2014. Acute-phase proteins in different pathological conditions at the lungs of buffaloes. Comparative Clinical Pathology 23(4), 823-828.
12. Doumas, B.T., Watson, W.A., Biggs, H.G., 1971. Albumin standards and the measurement of serum albumin with bromcresol green. Clinica chimica acta 31(1), 87-96.

13. Soltan, M., 2009. Influence of dietary glutamine supplementation on growth performance, small intestinal morphology, immune response and some blood parameters of broiler chickens. International Journal of Poultry Science 8(1), 60-68.
14. Fawcett, J., Scott, J., 1960. A rapid and precise method for the determination of urea. Journal of clinical pathology 13(2), 156-159.
15. Stein, E.A., Myers, G., 1987. Lipids, lipoproteins and apolipoproteins. Fundamentals of clinical chemistry 3, 478-479.
16. Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical biochemistry 95(2), 351-358.
17. Nishikimi, M., Rao, N.A., Yagi, K., 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochemical and biophysical research communications 46(2), 849-854.
18. Boom, R., Sol, C., Salimans, M., Jansen, C., Wertheim-van Dillen, P., Van der Noordaa, J., 1990. Rapid and simple method for purification of nucleic acids. Journal of clinical microbiology 28(3), 495-503.
19. Ghanema, I.I.A., Sadek, K.M., 2012. Olive leaves extract restored the antioxidant perturbations in red blood cells hemolysate in streptozotocin induced diabetic rats. World Academy of Science, Engineering and Technology, International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering 6(4), 124-130.
20. Sadek, K.M., Shaheen, H., 2014. Biochemical efficacy of vitamin D in ameliorating endocrine and metabolic disorders in diabetic rats. Pharmaceutical biology 52(5), 591-596.
21. Sadek, K., 2011. Hypoglycemic effect of green tea in experimentally induced diabetic rats. J. Pharmacol Toxicol 1, 7-9.
22. Taha, N., Korshom, M., Mandour, A., Sadek, K., 2013. Lipoic acid impedes markers of oxidative stress in the liver of streptozotocin-diabetic rats. Global Med. Plant Res. 1(2), 195-198.
23. Taha, N., Sadek, K., Korshom, M., Mandour, A., 2013. THE GREEN TEA IMPROVE THE OXIDATIVE STATUS IN STEREPTOZOTOCIN-INDUCED DIABETIC RATS. Journal of Current Research in Science 1(2), 79.
24. Ahmed, M.M., Ali, S.E., 2010. Protective effect of pomegranate peel ethanol extract against ferric nitrilotriacetate induced renal oxidative damage in rats. Journal of Cell and Molecular Biology 7(2), 35-43.
25. Al-Hussaini, J., 2014. Protective effect of Punica granatum peel extract against gastric mucosal erosions induced by ethanol in experimental rabbit models. Med. Sci 13(1).
26. Osman, H.F., Eshak, M.G., El-Sherbiny, E.M., Bayoumi, M.M., 2012. Biochemical and genetical evaluation of pomegranate impact on diabetes mellitus induced by alloxan in female rats. Life Sci J 9(3), 1543-1553.
27. Ramesh, B., Viswanathan, P., Pugalendi, K.V., 2007. Protective effect of Umbelliferone on membranous fatty acid composition in streptozotocin-induced diabetic rats. European journal of pharmacology 566(1), 231-239.
28. Kumar, V., Ahmed, D., Anwar, F., Ali, M., Mujeeb, M., 2013. Enhanced glycemic control, pancreas protective, antioxidant and hepatoprotective effects by umbelliferon-α-D-glucopyranosyl-(2 I→ 1 II)-α-D-glucopyranoside in streptozotocin induced diabetic rats. SpringerPlus 2(1), 639.