

Biochemical and Genetical Evaluation of Pomegranate Impact on Diabetes Mellitus Induced by Alloxan in Female Rats

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Abstract: Various food industries explored the possibility of developing a nutritional supplement rich in natural antioxidants from pomegranates. This study has focused on the ability of pomegranate peel and juice to study the antioxidant status. Thirty two rats were allocated in 4 groups as follows: Group I; control group without any treatment; Group II: diabetic animals injected with alloxan; Group III: diabetic peel group animals injected with alloxan and then feed on peel pomegranate; Group IV: diabetic juice group animals injected with alloxan and then gavage with pomegranate juice. After 4 weeks of treatment biochemical analysis were measured such as glucose, insulin, alpha-amylase, lipid profile (cholesterol, triglyceride HDL, LDL and total lipids), total protein, homocysteine, total antioxidant capacity and liver enzymes (AST&ALT). In addition, pancreas and liver tissues were separated for genetic analysis in which pancreatic tissues were used for RAPD-PCR analysis and liver tissues for DNA fragmentation assay. Results showed significant increase in glucose and alpha amylase levels in diabetic group, while insulin decreased. Peel and juice of pomegranate ameliorates this effect and decreased glucose, alpha amylase while insulin level increased. Cholesterol, triglycerides, LDL and total lipids increased while HDL decreased in diabetic group. Peel and juice of pomegranate prevented these changes. The more pronounced effect appeared in group III treated with peel pomegranate. Total protein was not affected by alloxan or pomegranate. Homocysteine was significantly increased while total antioxidant capacity decreased in diabetic group. After treatment by pomegranate peel and juice, these parameters become near to the control values. AST and ALT were significantly increased in diabetic group. But after treatment with peel and juice, AST and ALT levels decreased and become near to the control level especially ALT value. Furthermore, rate of DNA fragmentation and DNA band polymorphism increased significantly in diabetic group. While after treatment by peel and juice rate of DNA band polymorphism and DNA fragmentation were decreased significantly. Pomegranate peel and juice showed significant reduction in LDL oxidative susceptibility and an increase in total antioxidant status. Pomegranate is able to reduce the progression in atherosclerosis. The antioxidant content in foods decreased the oxidative stress related diseases. [Hala F. Osman, Mariam G. Eshak, Emad Mohamed El-Sherbiny, Mohamed M. Bayoumi, **Biochemical and Genetical Evaluation of Pomegranate Impact on Diabetes Mellitus Induced by Alloxan in Female Rats.** *Life Sci J* 2012;9(3):1543-1553] (ISSN:1097-8135). <http://www.lifesciencesite.com>. 225.

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1. Introduction

Diabetes is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. Hyperglycaemia is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body's systems (Rahimi *et al.*, 2005). Insulin stimulates muscle and fat cells to remove glucose from the blood and stimulates the liver to metabolize glucose, causing the blood sugar level to decrease to normal levels.

The burden of diabetes is increasing globally, particularly in developing countries. The causes are a complex, but are in large part due to rapid increases in overweight, obesity and physical inactivity (WHO, 2011).

A WHO fact sheet stated that, 346 million people worldwide have diabetes. In 2004, an

estimated 3.4 million people died from consequences of high blood sugar. More than 80% of diabetes deaths occur in low-and middle-income countries. WHO demonstrated that diabetes death will double between 2005 and 2030. The total number of persons with diagnosed and undiagnosed diabetes in Egypt will increase from 3.80 million in 2000 to 8.80 million by the year 2025 (Herman *et al.*, 1997).

Pomegranate (*Punica granatum*) is a small tree, belonging to the Punicaceae family. Pomegranate juice has become increasingly popular because of its important biological actions (Gil *et al.*, 2000 and Schubert *et al.*, 2002) including cardiovascular protection (Aviram *et al.*, 2002). Pomegranate juice has recently been demonstrated to improve lipid profiles in type II diabetic patients with hyperlipidemia (Esmailzadeh *et al.*, 2004).

Pomegranate has been known to possess considerable pharmacological properties because pomegranate peel is a rich source of antioxidants, especially polyphenols, such as ellagic acid, quercetin, anthocyanidins, punicalagin and many plant polyphenols (Guo *et al.*, 2007). It has been shown to act as anti-microbial, anti-viral, anti-cancer, potent anti-oxidant, anti-mutagenic (Aqil and Ahmad, 2007 and Lansky and Newman, 2007), antitumour (Afaq *et al.*, 2005), anti-diarrhoeal (Mathabe *et al.*, 2006), antifungal (Vasconcelos *et al.*, 2006) and anti-ulcer effects (Gharzouli *et al.*, 1999). Pomegranate has been used in the preparation of tinctures, juice, cosmetics and therapeutic formulae (Kim *et al.*, 2002). The pomegranate juice consumption has also shown to be effective for coronary heart disease (Fuhrman *et al.*, 2005) and chronic obstructive pulmonary disease (Cerdeira *et al.*, 2006). The presence of anti-oxidants has been reported from pomegranate in juice, peel, pulp and seed fractions (Li *et al.*, 2006).

For many years, scientists have been searching for clues in our genetic makeup that may explain why some people are more likely to get diabetes than others. "The Genetic Landscape of Diabetes" introduces some of the genes that have been suggested to play a role in the development of diabetes (Negi *et al.*, 2003).

Several extracts/constituents of *Punica granatum* have been found to prevent low-density lipoprotein oxidation and hence are anti-atherogenic (Wang *et al.*, 2004). Modulating the expression of oxidation-sensitive genes *in vitro* and in hypercholesterolemic mice (De Nigris *et al.*, 2005), as well as inhibiting the nuclear factor $\kappa\beta$, which is activated by reactive oxygen species (ROS) (Afaq *et al.*, 2005). Moreover, Guo *et al.* (2007) demonstrated *in vitro*, a powerful DNA damage prevention ability of *Punica granatum*.

Genetic toxicology tests are assays designed to detect direct or indirect genetic damage induced by chemical compounds. Fixation of DNA damage can result in gene mutations, loss of heterozygosity, chromosome loss or gain, and chromosome aberrations. These events may play an important role in many malignancies. Thus, identifying genotoxic/mutagenic effects is important for the risk/benefit assessment of substances, in particular those which are part of the dietary habits of any populations (Doppalapudi *et al.*, 2007).

Flowers of pomegranate have been used as an anti-diabetic medicine in Unani medicine and as a supplement in the diet therapy in many countries. The flowers can significantly lower the blood glucose level of type 2 diabetes animals with different possible mechanisms including enhancement of

mRNA expression, improvement of insulin receptor sensitivity, increment of peripheral glucose utilization, etc. Therefore, evaluation the protective role of several extracts from several parts of the pomegranate such as peel and fruit juice were studied in this work against alloxan induced diabetic female rats.

2. Materials and Methods

Animals:

Thirty-two female adult albino rats (*Rattus rattus*) weighting 270-290 g, were purchased from the animal house of National Research Center in Dokki, Egypt, and acclimated to the laboratory conditions for 2 weeks prior to the initiation of the experiment. Rats were maintained on a stock diet and tap water that were allowed *ad libitum*.

Chemical Treatments:

Alloxan was purchased from El-Gomhoriya Chemicals Company in Egypt and dissolved in saline for intraperitoneal injection as a single dose of 120 mg/kg body weight.

Fruit Preparation:

Pomegranate (*Punica granatum*) fruits were supplied from Agriculture Research Center, Giza, Egypt. It was made sure that it had no previous treatment with any pesticides. Peel was separated from seeds, washed by tap water, dried in sunlight then grinded to make powder (250 mg/kg b.w.). Seeds were mixed in an automatic mixer and filtered to obtain fresh juice (5ml/Kg b.w.).

Design of the Experiment:

Animals were divided into four groups as follows: GI-Control group: Normal control animals without any treatment, GII- Diabetic group: animals intraperitoneally injected with a single dose of 120 mg/kg b.w. of alloxan, GIII- Diabetic Peel group: animals intraperitoneally injected with a single dose of 120 mg/kg b.w. of alloxan then were fed on pomegranate peel powder (250 mg/kg b.w.) with the diet daily for 4 weeks, GIV- Diabetic Juice group: animals intraperitoneally injected with a single dose of 120 mg/kg b.w. of alloxan, then were gavage with pomegranate juice (5ml/Kg b.w.) daily each animal received for 4 weeks. Blood glucose level was measured 72 hours post-injection using one-touch glucometer, to confirm diabetes.

Biochemical Analysis:

At the end of the experiment, blood samples were withdrawn by cardiac puncture after anaesthetization of rats by ether. The blood was collected in the dried test tubes and then centrifuged at 3000 r.p.m. for 10 min. The collected sera were used for determination of glucose level according to Trinder (1969), insulin hormone level was evaluated by the solid phase radioimmunoassay (RIA) using ^{125}I

according to Sapin *et al.*, (1998). Alpha-amylase activity was measured according to the method described by Caraway (1959). Serum total cholesterol, high density lipoprotein (HDL) and low density lipoprotein (LDL) were determined according to Stein (1987) and triglycerides were evaluated according to Young (1990). Total lipids determination was carried out according to the method of Knight (1972). Total protein evaluated by Biuret reagent Tietz (1992). Homocystiene was estimated by the method of Vester and Rasmussen (1991). Total antioxidant capacity was determined by colorimetric method described by Koracevic (2001). AST and ALT were determined according to the method of Reitman and Frankel (1957). The pancreas and liver tissues were separated for genetic analysis.

Molecular analysis:

The genomic DNA was isolated using phenol/chloroform extraction and ethanol precipitation method with minor modifications (Sambrook *et al.*, 1989). The purity of the DNA preparation was determined by examining the absorbance ratio at 260 to 280 nm (Aquadro *et al.*, 1992).

I-RAPD-PCR analysis:

RAPD profiles were generated from rats DNA using oligodecamers (10-mer random primers) A and C kits from the Operon Technologies. DNA amplification reactions were performed under conditions reported by Williams *et al.* (1990) and Plotsky *et al.* (1995). PCR amplification was conducted in 25 μ l reaction volume containing 100 ng genomic DNA, 100 μ M dNTPs, 40 nM primer (Operon, Alameda, CA, USA), 2.5 units of Taq DNA polymerase and 5 μ l promega 10X Taq DNA polymerase buffer. The reactions were carried out in Thermocycler (Perkin-Elmer 9700) programmed with a first denaturation of 5 min at 94°C, followed by 45 cycles of 1 min at 94°C, 1 min at 36°C and 2 min at 72°C and finally, one cycle at 72°C for 5 min. The PCR product was analyzed by electrophoresing 15 μ l of the amplified mixture on agarose gel. The Gel-Pro Analyzer (Media Cybernetics) was used to document ethidium bromide DNA gels.

II-DNA fragmentation analysis:

Liver tissues of female rats were used to determine the quantitative profile of the DNA fragmentation using Diphenylamine reaction procedure. Briefly, liver samples were collected immediately after sacrificing the animals. The tissues were lysed in 0.5 ml of lysis buffer containing, 10 mM tris-HCl (pH 8),

1 mM EDTA, 0.2% triton X-100, centrifuged at 10 000 r.p.m. (Eppendorf) for 20 min at 4°C. The pellets were resuspended in 0.5 ml of lysis buffer. To the pellets (P) and the supernatants (S), 0.5 ml of 25% trichloroacetic acid (TCA) was added and incubated at 4°C for 24 hrs. The samples were centrifuged for 20 min at 10 000 r.p.m. (Eppendorf) at 4°C and the pellets were suspended in 80 ml of 5% TCA, followed by incubation at 83°C for 20 min. Subsequently, to each sample 160 ml of DPA solution [150 mg DPA in 10 ml glacial acetic acid, 150 ml of sulfuric acid and 50 ml acetaldehyde (16 mg:ml)] was added and incubated at room temperature for 24 hrs. The proportion of fragmented DNA was calculated from absorbance reading at 600 nm using the formula:

$$\% \text{ DNA fragmentation} = \frac{\text{OD}(s)}{\text{OD}(s) + \text{OD}(P)} \times 100$$

Statistical Analysis

All data were statistically analyzed as a one-way analysis of variance using the General Linear Model, SAS software (SAS Institute, 2004). Weight data were reported as least square means (LSM) \pm standard errors (SEM). Duncen, multiple range test was used to separate the means when significant differences exist. Statistical significance was set at 5% probability.

3. Results

As shown in table (1) Glucose level was significantly increased ($P \leq 0.05$) in diabetic group. After peel or juice administration, glucose level was significantly decreased ($P \leq 0.05$) compared to diabetic rats. The pronounced decrease in glucose level was appeared in group III compared to control group. While insulin level decreased significantly ($P \leq 0.05$) in group II compared to control. Pomegranate administration increased the level of insulin significantly ($P \leq 0.05$) in groups (III&IV) compared to diabetic group.

Table (1) obviously showed that the level of alpha amylase in group II was increased significantly ($P \leq 0.05$) compared to control. The activity of alpha amylase in group III and IV administered peel or juice of pomegranate was decreased compared to diabetic group and became close to control level. From the data represented in the table, an inhibition in alpha amylase activity was noticed in group IV more than that in group III.

Table (1): Serum Glucose, insulin and α -amylase in control rats (Gr.I), diabetic rats (Gr. II), diabetic rats treated with pomegranate peel (Gr. III) and diabetic rats treated with pomegranate juice (Gr. IV).

Parameters	Groups (each group contain 8 rats)			
	Gr.I Control	Gr.II Diabetic	Gr.III Diabetic+ Peel	Gr.IV Diabetic+juice
Glucose (mg/dl) Mean \pm S.E.	94.52 \pm 4.83 ^d	227.80 \pm 7.72 ^a	114.18 \pm 1.44 ^c	164.38 \pm 6.65 ^b
Insulin (mIU/ml) Mean \pm S.E.	4.46 \pm 0.28 ^a	2.10 \pm 0.19 ^c	3.96 \pm 0.28 ^{ab}	3.54 \pm 0.26 ^b
α-Amylase (U/L) Mean \pm S.E.	637 \pm 15.40 ^a	678.75 \pm 15.68 ^a	550.62 \pm 15.89 ^b	496.25 \pm 13.08 ^c

Data are represented as Mean \pm SE.

Means in the same raw followed by different superscripts are significantly different ($P \leq 0.05$).

Table (2): Serum cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol and total lipids in control rats (Gr.I), diabetic rats (Gr. II), diabetic rats treated with pomegranate peel (Gr. III) and diabetic rats treated with pomegranate juice (Gr. IV).

Parameters	Groups (each group contain 8 rats)			
	Gr.I Control	Gr.II Diabetic	Gr.III Diabetic+Peel	Gr.IV Diabetic+ juice
Cholesterol (mg/dl) Mean \pm S.E.	99.03 \pm 2.38 ^c	162.43 \pm 9.03 ^a	135.94 \pm 6.59 ^b	138.03 \pm 6.61 ^b
Triglycerides (mg/dl) Mean \pm S.E.	108.63 \pm 5.50 ^b	141.42 \pm 5.19 ^a	110.78 \pm 6.49 ^b	112.75 \pm 6.52 ^b
HDL-cholesterol (mg/dl) Mean \pm S.E.	46.25 \pm 2.49 ^a	29.68 \pm 1.12 ^c	36.45 \pm 1.86 ^b	31.62 \pm 1.10 ^{bc}
LDL-cholesterol (mg/dl) Mean \pm S.E.	30.45 \pm 1.74 ^c	106.58 \pm 5.89 ^a	78.71 \pm 3.31 ^b	85.96 \pm 4.42 ^b
Total lipids (mg/dl) Mean \pm S.E.	406.25 \pm 5.97 ^c	573.12 \pm 16.78 ^a	464.5 \pm 12.22 ^b	474.25 \pm 13.01 ^b

Data are represented as Mean \pm SE.

Means in the same raw followed by different superscripts are significantly different ($P \leq 0.05$).

As shown in table (2) Cholesterol and triglycerides levels increased significantly ($P \leq 0.05$) in diabetic groups. Cholesterol level decreased significantly ($P \leq 0.05$) in groups (III&IV) treated with pomegranate peel and juice comparing to group (II), but still increased and did not approach the value of control. While triglycerides levels decreased significantly ($P \leq 0.05$) in groups (III&IV) compared to diabetic group and levels approached that of the control group.

HDL-cholesterol level was significantly decreased ($P \leq 0.05$) in comparison of all groups to control. HDL -cholesterol was remarkably higher in group III ($P \leq 0.05$) compared to diabetic group. In

group IV, HDL-cholesterol was not significantly changed. Whereas LDL-cholesterol level increased significantly ($P \leq 0.05$) in comparison the groups with control. After pomegranate peel and juice administration, LDL-cholesterol levels were decreased in groups III & IV ($P \leq 0.05$) compared to diabetic group.

Total lipids were increased significantly ($P \leq 0.05$) in diabetic group compared to control. Administration of pomegranate peel and juice decreased these effects. The level of total lipids in groups III & IV decreased significantly ($P \leq 0.05$) than the diabetic group, but it was still higher than the control.

Table (3): Serum total protein, homocystiene and total anti-oxidant capacity (TAC) in control rats (Gr.I), diabetic rats (Gr. II), diabetic rats treated with pomegranate peel (Gr. III) and diabetic rats treated with pomegranate juice (Gr. IV).

Parameters	Groups (each group contain 8 rats)			
	Gr.I Control	Gr.II Diabetic	Gr.III Diabetic+Peel	Gr.IV Diabetic+Juice
Total protein (g/dl) Mean ± S.E.	6.57± 0.28 ^a	6.80± 0.32 ^a	7.15± 0.45 ^a	6.67± 0.15 ^a
Homocystiene (µmol/L) Mean ± S.E.	9.83± 0.32 ^c	14.68± 0.54 ^a	10.49± 0.43 ^c	11.92± 0.53 ^b
TAC (µmol/L) Mean ± S.E.	1.18 ± 0.05 ^a	0.74 ± 0.03 ^c	0.99± 0.05 ^b	0.96 ± 0.04 ^b

Data are represented as Mean ± SE.

Means in the same raw followed by different superscripts are significantly different ($P \leq 0.05$).

As shown in table (3) Total protein was not significantly affected ($P > 0.05$) by any of the treatments. Homocystiene level showed significant increase ($P \leq 0.05$) in diabetic rats group compared to control. Whereas pomegranate peel administration decreased the level of homocysteine significantly ($P \leq 0.05$), as well as juice administration decreased homocysteine level significantly ($P \leq 0.05$) compared to diabetic group. The pronounced effect of

pomegranate peel administration was much higher than that of juice.

Total antioxidant capacity (TAC) was significantly decreased ($P \leq 0.05$) in group II compared to control and improved significantly increase in groups III & IV ($P \leq 0.05$). A powerful effect of pomegranate was observed in both groups III and IV ($P \leq 0.05$) compared to diabetic one. However the TAC levels of both these groups did not return to the control level.

Table (4): Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in control rats (Gr.I), diabetic rats (Gr. II), diabetic rats treated with pomegranate peel (Gr. III) and diabetic rats treated with pomegranate juice (Gr. IV).

Parameters	Groups (each group contain 8 rats)			
	Gr.I Control	Gr.II Diabetic	Gr.III Diabetic+Peel	Gr.IV Diaetic+juice
ALT (U/I) Mean ± S.E.	71.25± 2.54 ^c	135.62± 3.66 ^a	79.75± 2.58 ^c	90.00± 3.41 ^b
AST (U/I) Mean ± S.E.	245.12± 4.72 ^c	388.5± 12.93 ^a	281.87± 8.84 ^b	282.50± 8.78 ^b

Data are represented as Mean ± SE.

Means in the same raw followed by different superscripts are significantly different ($P \leq 0.05$).

As shown in table (4) ALT and AST revealed significant increase ($P \leq 0.05$) in diabetic groups compared to control. Administration of pomegranate peel and juice showed significant decrease ($P \leq 0.05$) in ALT & AST activities compared to diabetic group. ALT activity in group (III) became close to that of the control value.

I -RAPD fingerprinting assay

The molecular genetic variability among the treated rats genomes and their controls were evaluated using 2 random primer kits (A and C).

Only four of these primers (10-mer random primers, Table 5) gave positive and detectable bands (Figure1).

These primers provided a total of 218 different bands with an average of 20.2±0.9 bands per primer (Table 5). Nearly the same results were obtained when the PCR assay was performed for each sample within each group (5 animals).

Untreated control group did not reveal damage on the DNA compared with other groups. Where, most of the bands (270 bands, 87%) resulted from all primers (A02, A03, A05 and C03) were

monomorphic for the control and alloxan treated animals (Figure 1).

However, most of the DNA of the samples treated with alloxan revealed polymorphic bands including the appearance of new bands or the loss of

some of the bands, which did not appear in the DNA samples of control rats (Figure 1). These new bands could be considered as “genus diagnostic” markers which are attributed to the alloxan treatment.

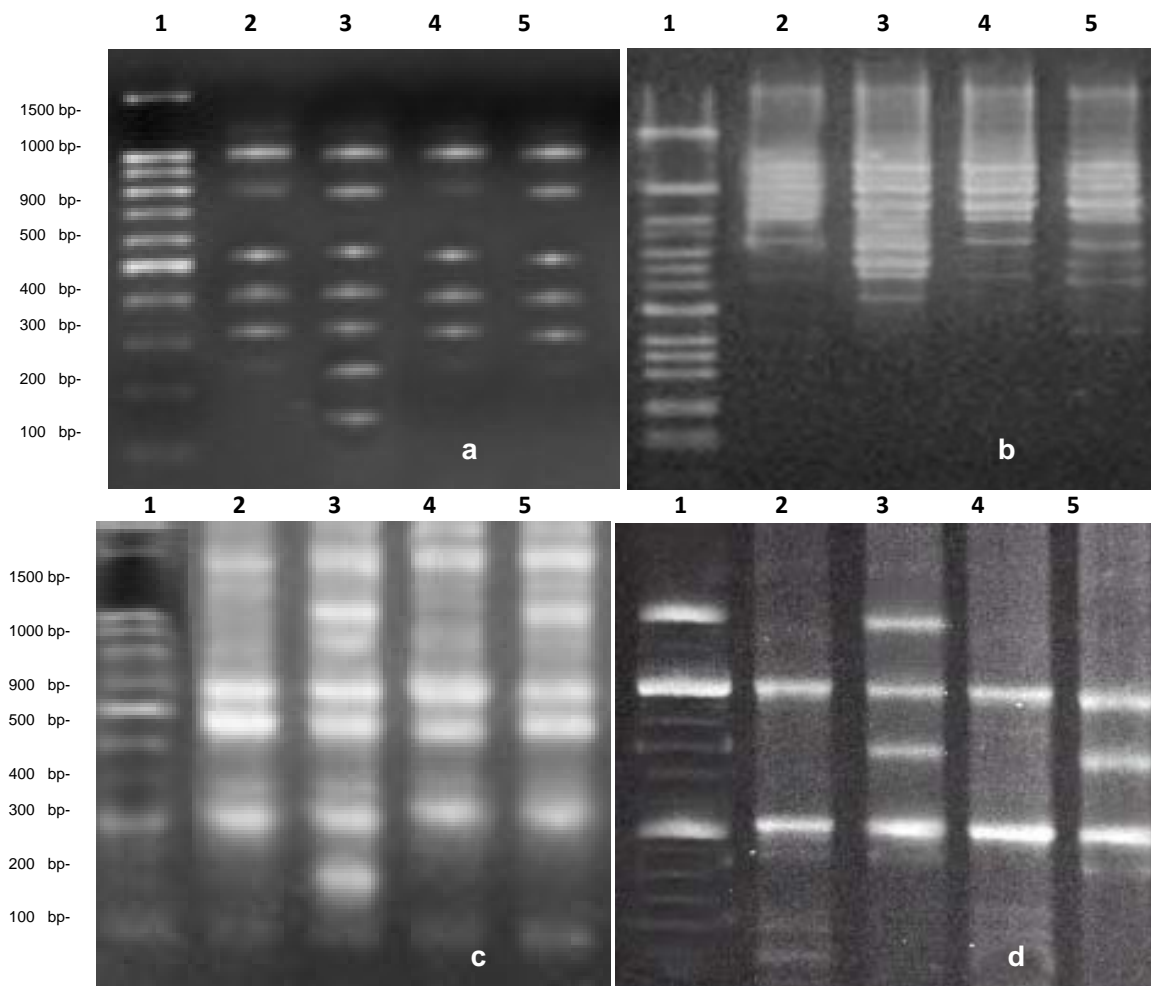


Figure 1: Comparison of RAPD fingerprinting profiles of different female rat genomic DNA treated with alloxan and pomegranate extracts (peel and juice). a) Represents PCR products with primer A02; b) represents PCR products with primer A03; c) represents PCR products with primer A05; and d) represents PCR products with primer C03. The DNA marker was in lanes 1. Lane 2 represents PCR products of untreated control samples; lane 3 represents rats treated with alloxan; lane 4 represents rat treated with alloxan plus pomegranate peel and lane 5 represents rats treated with alloxan plus pomegranate juice.

Table (5): Sequence of primers employed.

Primer	Sequence
A02	5'-TGCCGAGCTG-3'
A03	5'-AGTCAGCCAC-3'
A05	5'-AGGGGTCTTG-3'
C03	5'-GGGGTCTTT-3'

On the other hand, treatment with pomegranate peel after alloxan was able to inhibit the damage caused by alloxan treatment. Moreover,

treatment with pomegranate juice did slightly inhibit the DNA damage attributed to alloxan treatment. However, the effect of pomegranate juice in

decreasing the DNA damage was less than that of the pomegranate peel (Figure 1).

II-DNA fragmentation

The evaluation of the protective effect of pomegranate extracts (peel and juice) against alloxan induced diabetic in female rats was investigated by quantitative DNA fragmentation analysis. The DNA damage was examined in liver tissues collected from

diabetic rats using diphenylamine reaction procedure (Figure 2 and Table 6). The results revealed that DNA damage in untreated control rats was lower than all other treated groups. However, rate of DNA fragmentation in female rats treated with alloxan induced diabetic was significantly higher than control rats (Figure 2 & Table 6).

Table (6): Rate of DNA fragmentation in liver tissues of female rat treated with alloxan and pomegranate extracts (peel and juice).

Groups	Rate of DNA fragmentation
I) Control	9.8±0.2 ^b
II) Diabetic	17.8±0.4 ^a
III) Diabetic+ Pomegranate peel	11.2±0.4 ^b
IV) Diabetic+ Pomegranate juice	14.9±0.3 ^{ab}

Data are represented as Mean ± SE.

Means in the same row followed by different superscripts are significantly different ($P \leq 0.05$).

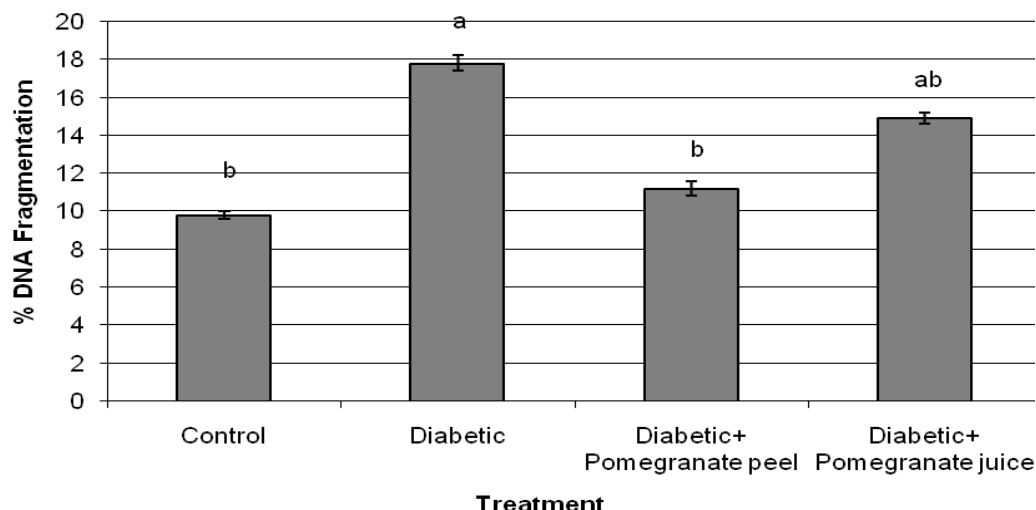


Figure 2. DNA fragmentation in liver tissues of female rats treated with alloxan and pomegranate extracts (peel and juice) analyzed by diphenylamine reaction procedure. Results are expressed as mean ± SEM of data from at least five samples, $P \leq 0.05$.

As shown in Fig. (2) and Table (6) treatment of alloxan-treated female rats with pomegranate peel decreased significantly the rate of DNA fragmentation compared with alloxan-treated female rats. Furthermore, treatment of alloxan-treated female rats with pomegranate juice slightly decreased the rate of DNA fragmentation compared with alloxan-treated female rats; however, this reduction was not significantly different.

4. Discussion

Punica granatum is widely used plant that has high nutritional value. This study assessed the effect of pomegranate peel and juice consumption on

rats treated with alloxan-induced diabetes mellitus. The obtained results are in accordance with data of Jafri *et al.* (2000) who found that oral administration of aqueous ethanolic extracts of *Punica granatum* flowers led to significant blood glucose lowering effect in normal, glucose-fed hyperglycaemic and alloxan-induced diabetic rats. In the study of Prashanth *et al.* (2001) the ethanolic extracts of *Punica granatum* was tested for their effect on alpha-amylase activity. It was found to exhibit interesting alpha-amylase inhibitory activity. Also Enas and Khalil, (2004) found that the diabetic rats treated with aqueous peel of pomegranate for 4 weeks displayed significantly lowered blood glucose level and

augmentation in insulin level. The action of alloxan is due to that alloxan induced free radical damage. *Punica grantum* peel possesses strong antioxidant property (Chidamabara *et al.*, 2004) and can act as free radical scavenger and protect cells from damage. Also it may increase insulin receptors. Thus, pomegranate can reduce blood glucose through regeneration of the cells. Parmar and Kar, (2007) found that peel of *Punica granatum* normalized all the adverse changes induced by alloxan. The dose of alloxan increased serum level of glucose and alpha amylase and decrease insulin level. Subsequent phytochemical analysis indicated that the high content of total polyphenols in the peel might be related to the antioxidant and antiperoxidative effects of the test peel. Also Joanne *et al.* (2011) found the same results for peel of *Punica granatum* in glucose, insulin and alpha-amylase.

The results of the current study are in agreement with that of Esmailzadeh *et al.* (2004), who found significant reductions were recorded in total cholesterol, LDL-cholesterol, whereas no change in HDL-cholesterol was noticed. It is concluded that concentrated pomegranate juice consumption may modify the risk factors in hyperlipidemic patients and its inclusion. Esmailzadeh *et al.* (2006) reported the same effect, whereas triglycerides were not changed in diabetic persons treated with concentrated pomegranate juice. Pomegranate juice was able to reduce the progression of atherosclerosis (De-Nigris *et al.*, 2005).

Moreover, a study of Bagri *et al.* (2009) found that the administration of pomegranate aqueous extract at doses of 250 mg/kg and 500 mg/kg for 21 days resulted in a significant reduction in fasting blood glucose, cholesterol, triglycerides and LDL-cholesterol in comparison with a diabetic group induced by streptozotocin. The results suggest that pomegranate could be used, as a dietary supplement, in the treatment of chronic diseases characterized by atherogenous lipoprotein profile, aggravated antioxidant status and impaired glucose metabolism and also in their prevention.

Also Mirimiran *et al.* (2010) reported that pomegranate seed oil (PSO) v. placebo adjusted the lipid profile for base line values. Serum cholesterol, LDL-cholesterol and glucose concentrations variables remained unchanged. It was concluded that administration of PSO for 4 weeks in hyperlipidaemic subjects had favorable effects on lipid profiles. Fenercioglu *et al.* (2010) indicated that the polyphenol antioxidant supplement containing pomegranate extract has important antagonizing effect on oxidative stress and lipid peroxidation in patients with type 2 diabetes mellitus and might be beneficial in preventing cardiovascular

complications. They showed a decrease in LDL and an increase in HDL.

On the other hand Changrani *et al.* (2006) reported that the concentration of total protein decreased significantly in case of diabetes. While Duman *et al.*, 2009 found that the incorporation of pomegranate extract rich in polyphenols did not influence the total protein.

Jacobs *et al.* (1998) found that an elevation in the concentration of total homocysteine is known to be an independent risk factor for the development of vascular disease. Alterations in homocysteine metabolism have also been observed in diabetic patients. Patients with two types of diabetes who have signs of renal dysfunction tend to exhibit elevated total homocysteine levels. Elias and Eng (2004) recorded that plasma homocysteine levels have been elevated in patients with diabetes. The plasma concentration of homocysteine in patients with diabetes is further confounded by the use of medication used to treat the disease and by the development of renal impairment. While Gursu (2001) found that Homocysteine level experimentally induced insulin-dependent diabetes mellitus is decreased. The insulin increases activities of enzymes transsulfuration and remethylation.

Our results are in agreement with Osama *et al.* (2010) who investigated the antioxidant effect of *Punica granatum* peel methanolic extract against oxidative damage in streptozotocin-induced diabetic rats. The results 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 antioxidant capacity. Kaur *et al.* (2006) found that pretreatment with pomegranate flower extract afforded up to 60% protection against hepatic lipid peroxidation. They found an inhibition in the modulation of liver markers (AST and ALT). These results indicated that pomegranate flowers possess a potent antioxidant and hepatoprotective properties. Toklu, *et al.* (2007) studied the effect of chronic administration of pomegranate peel extract on liver fibrosis induced by bile duct ligation. They showed that the elevated AST and ALT were significantly decreased after treatment. Thus chronic pomegranate peel extract treatment, with its antioxidant and antifibrotic properties may be of potential therapeutic value in protecting the liver from fibrosis and oxidative injury.

The concept that environmental chemical exposure could induce DNA damage has led to the introduction of requirements for testing mutagenic properties of new and/or frequently consumed substances, especially food. However, it has been documented, in the literature, that antioxidant intake can reduce cancer risk and may also mitigate the

effects of oxidative DNA damage (Watters *et al.*, 2007). In the current work evaluation the protective effect of the pomegranate peel and juice against alloxan induced diabetic was studied. The results revealed that pomegranate peel was more potent to inhibit the DNA fragmentation and DNA damage than pomegranate juice. These results are in accordance with the findings of Guo *et al.* (2007), which demonstrated *in vitro* that besides scavenging free radicals and reactive oxygen species (ROS), *Punica granatum* also prevents DNA damage. Therefore, our results confirm and extend our knowledge on the ability of *Punica granatum* to protect DNA in rats.

In recent years, attention has been focused on the antioxidant properties elicited by plants or food against ROS, lipid peroxidation, protein damage, and DNA strand breaking. Several plants can positively modulate biological systems against damaging effects produced by active oxygen species by several means, including free radical scavengers and enzymes such as superoxide dismutase (SOD) and catalase (CAT) (Srinivasan *et al.*, 2007).

The antioxidant activities of *Punica granatum* are associated with different bioactive components, mainly polyphenols, ellagitannins, condensed tannins, and anthocyanins (Noda *et al.*, 2002; Negi *et al.*, 2003 and Li *et al.*, 2006). In this regard, Watters *et al.* (2007) showed that polyphenols present in pomegranate protect neonatal mouse brain against hypoxic-ischemic injury. Moreover, Kaur *et al.* (2006) demonstrated that *Punica granatum* extract afforded up to 60% protection against hepatic lipid peroxidation due to the maintenance of the glutathione levels and activities of CAT, glutathione peroxidase, glutathione reductase, and glutathione-S-transferase.

Although the biochemical mechanisms underlying Pomegranate peel and Pomegranate juice activities are not yet clear, our results demonstrated that *Punica granatum* has an *in vivo* preventive effect against DNA fragmentation and/or damage due to alloxan, probably due to its free radical scavenging capability.

Conclusion: This study demonstrates that *Punica granatum* has a preventive effect against alloxan induced biochemical alterations and DNA fragmentation and damage in female rats. We suggest that the antioxidant content in *Punica granatum* extracts which decreased the oxidative stress related diseases may be due to its free radical scavenging capability. A little attention has been paid to pomegranate health promoting values.

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