Al-Azhar Med. J. *Vol. 46(4), October, 2017, 965-979*
DOI: 10.12816/0045179

EFFECTS OF POMEGRANATE (PUNICA GRANATUM L.) FRESH JUICE AND PEEL EXTRACT ON DIABETIC MALE ALBINO RATS

By

Nageh Mabrouk Gabr

Department of Medical Physiology, Faculty of Medicine,
Al-Azhar University, Cairo, Egypt

ABSTRACT

Background: Pomegranate is used in traditional medicine for its therapeutic properties.

Objective: Evaluation of the effectiveness of pomegranate fresh juice and peel extract on diabetes mellitus.

Material and Methods: Sixty adult male albino rats of local strain were distributed randomly into 6 equal groups: normal control group, control group received Pomegranate fresh juice (500 mg/kg body weight per rat orally), control group received Pomegranate peel extract (500 mg/kg body weight per rat orally), diabetic group received alloxan (140 mg/kg body weight intraperitoneally), diabetic group received Pomegranate fresh juice (500 mg/kg body weight per rat orally), and diabetic group received Pomegranate peel extract (500 mg /kg body weight per rat orally).

Results: Oral administration of Pomegranate juice and peel extract to diabetic rats for 4 weeks significantly decreased serum levels of TC, TG, low density lipoproteins cholesterol (LDL-c), and liver enzymes when compared to the control group. Levels of high density lipoprotein cholesterol (HDL-c) and antioxidant enzymes significantly increased as compared to the control group. Histopathological examination of liver and pancreas of Pomegranate juice-treated groups showed amelioration of histological changes caused by high level of cholesterol in diabetic group. These results were more prominent by Pomegranate fresh juice than peel extract.

Conclusion: Pomegranate has antioxidant and lipid lowering effects and improves the health of pancreatic islets of Langerhans in diabetic rats.

Key words: Pomegranate, alloxan, diabetogenic, antiatherogenic, antioxidants, dyslipidemia.

INTRODUCTION

Many metabolic disturbances accompany diabetes mellitus including hyperglycemia, hyperlipidemia, relative or absolute deficiency of insulin, and increased oxidative stress. These abnormalities represent the backbone in the pathogenesis of diabetic complications **(American Diabetes Association, 2016).**

Pomegranate (Punica granatum L.) is a member of the family of Punicaceae, one of the most ancient edible fruits and they are widely grown in Mediterranean regions including Iran, Egypt, Iraq and India, but sparsely cultivated in the USA, China, Japan and Russia **(Matthaeus and Ozcan, 2016).** The Pomegranate fruit has valuable compounds in different parts of

the fruit. These parts can be divided into several anatomical origins: peel, seeds, and arils **(Zhang et al., 2010).**

An important product obtained from Pomegranate fruit is the juice that can be obtained from arils or from whole fruit. The edible part of Pomegranate fruit represents 52% of total fruit weight, comprising 78% juice and 22% seeds. **(Amri et al., 20017).** Both pomegranate juice and Pomegranate peel extract (PPE) are rich in bioactive compounds such as polyphenols, anthocyanidins, tannic acid, gallic acid, and ellagic acid that exert anti-oxidant activities and prevent oxidative stress **(Faghihimani et al., 2017).**

Dietary intake of antioxidants can inhibit or delay the oxidation of susceptible cellular substrates so prevent oxidative stress. Therefore, it is important to enrich our diet with antioxidants to protect against many chronic diseases **(Rouhi et al., 2017).**

The aim of the present work was to evaluate the effects of Pomegranate fresh juice and peel extract on diabetic adult male albino rats.

MATERIALS AND METHODS

- Pomegranate fresh juice: The fruits of fresh Pomegranate were washed and manually peeled, without separating the seeds. Pomegranate juice was obtained using a commercial blender (Moulinex) and filtrated with a Buchner funnel to remove water insoluble materials and immediately diluted with distilled water to volume of (1:10 water/volume). The juice was stored at -20°C for further use in a dose of 500 mg/kg body weight per rat by nasogastric tube **(Al-Olayan et al., 2014).**

- Pomegranate peel extraction (PPE): Pomegranate peels were separated from the fruit manually and were cut into small pieces (2 cm×2 cm). The cut pieces were dried for 5 days. The dried samples were ground into fine powder by a grinder (Moulinex). 500 grams of pomegranate peel powders were separately extracted in water (1:10 water/volume). PPE were stored in a deep freezer (-18 °C) until use and administered orally at a dose of 500 mg/kg body weight per rat by nasogastric tube **(Mesgari et al., 2016).**

- Animals: This experimental study was performed at Medical Physiology department, Al-Azhar Faculty of medicine, Cairo. A total of sixty adult male albino rats of local strains were used in this study ranging in weight from 155-170 grams at the time of the research. The animals were housed under similar standard environmental conditions in suitable cages (20 x 32 x 20 cm for every 3 rats) with wide meshed raised floors to prevent coprophagia. They were kept ten days on basal diet before starting experimental diet for adaptation. They were also kept at room temperature and normal light/dark cycle. Animals were divided randomly and equally into 6 groups as follows:

Group I (Control Group): Rats fed on normal standard rat chow with free water supply and served as a control group.

Group II (Pomegranate fresh juice - treated group): Rats received 500 mg /kg body weight per rat for 4 weeks orally.

Group III (Pomegranate peel extract - treated group): Rats received 500 mg /kg body weight per rat for 4 weeks orally.

Group IV (Alloxan-treated diabetic control Group): Rats were subjected to induction of diabetes by a single intraperitoneal injection of alloxan (140 mg/kg body weight) in normal saline and fed on normal standard rat chow diet.

Group V (Diabetic pomegranate fresh juice-treated group): Rats were subjected to induction of diabetes and received pomegranate fresh juice 500 mg /kg body weight per rat for 4 weeks orally.

Group VI (Diabetic Pomegranate peel extract -treated group): Rats were subjected to induction of diabetes and received 500 mg /kg body weight per rat for 4 weeks orally.

- Determination of body weight gain percentage (BWG %): The biological values of diets were assessed by the determination of body weight gain percent (BWG %) which was calculated at the end of the experimental period. It was calculated using the equation of **Lei et al. (2007)**:

$$\frac{\text{Final body weight} - \text{Initial body weight}}{\text{Initial body weight}} \times 100.$$

- Induction of diabetes mellitus: Alloxan (Sigma Pharmaceuticals Company) was dissolved in 0.9% NaCl and injected intraperitoneally at a dose of 140 mg/kg body weight after overnight fasting. Just before alloxan injection, 2 ml of glucose (5%) were given orally. Blood samples was collected on the 3rd day ensure production of diabetes. Rats with blood sugar higher than 200 mg/dl were considered diabetic **(Ezazul et al., 2012).**

- Collection of Blood Samples: Blood was collected (4 ml of blood each) from the retro-orbital plexus using heparinized capillary tube (0.75 - 1.0 mm internal diameter) inserted in the medial canthus. To obtain serum, the blood was collected into a dry clean graduated glass centrifuge tube. It was rapidly set to centrifuge at 3000 r.p.m. for 15 minutes. About half of the supernatant serum was sucked out into Eppendorf tubes and stored frozen at -20?C **(Margoni et al., 2011).**

- Histopathological studies: At the end of the 4th week and under ether anesthesia, abdomen of the animal was opened after reaching the stage of surgical anesthesia, as evident by loss of withdrawal reflex. Pancreas and the liver were excised for histopathological studies. Specimens from both tissues were taken immediately and stained with Hematoxylin and eosin (H and E) and examined microscopically according to **William (2010).**

- Statistical Analysis: Data input and analysis were done using SPSS computer program. All results were expressed as the mean ± standard deviation (SD). Mean values of the different groups were compared using a one way analysis of variance (ANOVA). Least significant difference (LSD) post hoc analysis was used to identify significantly different mean values. *P* Value < 0.05 was accepted to denote a significant difference**.**

RESULTS

In this study, body weight gain percentage (BWG %) was 13.29 ± 1.15, 13.30 ± 1.15, 13.31 ± 1.15, 28.16 ± 1.30, 16.76 ± 1.9, and 17.01 ± 3.05 in groups I, II, III, IV, V and VI respectively. Groups II and III showed insignificant changes in respect to the control group I. There was a significant increase in this parameter in

diabetic group IV when compared with the control group. Pomegranate (fresh juice and peel extract)-treated groups showed a significant decrease in body weight gain percent (BWG %) in relation to group IV. There were no significant differences in body weight between group II and III when compared to group I. there were no significant changes also between group V and VI when compared with diabetic group (Table 1).

The mean ± standard deviation of blood glucose was 95.3 ± 8.9, 92.3 ± 11.47, 90.35 ± 11.3, 379.2 ± 23.9, 287.4 ± 12.58 and 293.9 ± 19.4 mg/dl in in groups I, II, III, IV, V and VI respectively. Groups II and III showed insignificant changes in respect to the control group I. Diabetes induced by alloxan resulted in a significant elevation in the levels of fasting blood glucose (FBG) in group IV (diabetic group) in respect to control group I. while the treatment with Pomegranate fresh juice and peel extract reduced the elevated fasting blood glucose in groups V and VI respectively in respect to untreated alloxan-induced diabetic group, but still significantly higher than that of groups I, II and III. Also, there was no significant difference in fasting blood glucose levels between group V and group VI (Table 2).

It is noted that Pomegranate fresh juice has no significant influence on plasma glucose level and lipid profile of normal rats. Also, these results were prominent changes in treatment with Pomegranate juice than treatment with Pomegranate peel extracts (Table 2).

The mean ± standard deviation of total serum cholesterol was 110.4 ± 10.3, 107.74 ± 7.98, 197.1± 13.7, 190.5 ± 7.15, and 186.6 ± 11.5 mg/dl in groups I, II, III, IV, V and VI respectively. Whereas the mean ± standard deviation of triglycerides (TG) was 160.2 ± 16.7, 159.3 ± 32.4, 146.9 ± 15.2, 269.2 ± 35, 149.2 ± 23.5 and 164.2 ± 35.2 mg/dl in groups I, II, III, IV, V and VI respectively. Groups II and III showed insignificant changes in both total cholesterol and triglycerides in respect to the control group I. Treatment with Pomegranate fresh juice and peel extract significantly decreased the total serum cholesterol and triglycerides levels when compared to group IV (Table 2).

As regard LDL levels, the mean ± standard deviation was 4.3 ± 0.9, 5.9 ± 1.5, 4.8 ± 1.2, 76.8 ± 8.7, 60.7 ± 14.5 and 69.9 ± 8.1 mg/dl in groups I, II, III, IV, V and VI respectively. Groups II and III showed insignificant changes in LDL level in respect to the control group I. Treatment with Pomegranate fresh juice and peel extract significantly decreased LDL levels when compared to group IV. Also, there was no significant difference in LDL levels between group V and group VI (Table 2).

The mean ± standard deviation of HDL was 74.6± 4.2, 75± 6.2, 76.3± 9.3, 63.9± 7.2, 94.7± 9.4 and 92.3± 17.1 mg/dl in groups I, II, III, IV, V and VI respectively. Groups II and III showed insignificant changes in HDL in respect to the control group I. There was a significant decrease in the level of HDL levels in diabetic group (group1V) when compared to the normal control group I. Treatment with Pomegranate fresh juice and peel extract significantly increased HDL levels when compared to group IV. Also, there was no significant difference in HDL levels between group V and group VI (Table 2).

It is noted that Pomegranate fresh juice has no significant influence on plasma glucose level and lipid profile of normal rats. Also, these results were prominent changes in treatment with Pomegranate juice than treatment with Pomegranate peel extracts (Table 2).

The mean ± standard deviation of AST was 55.24 ± 2.33, 54.24 ± 1.22, 55.94 ± 3.03, 106.38 ± 4.33, 90.02 ± 0.22 and 92.72. ± 1.59 U/L in groups I, II, III, IV, V and VI respectively. Whereas the mean ± standard deviation of ALT was 26.74 ± 0.88, 25.34 ± 0.55, 24.74 ± 0.72, 50.00 ± 1.01, 38.50 ± 1.10 and 40.50 ± 1.1 U/L in groups I, II, III, IV, V and VI respectively. Groups II and III showed insignificant changes in both AST and ALT in respect to the control group I. there were significant increase in both AST and ALT levels in diabetic groups in respect to control group. Results of liver function tests of diabetic rats orally given Pomegranate fresh juice and peel extract showed significant decreases of AST and ALT levels in respect to control group. These results were also more significant in ALT than AST and in treatment with Pomegranate fresh juice than treatment with Pomegranate peel extracts (Table 2).

Table (1): Effects of diabetes and pomegranate on initial body weight, final body weight and body weight gain %(BWG %) in different groups..

Groups	Body weight gain (%)	P value
Control normal (Group I)	13.29 ± 1.15	P < 0.05 P < 0.05@
Pomegranate fresh juice (Group II)	13.30 ± 1.15	P > 0.05* P < 0.05@
Pomegranate peel extract (Group III)	13.31 ± 1.15	P > 0.05* P < 0.05@ P > 0.05Ω
Diabetic (Group IV)	28.16 ± 1.30	P < 0.05*
Diabetic + Pomegranate fresh juice (Group V)	16.76 ± 1.99	P < 0.05* P < 0.05#
Diabetic + Pomegranate peel extract (Group VI)	17.01 ± 3.05	P < 0.05* P < 0.05# P > 0.05¶¶

Number of rats in each group = 10. **@** Groups I, II, and III were compared to control group IV.
*****All groups were compared to control group1. **Ω** Groups II was compared to control group III
Groups V and VI was compared to control group **¶** Groups V and VI were compared to group IV..

Table (2): Effects of diabetes and Pomegranate in different groups (Mean ±SD)

Groups / Para-meters	Control normal (Group I)	Pomegranate fresh juice (Group II)	Normal + Pomegranate peel extract (Group III)	Diabetic (Group IV)	Diabetic + Pomegranate fresh juice (Group V)	Diabetic + Pomegranate peel extract (Group VI)
Fasting blood glucose (mg/dl)	95.3 ± 8.9	92.3 ± 11.47	90.35 ± 11.3	379.2 ± 23.9	287.4 ± 12.58	293.9 ± 19.4
	P < 0.05@	P > 0.05* P < 0.05@	P > 0.05* P < 0.05@ P > 0.05Ω	P < 0.05*	P < 0.05* P < 0.05#	P < 0.05* P < 0.05# P > 0.05¶¶
Cholesterol (mg/dl)	110.4 ± 10.3	107.74 ± 5.5	104.75 ± 7.98	197.1 ± 13.7	190.5 ± 7.15	186.6 ± 11.5
	P < 0.05@	P > 0.05* P < 0.05@	P > 0.05* P < 0.05@ P > 0.05Ω	P < 0.05*	P < 0.05* P < 0.05#	P < 0.05* P < 0.05# P > 0.05¶¶
TG (mg/dl)	160.2 ± 16.7	159.3 ± 32.4	146.9 ± 15.2	269.2 ± 35	149.2 ± 23.5	164.2 ± 35.2
	P < 0.05@	P > 0.05* P < 0.05@	P > 0.05* P < 0.05@ P > 0.05Ω	P < 0.05*	P < 0.05* P < 0.05#	P < 0.05* P < 0.05# P > 0.05¶¶
LDL (mg/dl)	4.3 ± 0.9	5.9 ± 1.5	4.8 ± 1.2	76.8 ± 8.7	60.7 ± 14.6	69.9 ± 8.1
	P < 0.05@	P > 0.05 P < 0.05@	P > 0.05* P < 0.05@ P > 0.05Ω	P < 0.05*	P < 0.05* P < 0.05#	P < 0.05* P < 0.05# P > 0.05¶¶
HDL (mg/dl)	74.6 ± 4.2	75 ± 6.2	76.3 ± 9.3	63.9 ± 7.2	94.7 ± 9.4	92.3 ± 17.1
	P < 0.05@	P > 0.05* P < 0.05@	P > 0.05* P < 0.05@ P > 0.05Ω	P < 0.05*	P < 0.05* P < 0.05#	P < 0.05* P < 0.05# P > 0.05¶¶
AST(U/L)	55.24 ± 2.33	54.24 ± 1.22	55.94 ± 3.03	106.38 ±4.33	90.02 ± 0.22	92.72 ± 1.59
	P < 0.05@	P > 0.05* P < 0.05@	P > 0.05* P < 0.05@ P > 0.05Ω	P < 0.05*	P < 0.05* P < 0.05#	P < 0.05* P < 0.05# P > 0.05¶¶
ALT(U/L)	26.74 ± 0.88	25.34 ± 0.55	24.74 ± 0.72	50.00 ± 1.01	38.50 ± 1.10	40.50 ± 1.10
	P < 0.05@	P > 0.05* P < 0.05@	P > 0.05* P < 0.05@ P > 0.05Ω	P < 0.05*	P < 0.05* P < 0.05#	P < 0.05* P < 0.05# P > 0.05¶¶
Catalase (U/ml)	29.9 ± 3.03	33.1 ± 3.0	32.7 ± 2.23	21.7 ± 2.83	39.5± 2.27	38.20 ± 3.08
	P < 0.05@	P > 0.05* P < 0.05@	P > 0.05* P < 0.05@ P > 0.05Ω	P < 0.05*	P < 0.05* P < 0.05#	P < 0.05* P < 0.05# P > 0.05¶¶
MDA (µmol/l)	16.1 ± 3.62	15.05 ± 2.68	15.73 ± 3.29	23.73 ± 3.29	16.37 ± 2.77	14.1 ± 2.54
	P < 0.05@	P > 0.05* P < 0.05@	P > 0.05* P < 0.05@ P > 0.05Ω	P < 0.05*	P < 0.05* P < 0.05#	P < 0.05* P < 0.05# P > 0.05¶¶

Number of rats in each group = 10. @ Groups I, II, and III were compared to control group IV.
*All groups were compared to control group1. Ω Groups II was compared to control group III
Groups V and VI was compared to control group1. ¶ Groups V and VI were compared to group IV.

There was a significant decrease in the level of catalase as an antioxidant (21.7 ± 2.83 U/ml) and a significant increase in the level of MDA as an oxidant (23.73 ± 3.29 µmol/l) in diabetic group in comparison to control group (Table 2). On the other hand, after treatment of alloxan-induced diabetic rats with Pomegranate juice, the level of catalase was significantly increased (39.5 ± 2.27 U/ml), and MDA level significantly decreased (16.37 ± 2.77 µmol/l) in respect to untreated alloxan-induced diabetic group. After treatment of alloxan-induced diabetic rats with Pomegranate peel extract, the level of catalase was significantly increased (38.20 ± 3.08 U/ml), and MDA level significantly decreased (14.1 ± 2.54 µmol/l) in respect to untreated alloxan-induced diabetic group (Table 2).

These results were also more significant in treatment with Pomegranate fresh juice than treatment with Pomegranate peel extracts.

Histopathological examination of the pancreas showed normal pancreatic islets, pancreatic acini and normal blood vessels in groups I, II and III (Figure 1). On the other hand, pancreatic islets decreased in number and size, normal pancreatic acini and thick wall of the blood vessels, in diabetic group (Figure 2). The pancreas in diabetic group received Pomegranate juice and that received Pomegranate peel extracts showed normalized number of pancreatic islets and decreased size, some normal pancreatic acini and partial improvement of the thick walled blood vessels (thinner than diabetic group but thicker than normal group) (Figure 3).

Histological examination of liver of the control groups I,II and III demonstrated normal histological pattern where hepatic lobules appeared as hexagonal masses of hepatocytes radiating form a central vein. Blood sinusoids appeared between cords of hepatocytes. The hepatocytes had a hexagonal outline with central rounded nucleus. The cytoplasm showed some vacuoles (Figure 4). Diabetic group revealed marked impairment of the normal structure of hepatic lobules in many areas and deposition of large lipid droplet in cells, vacuolar degeneration and swollen cells (Figures 5a and 5b). Diabetic rats treated by Pomegranate juice showed more improvement in histological structures comparing with sections of diabetic rats that orally given Pomegranate peel extract. The examined sections showed almost normal structure with regular arrangement of hepatocyte cell cords and exhibited a reduction in fat accumulation. The hepatocytes around the central vein (CV) showed rounded nuclei and vesicular indicating active cells. Blood sinusoids between the cells had also normal appearance (Figure 6).

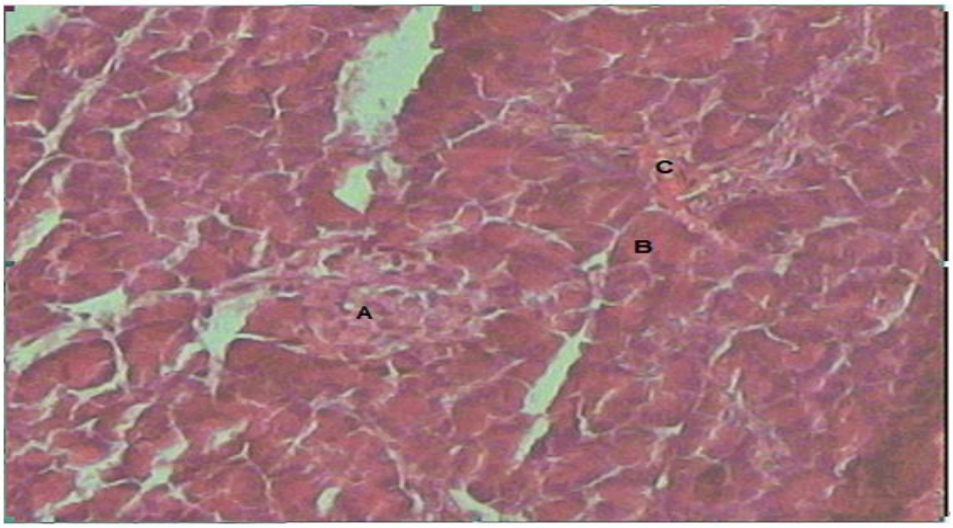


Figure (1): Section in the pancreas of group I, II and III (normal control groups) showing some normalized pancreatic islets (A), some normal pancreatic acini (B) and normal blood vessels (C). Hx&E, 400X.

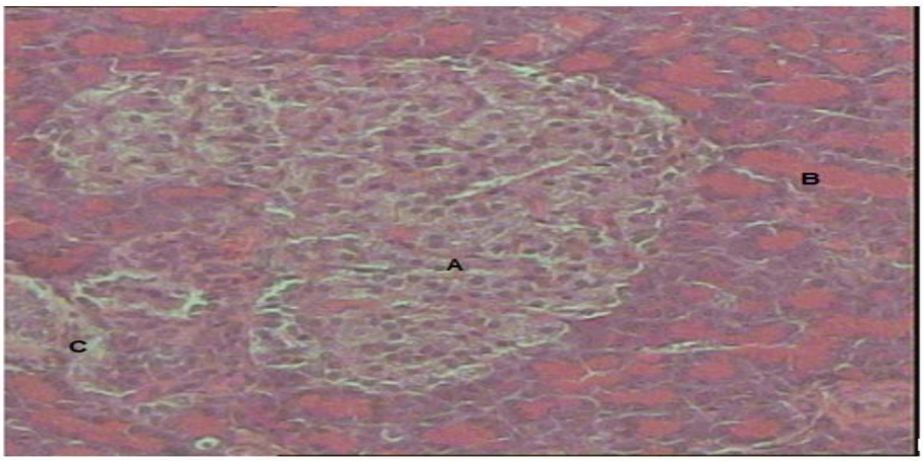


Figure (2): Section in the pancreas of group IV (diabetic-group) showing pancreatic islets decreased in number and size (A), normal pancreatic acini (B) and thick wall of the blood vessels (C). Hx&E, 400X.

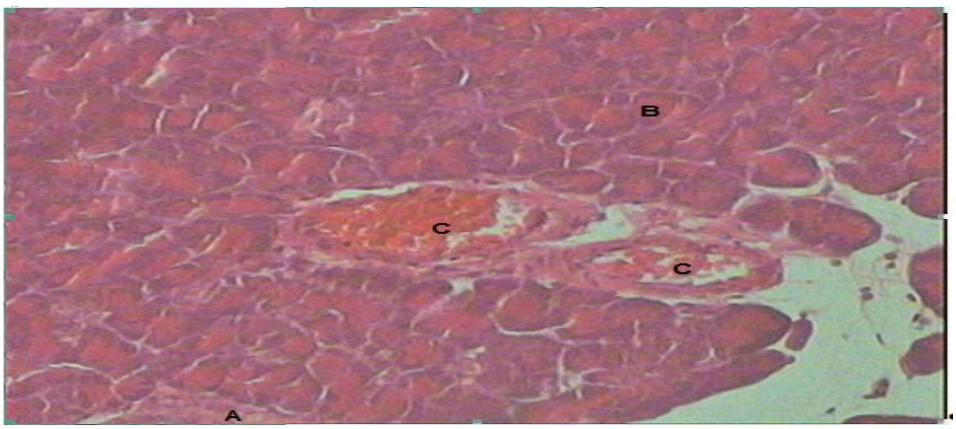


Figure (3): Section in the pancreas of group V and VI- diabetic-received Pomegranate juice and Pomegranate peel extracts respectively showing normalized number of pancreatic islets and decreased size (A), normal pancreatic acini (B) and improvement of the thick walled blood vessels (C). Hx&E, 400X.

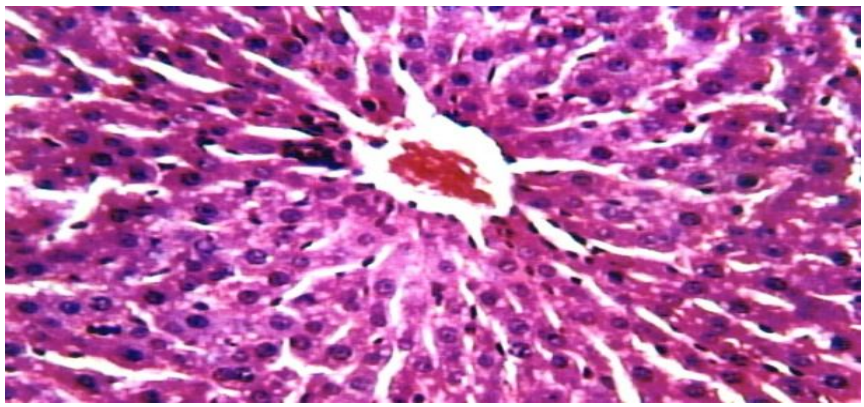
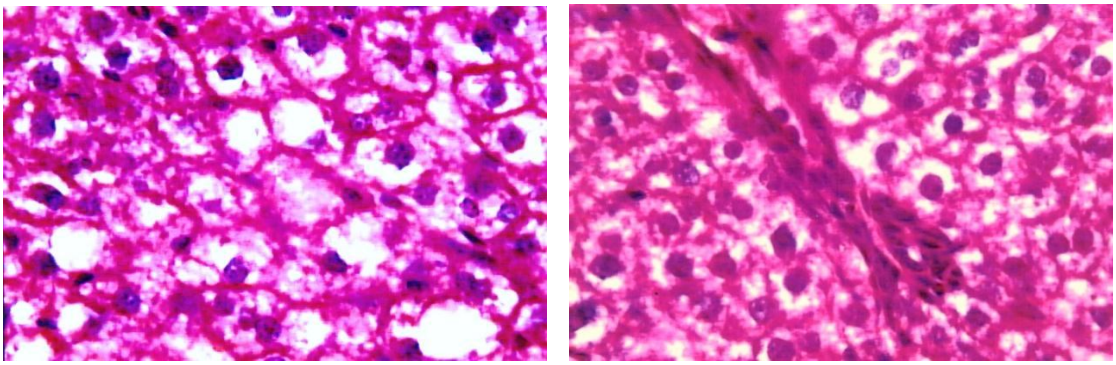


Figure (4): Section in the liver (Hx&E, 400X) of group I, II and III (normal control groups) showing Normal hepatocytes arranged in plates.



a **b**

Figure (5): Section in the liver of group IV (diabetic-group) showing vacuolar degenerated hepatocytes, swollen and vacuolated cells with lymphocytic infiltration (a). Degenerated hepatocytes showing ballooning (b). Hx&E, 400X.

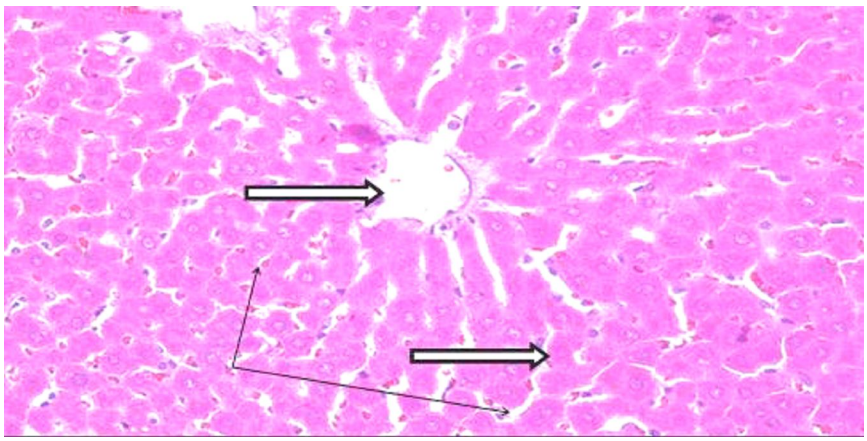


Figure (6): Section in the liver of group V and VI- diabetic-received Pomegranate juice and Pomegranate peel extracts respectively showing almost normal structure with regular arrangement of hepatic cell cords (black thin arrows) around the central vein. Hepatic sinusoids between the cells showed normal appearance (white arrows). Hx&E, 400X.

DISCUSSION

In spite of the presence of known antidiabetic medicines in the pharmaceutical market, remedies from medicinal plants are used with success to treat diabetes and its complications.

Results of the present study revealed incidence of significant increases in BWG % of diabetic rats when compared to the control rats. These findings were in agreement with those obtained by **Amin et al. (2011) and Nwozo et al. (2011)** who confirmed our results. The increase in body weight of diabetic rats might be due to the increase of feed and caloric intake by rats.

In the present study, there was a significant increase in blood glucose level in diabetic group (IV) when compared with the control group (I). In treated groups with Pomegranate fresh juice and peel extract (V and VI), there were significant decrease in blood glucose levels when compared with diabetic group (IV). The mechanisms by which alloxan brought about its diabetic state included selective destruction of pancreatic insulin secreting ?-cells, which make cells less active and lead to poor glucose utilization by tissues (**Papaccio et al., 2017)**. **Amri et al. (2017)** attributed the anti-diabetic effect of pomegranate contents as phenolic compounds, tannic acids, gallic acid, ellagic acids and flavonoides which have hypoglycemic effects.

The result of this study revealed that treatment of alloxan-induced diabetic rats with pomegranate (fresh juice and peel extract) significantly reduced blood glucose level, and this triggered the liver to revert to its normal homeostasis during experimental diabetes **(Rouhi et al., 2017).** The anti-hyperglycemic activity of pomegranate may be through a stimulatory effect on insulin secretion or through improvement of insulin action. Also, pomegranate may have extra pancreatic mechanism of action which improves pancreatic ?-cell function, and thus enhance insulin secretion (**Papaccio et al., 2017)**.

Results of the present study revealed that diabetic rats showed significant increases in serum concentrations of TC, TG and LDL-c and the reduction in serum HDL-c. The present findings were in the same line as with those reported by **Frantz et al. (2012)** who demonstrated that lipid metabolic disorders and levels of serum TC and TG increased significantly when compared with control group.

Concerning serum TG level, the present findings agreed with the study of **Rouhi et al. (2017)** who demonstrated that plasma TG level increased significantly in diabetic rats.

Regarding to serum LDL-c and HDL-c levels in diabetic group, the current results were in agreement with those of **Kumar et al. (2010)** who concluded that oxidation of LDL-c resulted in formation of a wide range of biologically active products, including peroxides and malondialdehyde. Moreover, **Sezer et al. (2011)** demonstrated that the oxidative modified lipids and their degradation products are believed to have adverse effects such as pro-inflammatory, immunogenic and cytotoxic activities which contribute to both the initiation and progression of atherosclerotic lesions.

The present study showed that serum HDL-c level was decreased significantly

in diabetic group in respect to the control group. These results were well documented by the study of **Farideh et al. (2017)**. It has been reported that cholesterol transport to extra-hepatic tissues is primarily ensured by LDL-c (bad cholesterol); while HDL-c (good cholesterol) has an important role in reversing the cholesterol transport process **(Faghihimani et al., 2017).**

The present study showed that oral administration of Pomegranate juice significantly decreased serum level of TC, TG and LDL-c but increased HDL-c as compared to the control group. These findings correlated with those obtained by **Tezcan et al. (2016)** who reported that Pomegranate juice consumption by diabetic rats significantly reduced cholesterol accumulation and foam cell formation in tissues. Pomegranate juice treatment significantly inhibited the progression of atherosclerotic lesions by inhibition of atherogenic modifications of LDL-c including its retention, oxidation, and aggregation.

Rouhi et al. (2017) reported that diabetic rats with elevated blood lipids treated with Pomegranate juice experienced significant reductions in their TC and LDL-c.

The results of **Farideh et al. (2017)** demonstrated that Pomegranate juice can inhibit LDL-c oxidation by polyphenols content that inhibit copper ion-induced LDL-c oxidation, and thus reduce the oxidized LDL content. Pomegranate juice polyphenols also increase the activity of serum HDL-c associated paraoxonase 1 (PON1) which hydrolyze lipid peroxides and convert them to a less atherogenic LDL-c thus causing further reduction in LDL content **(Czerska et al., 2015).**

Plasma AST and ALT, alone or in combination are primarily recommended for the assessment of hepatocellular injury. They are sensitive markers for drug-induced liver damage, and the elevated activities of these marker enzymes in plasma are indicative of cellular leakage and loss of the functional integrity of cell membranes in the liver **(Gurbet et al., 2013).**

Results of the present study showed that there were significant decreases in serum levels of AST and ALT enzymes in diabetic rats orally given Pomegranate juice compared to the control group. The present results agreed with the results obtained by **Osman et al. (2012)** who examined the antioxidant effect of Pomegranate juice and peel on diabetes mellitus induced by alloxan in Female Rats. The results showed that 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. This effect is due to antioxidant content of Pomegranate juice and peel extract. ROS stimulate glutathione and superoxide dismutase enzymes depletion, accumulation of lipid peroxides and oxidative damage of different organelles in liver tissue (**Daftardar et al., 2014).** Antioxidant supplements may have a role in preventing or treating hepatic lesion in patients with diabetes **(Siham et al., 2017).**

The ameliorative effect as anti-oxidant was in agreement with **Messarah et al. (2012)** who revealed that stimulation of

anti-oxidant defense system reversed hepatic stress.

The biochemical results of our study were confirmed by histopathological findings, which seen in liver sections. The histological findings of liver of the treated rats showed almost completely normal structure with regular arrangement of hepatocyte cell cords and exhibited reduction in fat accumulation. The nuclei of hepatocytes around the central vein (CV) were rounded and vesicular indicating active cells. Blood sinusoids between the cells had also normal appearance when compared to the positive control group. These histological findings agreed with the study of **Fyiad et al. (2012)** who investigated the effect of Pomegranate juice on nucleic acids alterations and oxidative stress in experimentally hepatitis rats.

As indicated in the present study, the untreated diabetic rats had significant decrease in the level of antioxidant enzyme system as catalase enzyme. These findings agreed with **Illana et al. (2014)** who reported that diabetes mellitus enhanced the free radical generation in various ways. Several studies suggested that disorders of lipid metabolism, hyperlipidemia and obesity are associated with overproduction of oxygen free radicals **(Issaoui et al., 2010).** The enhanced accumulation of these free radicals and dysfunction of antioxidant defense system resulted in oxidative stress **(Gouda et al., 2016).**

Pomegranate juice and peel extract were shown to have significant higher levels of antioxidants in comparison to commonly consumed fruit juices, such as grape, cranberry, grapefruit or orange juice **(Tapias et al., 2014).** The principal antioxidant polyphenols in Pomegranate juice extract include tannins and anthocyanins which have been shown to be the antioxidant responsible for the free radicals scavenging ability of Pomegranate juice **(Illana et al., 2014). Farideh et al. (2017)** concluded that Pomegranate has also been shown to protect the antioxidant enzymes CAT from the effects of toxic chemicals.

Turk et al. (2008) reported that there was a significant decrease in malondialdehyde (MDA) level and marked increase in catalase (CAT) activities in rats treated with Pomegranate seeds juice.

In the present study, Oral administration of Pomegranate juice and peel extract caused a significant increase in the activity of CAT enzymes when compared to the control group. The improvement of CAT enzyme activities could be explained by antioxidant properties of Pomegranate seeds extract due to presence of bioactive polyphenolic compounds which play a role in scavenging free radicals and also prevent DNA damage **(Rom et al., 2016).**

Valadares et al. (2010) confirmed the ability of Pomegranate to protect DNA and prevent chromosomal damage in mice. In addition, **Rouhi et al. (2017)** demonstrated that Pomegranate afforded up to 60 % protection against hepatic lipid peroxidation due to maintenance of the GSH and serum levels and activities of CAT, GPx and glutathione reductase (GR) enzymes.

ACKNOWLEDGEMENT

I would like to express my deep appreciation and utmost thanks to **Assist.**

Prof. Dr. Amr El-Karef, Assistant Professor of Medical Pathology, Faculty of Medicine, Mansoura University, for his great help in this work

REFERENCES

1. **Al-Olayan, E.M., El-Khadragy, M.F., Metwally, D.M. and Abdel Moneim, A.E. (2014):** Protective effects of pomegranate (Punica granatum) juice on testes against carbon tetrachloride intoxication in rats. Compl. Alter. Med., 14 (164): 1 - 9.
2. **American Diabetes Association (2016):** Diagnosis and classification of diabetes mellitus. Diabetes Care, 39(1): 81-90.
3. **Amin, K., Kamel, H. and Abd Eltawab, M. (2011):** The relation of high fat diet, metabolic disturbances and brain oxidative dysfunction: modulation by hydroxy citric acid. Lipids in Health and Disease, 14(10):74-80.
4. **Amri, Z., Houda, L., Manel, M., Sinda, G., Olfa, D., Beligh, M. and Mohamed, H. (2017):** Oil Characterization and Lipids Class Composition of Pomegranate Seeds. Bio. Med. Research International, 2037341- 49.
5. **Czerska, M., Mikolajewska, K., Zielinski M., Gromadzinska, J. and Wasowicz, W. (2015):** Today's oxidative stress markers. Med. Pr; 66(3):393-405
6. **Daftardar, S., Kaur, G. and Addepalli, V. (2014):** Nutraceutical approaches in the management of cardiovascular dysfunctions associated with diabetes mellitus.377-96.
7. **Ezazul, H., Subboroto, K.K., Dipa, I. and Rezuanul, I. (2012):** Comparative study between the effect of coccinia cordifolia (Leaf and Root) powder on hypoglycemic and hypolipidemic activity of alloxan-induced type II diabetes long-Evan rats. Journal of Diabetes and endocrinology, 3(4):37-43).
8. **Faghihimani, Z., Parvin, M., Golbon, S., Bijan, I. and Elham, F. (2017):** Effects of Pomegranate Seed Oil on Metabolic State of Patients with Type 2 Diabetes Mellitus. Complementary and Alternative Medicine, 17:156-167.
9. **Farideh, D., Roxana, V., Parvin, Z., Aliasghar, P., Rogayeh. And Mehran, M. (2017):** Effects of Pomegranate (Punica Granatum L.) Seed and Peel Methanolic Extracts on Oxidative Stress and Lipid Profile Changes Induced by Methotrexate in Rats. Advanced Pharmaceutical Bulletin, 7(2): 269-274.
10. **Frantz, E., Menezes, H., Lange, K., Abegg, M., Correa, C., Zangalli, L., Vieira, J. and Zettler, C. (2012):** The effect of maternal hypercholesterolemia on the placenta and fetal arteries in rabbits. Acta. Cirurgica. Brasileira., 27(1):7-12.
11. **Fyiad, A., Abd El-Kader, M. and Abd El-Haleem, A. (2012):** Modulatory Effects of Pomegranate Juice on Nucleic Acids Alterations and Oxidative Stress in Experimentally Hepatitis Rats. Life Science Journal, 9(3):676-82.
12. **Gurbet, C., Asij, S., Serdar, K., Sevki A., Orhan, A. and Alaattin, S. (2013):** A Comparative Study for the evaluation of two doses of ellagic acid on hepatic drug metabolizing and antioxidant. Bio. Med. Research International, 9: 358945-55.
13. **Gouda, M., Moustafa, A., Hussein, L. and Hamza, M. (2016):** Three week dietary intervention using apricots, pomegranate juice or/and fermented sour sobya and impact on biomarkers of anti-oxidative activity, oxidative stress and erythrocytic glutathione transferase activity among adults. Nutr. J., 15(1):52 61.
14. **Illana, L., Eliane, B., Jorge, M. (2014):** Pomegranate seed oil (Punica Granatum L.): A Source of Punicic Acid (Conjugated α-Linolenic Acid. J. Hum. Nutr. Food Sci., 2 (1): 1024-35.
15. **International Journal of Pharma Tech Research (2014):** Antidiabetic effect of Rosella Extract in Streptozotocin induced Mice. CODEN (USA): ISSN: 0974 4304; 6 (5):1703-1711.
16. **Issaoui, M., Flamini, G. and Brahmi, F. (2010):** Effect of the growing area conditions on differentiation between Chemlali and Ch´etoui olive oils. Food Chemistry, 119 (1) 220–225.
17. **Kumar, D. S., Muthu, A. K. Smith, A. A. and Manavlan, R. (2010):** Hypolipidemic effect of various extracts of whole plant of Mucuna pruriens (Linn) in rat fed with high fat diet. European Journal of Biological Sciences, 2:32-8.
18. **Lei, F., Zhang, X. and Wang, W. (2007):** Evidence of anti-obesity effects of the pomegranate leaf extract in high-fat diet

induced obese mice. Int. J. Obes., 31:1023-1029.

19. **Margoni, A., Perrea, D. N., Vlachos, I., Prokopaki G., Pantopoulou A., Fotis L., Kostaki M. and Papavassiliou A. (2011):** Serum Leptin, Adiponectin and Tumor Necrosis Factor-α in Hyperlipidemic Rats with/without Concomitant Diabetes Mellitus. The Feinstein Institute for Medical Research, 17(2): 36-40.

20. **Matthaeus, B. and Ozcan, M. (2016):** Pomegranate plant (Punica granatum L.) composition, antioxidant activity, therapeutic effect and antimicrobial activity. Journal of Applied Pharmaceutical Science, 21: 160-167.

21. **Mesgari, A., Heidari R., Amini, R., Zakeri, P. and Ghamarzad, S. N. (2016):** Effects of pomegranate seed methanolic extract on methotrexate-induced changes in rat liver antioxidant compounds. Curr. Top Nutraceutr., 13(3):153-9.

22. **Messarah, M., Klibet, F. and Boumendjel, A. (2012):** Hepatoprotective role and antioxidant capacity of selenium on arsenic-induced liver injury in rats. Exp. Toxicol. Pathol., 64:167–174.

23. **Nwozo, S., Orojobi, B. and Adaramoye, O. (2011):** Hypolipidemic and antioxidant potentials of Xylopia aethiopica seed extract in hypercholesterolemic rats. Journal of Medicinal Food, 14(2):114-9.

24. **Osman, H. F., Eshak, M. G., El-Sherbiny, E. M. and Bayoumi, M. M. (2012):** Biochemical and Genetical Evaluation of Pomegranate Impact on Diabetes Mellitus Induced by Alloxan in Female Rats. Life Science Journal, 9(3): 1543-53.

25. **Papaccio, G., Pisanti, F.A., Latronico, M.V., and Ammendola, E. and Galdieri, M. (2017):** Multiple low-dose and single high-dose treatments with streptozotocin do not generate nitric oxide. J. Cell Biochem., 77: 82–91.

26. **Rom, O., Volkova, N., Nandi, S., Jelinek, R. and Aviram, M. (2016**): Pomegranate juice polyphenols induce macrophage death via apoptosis as opposed to necrosis induced by free radical generation. Cardiovasc. Pharmacol. J., 68(2):106-14.

27. **Rouhi, S. Z., Moklesur, R., Asmah, R., Saad, A. and Fauziah, O. (2017):** The effect of pomegranate fresh juice versus pomegranate seed powder on metabolic indices, lipid profile, inflammatory biomarkers, and the histopathology of pancreatic islets of Langerhans in streptozotocin-nicotinamide induced type 2 diabetic Sprague–Dawley rats. Complementary and Alternative Medicine, 17:156-69.

28. **Sezer, E., Akcay, Y., Ilanbey, B., Yƚdƚrƚm, H. and Sozmen, E. (2011):** Pomegranate wine has greater protection capacity than red wine on low-density lipoprotein oxidation. Journal of Medicinal Food, 10(2):371-4.

29. **Siham, N. K., Mostafa, I., Lyutha, S., Mohamed, H., Nada, M. and Mohammad, S. (2017):** Ameliorative Effects of Pomegranate Peel Extract against Dietary-Induced Nonalcoholic Fatty Liver in Rats. Prev. Nutr. Food Sci., 21(1):14-23.

30. **Tapias, V., Cannon, J. and Greenamyre, J. (2014):** Pomegranate juice exacerbates oxidative stress and nigrostriatal degeneration in Parkinson's disease. Neurobiol. Aging. 35(5):1162-76.

31. **Tezcan, F., Gultekin, M., Diken, T., Ozcelik, B. and Erim, F. B. (2016):** Antioxidant activity and total phenolic, organic acid and sugar content in commercial pomegranate juices. Food Chemistry, 115(3):873–7.

32. **Turk, G., Sonmez, M., Aydin, M., Yuce, A., Gur S., Yuksel, M., Aksu, E. and Aksoy, H. (2008):** Effects of pomegranate juice consumption on sperm quality, spermatogenic cell density, antioxidant activity and testosterone level in male rats. Clinical Nutrition, 27(2):289-96.

33. **Valadares, M. C., Pereira, E. R. T., Benfica, P. L. and Paula, J. R. (2010):** Assessment of mutagenic and antimutagenic effects of Punica granatum in mice. Brazilian Journal of Pharmaceutical Sciences, 46(1): 121-7.

34. **William, E. G. (2010):** Models of Fixation and Tissue Processing. Biotech. Histochem., 84 (5): 185–193.

35. **Zhang, L., Yang, Y., Zu, X., Chen, F., Wang, Z. and Liu, F. ((2010):** Oxidative stability of sunflower oil supplemented with carnosic acid compared with synthetic antioxidants during accelerated storage. Food Chemistry, 118 (3): 656-662.

ناجح مبروك محمد جبر

قسم الفسيولوجيا الطبية- كلية الطب - جامعة الأزهر

خلفية البحث: إن الرمان هو ثمرة يستخدم في الطب التقليدي لخواصه العلاجية.

الهدف من البحث: تقييم تأثير عصير الرمان الطازج ومستخلص قشرته المائية على ذكور الجرذان البيضاء المصابة بمرض السكر من حيث نسب كلا من السكر والدهون بالدم ووظائف الكبد وعوامل الأكسدة ومضاداتها.

طرق ومواد البحث: تم تطبيق البحث على ستين جرذ ذكر بالغ من فصيلة الألبينو من سلالة محلية كنموذج للدراسة. وقد قسمت الجرذان عشوائيا إلي ست مجموعات متساوية كالآتي: مجموعة ضابطة ومجموعة ضابطة تم إعطاؤها عصير الرمان الطازج بجرعة 500 مليجرام/كجم من وزن الجسم لكل جرذ يوميا ومجموعة ضابطة تم إعطاؤها مستخلص قشر الرمان بجرعة 500 مليجرام/كجم من وزن الجسم لكل جرذ يوميا ومجموعة مصابة بالسكر تم إعطاؤها الألوكسان بجرعة140 مليجرام لكل جرذ بالبطن مرة واحدة ومجموعة مصابة بالسكر تم إعطاؤها عصير الرمان الطازج ومجموعة مصابة بالسكر تم إعطاؤها مستخلص قشر الرمان بنفس الجرعات السابق ذكرها.

وتم سحب عينات الدم في آخر التجربة بعد أربعة أسابيع وذلك لقياس نسبة كلا من : السكر بالدم (صائم)، الكوليستيرول، الدهون الثلاثية، البروتين الدهني عالي الكثافة، البروتين الدهني منخفض الكثافة، ألانين أمينو ترانسفيريز، أسبرتيت أمينوترانسفيريز، إنزيم المالون داى الدهيد في الدم و إنزيم الكاتاليز في الدم. كما تم أيضا أخذ عينات من أنسجة البنكرياس والكبد وعمل فحص مجهري لها.

نتائج البحث: أظهرت النتائج أن تناول عصير الرمان ومستخلص قشرته المائية عن طريق الفم للجرذان المصابة بالسكر قد أديا إلى نقص ذا دلالة إحصائية في الوزن النسبي للجسم ، مستويات إنزيمات الكبد، الكولسترول الكلي، الدهون الثلاثية والبروتين الدهنى المنخفض الكثافة، بينما أدى إلى زيادة البروتين الدهنى المرتفع الكثافة ذات دلالة إحصائية. وكذلك أديا ايضا الى زيادة في نشاط الإنزيمات المؤكسدة ونقص الإنزيمات المضادة للأكسدة. وقد أظهر الفحص الهستوباثولوجى لأنسجة البنكرياس والكبد وجود تحسن واضح في التغيرات الهستوباثولوجية التي سببها الكولسترول المرتفع بهذه الأنسجة.

الإستنتاج: إستخدام عصير الرمان وكذلك مستخلص قشرته المائية يمكن أن يكونا ذا فائدة تطبيقية وعاملان مساعداًن في علاج مرض السكر وفي حالات الإجهاد التأكسدي والوقاية من مضاعفاتها وقد يرجع ذلك إلى قدرتهما علي تحسن نسبى فى أنسجة البنكرياس وتحسن وظائف الكبد وقلة إمتصاص الدهون وزيادة مضادات الأكسدة مع الأخذ فى الاعتبار أن عصير الرمان الطازج قد يكون أكثر تأثيرا من مستخلص قشره.