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EFFECTS OF *PUNICA GRANATUM* PEELS EXTRACT ON THE INTESTINAL α-GLUCOSIDASE ACTIVITY AND THE HISTOPATHOLOGY OF THE PANCREAS OF ALLOXAN –INDUCED DIABETIC RATS

By

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ABSTRACT

Pomegranate (*Punica granatum*) had been detected to have hypoglycemic effects. This study was done to evaluate the effects of Pomegranate peel ethyl acetate extract (PGE) on the intestinal α-glucosidase enzyme activity and on the pancreas of the Alloxan-induced diabetic rats. Forty adult male albino rats were divided into: Group I: (Normal control group); Group II (Diabetic control group); Group III (Diabetic+ PGE (200mg/kg) and Group IV (Diabetic+ PGE (400 mg/kg). The results showed that the administration of PGE to the diabetic rats in a dose of 400 mg/kg for three weeks significantly reduced the blood glucose level and the intestinal α-glucosidase enzyme activity. Histological and morphometric studies of the pancreas showed a significant increase in the diameter and the number of the islets of Langerhans and the percentage of insulin immune positive cells/ islet in the Diabetic+ PGE (400 mg/kg) group. These data indicate that PGE at a dose of 400 mg/kg has anti-diabetic effects which may be achieved by two mechanisms: 1. Helping the regeneration of β-cells in the pancreas. 2. Inhibiting intestinal α-glucosidase activity thus decreasing carbohydrates absorption in the small intestine. Therefore, Pomegranate peels extract can be used as a supplement for the regulation of hyperglycemia.

INTRODUCTION

Diabetes is a chronic endocrine disorder associated with several secondary complications (Mohan et al. 2013; Wild et al**.** 2004). Despite the currently available anti-diabetic drugs, there is a need for adjuvants which are economical and safe (Kapoor et al. 2014). Medicinal plants are good sources of hypoglycemic compounds, this fact lead to the use of these plants as adjuncts to the existing

therapies for the treatment of diabetes mellitus (Sharma et al. 2008).

Medicinal plants may act on blood glucose through different mechanisms, some of them may have insulin-like substances (Gray and Flatt, 1999; Collier et al, 1987) some may inhibit insulinase activity, others may cause increase in the numbers of beta cells in the pancreas by activating regeneration of these cells (Arya et al. 2015; Abdel-Rahman and Nahata 2007). The fiber of plants may also interfere with carbohydrate absorption; thereby affecting blood glucose (Subhasree et al. 2015; Nelson et al, 1991).

Pomegranate juice has a potential anti-atherogenic effects in humans and anti-atherosclerotic effects in mice (Aviram et al., 2004). Different studies have shown that pomegranate juice contains high levels of antioxidants (Bakir et al. 2015; Aslan et al. 2014; Türk et al. 2008; Gil et al., 2000). It has been reported that *pomegranate* juice is an important source of anthocyanins (cyanidin, delphinidin, pelargonidin), phenolics and tannins (punicalin, pedunculagin, punicalagin, ellagic acid) which have many nutritional and health advantages (Kulkarni and Aradhya, 2005).

Scientific studies reported an anti-diabetic activity of the flower and the juice of the pomegranate seeds (Katz et. al. 2007; Parmar et. al. 2007; Huang et. al. 2005). The hypoglycemic activity of pomegranate seeds has been reported in humans (Katz et al., 2007) and rats (Das and Barman 2012). However, only few studies have evaluated the anti-diabetic properties of *Punica granatum* fruit peel extract. Hence, the present study was designed to scientifically estimate the effects of the fruit peel extract on the blood glucose level, on the pancreatic β-cells and on the intestinal α-glucosidase activity.

MATERIAL AND METHODS

Animals:

Forty adult male albino Westar rats aged 12-14 weeks and weighted 180-200 gm were used in this study. The study was conducted according to the National Institutes of Health guidelines for the care and use of laboratory animals. All animal care and experimental procedures were carried out with the ethics approval of the local regulatory authority. The animals were kept at room temperature with a 12 h/12 h dark/ light cycle in ventilated animal house of Batterjee medical college, Jeddah, KSA. The rats were habituated to laboratory conditions and received a standard diet and water.

Drugs and chemicals:

Alpha- Glucosidase assay kits and Alloxan monohydrate were purchased by Sigma-Aldrich, USA. Acarbose was obtained from Bayer, Saudi Arabia. All other chemicals and reagents were of analytical grade.

Preparation of Pomegranate (*Punica granatum Linn*.) extract (PGE):

Pomegranate fruits (3 kg) were purchased from the local market in Abhur, Jeddah, KSA and authenticated by Dr. Samah Shabana, Pharmacognosy Department, Pharmacy Program, Batterjee medical college. The fruits were cleaned, shadow dried and stored. The peel and the seed powders were extracted with 70% ethanol for one week concentrated under vacuum at 60◦C and then dried. Vacuum-dried ethanolic extract

(800 g) was macerated with ethyl acetate in the solvent: solute ratio of 3: 1 for 48 h with frequent shaking. The ethyl acetate fraction was dried under vacuum using rotary evaporator IKA- RV10, USA. The dried extract was weighted and dissolved in a definite amount of distilled water to make concentration of 40 mg/ml.

Induction of Diabetes by Alloxan

Alloxan monohydrate was dissolved in sterile normal saline. Diabetes was induced in 30 rats by a single intraperitoneal injection of alloxan (5%) 185 mg/kg. The rats were kept fasting for 12 h before and after Alloxan injection. Fasting plasma glucose was measured by obtaining blood samples from the tails of animals. The rats which showed plasma glucose level of 200 mg/dl or more were considered diabetic (Kanter et al., 2004).

Experimental design

The rats were randomly divided into 4 groups as follows:

- **Group I (Normal control group):** Ten rats were injected with the same volume of normal saline as that used as a vehicle in the diabetic group.
- **Group II (Diabetic control group)**: Overnight-fasted ten rats received a single intraperitoneal injection of Alloxan (185 mg/kg).
- **Group III (Diabetic+PGE (200mg/ kg):** Ten rats received a single intraperitoneal injection of Alloxan 185 mg/kg then the rats were treated with PGE (200 mg/kg/day) given orally by gavages for 3 weeks.
- **Group IV (Diabetic+PGE (400mg/ kg):** Ten rats received a single intraperitoneal injection of Alloxan 185 mg/kg then the rats were treated with PGE (400 mg/kg/day) given orally by gavages for 3 weeks.

Determination of blood glucose level

Blood glucose levels were tested on the 0 day, 1st, 7th . 14th and 21st days from the start of the experiment. Blood samples were collected from the tail of the fasting animals. The tail was embedded in 45 °C water bath and about one millimeter of its end was cut and a drop of blood was used for blood glucose test using advanced glucometer (Roche, USA). The accuracy of glucometer was checked with O-toluidine method (Mukherjee 1988).

Effects of PGE on the intestinal α-Glucosidase enzyme activity

The in vivo inhibitory effect of PGE on α-glucosidase enzyme was investigated using five rats from each of the studied groups. At the end of the experiment the animals were scarified under anesthesia to collect small intestine for assay of α-glucosidase activity.

Preparation of crude α-glucosidase from rat small intestine: The rat small intestine was prepared according to the method described by Lossow et al. (1964) with slight modifications. In summary, the small intestine was removed and washed with 30ml of 0.9% NaCl and placed in ice cold 0.9% NaCl. The intestine was minced with a surgical knife and homogenized using advanced type of homogenizer in 50 ml of 0.1 M potassium phosphate buffer of pH 6.8. After 30 min, it was centrifuged for 30 min at 10,000 rpm at 4˚C.The supernatant was used as crude enzyme source (Kathirvel et al, 2012).

α- Glucosidase inhibitor assay: The α-glucosidase inhibitory activity was determined at the end of the experiment (after three weeks of the onset) in the studied groups. Chromogenic assay was done according to McCue et al. (2005) by measuring the release of 4-nitrophenol from P- nitrophenyl α-D glucopyranoside. The assay mixtures contained 0.3 ml of 10mM p- nitrophenyl α-glucopyranoside, 1.0ml of potassium phosphate (0.1M, pH: 6.8), 0.2 ml of crude enzyme solution and 0.2 ml of inhibitory extract, all in a final volume of 1.7 ml. The Reaction mixture was incubated for 30 min at 37^{o}C, and it was terminated by the addition of 2.0 ml of 100 mM sodium carbonate. The liberated p-nitrophenol was determined at 400*nm* using spectrophotometer (Beckman coulter DU 530 model). The system without α-glucosidase was used as blank, and acarbose was used as positive control. Each experiment was conducted in triplicate. The % inhibitory rates were calculated using the formula, % inhibition = (Absorption $_{control}$- Absorption $_{test}$) / Absorption $_{control}$. X 100

Histological Effects of PGE on the pancreas

After three weeks of the start of the experiment, five rats from each group were anaesthetized by ether and sacrificed. The abdominal cavity was opened, the pancreas was dissected and blood sample was obtained. Two pancreases from each group were fixed in formalin 10% for hematoxylin and eosins (H&E) staining while three freshly taken pancreases were used for anti-insulin immunohistochemistry.

Hematoxylin & Eosin (H&E) staining: According to Kiernan (2001) the fixed pancreases were dehydrated in different grades of ethanol: 70%, 80%, 90% and finally100%. Then the whole pancreases were mounted with hot paraffin then left to be solid to form paraffin blocks. Horizontal sections were done and slides (of 5 um thickness) containing paraffin were placed in a slide holder (glass or metal) and de-paraffinized then mounted in Xylene. Then the slides were rehydrated in different grades of 100% ethanol, 95% ethanol, 80% ethanol. Then the slides were mounted in deionized H2O. Hematoxylin staining were done by Hematoxylin dye (Poly Scientific, Bayshore, NY, #s212A; Harris hematoxylin with glacial acetic acid), then rinsed by deionized water, then tap water. Then dipped in 12x (fast) Acid ethanol (to destain) then rinsed in tap water. Eosin staining were done by mounting 30 sec in Eosin (Poly Scientific, Bayshore, NY; #s176; Eosin Phloxine stain,) and then dehydrated by 95% ethanol, 100% ethanol finally mounted in xylene Then the slides were covered using Permount (Fisher Scientific #SP15 -100; Histological mounting medium).

Immunohistochemistry: Serial sections of the whole pancreases of three rats from each group were done and then every 6 th section were immuno-stained by anti-insulin antibody for the detection of β- cells according to Bancroft and Cook (1994). In summary: The 30-µm-thick fresh pancreatic sections in horizontal plane were cut using a cryostat (Leica, Wetzlar, Germany). Slices were kept in cryoprotectant (30% ethylene glycol and 25% glycerin in 1× PBS) at −20 °C until processed for immunostaining. The sections were sequentially treat-

ed with 0.3% hydrogen peroxide (H2O2) in PBS for 30 min and 10% normal goat or rabbit serum in 0.05 M PBS for 30 min. They were next incubated with diluted rabbit anti-insulin (1:1, 000, Abcam, Cambridge, UK) overnight at room temperature and subsequently exposed to biotinylated rabbit anti-goat (diluted 1:200, Vector, Burlingame, CA, U.S.A.) and streptavidin peroxidase complex (diluted 1:200, Vector). Then, the sections were visualized with reaction to 3,3'-diaminobenzidine tetrachloride (Sigma) in 0.1 M Tris-HCl buffer (pH 7.2), counter stained with hematoxylin and mounted on gelatin-coated slides. Insulin+ve cells showed reddish brown deposits under the microscope

Morphometric studies: In all immunostained sections the islet size in µm were measured and the percentage of immune-positive insulin cells were measured and analyzed automatically by "Leica Qwin 500 C" Microscope with Image Analyzer Computer System Ltd. (Cambridge, England). Counting the mean number of islets/slide were also done from each section by counting islets in 10 non overlapping fields using an objective lens ×10 of all immuno-stained sections then dividing the total number by the total number of immuno-stained slides (total magnification ×100) of the light microscope (Danial et al, 1988)

Statistical analysis

The mean value for diameter of the islets, the mean number of islets/slide and the mean percentage (%) of insulin positive immuno-reactive cells/islet were calculated for all the groups. The results are expressed as mean± SE. The significance of the differences between the values was performed by one-way ANOVA test and Dunnett's Multiple Comparison Test using GraphPad Prism software. $P < 0.05$ was considered to be a significant difference.

RESULTS

PGE improves the fasting blood glucose level in alloxan- treated rats

The Diabetic control group showed a significant ($p < 0.05$) rise in blood glucose level as compared to the Normal control Group. On repeated administration of the PGE extracts, a significant ($p < 0.05$) decrease in blood glucose by time was found in doses of (200 mg/kg) and (400 mg/kg) respectively as compared to the Diabetic control group. After three weeks of PGE ingestion the hypoglycemic activity of PGE (400 mg/kg) was significantly higher than that of the treated by PGE in a dose of (200mg/kg) ($p < 0.05$) (Table 1).

PGE inhibits the intestinal α- glucosidase activity in alloxan -treated rats

To clarify the role of PGE on intestinal enzymes, that is responsible for digestion of carbohydrates, the activity of α- glucosidase was investigated in the small intestine of the rats which was treated with PGE. At 200 mg/kg dose, there is little inhibitory effect on α-glucosidase activity (mean 32.2% ± 1.2) of the control, but at a dose of 400 mg/kg, the reduction in this intestinal enzyme activity was 61.2% ± 1.3) which is significantly high. In similar conditions Acarbose treatment of diabetic rats at a dose of (1 mg/kg) showed reduction of α-glucosidase activity by 75.1% ± 1.2). Treatment of some diabetic rats with Acarbose enzyme, which is well known inhibitor of intestinal α- glucosidase, was used as a positive control in this study (Table 2).

PGE ameliorates the diabetic changes of alloxan- treated pancreas

Morphomteric studies: Morphometric analysis of the pancreatic islets of the studied groups showed that all the three measured parameters which are the mean diameter of the islet in µm, the mean number of islets/slide and the mean percentage of insulin immune- positive cells/islet) are all significantly much lower in the Diabetic group in comparison to that of the Normal control group ($P< 0.05$). The Diabetic+ PGE (200mg/kg) group showed increased values of the three parameters of the pancreas in comparison to that of the Diabetic control. However, there is still significant differences in these parameters between the Diabetic+ PGE (200mg/kg) group and the Normal control group. The diabetic rats that were treated with the higher dose of PGE (400 mg/kg) showed much increase in all the three analyzed parameters of the pancreas and there were non-significant differences between this group and the Normal control. This means that when treating diabetic rats with a dose of PGE of 400 mg/kg the islets size, islet number/slide and the percent of β cells/islet become more or less similar to that of the normal pancreas (Table 3).

H&E staining and immunohistochemical studies: Using H&E stain**,** the Normal control group showed the lobular architecture of the pancreas: The pancreas had abundant islet of Langerhans interspersed among the pancreatic exocrine acini. The islets appeared lightly stained than the surrounding acinar cells, with intact interlobular connective tissue (Figure 1A). Each islet consisted of lightly stained polygonal cells arranged in cords separated by a spaces which represent a network of blood capillaries. The acinar cells were characterized by its basal basophilia and apical acidophilia (Figure 1A). Anti-insulin stained pancreatic sections of the Normal control group (Fig. 1B) revealed dense reddish brown cytoplasmic immunoreactivity that was detected in the cytoplasm of the cells of the pancreatic islets of Langerhans. The immunoreactivity was observed in most of the islet cells except some cells which are insulin negative and represent other islet cell types than β - cells (Figure 1B). The mean percentage of the immuno-reactive insulin cells/islet was 90% ± 0.6 in the Normal control group (table 3).

By H&E stain, the islets of the diabetic group showed a decreased cellular density in most of the islets of the Langerhans. There were lots of gaps that indicated the presence of degenerated cells. Some islet cells were showing nuclear shrinkage and pyknosis mostly in the center of the islet (Figure 2A). Immuno-stained sections for insulin cells of the Diabetic group revealed markedly decreased immunoreactivity in most of the islets (Figure 2B). The apparent decrease in the insulin positive cells in the Diabetic group was confirmed by the analysis of the mean percentage of β - cells/islet which was detected to be 20% ± 0.5. This percentage was significantly much lower than that of Normal control ($P<0.05$) (table 3)

Sections of the Diabetic+ PGE (200mg/kg) group after 3 weeks of administration showed by H&E stain that there is an improvement of histopathological changes in a large number of islets. The border between exocrine and

endocrine portions became distinct. Many islets showed an apparantly increased cellular density (Figure 3A). Immunostaining for insulin of this group revealed apparently considerably higher immuno-reactive insulin cells in the islet of Langerhans than that of the Diabetic group (Fig. 3B). This was confirmed by the calculated precentage of the number of β -cells/islet which was 62% ± 0.1. But this number still significantly differ from that of Normal control group (Table 3).

H&E stained sections of the Diabetic+ PGE (400 mg/kg/d) group showed apparently normal architecture of most of the islets with good cellularity (Figure 4A). By immunohistochemistry there is apparently much increased insulin immuno-reactivity in most islets of this group. The β-cell number was apparently higher than the previous group (Fig. 4B). The percentage of β- cells/islet was 79 % ± 0.9 in the Diabetic+ PGE (400 mg/kg) group. This percentage showed a non-significant difference in comparison to that of the Normal control (Table 3). This means that treatment with a dose of 400 mg/kg PGE improves the diabetic changes of alloxan treated pancreas to be more or less similar to the normal one.

Table (1) Effect of *Punica Granatum* peel ethyl acetate extract on the blood glucose level in the studied groups

Mean blood glucose level in mg/dl ± SE					
Groups	**0 day (base line)**	**1st day (after 72 hours)**	**7 th day**	**14th day**	**21th day**
Normal control	89 ± 1.00	112 ± 0.93	109 ± 1.34	111 ± 0.86	114 ± 1.53
Diabetic Control	83 ± 0.79	296 ± 2.32*	337 ± 1.00*	369 ± 1.31*	357 ±2.81*
Diabetic + PGE; 200mg/kg/d)	103 ± 0.79	319 ± 2.32*	247 ± 1.00*	208 ± 1.31*[a]	187 ±2.81*[a]
Diabetic + PGE; 400mg/kg/d)	92 ± 1.21	313 ± 2.37*	208 ± 2.11*[a]	147 ± 1.62[a]	142 ±1.21[a]

The values are expressed as Mean ± SE ; One-way ANOVA followed by Dunnett's multiple comparison test was done. *= $p<0.05$ when compared to Normal Control Group. a = $p<0.05$ when compared to Diabetic Control Group.

Table (2): The percentage of inhibition of intestinal α- Glucosidase activity in the diabetic groups

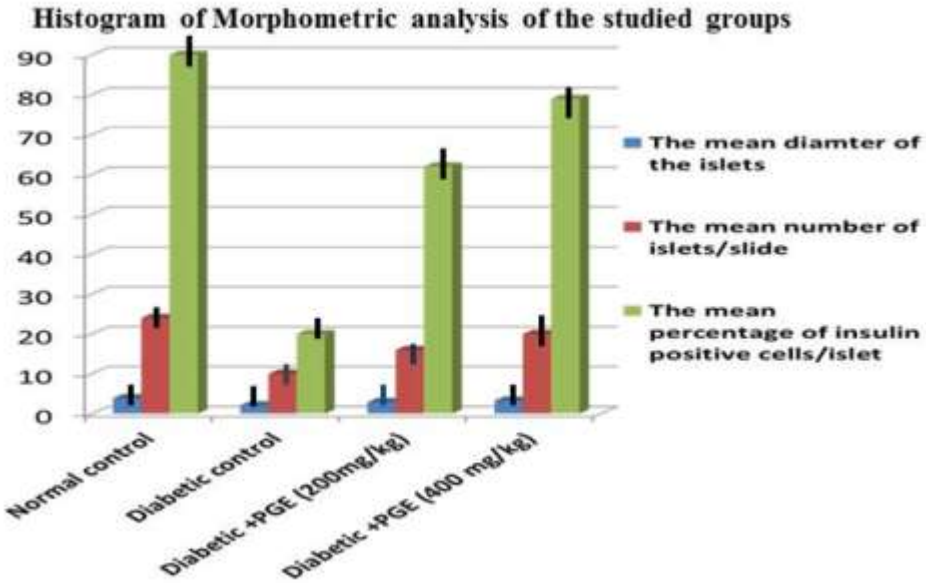
Groups	Percentage Inhibition of α- glucosidase (%)
Diabetic+ PGE (200mg/kg)	32.2 ± 1.2
Diabetic+ PGE (400mg/kg)	61.2 ± 1.3 *
Diabetic+ Acarbose (positive control)	75.1 ± 1.2 *

* significant at P<0.05. Results are expressed in the term of % inhibition. Acarbose enzyme treatment of some diabetic rats, was used as a positive control.

Table (3) Morphometric Analysis of the islets in the studied groups

Groups	Number of rats	Mean Diameter of islet (µm)	Mean Number of Islets/slide	Mean Percentage of the number of insulin immune- positive cells / islet (%)
Normal control	5	3.9± 0.9	24 ± 0 .7	90 ± 0.6
Diabetic control	5	2.1± 0.6*	10 ± 0.3*	20 ± 0.5*
Diabetic+ PGE (200mg/kg)	5	2.9± 0.6*	16 ± .8*	62 ± 0.1*
Diabetic+ PGE (400mg/kg)	5	3.3 ± 0.4 NS	20 ± 0. 5 NS	79 ± 0.9 NS

* = Significant at P<0.05. NS = non significant. Data are compared to the normal control and expressed as Mean ± SE



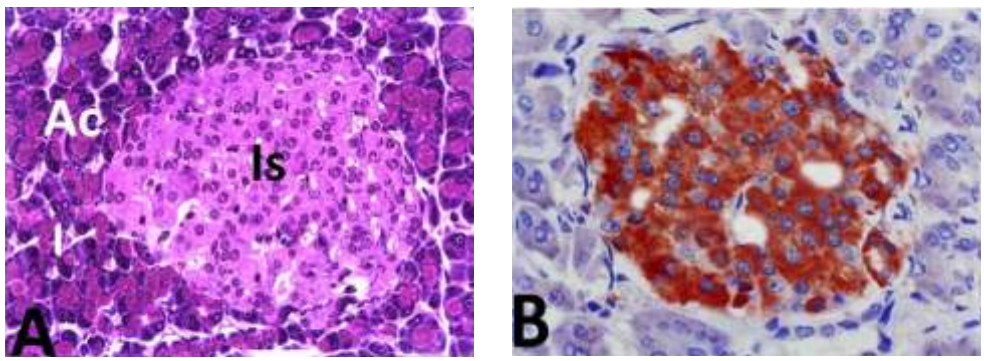


Fig. (1): Photomicrograph of the normal rat pancreas showing: **(A):** The normal structure of the pancreatic islet (Is) and acini (Ac). (H&E stain; X400) . **(B):** The normal islet of Langerhans contains a large number of immuno-stained cells for insulin (β-cells) while small number of cells are showing insulin negative immuno-reactivity and these cells represent other non β- cell types (anti-insulin immunohistochemistry counter stained with hematoxylin; X600).

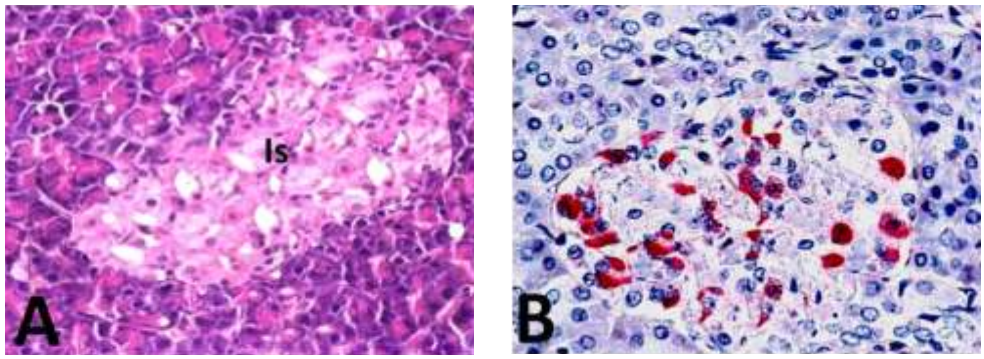


Fig. (2): Photomicrograph of rat pancreas of the Diabetic group showing: **(A)** The normal architecture of the islets is disrupted. The islets of Langerhans (Is) are hypocelullar. In addition there are hydrophobic cells, necrotic cells, vacuolizations and irregular hyperchromic nuclei (H& E; x 400). **(B)** Most beta-cells are damaged by alloxan administration as indicated by the few number of cells which are positive for anti-insulin (anti-insulin immunostaining counter stained with hematoxylin; X400).

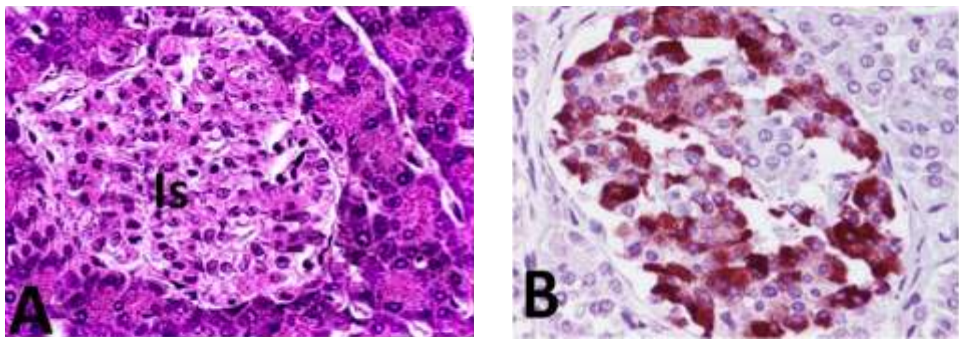


Fig. (3) A Photomicrograph of rat pancreas of the Diabetic+ PGE (200mg/kg) group showing: (A): An increase of intact islet (Is) cell density by administration of PGE (200 mg/kg/d) of alloxan induced- diabetic pancreas. (H&E stain; X600). **(B):** Apparent increase of β-cell number is seen in this group which is higher than the Diabetic group, as indicated by the increase in the number of insulin immuno-reactive cells in the islets (anti -insulin immunohistochemistry counter stained with hematoxylin; X400).

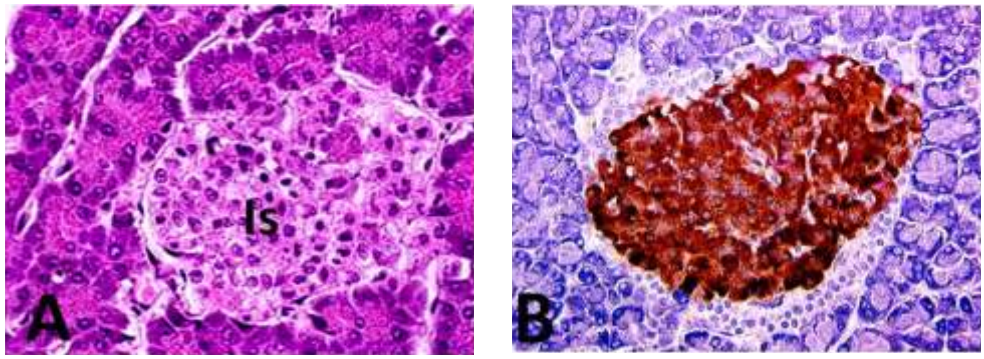


Fig. (4): A Photomicrograph of rat pancreas of the Diabetic +PGE (400 mg/kg) group showing: (A): The cellularity of the islet cells (Is) are increased by administration of PGE (400 mg/kg/d) of Alloxan treated pancreas. The architecture of the islet (Is) is apparently more or less similar to that of the normal pancreas. (H&E stain; X600). **(B)**: The number of anti-insulin immuno-positive cells (β- cells) are apparently high in comparison to that of the diabetic pancreas (anti-insulin immunohistochemistry counter stained with hematoxylin; X 400).

DISCUSSION

In this study the pancreatic beta cells were destroyed with the use of Alloxan. Alloxan and streptozotocin are the most usual applicable substance for induction of diabetes mellitus (Szkudelski, 2001). The rats were fasted for 12h before and after injection of alloxan, as unfed animals are more susceptible for alloxan induction of diabetes (Szkudelski et al., 1998; Katsumata et al., 1992).

The hypoglycemic effect of medicinal plants may be due to the presence of insulin-like substances in the plants (Gray and Flatt, 1999; Collier et al., 1987), stimulation of β cells to produce more insulin (Khan et al, 1990; Chang and Johnson 1980), or improving insulin action and binding (Khan et al., 1990). On the other hand some medicinal plants have high amount of fibers which interfere with carbohydrate absorption (Nelson et al., 1991). Others increase glucose metabolism (Broadhurst, 1997), or have a regenerative effect on pancreatic tissue (Arya et al., 2015).

In the present study, Alloxan-induced diabetic rats that were treated with PGE for 3 weeks, showed significant reduction in the high blood glucose levels as compared to the Diabetic control rats. These results suggested a significant anti-hyperglycemic effect of PGE in diabetic rats. These data were in agreement with the results of Das et al. (2001) who found that pomegranate seed extract led to significant reduction of blood glucose level in streptozocin induced diabetic rats. Another study supported the notion that the use of *P. granatum* especially the peel in diabetes is having anti-diabetic, hypolipedimic and antioxidant activity (Salwe et al., 2015).

The anti-diabetic effect of *pomegranate* seeds may be, partly, due to their positive effect on glycogen synthesis in the liver and the skeletal and the heart muscles, in combination with insulin-like or insulin releasing ingredients that existed in pomegranate seeds as detected by Das and Sarma (2009). The known compounds that existed in *pomegranate* seed, such as ellagic, gallic and ursolic acid, were detected as anti-diabetic compounds (Banihani et al., 2013). The presence of one or more bioactive anti-hyperglycemic principles, such as flavonoids, isoflavones, and their synergistic effects, are also involved in this hypoglycemic effect of PGE (Manoharan et al., 2009).

In the present study, the histological studies of the pancreas had been used to clarify the effect of applied plant on the pancreatic β cells. The diabetic untreated rat pancreas showed marked hypocellular islets of Langerhans due to the destruction of β-cells as shown by H&E stain and anti-insulin immunohistochemistry. There were also a decrease in the number of islets as well as the percentage of β- cells in the islets of the diabetic rats. Reduction in the numbers and insulin cells percentage of the islets by 70% was also reported previously in the diabetic dogs (Sudha Rastogi et al., 1990). In this study, the histological study of diabetic groups treated with PGE revealed significant increase in the size and number of the islets in the group received PGE at dose of 400 mg/kg, which may be a sign of regeneration of beta cells. However, a previous study revealed that there may be a reduction of β cells with an increase in size and number of islets and this was suggested to be due to an increase of other types of pancreatic cells specially α- cells (Sudha Rastogi et al., 1990). Thus, in the present study we perform an anti-insulin immunostaining to detect whether there were an actual increase in the number of β- cells of the Islets in the PGE treated pancreases or not. Thus the present study detected an increase in the number of insulin positive immuno-reactive cells in the Islets of diabetic rats treated with 200 mg/kg and 400 mg/kg PGE. But, It was noted that the rats treated with a doseof 400 mg/kg PGE showed more increase in the percentage of β-cells/islet which indicate that the larger dose had more protective effect than the smaller dose Our data suggest the presence of replicative effects of PGE treatment (especially with the high doses of 400 mg/kg/d) on the β-cells of the diabetic pancreas. A previous study found that the fruit peel of pomegranate had antioxidant effects (Elfalleh et al., 2012). This antioxidant action of PGE protect the existing β-cells from dying by their free radicals (Wang et al., 2015; Kaneto et al., 1999).

In the present study, investigation of the intestinal α-glucosidase activity of the diabetic groups revealed that the group that was treated with PGE in a dose of 400 mg/kg/d for 3 weeks showed a significant inhibition in activity of the enzyme. Ideally, any α-glucosidase inhibitor decreases oligosaccharides hydrolysis, thus decreases the digestion and subsequent absorption of the carbohydrates in the small intestine (Nakamura et al., 2012; Sim et al., 2008). Therefore, in the present study the inhibition of intestinal α-glucosidase contributes with the mechanism of hypoglycemic effect of PGE in the diabetic rats treated with the peels extract in a high dose (400 mg/

kg). These data were in agreement with the previous studies which detected that the *Punica Granatum* peel extract strongly inhibited the rat intestinal α-glucosidase *in vitro* (Bellesia et al., 2015; Salah El Din et al., 2014; Kam et al., 2013).

CONCLUSION

This study concluded that the ethyl acetate extract of the *Punica Granatum* (peels) had an antidiabetic and hypoglycemic effects. This hypoglycemic action could be via two mechanisms: by its contents of natural antioxidants that can protect existing β-cells and help the regeneration of islets. And by inhibiting the activity of intestinal α-glucosidase enzyme, thus decreasing the digestion and the absorption of the carbohydrates. Therefore, *Punica granatum* (peels) can be used as a food supplement for the regulation of hyperglycemia.

Conflict of interest: Non

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تأثيرات مستخلص قشر الرمان على نشاط الألفا – جلوكوزيديز المعوى وعلى الهستوباثولوجي للبنكرياس في الفئران المصابة بمرض السكر المستحدث بالألوكسان

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تم التعرف على الرمان على ان له تأثيرات مخفضة للسكر في الدم. وتمت هذه الدراسة لتقييم آثار المستخرج الإيثيلى لقشر الرمان (PGE) على نشاط أنزيم الامعاء ألفا– جلوكوزيديز وعلى البنكرياس في الفئران المصابة بالسكر المستحدث بواسطة الألوكسان. تم استخدام اربعين فأرا ذكرا بالغا وتقسيمهم الى: مجموعة (١) وهى المجموعة الطبيعية الضابطة. ومجموعة (٢) وهيا المجموعة المصابة بالسكر الضابطة ومجموعة (٣) وهيا مصابة بالسكر + مستخلص قشر الرمان (٢٠٠ ملجم / كج) ومجموعة (٤) مجموعة مصابة بالسكر + مستخلص قشر الرمان (٤٠٠ ملجم/ كج). أظهرت النتائج ان تناول مستخلص قشر الرمان بواسطة الفئران المصابة بالسكر وبجرعة ٤٠٠ ملجم/ كج ولمدة ثلاث أسابيع قلل بصورة إحصائية ذات دلالة من نسبة الجلوكوز في الدم وكذلك من نشاط أنزيم الألفا– جلوكوزيديز المعوى. أظهرت الدراسات الهستولوجيه والكمية للبنكرياس زيادة ذات دلالة احصائية في قطر

وعدد جزر لانجرهانز وكذلك زيادة النسبه المئوية للخلايا المتصبغة بالأنسولين لكل جزيرة (من جزر لانجرهانز) في مجموعة الفئران المصابة بمرض السكر ومعالجة بمستخلص قشر الرمان بجرعة ٤٠٠ ملجم/كج. تشير هذه المعلومات إلى أن تناول مستخلص قشر الرمان بجرعة ٤٠٠ ملجم / كج له تأثيرات مضادة لمرض السكر وذلك من خلال آليتين: ١. مساعدته على تجديد خلايا بيتا في البنكرياس . ٢. تثبيطه لنشاط الألفا – جلوكوزيديز المعوى وبالتالي تقليل إمتصاص الكربوهيدرات في الأمعاء الدقيقة . ولذلك فإن مستخرج قشر الرمان يمكن استخدامه كمكمل لتنظيم ارتفاع السكر في الدم.

١٥. المجلة المصرية للعلوم الطبية ٣٦(١) يونيو ٢٠١٥: ٢٥٥–٢٧٢.