Original Article

Ameliorative Effect of Watermelon rind ingestion on the Pancreas of Diabetic Female Albino Rat (Histological, immunohistochemical and morphometric study)

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ABSTRACT

Introduction: Type II diabetes is a multifactorial metabolic disorder that affects more than 348 million people worldwide. The pathway of type II diabetes is characterized both by insulin resistance in muscle, fat, and liver in addition to a relative failure of pancreatic cells. CitrullusLanatus (Watermelon) is one of the few foods naturally rich in amino acids citrulline and lycopene which decrease serum glucose concentration in diabetic rats.

Aim of the study: To study the structural changes in diabetic pancreas and the possible role of watermelon rind ingestion in ameliorating the pancreatic changes in experimentally-induced type II diabetes mellitus in rats.

Materials and methods: Eighteen female adult albino rats weighing 170g-200g were randomly divided into three equal groups. Diabetes was induced in all the rats except GI by intraperitoneal injection of 120 mg/kg.b.wnicotinamide, followed by streptozotocin (STZ) at a dose of 60mg/kg.b.w. 15 minutes later, to induce type 2 diabetes. Rats were divided into three groups: namely GI control, GII diabetic, GIII diabetic watermelon rind juice treated. The mean values of body weight and the blood glucose levels were assessed. Histological study of the different experimental groups was done using H&E, Mallory trichrome, Orcein, and immunohistochemical stain for detection of Endothelial Nitric oxide Synthase eNOS positive endothelial cells. Data obtained by morphometric and statistical studies were discussed in relation to the microscopic findings.

Results: Diabetes caused harmful effects on the structure of the pancreas. In addition, watermelon rind improved the histological findings if compared with the diabetic untreated group. Also, watermelon rind treatment showed strong immunohistochemical expression for eNOS.

Conclusion: Watermelon rind ingestion in Streptozotocin induced diabetic female albino rat resulted in ameliorating the structural changes in the pancreas.

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Key Words: CitrullusLanatus, diabetes, eNOS, nicotinamide, pancreas, rats, streptozotocin.

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INTRODUCTION

Diabetes mellitus is a major disorder of the human race implicated with numerous clinical manifestations. It is a clinical syndrome manifested with chronic hyperglycemia associated with impaired carbohydrate, fat and protein metabolism resulting from either insulin deficiency, or decreased sensitivity of the tissues to insulin. It has been reported that the diabetic chronic hyperglycemia is usually followed with complications like renal failure, coronary artery disorder, neurological complications, cerebro-vascular disease, blindness, limb amputation, organs dysfunctions and eventually premature death[1].

Diabetes is also associated with severe long-term micro-and macro vascular complications, that carries a high rate of morbidity and mortality. Type I and II diabetes is a significant public health concern leading to a constant increase in treatment costs[2].

Different regimens of insulin therapy failed to offer complete cure and could not prevent associated diabetic complications. Moreover, treatment of diabetes with insulin was frequently associated with severe hypoglycemic episodes[3,4]. Conventional antidiabetic drugs have relatively high incidence of side effects. The management of diabetes without any side effect is still therefore a major challenge. According to the World health organization (WHO), approximately 80% of the world's population currently uses herbal medicines in healing different ailments[1].

Herbal products are characterized by their low side effects, accessibility and affordability when compared with conventional drugs[5]. Watermelon rind is one of the few foods naturally rich in amino acid citrulline that can be metabolized to arginine which decreases serum glucose concentration in diabetic rats. Moreover, watermelon rind is rich in lycopene, a carotenoid of antioxidant capacity and potential health benefits. Therefore watermelon has antioxidant, cardioprotective, antidiabetic and anti-inflammatory activities[6].

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The current study was designed to study the structural changes in diabetic pancreas and the possible role of watermelon rind juice ingestion in ameliorating the pancreatic changes in case of experimentally-induced diabetes mellitus.

MATERIALS AND METHODS

Materials

1- Streptozotocin (STZ):

Streptozotocin was purchased from Sigma-Aldirch Company (St. Louis, Mo, USA). Rats were injected by a single intraperitoneal (i.p) dose of STZ 60 mg/kg body weight (b.w), which was freshly dissolved in 1ml citrate buffer (PH 4.5)[7].

2- Nicotinamide (NA):

NA was purchased from Sigma–Aldirch Company (St. Louis, MO, USA). It was used to protect theβ cells from the hazardous effect of STZ in induction of type II diabetes mellitus. Nicotinamide was dissolved in normal saline and administered as a single dose. The dose was 120 mg/kg.b.w injected intraperitoneally 15 min before STZ. Nicotinamide was freshly prepared, each 20 mg nicotinamide were dissolved in 1 ml saline[7].

3- Citrulluslanatus (watermelon rind):

Watermelon was purchased from the market, the fresh rind of watermelon was peeled off with a peeler, then the rind was cut into small pieces, and the rind pieces were minced in a mixer. Liquid was collected in a beaker, preserved in the refrigerator and used for 3 days. Each 100g of watermelon rind gave 70ml juice, the used dose was 1ml of rind juice given daily/animal by orogastric tube.

4- Animals:

This study included eighteen adult female RattusNorvogicus albino rats, 8-10 weeks of age and weighed 170-200 g. They were housed in the animal house at the Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt. Rats were housed in conventional stainless steel meshed cages at room temperature under strict care and hygienic conditions. Environmental conditions such as humidity, heat, light and ventilation were kept constantly for 24 hours daily during the period of the study. The animals were supplied with water and classic rat chow adlibitum and they were kept under observation for 7 days before the start of the experiment in order to acclimatize them and to exclude any intercurrent infection. Then the animals were divided into three groups, 6 rats each.

Control Group, Group I (GI):

Half of the animals of this group received 1 ml citrate buffer, subgroup (1a), and the other half 1 ml saline, subgroup (1b), single dose intraperitoneal.

Diabetic Group, Group II (GII):

Animals were given 120 mg/kg. b.w nicotinamide intraperitoneally, then after 15 minutes, intraperitoneal injection of STZ at a dose of 60 mg/kg. b.w was performed to induce type 2 diabetes, then this group was left till the end of experiment[8].

Diabetic-Watermelon Group, Group III (GIII):

Rats were given nicotinamide followed by STZ as the previously mentioned regimen. Induction of diabetes was confirmed by estimation of blood glucose level after 72 hrs. Diabetic rats ingested daily 1ml watermelon rind juice/ animal using an orogastric tube till the day of sacrifice.

Blood and histological samples after three weeks were collected from all animals after 3 weeks from the injection.

The body weight of rats of all groups was measured at the beginning and at the end of the experiment.

Methods

Blood sampling

Blood samples were collected to estimate the blood glucose levels at the beginning 3days after injection of STZ and at the end of present experiment. The blood samples were collected from the tail vein of the overnight fasted rats it. Estimation of blood glucose level was performed using a glucometer (One Touch Select Mini Blood Glucose Monitoring System, Life Scan Europe Division of Cilag GmbH international 6300 Zug Switzerland).Only rats with a basal blood glucose level above 200 mg/dL were considered diabetic[9].

Histological Study

After proper fixation of pancreatic specimens form the tail using 10% neutral buffered formalin the specimens were dehydrated, cleared in xylol, impregnated and then embedded in paraffin wax. Five microns sections were cut and mounted on glass slides. For immunohistochemical studies, sections were mounted on positively charged slides. The consecutive slides were subjected to the following stains:

Haematoxylin and Eosin (H&E) stain for routine histological examination of the pancreas Mallory's Trichrome stain to demonstrate the collagen fibers[10] and Orcein stain to demonstrate the elastic fibers[11].

Immunohistochemical technique was used to localize endothelial nitric oxide synthase (e-NOS) in the pancreatic vasculature[12]. Immunohistochemical protocol (horse-radish-peroxidase) was used to visualize the intensity and distribution of eNOS immune staining in the pancreas of the experimental rats. The sections were counterstained with hematoxylin and mounted.

To validate the specificity of the immune staining, the positive and negative controls were carried out. External positive tissue control was a human full term placenta tissue (according to data provided by the antibody manufacturer). For negative controls, slides were incubated similarly to the above protocol, by replacing the primary antibody

with PBS (Phosphate- Buffered Salaine). Immuno freshly prepared histochemical staining was evaluated using a light microscope at ×400 magnification. The final results of study were expressed as percentage of optical density.

Morphometric study

Measurement were performed using the image analyzer computer system (Leica Qwin 500; Leica, Cambridge, UK) at the Pathology Department, Faculty of Dentistry, Cairo University. For each parameter ten different non overlapping fields from five different sections were examined in each group. The results were expressed as mean ± SD[13].

The following parameters were measured:

Mean area percentage of collagen fibers in Mallory's trichrome stained sections (At magnification X100).

Optical density of eNOS immunohistochemical stained sections of pancreatic endothelium (at magnification X400).

Statistical analysis

Body weight, blood glucose level, area percentage of collagen fibers and optical density of eNOS immune positive cells were estimated in all studied groups.

Data evaluation was expressed as means ± Standard Deviation (SD) and analysis was performed using student t-test for data analysis between different experimental groups[14]. P≤0.05 was considered statistically significant. Data were tabulated and represented graphically.

RESULTS

Body weight and blood glucose levels:

The baseline weight in all the groups was nearly similar ranging from 170-200 gm. Rats of the control group showed an increase in body weight at the end of the experimental period (206.66gm±10.32). Highly significant decrease in the body weight of the diabetic rats (GII) (163.33gm±13.66) was observed when compared with the corresponding control group (GI). However, watermelon rind effectively ameliorated STZ-induced weight loss. In watermelon rind treated diabetic group (GIII), the body weight was higher than STZ group (GII), but not statistically significant (168.33gm±11.69) (Table 1) and (Histogram 1).

Fasting blood glucose (FBG) concentration increased significantly following STZ injection compared with the control group. The diabetic rats in group III, which received watermelon rind juice showed a significant decrease (P ≤ 0.05) in FBG as compared with the rats of the untreated diabetic group II (Table 2) and (Histogram 2)

Morphometric results:

Area % of collagen fibers

Comparing the means of area % of collagen fibers/µm² surface area in the pancreatic tissue among the experimental groups stained by Mallory's trichrome stain revealed that the area occupied by collagen fibers was higher in STZ group (GII) if compared to the control group (GI) and it was lower in group III if compared to the STZ untreated group. The least mean was in the control group (GI) (8.55±2.48) followed by watermelon rind treated diabetic group (GIII) (11.55±2.28).Finally the highest mean was recorded in diabetic rats (GII) (15.52±3.61). These findings were of statistically significant values (P ≤ 0.05). All these data were presented in (Table 3) and (Histogram 3).

Optical density of eNOS immunohistochemical stained sections

The present study showed that in all the experimental groups, the endothelial lining of blood vessels had positive staining for antibody to e-NOS. Statistical study of the Optical density of e-NOS immunohistochemically, stained endothelial cells of the pancreatic blood vessels showed that the highest mean was recorded in the control group (GI) (0.65±0.014) followed by watermelon rind treated diabetic group (GIII) (0.63±0.01).The least mean was recorded in diabetic rats (GII) (0.55±0.02).

However, these findings were of statistically significant values (P≤0.05), and the data were presented in (Table 3) and (Histogram 4).

Histological results

• Haematoxylin and Eosim stain

No histological differences were observed in the two subgroups of the control. Histological sections stained by H&E of the control group (GI) showed that the parenchymatous tissue of the pancreas was divided into exocrine and endocrine part. (Fig.1).The pancreatic acini appeared as small rounded structures with narrow lumens. The lining cells were roughly pyramidal with indistinct cell boundaries and rounded basal nuclei. Their cytoplasm showed basal basophilia and apical acidophilic zymogen granules. (Fig. 2).

The endocrine component (islets of Langerhans) were scattered as lightly stained areas between the darkly stained exocrine glandular tissue. The islets of Langerhans varied in size and were composed of masses and cords of secretory cells with numerous fenestrated capillaries in between. Some endocrine cells exhibited pale acidophilic cytoplasm and pale prominent nuclei mostly situated at the center. Other cells had strongly acidophilic cytoplasm and dark nuclei were found mainly at the periphery of the islets. The blood capillaries within the islet were recognized by the flat nuclei of the capillary endothelium (Fig. 2).

On the other hand, the diabetic untreated group (GII) showed marked structural variations particularly in the pancreatic vasculature and islets of Langerhans. Most of the blood vessels were congested. (Figs. 3, 4).

The islet cells were separated by empty spaces.

Some islet cells appeared to have very small pyknotic or disintegrated nuclei, while other cells were devoid of their nuclei. Most of the cells at the periphery of the islets were intact and contained darkly stained nuclei. (Fig. 5).

Pancreatic sections of the diabetic-watermelon rind treated group (GIII) showed that the islets appear more or less comparable to the control. Some scattered blood vessels that in between the acini were congested. (Figs. 6, 7).

• *Mallory Stain:*

Pancreatic sections of the control group stained with Mallory revealed usual distribution blue collagen fibers within the connective tissue septa, around pancreatic acini, ducts and blood vessels. Some collagen fibers were scattered in between the islet cells. Large vessels were surrounded with dense amount of collagen, while small ones were only surrounded with thin collagen fibers. (Fig. 8).

The β-cells were distributed all over the islets, and stained blue with Mallory's trichrome, while the α-cells were stained deeply pink and tend to be at the periphery of the islet. The blood capillaries in between the islet cells were recognized by the presence of intraluminal blood. (Fig. 8).

On the other hand, the diabetic group showed dense collagen fibers around the islets of Langerhans, in between the islet cells and also around the pancreatic acini. The islets of Langerhans contained many vacuoles, the β-cells which stained blue were rarely seen while the α-cells which stained deeply pink were more numerous (Fig.9). Collagen fibers were thickened in the interstitial tissue or between and around the blood vessels. Some of these vessels were congested. (Fig. 10).

The diabetic-watermelon rind treated group showed blue collagen fibers around the pancreatic acini, scattered in between the islet cells and forming the main constituents of the blood vessels' wall (Figs. 11, 12). Generally collagen fibers were more condensed if compared to the control group but less condensed if compared with the diabetic group. These findings were confirmed by the statistical results. The islet contained some blueβ-cells present at the islets' center, while red α-cells were detected at the periphery (Fig.11).

• *Orcein Stain:*

Histological sections stained by orcein stain in the control group showed dark purple to dark brown elastic fibers, usually forming continuous wavy well developed layer delineating the inner layer of the vessel lumen (Fig. 13).

However, in the diabetic group the wall of the blood vessels showed irregular wavy elastic fibers. Some of the elastic fibers were fragmented. Subintemal vacuolations were also seen (Fig. 14).

The diabetic-watermelon rind treated group showed the usual appearance of the elastic fibers of the pancreatic blood vessels which were stained deeply brown. Elastic fibers appeared more corrugated, and forming continuous line if compared with the diabetic group. Some vacuoles within the vessel wall were still recognized (Fig. 15).

Endothelial Nitric oxide Synthase- immunostained pancreatic sections

Nitric oxide synthase stained the cytoplasm of endothelial cells of blood vessels by brown colour (positive reaction). This positive reaction was seen in the pancreatic histological sections of the control group, along the inner aspect of the vessel wall. (Fig. 16).

The immunohistochemical reaction of eNOS in the diabetic group was weak +ve reaction identified in the endothelium of the blood vessels compared to the control group (Fig. 17).

Examination of histological sections stained by eNOS antibody in the diabetic-watermelon rind treated group showed that pancreatic endothelial cells appeared with marked positive staining if compared with the diabetic group, if compared with the control group (Fig. 18).

Table 1: Means of body weights at the end of the experiment in the different experimental groups

Variable / Studied groups	At the end of the experiment Mean±SD
Control group (GI)	206.66±10.32
Diabetic group (GII)	163.33±13.66[*a]
Diabetic watermelon rind treated group (GIII)	168.33±11.69[b]

[*] $P \leq 0.05$ = statistically significant.

[a] compared with control group (GI)

[b] compared with diabetic group (GII)

Histogram 1: Means of body weight at the end of the experiment in different experimental groups.

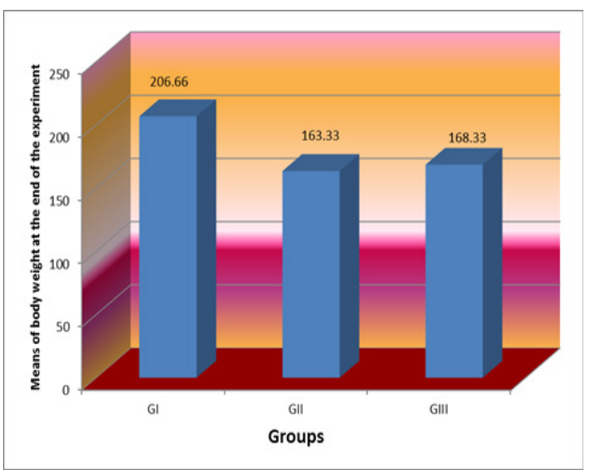


Table 2: Means of blood glucose levels on the 3rd day of induction of diabetes and at the end of the experiment in different experimental groups

Variable / Studied groups	On the 3rd day of induction Mean ±SD	At the end of the experiment Mean ±SD
Control group (GI)	88±9.87	88±7.29
Diabetic group (GII)	471.33±111.21[*a]	505.16±148.17[*a]
Diabetic watermelon rind treated group (GIII)	369.83±7.49[*b]	171.33±10.48[*b]

[*] $P \leq 0.05$ = statistically significant.

[a] compared with control group (GI)

[b] compared with diabetic group (GII)

Histogram 2: Means of blood glucose on the 3rd day of induction and at the end of the experiment in different experimental group

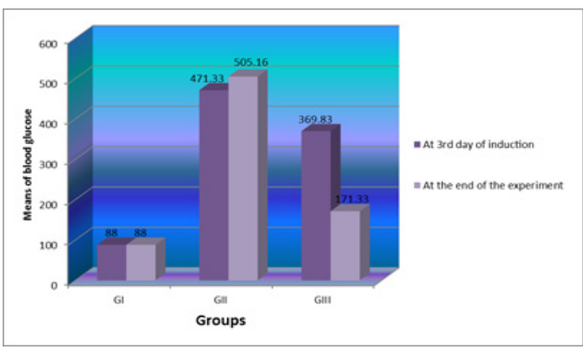


Table 3: Mean values of collagen area % &optical density of eNOS in different experimental groups

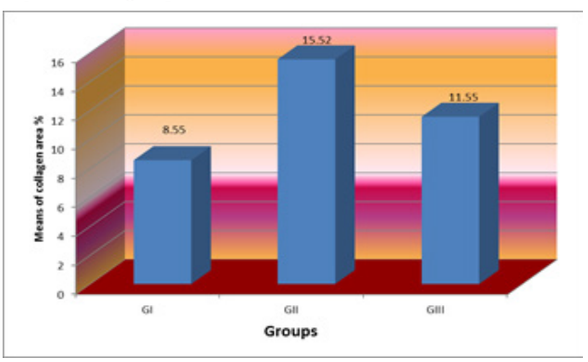
Variable / Studied groups	Collagen area % Mean ±SD	Optical density of eNOS Mean ±SD
Control group (GI)	8.55±2.48	0.65±0.014
Diabetic group (GII)	15.52±3.61[*a]	0.55±0.02[*a]
Diabetic watermelon rind treated group (GIII)	11.55±2.28[*b]	0.63±0.01[*b]

[*] $P \leq 0.05$ = statistically significant.

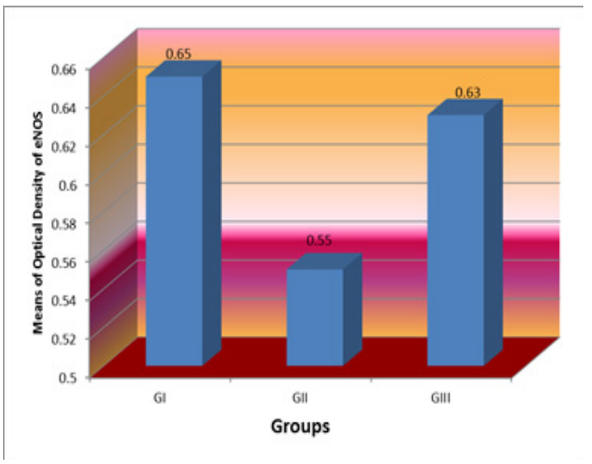
[a] compared with control group (GI)

[b] compared with diabetic group

Histogram 3: Showing the means of collagen area % in different experimental groups



Histogram 4: Showing the means of optical density of eNOS in different experimental groups



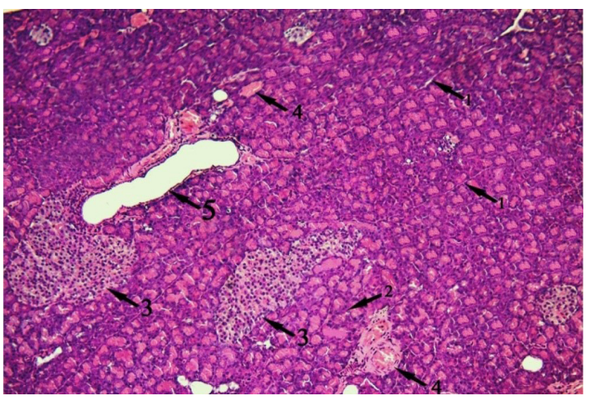


Fig. 1: A photomicrograph of a pancreatic section (GI) showing: pancreatic septa (arrow1). Deeply stained pancreatic acini (arrow2). Lightly stained islets (arrow3). Some blood vessels are distributed throughout the field (arrow4) and Interlobular duct (arrow5). (H&E, x100)

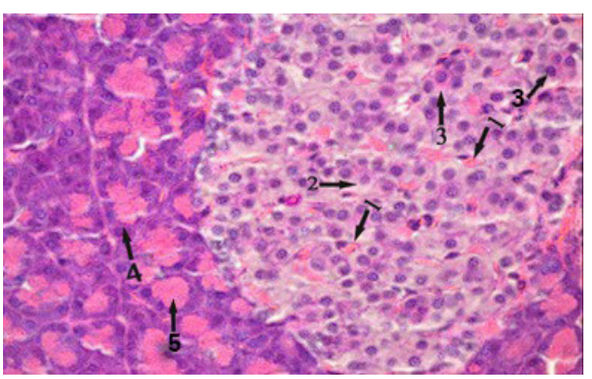


Fig. 2: A photomicrograph of a pancreatic section (GI) showing: One pale stained islet rich in capillaries (arrow1). Some endocrine cells have pale acidophilic cytoplasm and pale prominent nuclei at the center (arrow2), while other cells have strongly acidophilic cytoplasm and dark nuclei found mainly at the periphery of the islet (arrow3). Notice the basal basophilia and the apical acidophilic (arrow4) and granules within the acinar cells (arrow5). (H&E, x400)

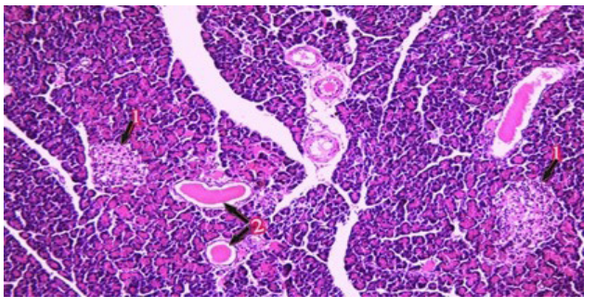


Fig. 3: A photomicrograph of a pancreatic section (GII) showing: Islets of Langerhans (arrow1) and many congested blood vessels (arrow2).

(H&E, x100)

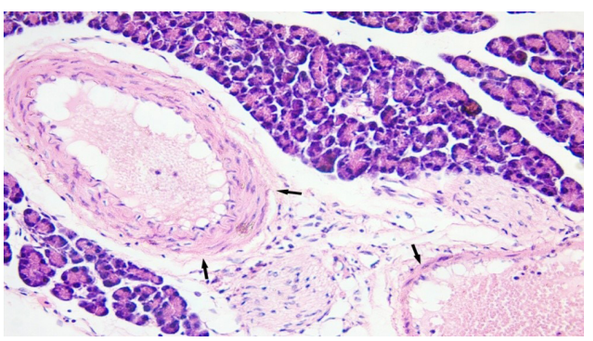


Fig. 4: A photomicrograph of a pancreatic section (GII) showing: Wide congested blood vessels (arrows). (H&E, x200)

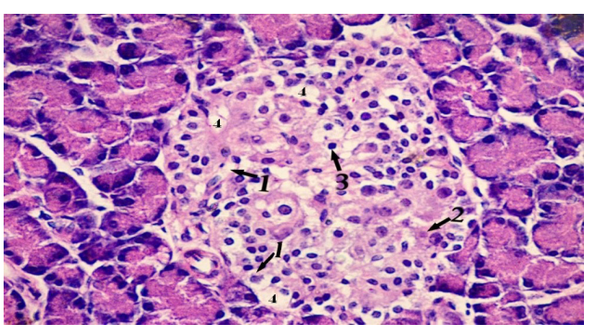


Fig. 5: A photomicrograph of a pancreatic section GII showing: Blood capillaries (arrow1), Cells with pale nucleus and pale cytoplasm (arrow2), Cells with dark pyknotic nucleus (arrow3) and Vacuolations[4].

(H&E, x400)

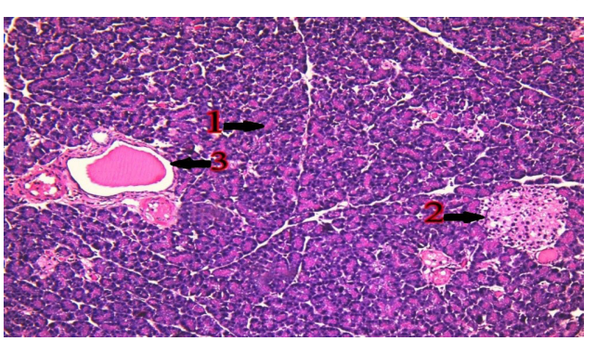


Fig. 6: A photomicrograph of a pancreatic section (GIII) showing: The pancreatic acini are generally similar to the control group (arrow1). The islet of Langerhans is paler than the surrounding tissue (arrow2). Some blood vessels are congested (arrow3). (H&E, x100)

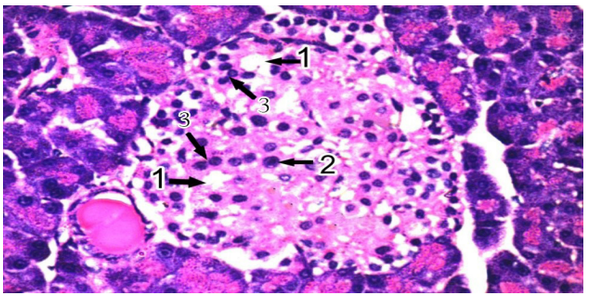


Fig. 7: Photomicrograph of a pancreatic section (GIII) showing: Presence of some vacuolations in the islet (arrow1). Some of the islet cells contain large hyperchromatic nuclei (arrow2). Mitotic figures are seen (arrow 3).

(H&E, x400).

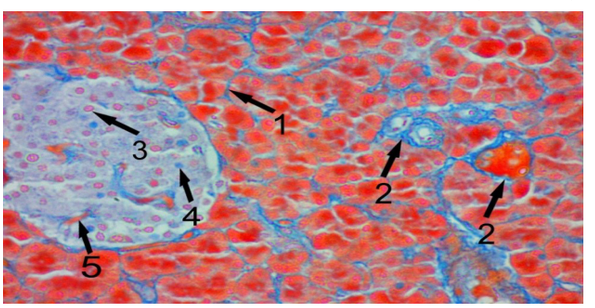


Fig. 8: A photomicrograph of a pancreatic section (GI) showing: Collagen fibers (stained blue) around pancreatic acini (arrow 1) and around small and large vessels (arrow2). Islets of Langerhans contain α-cells (arrow3), β-cells (arrow4), and blood capillaries (arrow5).

(Mallory's Trichrome, x400)

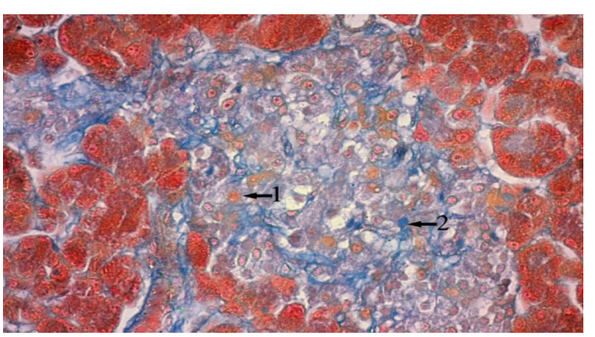


Fig. 9: A photomicrograph of a pancreatic section (GII) showing: Islet contains α-cells (arrow1). Rarely seen β-cells (arrow2). Dense collagen fibers around and in between islet cells. (Mallory's Trichrome, x400)

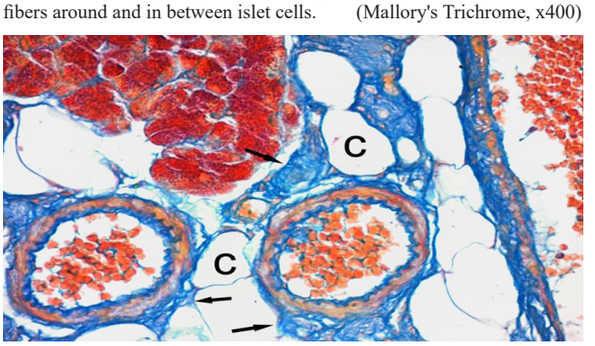


Fig. 10: A photomicrograph of a pancreatic section (GII) showing: Congested blood vessels (B). Condensed collagen fibers around and in between the blood vessels (arrows). Large empty (fat-like spaces) are noticed (C). (Mallory's Trichrome, x400)

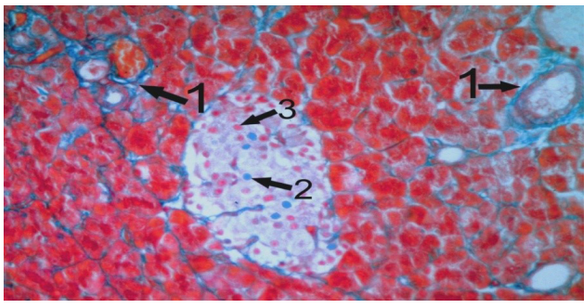


Fig. 11: A photomicrograph of a pancreatic section GIII showing: collagen fibers(stained blue) around pancreatic acini, scattered in between the islet cells, and forming the main bulk of the blood vessels (arrow 1). Islet contains β-cells (arrow2) and α-cells (arrow3).

(Mallory's Trichrome, x400)

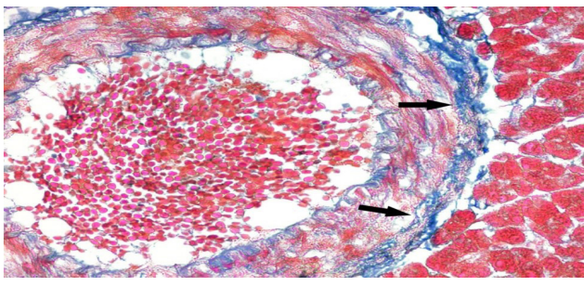


Fig. 12: A photomicrograph of a pancreatic section (GIII) showing: collagen fibers around the blood vessel (arrows).

(Mallory's Trichrome, x400)

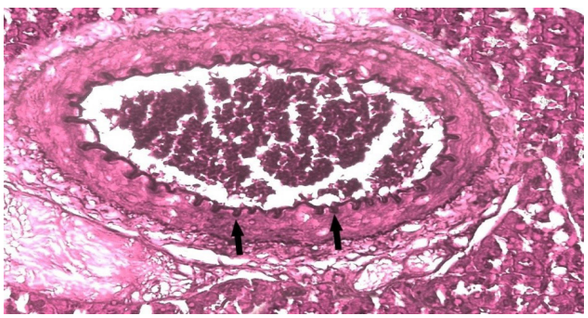


Fig. 13: A photomicrograph of a pancreatic section (GI) showing: A large blood vessel filled with blood, its wall contains continuous wavy layer of elastic fibers which appears deep brown (arrows). (Orcein, x400)

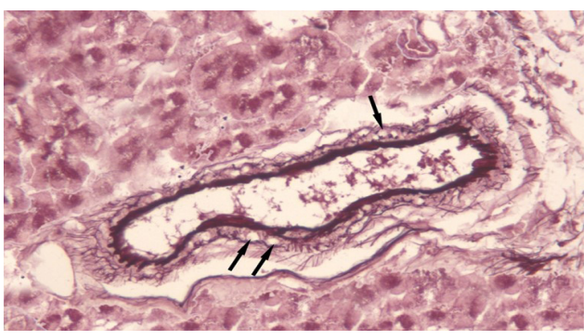


Fig. 14: A photomicrograph of a pancreatic section (GII) showing: The elastic fibers appear to lose their wavy appearance and are straightened at a certain area. Also, subintimal vacuolations (arrows) can be observed.

(Orcein, x400)

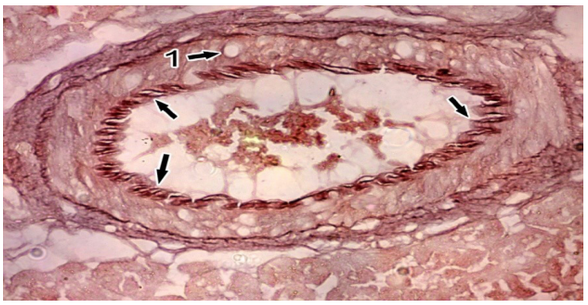


Fig. 15: A photomicrograph of a pancreatic section (GIII) showing: The elastic fibers of the blood vessel are stained deep brown. They appear wavy (arrow). Some vacuolations within the vessel wall are recognized (arrow1). (Orcein, x400)

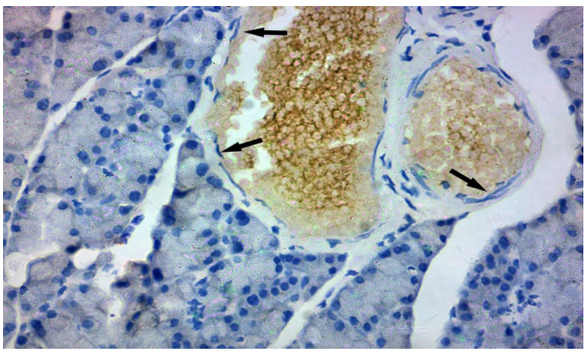


Fig. 16: A photomicrograph of a pancreatic section (GI) showing: Immuno-positive stained endothelial cells of the blood vessels which appear brown in color (arrows). (eNOS antibody, x400)

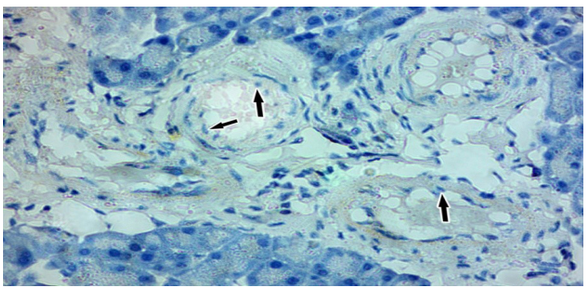


Fig. 17: A photomicrograph of a pancreatic section (GII) showing: Weak +ve reaction of the eNOS antibody of the endothelial cells (arrows).

(eNOS antibody, x400)

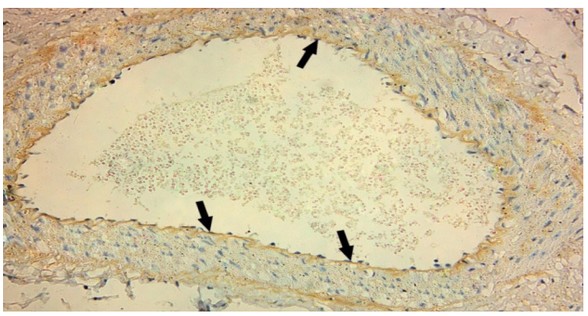


Fig. 18: A photomicrograph of a pancreatic section (GIII) showing: Marked immuno-positive staining of the endothelial cells of the blood vessels which stained brown (arrows). (eNOS antibody, x400).

DISCUSSION

Diabetes is the most common metabolic disorder in the world. According to the World Health Organization, it is the third most prevalent disease after cardiovascular and oncological diseases. Nowadays, it is widely accepted that the beneficial health effects of fruits and vegetables in the prevention of diseases could be related to their bioactive ingredients[15].

One of the ways to control diabetes mellitus is through the diet and fruits that can have a crucial role[1]. So the current study was done to demonstrate the modulating effect of watermelon rind juice ingestion as a natural therapeutic agent on the pancreas in a model of nicotinamide streptozotocin-induced type II diabetic rats.

In our study, measurements of the diabetic rats' body weight revealed a highly significant decrease in their body weight in comparison with the control group. These results were in agreement with the results of[16, 17, 18], who attributed the decrease of body weight either to the increase of the urine output causing dehydration and loss of valuable fluids or to the breakdown of muscles caused by high blood sugar. Also[19] mentioned that muscle wasting and loss of adipose tissues were responsible for the weight loss in diabetes and this is due to the increased rate of proteolysis and lipolysis in diabetic state.

On the other hand, treatment of the diabetic rats with watermelon rind protected the rats against characteristic diabetic weight loss as there was insignificant increase in their body weight when compared with that of the diabetic non treated group. This was in agreement with[6]. They attributed this effect to the hypoglycemic potential of watermelon rind.

In this research, the blood glucose level showed a highly significant increase after STZ injection till the end of the experiment in (GII). This is in accordance with the researches of[20, 21] who referred this elevation the reduction in the plasma insulin levels caused by selective necrosis of pancreatic beta cells.

Watermelon rind ingestion treatment was found to have beneficial effect on hyperglycemia. Blood glucose level returned to a relatively normal value. This result is in agreement with[22]. They added that rind extract was found significantly reduce the blood glucose level of rats, similar to the standard anti-diabetic agents used.

They also explained the antidiabetic activity of watermelon rind by the presence of plant secondary metabolites like flavonoids and polyphenols, known for their antioxidant and possible hypoglycemic activities.

Furthermore, watermelon is one of the few foods naturally rich in citrulline, an effective precursor for arginine synthesis. Arginine was reported to have various biological activities. Dietary arginine supplementation decreases serum glucose concentration in diabetic rats. Interestingly, it was reported that watermelon consumption increased plasma arginine concentration in humans[6].

In the present study, H&E stained sections showed that the pancreatic islets of the diabetic group (GII) displayed marked morphological changes in the form of marked degeneration of islet cells. These degenerated cells appeared with nuclear pyknosis, fragmentation, while others showed cytoplasmic vacuolations.

The present findings are in accordance with[23] who reported that STZ injection caused β cell damage in rat pancreas. Also[24] demonstrated a histological picture of the pancreas of diabetic rats more or less similar to the results of the present study.

Some of the major morphological changes that occur with necrosis were deduced by[25]. These include cell swelling with formation of cytoplasmic vacuoles, while other cells exhibited small darkly stained nuclei with condensed chromatin together with darkly acidophilic cytoplasm probably denoting apoptosis.[26] described pyknosis as condensation of chromatin in the nucleus of a cell undergoing necrosis, while[27] referred pyknosis to apoptosis.

Vacuolation was described by[28] as one of the structural indications of permeability disorders of the membranes, which results in an enhanced transport of water and electrolytes into the cell. The permeability disorder could be attributed to many cellular membrane insults caused by reactive oxygen species mediated lipid peroxidation.

In the current study, congestion of some pancreatic blood vessels was detected in diabetic group.[29]documented that nitric oxide is an important molecule that involves many vascular functions. Diabetes induced inactivation of nitric oxide leading to diabetic dilatation and diabetic vascular complications.

Islet cells of group III (watermelon rind treated diabetic group) had regenerated considerably. This may be due to the presence of stable cells in the islets with the ability of regeneration. This also suggests that the watermelon rind has the ability of inducing the quiescent cells to proliferate and replace the lost cells. The exact mechanism is not known but it has been proved that, its active constituents (flavonoids and polyphenols, etc) acting singly or synergistically cause regeneration of pancreatic β cells[22].

Histological examination of diabetic rat pancreatic sections (GII) stained with Mallory trichrom stain revealed, significant increased amount of collagen fibers around the islands of Langerhans, in between the islet cells, around the pancreatic acini and in the wall of blood vesseles compared to the control group (GI).On the other hand, there was significant decreased amount of collagen fibers in (GIII) as compared to the diabetic group (GII).This finding is in agreement with[30, 31] who found that the collagen fibers were seen around the blood capillaries and between the endocrine and the exocrine portion of the pancreas causing insufficient oxygen to reach the tissue, which resulted in degenerative changes and necrosis.

It was suggested that pancreatic stellate cells might participate in the development of pancreatic fibrosis. These cells are similar to hepatic stellate cells. High concentrations of glucose and insulin as in case of type II diabetes contribute to pancreatic stellate activation leading to the fibrotic changes. Also, invasions of the pancreatic islets by stellate cells result in fibrotic islet destruction which leads to the limited capacity of beta-cell proliferation and the accelerated apoptosis in diabetic patients[32].

Pancreatic sections (GII) stained with orcein stain revealed, irregularity and fragmentation of the elastic fibers with sub intimal vacuolations, while elastic fibers appeared wavy and forming continuous line in watermelon rind treated group (GIII) if compared with the diabetic group. However, some vacuoles within the vessel wall were still recognized.

Our results are partially in accordance with[33] who stated that, the morphological changes were in the form of intimal thickening, migration and proliferation of smooth muscles, intracellular lipid accumulation, fragmentation of elastic fibers and increase in collagen deposition.[34, 35] suggested that migration and proliferation of smooth muscle cells from the tunica media to the tunica intima and the parallel increase in collagen synthesis in diabetic blood vessels was due to platelet derived growth factors (PDGF) present in diabetic environment or released from injured endothelial cells. These growth factors were reported to stimulate smooth muscle cell migration and proliferation[36].

Irregularity and fragmentation of elastic fibers had been explained by decreased elastin synthesis by smooth muscle cells as a results of oxidative damage[37] or due to the increased activity of matrix metalloproteinase from the vascular endothelium as well as oxidative stress that break down collagen and elastin[38].

On the other hand, the subintemal vacuolations may be explained by the work of[39] who mentioned that, macrophages and smooth muscle cells absorbed lipid and their cytoplasm became swollen.[40] Added that lipids have been oxidized by the endothelium or by smooth muscle cells and macrophages. Lipid Oxidation enhances its uptake by vessel intimal cells and monocyte-derived macrophages with stimulation of foam cells formation[41].

Histological examination of diabetic rat pancreatic sections (GII) using the eNOS antibody immunostaining technique revealed, a significant decrease in the immunostaining of endothelial blood vessels as compared with the control group(GI). This may be due to reduced plasma concentrations of insulin and arginine in diabetic rats.[42] Stated that insulin can modulate eNOS activity by increasing tetra hydro biopterin (BH4) synthesis. They also added that, functional impairment of the vascular endothelium is found in people with insulin resistance, obesity and type 2 diabetes.

The work of[43] may also explain the reduced eNOS activity in the diabetic rats due to reduction of plasma concentrations of arginine. The relative deficiency of arginine in STZ-diabetic rats may result from an increase in arginine catabolism and/or a decrease in the extra intestinal conversion of citrulline to arginine. Whatever the mechanism, dietary arginine supplementation increased both arginine concentration and NO synthesis in endothelial cells.

Ingestion of watermelon rind to diabetic rat (GIII) resulted in significantly a increased immunostaining eNOS activity in endothelium of the blood vessels as compared with the diabetic group (GII). This may be due to L-arginine content of watermelon rind. This result is in agreement with[42] who mentioned that L-arginine–NOS pathway could increase NO bioavailability which in turn augments NO production by eNOS. The simplest way to modulate eNOS is administration of its substrate L-arginine which is one of watermelon rind component.

Obvious improvement and restoration of islet architecture were encountered in the watermelon rind treated diabetic group (GIII). Some cells have mitotic figures, fewer cells with dark nuclei together with regression of the vacuolated cells. These findings were accompanied by significant decrease of blood glucose level if compared to diabetic group. These results were consistent with[22].

One of the constituent of watermelon, Lycopene protects humans from various pathogenic attacks responsible for an array of diseases e.g numerous metabolic syndromes. The protective aspects are ascribed to the singlet oxygen scavenging ability[44, 45].

Furthermore, several authors have reported that lycopene holds nutraceutical potential and provides protection against free radicals and oxidative damage[46, 47 and 48]. Lycopene plays a role in maintaining normal cellular differentiation and division[49, 50].This may explain the presence of mitotic figures in between islet cells in this group of animals (GIII).[51] Concluded that, lycopene have the ability to improve insulin sensitivity and glucose metabolism. Also[6] added that watermelon rind extract is considered as a concentrated source of nutrient as well as it is considered hyperinsulinemic and hypoglycemic.

CONCLUSION

The current study suggests that watermelon rind ingestion in streptozotocin induced diabetic female albino rat resulted in a decrease in blood glucose level and ameliorate the diabetic pancreatic structural changes. Further long-term studies are needed to elucidate exactly the mechanism of action of watermelon rind ingestion in treatment of diabetes mellitus.

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الملخص العربى

التاثير المحتمل لقشرة البطيخ البيضاء على تركيب بنكرياس أنثى الجرذ الأبيض البالغ المصابة بمرض السكرى (هيستولوجيا – هيستوكيميائيا مناعيا – مورفوميتريا).

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مرض السكرى من النوع الثانى يعتبر مشكلة صحية متعددة المضاعفات يصيب حوالى 348 مليون نسمة تقريبا من سكان العالم. ويتميز هذا المرض بوجود مقاومة للانسولين فى خلايا العضلات والانسجة الدهنية والكبد بالاضافة الى فشل نسبى فى خلايا البنكرياس (خلايا بيتا بجزر لانجرهانز) ... فيما يعتبر البطيخ (سترولس لانيتس) من احد الاطعمة القليلة الغنية بالاحماض الامينية (سترولن و ليكوبين) الطبيعية اللذان يؤديان الى انخفاض تركيز مستوى السكر بالدم فى حالات الاصابة بمرض السكرى.

وقد خطط هذا العمل لتقييم دور تناول عصير قشر البطيخ في تحسين النوع الثاني من داء السكري والذى تم إحداثه تجريبيا بالإستربتوزوتوسين مع نيكوتيناميد في الجرذان البيضاء.

وقد أجريت هذه الدراسة على ثمانية عشر من إناث الجرذان البيضاء وزنها يتراوح بين 200-170 جرام وتم تقسيمهم إلي ثلاث مجموعات متساوية.

المجموعة الأولى: (المجموعة الضابطة). لم تتلقى الجرذان أي علاج

المجموعة الثانية: (مجموعة مرضى السكري). تم إحداث مرض السكري عن طريق حقن عقار ستربتزوتوسين داخل الغشاء البريتونى بعد حقن نيكوتيناميد داخل الغشاء البريتونى ثم تركت هذه المجموعة بدون علاج. تم التأكد من حدوث مرض السكري عن طريق قياس مستوى السكر في الدم في اليوم الثالث.

المجموعة الثالثة: (مجموعة السكري - معالجه بعصيرقشرة البطيخ) . في هذه المجموعة من الحيوانات أعطيت الجرذان نيكوتيناميد يليها ستربتزوتوسين، تم عمل اختبار السكر في الدم وبعد التأكد من أن الجرذان أصبحت مصابة بالسكرى (ثالث يوم تقريبا) تلقت الجرذان (عصير قشرة البطيخ 1 مل / جرذ) و أعطى عن طريق تزقيم المعدة يوميا حتى يوم التضحية بها.

استمرت التجربة لمدة 3 أسابيع، في نهاية التجربة تم التضحية بهذه الحيوانات وتم استخراج البنكرياس بأكمله وتجهيز العينات وفحصها هيستولوجيا، وهيستو كيميائيا مناعيا.

تم تقدير أوزان الجسم في بداية ونهاية التجربة. وتقدير مستويات السكر الصائم في الدم للجرذان في جميع المجموعات 3 مرات، قبل إحداث السكري، في اليوم الثالث بعد إحداث السكري وأخيرا في نهاية التجربة. تم قياس التغيرات في وزن

الجسم ومستويات السكر الصائم في الدم وبعد ذلك تحليلها إحصائيا. أجريت قياسات كميه أيضا لقياس متوسط منطقة ألياف الكولاجين والكثافة الضوئية البطانيه لأكسيد النيتريك سينسيز.

أظهرت التجربة التـأثير الضار لداء السكرى على تركيب وكتلة خلايا بيتا بجزر لانجرهانز فى بنكرياس جرذان المجموعة (ج 2) المصابة بالسكرى. بالاضافة الى التحسن الملحوظ فى خلايا بنكرياس جرذان المجموعة (ج 3) المعالجة بعصير قشرة البطيخ البيضاء اذا ما قورنت بالجرذان المصابه بداء السكرى (ج 2).

بالاستنتاج، فإن تناول قشرة البطيخ في إناث الجرذان البيضاء المصابة بداء السكرى المحدث بالستربتوزوتوسين أدى إلى انخفاض في مستوى السكر في الدم مرتبط بزيادة نمو جذر لانجرهانز. هناك حاجة لدراسات أخرى يتعين القيام بها لفهم آلية تناول قشرة البطيخ في علاج مرضى السكرى.