



Effect of green tea on pancreas of streptozocin induced diabetes mellitus in adult albino rats (Electron microscopic study)

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Abstract

Background: green tea is one of the most famous beverages in the world. Green tea is a non-fermented tea that has a big amount of non oxidized polyphenols, named catechins which has been found to be effective in preventing many diseases. Many studies have been done to detect the effects of green tea extracts on various body systems. **Aim of work:** Our research aimed to study the possible effect of green tea in treatment of streptozotocin induced diabetes mellitus by studying its effect on rat pancreatic cells. **Material and methods:** A number of 15 adult male albino rats (group I) then subdivided in to three groups group I control, group II STZ induced diabetics, group III STZ induced diabetics then treated with green tea. Then animals were sacrificed after six weeks and samples of pancreas were taken and prepared for Electron microscopic examination. **Results:** various histopathological changes are seen on streptozotocin induced diabetes pancreatic cells and these changes become less or near normal on group III treated with green tea after streptozotocin. **Conclusion:** green tea has protective and therapeutic effects on STZ induced diabetes in rats. So, further investigations on the effect of green tea are recommended.

Key word: Insulin ,Green tea ,Pancreas ,Streptozotocin,Diabetes.

Introduction :

Diabetes mellitus is considered to be a worldwide epidemic and without good prevention, the epidemic will continue increasing ⁽¹⁾.

Aging has an important role in inducing diabetes and its complications remain major cause of morbidity and mortality .Almost one third of the elderly have diabetes and three quarters are prediabetics ⁽²⁾.

Streptozotocin (STZ, 2-deoxy-2-3-(methyl-3-nitrosoureido)-D-glucopyranose) is synthesized by *Streptomyces achromogenes* and is used to Promote both insulin dependent and non insulin dependent diabetes mel-

litus (IDDM and NIDDM, respectively). The range of the STZ dose is not as narrow as in the case of alloxan. The frequently used single intravenous dose in adult rats to induce IDDM is between 40 and 60 mg/kg b.w., but higher doses are also used ⁽³⁾.

The World Health Organization Expert Committee on diabetes recommended that traditional medicinal herbs have less side effects on the patients. In general, herbal medicines are complex mixtures of different compounds that often act synergistically to exert their full beneficial effect on diabetes mellitus and other disease ⁽⁴⁾.

Green tea is one of the most famous beverages in the world. Green tea is a non-fermented tea that has a big amount of non-oxidized polyphenols, named catechins⁽⁵⁾. These polyphenols have been found to be effective in protecting from different diseases⁽⁶⁾. Multiple studies on humans have also indicated that green tea has different health benefits in protecting from cancer, cardiovascular diseases, multiple infections, non-alcoholic fatty liver (NAFLD) and maintaining glucose homeostasis⁽⁷⁾.

Aim of the Work:

The aim of the present work is to study the possible protective role of green tea in treatment and prophylaxis of streptozocin induced diabetes mellitus by studying its effect on rat pancreatic cells.

Material and methods:

A total number of 15 adult male albino rats were included in the study

Group I (control): Includes 5 rats injected subcutaneously with distilled water.

Group II (STZ induced diabetics): includes 5 rats injected STZ intravenously as 50mg/kg as single dose intravenously⁽³⁾.

Group III (STZ followed with green tea.): includes 5 rats injected STZ intravenously as previous followed by administration of green tea orally for 6 weeks

The green tea tablets, each tablet contain green tea extract 200 mg manufactured by El Obour City pharmaceutical industries. Following grinding, the obtained green tea powder dissolved in distilled water. This solution was provided to rats orally by using a stomach tube as 200 mg/kg⁽¹⁸⁾

Then animals were sacrificed after six weeks and samples of pancreas were taken and prepared for Electron microscopic examination.

Results:

Control group:

Electron microscopic examination of the pancreas of the control rats showed the normal apical-basal polarity of the exocrine acinar cells. The acinar cells appear pyramidal in shape and have basal euchromatic oval nuclei with prominent nucleoli. The cytoplasm contains numerous apical secretory (zymogen) granules of high electron density and variable sizes (**fig 1**). Each zymogen granule is round in outline and contains a homogenous electron dense material. The cytoplasm exhibits regular arrangement of parallel strands of rough endoplasmic reticulum (RER) in its basal part. Mitochondria are elongated or ovoid and scattered throughout the cytoplasm. **Ultra structurally**, B cells show euchromatic rounded nuclei. Their cytoplasm contains mitochondria, strands of RER, Golgi apparatus and numerous secretory granules. These granules consist of electron dense core of variable electron density (light and dark) granules surrounded by a wide electron-lucent halo under the limiting membrane (**figure.2**).

Group II (streptozocin induced diabetes):

Diabetic rat pancreas show preserved apical-basal polarity of the acinar cells which have basal euchromatic nuclei with localized chromatin condensation. The cytoplasm contains numerous apical spherical secretory (zymogen) granules of variable electron density and variable sizes with occasional fusion between some of them (zg-zg fusion). The cytoplasm exhibits dilated cisternae of rough endoplasmic reticulum (RER), some vacuolations and pleomorphic mitochondria (**fig. 3**).

Ultra structurally, B cells appear with hyperchromatic nuclei. The cytoplasm shows marked vacuolations, dilated cisternae of RER and some mitochondria with disrupted cristae. Apparent reduc-

tion of the amount of secretory granules with fusion of the limiting membrane of some granules is noticed (figs.4).

Group III (STZ and green tea):

Electron microscopic examination of this group shows that the acinar cell had basal rounded nuclei. The cytoplasm contains numerous apical secretory (zymogen) granules of high electron density and variable sizes. Each zymogen granule is round in outline and contains a homogenous electron dense material. The cytoplasm exhibits parallel strands of rough endo-

plasmic reticulum (RER) , supranuclear Golgi, minimal cytoplasmic vacuoles and scattered mitochondria (fig.5). Ultra structurally, B cells has more or less heterochromatic rounded nuclei with prominent nucleoli. Their cytoplasm contains scattered mitochondria, dilated perinuclear strands of RER, minimal cytoplasmic vacuoles and numerous secretory granules . These granules consist of electron dense core of variable electron density (light and dark) with clear membrane bound regions (figs.6)

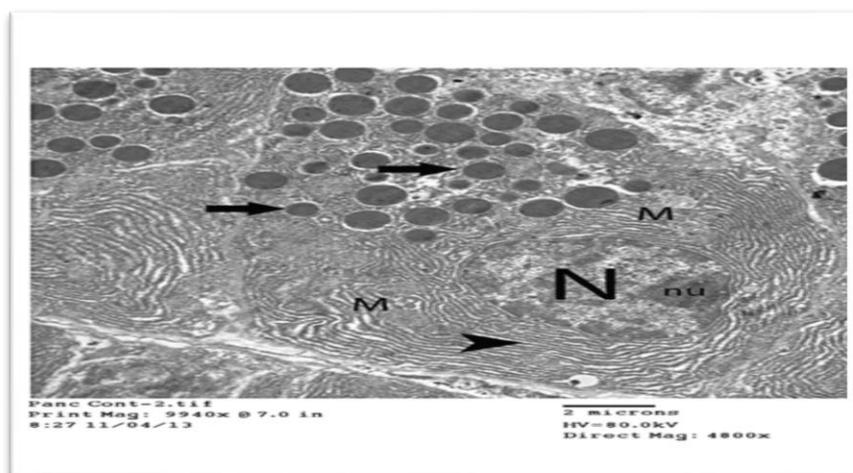


Fig.1 An electron micrograph of the acinar cells of rat pancreas in control group showing normal apical-basal polarity of the exocrine acinar cells. The pyramidal cell has basal euchromatic nucleus (N) with prominent nucleolus (nu) and apical secretory cytoplasmic zymogen granules of high electron density and variable sizes (thick arrows). Note the parallel strands of rough endoplasmic reticulum in its basal part (arrow head) and mitochondria (M). X 4800

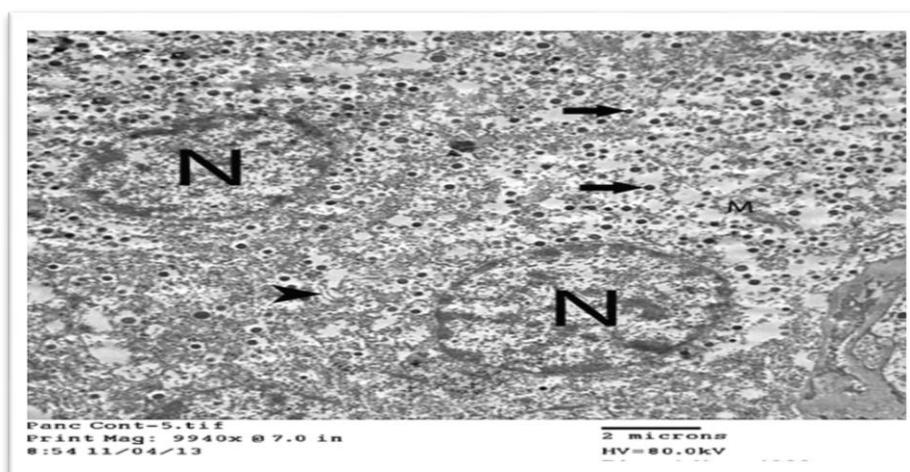


Fig.2 An electron micrograph of islet B cells in rat pancreas of control group showing adjacent beta cells with normal rounded nuclei (N) with abundant cytoplasmic granules (thick arrows) , strands of rough endoplasmic reticulum (arrow head) and scattered mitochondria (M)..X4800

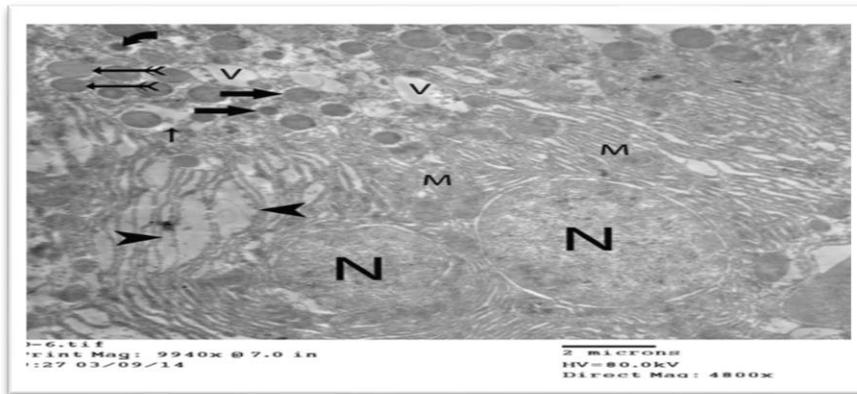


Fig. 3 An electron micrograph of the acinar cells in rat pancreas of group II showing preserved apical-basal polarity , basal euchromatic nuclei (N) , pleomorphic mitochondria(M), dilated cisternae of rough endoplasmic reticulum (arrow heads) and accumulation of apical spherical zymogen granules of variable sizes (thick arrows) and electron density (double tailed arrows).Occasional fusion between zymogen granules is noticed (thin arrow).Autophagic vacuoles (curved arrow) and cytoplasmic vacuolations (V) can be noticed. x4800

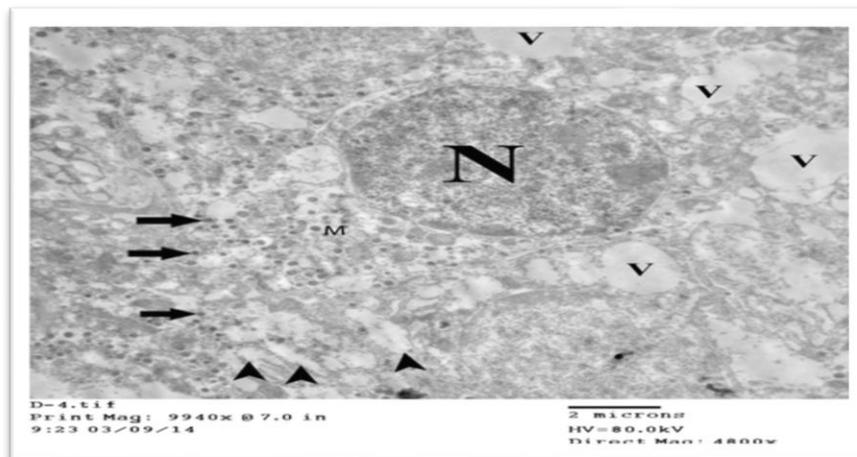


Fig. 4 An electron micrograph of Islet B cells in rat pancreas of group II showing hyperchromatic nuclei (N), marked vacuolations (V) and apparent reduction in the amount of secretory granules (thick arrows). Note dilated strands of rough endoplasmic reticulum (arrow head) and mitochondria with disrupted cristae (M).,X4800

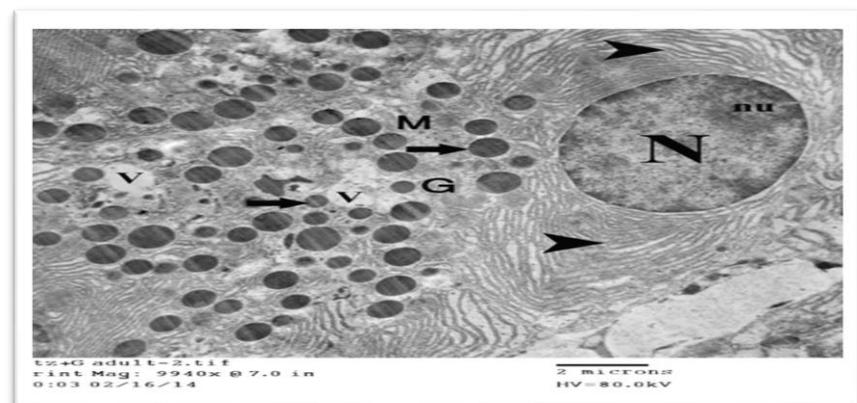


Fig.5 An electron micrograph of the acinar cells in rat pancreas of group III showing more or less basal rounded nucleus(N) ,abundant apical cytoplasmic secretory granules with high electron density and variable sizes (thick arrows), parallel strands of rough endoplasmic reticulum (arrow heads), subnuclear Golgi complex (G) and scattered mitochondria (M). Minimal cytoplasmic vacuoles are noticed (V). X 4800

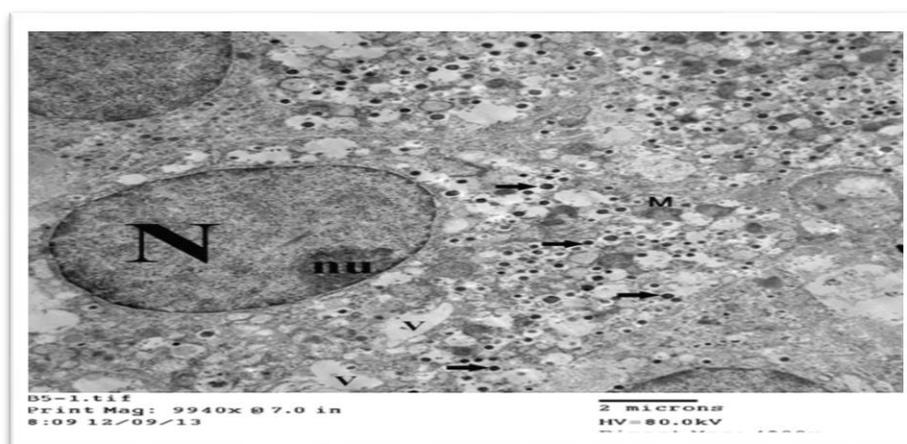


Fig.6 An electron micrograph of Islet B cells in rat pancreas of subgroup Ic showing more or less heterochromatic rounded nucleus (N) with prominent nucleolus (nu). The cytoplasm reveals minimal vacuolations (V), scattered mitochondria (M) and noticeable increase in secretory granules (thick arrows). , X 4800

Discussion:

The prevalence of diabetes mellitus (D.M) is increasing at dramatic rate. So effective nutritional strategies for control of this disease are required. Specific dietary components with anti diabetic effect could be one aspect of these strategies. In this study the role of green tea in controlling experimentally induced hyperglycemia through its effect on B-cells studied. Green tea has been found to be effective in protecting from multiple diseases.

Regarding the pancreatic acinar cells in group II the present work revealed decreased apical zymogen granules in comparison to control group. Occasional fusion between zymogen granules (zg-zg fusion) was noticed in the cytoplasm of acinar cells. The cytoplasm contained pleomorphic mitochondria & dilated rough endoplasmic reticulum (RER) with vacuolations.

In harmony with the present study, ⁽⁸⁾ found that there was several degenerative changes in some acinar cells appeared as low number of secretory granules, multiple vacuoles and degenerated mitochondria.

Another study done by ⁽⁹⁾ showed similar results as regards the histopa-

thological changes in the pancreatic tissue of rats.

B cells of group II appeared with hyperchromatic nuclei. The cytoplasm showed marked vacuolations, dilated strands of RER, some mitochondria with disrupted cristae. Apparent reduction of the amount of secretory granule with fusion of the limiting membrane of some granules was noticed.

The present study is in accordance with the previous results of ⁽¹⁰⁾ who showed that diabetes made massive β -cell degradation and lowered cellular density in pancreatic islets. Selective destruction of insulin producing β cells could be explained by ⁽¹¹⁾, who reported that type one D.M is characterized by inflammation around the islets followed by β cell destruction.

In agreement with the present results, ⁽¹²⁾ showed a decrease in cell proliferation and abnormal mitochondria. STZ treated pancreatic cells with increased expression of multiple markers of apoptosis, such as caspase-9, caspase-3, and the ratio of Bax/Bcl-2.

The present results were similar to that stated by ⁽¹³⁾ who revealed that the pancreas of diabetic rats induced by STZ revealed destroyed beta cells with many nuclear vacuolization and

ballooning appearances of mitochondria as well as dilated rough endoplasmic reticulum. Beta cells showed small pyknotic nuclei, serrated nuclear membrane with electron-translucent areas in the cytoplasm and decreased insulin secretory granules.

The present results showed that green tea improved structural changes of pancreatic acinar cells in subgroup Ic and these cells revealed apical electron dense zymogen granules with parallel strands of RER. Moreover in the same group there were marked improvement of islet cells in the form of normal architecture and morphology with minimal vacuolations and noticeable increase in both light and dark secretory granules. Most of the islets retained their normal size.

These findings were as observed by ⁽¹⁴⁾ ⁽¹⁵⁾ who described normal histological appearance of pancreatic tissue.

A study done by ⁽¹⁶⁾ showed that administration of green tea in diabetic rats improved their diabetes and reduced their serum blood glucose. However, other studies concluded that the efforts to manage type 2 diabetes and obesity by natural green tea treatment appeared to be taken cautiously because the systemically absorbed green tea extract (GTE) may block the cellular glucose uptake and thereby increases blood glucose ⁽¹⁷⁾.

Conclusion :

In conclusion, green tea has protective and therapeutic effects on STZ induced diabetes in rats. So, further investigations on the role of green tea are recommended.

References:

1- Mirzaei K, Hossein-Nezhad A, Karimi M, Hosseinzadeh-Attar MJ, Jafari N, Najmafshar A, Larijani B. Effect of green tea extract on bone turnover markers in type 2 diabetic patients; A double-blind, placebo-controlled clinical trial study. *DARU*. 2009;17: 38-44.

- 2- Cowie CC, Rust KF, Ford ES, Eberhardt MS, Byrd-Holt DD, Li C, Williams DE, Gregg EW, Bainbridge KE, Saydah SH, Geiss LS. Full accounting of diabetes and pre-diabetes in the U.S. population in 1988-1994 and 2005-2006. *Diabetes Care*. 2009 Feb;32(2):287-94.
- 3- Ganda OP, Rossi AA, Like AA: Studies on streptozotocin diabetes. *Diabetes* 25: 595-603, 1976.
- 4- Vinson JA, Zhang J. Black and Green Teas Equally Inhibit Diabetic Cataracts in a Streptozotocin-Induced Rat Model of Diabetes. *J Agric Food Chem*. 2005; 53: 3710-3713
- 5- Alexopoulos N, Vlachopoulos C, Stefanadis C. Role of green tea in reduction of cardiovascular risk factors. *Nutrition and Dietary Supplements* 2010; 2: 85-95
- 6- Alipoor B, Rad AH. A review on therapeutic effect of tea. *Asian J Clin Nutr*. 2012; 4: 1-15.
- 7- Suliburska J, Bogdanski P, Szulinska M, Stepien M, Pupek-Musialik D, Jablecka A. Effects of Green Tea Supplementation on Elements, Total Antioxidants, Lipids, and Glucose Values in the Serum of Obese Patients. *Biol Trace Elem Res*. 2012.
- 8- Abo Gazia M and Hasan NM. Effect of Glabridin on the Structure of Ileum and Pancreas in Diabetic Rats: A histological, Immunohistochemical and Ultrastructural Study. *Nature and Science*, 2012;10(3):78-90
- 9- Ahmadi S, Karimian SM, Sotoudeh M, and Bahadori M. Histological and immunohistochemical study of pancreatic islet beta cells of diabetic rats treated with oral vanadylsulphate. *Medical Journal of the Islamic Republic of Iran*. 2002;16(3):173-8.
- 10- Adewole SO, Caxton-Martins EA, Ojewole JA:) Protective effect of quercetin on the morphology of pancreatic β -cells of streptozotocin-

- treated diabetic rats. Afr J Tradit Complement Altern Med 2006 ,4: 64-74.
- 11-** Blair LA, Maggi LB Jr, Scarim AL, Corbett JA :Role of interferon regulatory factor-1 in double-stranded RNA-induced iNOS expression by mouse islets. J Biol Chem 2002 ,277: 359-365.
- 12-** Zhang X. The effect of hexarelin in streptozotocin (STZ)-induced rat model through protective actions on islet beta cells and cardiomyocytes. A thesis submitted for the degree of Doctor of Philosophy at The University of Queensland in 2014
- 13-** Mohammadi MT, Pirmoradi L, Mesbah F, Safaei A, Dehghani GA. Trophic Actions of Oral Vanadium and Improved Glycemia on the Pancreatic Beta-Cell Ultrastructure of Streptozotocin-Induced Diabetic Rats. JOP. J Pancreas (Online) 2014 Nov 28; 15(6): 591-596.
- 14-** Karaca T, Yoruk M, Ibrahim H. Yoruk and Uslu S. Effects of Extract of Green Tea and Ginseng on Pancreatic Beta Cells and Levels of Serum Glucose, Insulin, Cholesterol and Triglycerides in Rats with Experimentally Streptozotocin-Induced Diabetes: A Histochemical and Immunohistochemical Study. Journal of Animal and Veterinary Advances. 2010;9(1):102-7
- 15-** Al-Hilfy JHY. Effect of Green Tea Extract on Histological Structure of Kidney, Pancreas and Adrenal Gland in Alloxan-Induced Diabetic Male Albino Rats. Journal of Al-Naharin University. 2013;16(1):156-165.
- 16-** Suzuki T, Takagi A and Takahashi M.. Catechin-rich green tea extract increases serum cholesterol levels in normal diet- and high fat diet-fed rats; BMC Proceedings, 2012. 6(Suppl3):P47
- 17-** Park JH, Jin JY, Baek WK, Park SH, Sung HY, Kim YK, Lee J and Song DK.. Ambivalent role of gallated catechins in glucose tolerance humans: A novel insight into non-absorbable gallated catechin-derived inhibitors of glucose absorption. J Physiol Pharmacol 2009., 60 (4): 101-109.
- 18-** Abolfathi AA, Mohajeri D, Rezaie A, Nazeri M. Protective effects of green tea extract against hepatic tissue injury in streptozotocin-induced diabetic rats. Evidence-Based Complementary and Alternative Medicine. 2012; 2012: 740671–81