http://www.pjbs.org

PJBS

ISSN 1028-8880

# Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 12 (4): 301-314, 2009
ISSN 1028-8880


# Histological and Electron Microscopic Studies of the Effect of β-Carotene on the Pancreas of Streptozotocin (STZ)-Induced Diabetic Rats

Azza A. Attia
Department of Zoology, Faculty of Science, Alexandria University, Egypt

**Abstract:** To evaluate the protective effect of β-carotene on induction of diabetes by streptozotocin (STZ), 45 albino rats, weighed about 110-130 g were used. They were divided randomly into six groups. GI rats used as control; GII rats were injected i.p. with a single dose of 40 mg streptozotocin (STZ) to become diabetic; GIII and GIV, the diabetic rats were injected i.p. with 0.3 and 0.1 mg β-carotene, respectively; GV and GVI rats were injected i.p. only with 0.3 and 0.1 mg β-carotene respectively. At the end of the experiment, the final body weights, blood glucose and insulin levels were determined and the values were statistically analyzed. Histological, semithin and ultrathin sections were prepared for pancreatic tissues. In the diabetic rats (GII), there was significant loss in body weight accompanied by significant increase in blood glucose levels. In addition, many light and electron microscopic changes were observed in the acinar and endocrine β-cells of islets of pancreas. These changes were summarized as disturbance of acini arrangement, shrinkage and pyknotic nuclei, vacuolation and dissolution of mitochondria and Golgi elements, degranulation of β-cells. In addition to the significant decrease in blood glucose levels, 0.3 mg β-carotene (GIII) had decreased most of these changes than 0.1 mg of it (GIV). So, GIII provides more protection for the pancreatic tissue more than GIV. Also, the results revealed that injection of rats only with 0.3 and 0.1 mg β-carotene (GV and GVI) had no observable changes in the pancreatic tissues, except that there was an increase in number of the vacuolized mitochondria in most acinar and β-cells of islets. In conclusions, 0.3 mg β-carotene could normalize the biochemical disorders of diabetes and provides more protection for the pancreatic tissues than 0.1 mg from the damaging effect of STZ to a greater extent.

**Key words:** Streptozotocin, diabetes mellitus, β-carotene, islets of langerhans, oxidative stress, antioxidants, pancreas

## INTRODUCTION

It is well known that Diabetes Mellitus (DM) is a universal health problem affecting human societies at all stages of development, so detecting diabetes as early as possible helps to control the disease and protect against additional serious health complications. Diabetes Mellitus (DM) is characterized by wide spread of damage in almost every tissue in the body (Olefsky, 1992; World Health Organization, 2002). Chakrabarty *et al.* (2002) stated that DM is a common metabolic disorder, characterized by elevated serum glucose levels resulting from defects in insulin production, insulin action or a combination. The hormone insulin is produced from β-cells of islet of Langerhans and the body needs it to use simple sugars from digested foods to regulates blood glucose levels (Sylvia, 1993).

There is a growing consensus that diabetes is usually accompanied by an increased production of free radicals, or by impaired antioxidant defenses which is accompanied by development and progression of diabetic complications (Maritim *et al.*, 2003; Hong *et al.*, 2004; Brownlee, 2005; Arulselvan and Subramanian, 2007). All tissues in the body contain adequate amounts of antioxidants to protect them against the toxic actions of free radicals. A deficiency of these antioxidants can result in tissue and organ damage (Krishna and Das, 2001).

Only few human studies have investigated the role of antioxidants in the pathogenesis of diabetes.

DM is classified into two main types, type 1 (insulin-dependent) that results from destruction of pancreatic β-cells which produce insulin; whereas type 2 diabetes (non-insulin dependent) results from resistance of the body to use insulin that it produces (Chakrabarty *et al.*, 2002).

STZ is the most commonly used diabetogenic agent for experimental animals, it is used in medical research to produce an animal model for type 1 diabetes by selectively destroying pancreatic β-cells (Mansford and Opie, 1968; Szkudelski, 2001). STZ is also toxic to the β-cells of pancreas in mammals. One of the primary effects of it on pancreatic-β cells is the damage caused by free

radicals formed when it decomposes inside the cell (Gandy *et al*., 1983). Kakkar *et al*. (1998) found an increased oxidative stress in rat liver and pancreas during progression of STZ-induced diabetes.

Many researchers have studied the ultrastructure effect of STZ on the pancreatic islets of different experimental animals (Flament and Remacle, 1987; Aughsteen, 2000; Howarth *et al*., 2000; Mythili *et al*., 2004).

Certain dietary components have been reported to potentially suppress the initiation of experimental diabetes induced by STZ in animal models.

Evidence is mounting about the potential protective role of carotenoids as an antioxidants in the development of chronic diseases, especially atherosclerosis, inflammatory joint disease, cancer, degenerative eye disease and Diabetes Mellitus (DM) (Seddon *et al*., 1994; Florence, 1995; Kohlmeier and Hastings, 1995; Mayne, 1996). Carotenoids are an extremely diverse group of compounds found in plants and include the compounds that give flowers their color (Olson and Krinski, 1995). It also represents of the one most widespread groups of naturally occurring pigments. β-carotene is one of these carotenoids which presents in abundance in green and yellow vegetables and has the highest provitamin-A activity, which is converted in the body into retinol (the active form of vitamin A).

Colditz *et al*. (1992) found that the protective role of β-carotene comes from several observational studies relating to the increased intake of vegetables that are rich in carotenoids with a lower risk of type 2 DM. Ford *et al*. (1999) tried to suggest new opportunities for research that include exploring a possible role for carotenoids in the pathogenesis of insulin resistance and diabetes. Liu *et al*. (1999) examined the effect of long term β-carotene supplementation on the incidence of type 2 DM in physician male. Also, Frusho *et al*. (2002) found that administration of β-carotene suppressed lipid peroxidation in tissues and improved the glucose tolerance ability of STZ-induced diabetic rats. Lee and Park (2000) and Lee *et al*. (2003) studied the cytoprotective effect of soybean diet or its extraction (PPC) on β-cells of STZ-diabetic rats. Kataja-Tuomola *et al*. (2008) studied the effect of α-tocopherol and β-carotene supplementation on the incidence of type 2 diabetes.

The present investigation was undertaken to evaluate the possible protective effect of β-carotene on the pancreatic tissue of the diabetic rats induced by STZ. Two doses of β-carotene are used to investigate the possible effective one in its protection. The study includes determination of serum glucose and insulin levels in control rats, diabetic as well as after the treatment with β-carotene. Also, histological and ultrastrucural studies were done to examine the alterations occurred whether in β-cells of islet of Langerhans or in the acinar cells of control, diabetic and after β-carotene treated rats. Moreover, it was of an important to study any side effect of β-carotene on the control pancreatic tissues.

## MATERIALS AND METHODS

Forty five albino adult male rats, weighting from 110 -130 g were used. All animals were kept under the same laboratory conditions of temp, humidity and were fed on the same balanced standards diet throughout the experiment. They were divided into six groups, each with random number as follows:

**GI:** Rats served as control and had free access to food, milk and bread.

**GII:** Rats were injected intraperitoneally with a freshly prepared single dose of STZ (40 mg $kg^{-1}$) to become diabetic. Streptozotocin was dissolved in citrate buffer, adjusted to pH 4.5 (Ganda *et al*., 1976), it was purchased from Sigma Chemical Co., USA. The diabetic rats were defined by the appearance of glucosouria within three days, using Pasteur lab test strips for rapid determination of glucose in urine and to confirm that the animals become positively diabetic. The animals who failed to become diabetic were excluded from the experiment.

The already diabetic rats were divided into two subgroups as follows:

**GIII:** Rats were injected i.p. with 0.3 mg β-carotene, three times/week on alternate days for two months. β-carotene was purchased from Sigma Chemical Co., USA and was dissolved in olive oil.

**GIV:** Rats were injected i.p. with 0.1 mg/kg/rat β-carotene by the same earlier manner as GIII.

**GV and GVI:** rats were injected i.p. only with 0.3 mg and 0.1 mg $kg^{-1}$ β-carotene, respectively, three times/week on alternate days for 2 months.

At the end of the experiment, all animals were weighed and shown in Table 1. Then, they were fasted overnight and killed by cervical dislocation. Blood samples were collected by cardiac puncture, allowed to clot and sera of all different group rats were separated for determination of glucose and insulin levels by available kits, BioSystems SA (glucose) and cobas e analyzer (insulin).

Table 1: The statistical means of body weight, glucose levels and insulin levels in various experimental groups

| Groups | Treatment | Body weight (g) | Glucose levels (mg $dL^{-1}$) | Insulin levels (μu $mL^{-1}$) |
|---|---|---|---|---|
| I | Control | 102.67** | 75.0** | 0.20 |
| II | Diabetic | 88.67*** | 225.0* | 0.17 |
| III | Diabetic+0.3 mg β-carotene | 161.67* | 107.0** | 0.19 |
| IV | Diabetic+0.1 mg β-carotene | 113.33** | 198.0* | 0.18 |
| V | 0.3 mg β-carotene | 115.33** | 100.0** | 0.20 |
| VI | 0.1 mg β-carotene | 109.67** | 67.5** | 0.21 |

*Mean values are significantly different at 5%

For the histological studies, pieces of pancreas were dissected out quickly from the control and the different experimental group animals, fixed in Bouin's fluid for routine H and E-stain (Drury and Wallington, 1980). Other specimens were fixed in 3% phosphate buffer glutaraldehyde for 3 h at -4, post fixed in buffered Osmium tetroxide, dehydrated in ethanol and embedded in araldite (Glauert and Glauert, 1958). Ultrathin sections, cut with LKB ultramicrotome were prepared for semithin (1 μm) thick sections, stained with toluidine blue examined by light microscope and photographed. Other ultrathin sections were placed on copper grids, doubly stained with uranyl acetate followed by lead citrate (Reynolds, 1963) and examined with JEOL 100cX electron microscope.

Statistical analysis was conducted with SAS (Statistical Analysis System, Cary, North Carolina) for the body weight results, blood glucose and insulin levels of the control and the different experimental group animals. All values were expressed as means±SE and values of $p>0.05$ were considered statistically non significant, while values of $p<0.05$, 0.01 were considered significant (Table 1).

## RESULTS

The present results showed significant loss in body weights of STZ-induced diabetic rats (GII) more than the control (GI), while it was significantly increased in GIII, indicating that the diabetic rats had regained their weights after giving 0.3 mg β-carotene. In addition, there were no significant differences in body weights of other groups (GIV, GV and GVI) comparing with the control (GI) (Table 1).

The mean values of glucose level was significantly higher in rats developed hyperglycemia (GII) compared with those of the control (GI), while it was nearly similar to the control in GIII, GV and GVI. However, GIV where the diabetic rats were given 0.1 mg β-carotene, the level of glucose was nearly similar to that level in the diabetic rats (GII) (Table 1), indicating that 0.3 mg β-carotene could normalize the biochemical abnormalities caused by the diabetes. No significant differences in insulin levels were detected in the different experimental groups. However, slight increase of it was determined in both GIII and GIV than GII, while it was nearly similar to that of the control in both GV and GVI (Table 1).

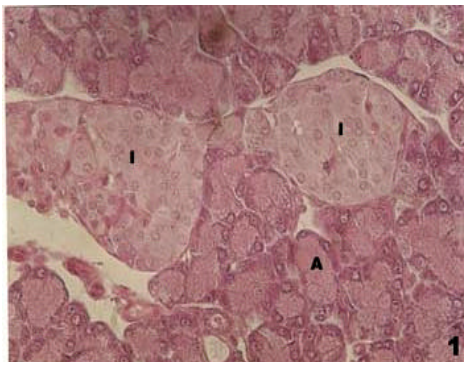


Fig. 1: Light micrograph of control pancreas of rat (GI) showing pale staining islet (I) of Langerhans among dark acini (A) which of pyramidal-shaped appearance. H and E. X400

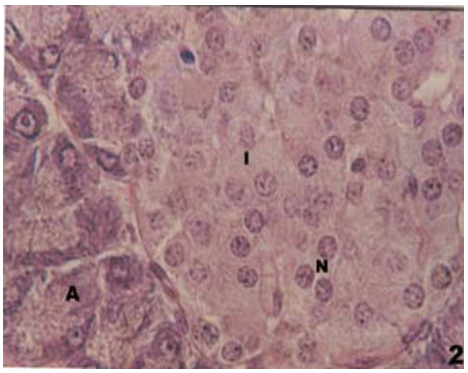


Fig. 2: Higher magnification of the earlier figure showing islet (I) cells with rounded shaped nuclei (N) and prominent nucleoli; pyramidal-shaped acini (A). H and E. X1000

### Histological results

**The control acinar cells:** Light micrographs of sections of control pancreas of rats showed that the bulk of pancreas is considered as exocrine and the islets of Langerhans are scattered throughout its substance. The exocrine tissue consists of closely packed acini with very little connective tissue in between. The acinar cells of these acini were pyramidal in shape, having very small acinar lumen. They have rounded, basally located nuclei with dispersed and prominent nucleoli (Fig. 1-3). In TB-stained semithin sections, the secretory or zymogen granules were seen in the apical cytoplasm of these cells (Fig. 3).

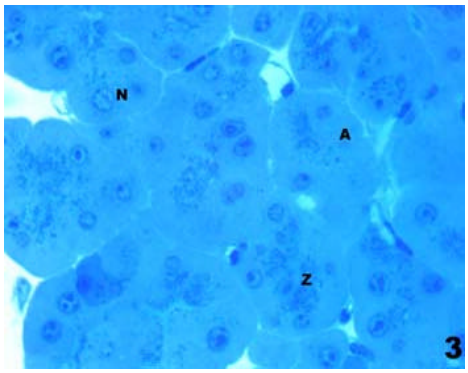


Fig. 3: Semithin section of control pancreas of rat (GI) showing the pyramidal shaped appearance of the acini (A); the nuclei (N) are basally located, having prominent nucleoli (arrows) and zymogen (Z) granules. TB X1000

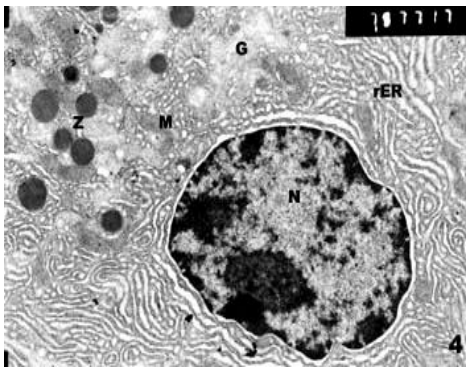


Fig. 4: Electron micrograph of control pancreatic acinar cell of rat (GI) showing basally located nucleus (N); zymogen (Z) granules; arrays of rough endoplasmic reticulum (rER); mitochondria (M) and Golgi element (G). X7500

Ultrastructurally, the acinar cells showed basally located rounded nuclei relatively with large nucleolus. Dense zymogen granules are scattered in the apical cytoplasm, the basal cytoplasm is crammed with lamellar profiles of rough endoplasmic reticulum within which are scattered rounded and oval–shaped mitochondria. The mitochondria have an electron dense matrix and more closed cristae. Supranuclear Golgi area is usually located near zymogen granules (Fig. 4).

**The control endocrine cells:** Figure 1 and 2 showed the presence of pale- staining lobules represent islets of Langerhans, containing alpha and β-cells. They have large rounded and basophilic nuclei and prominent nucleoli (Fig. 2). There were no obvious differences

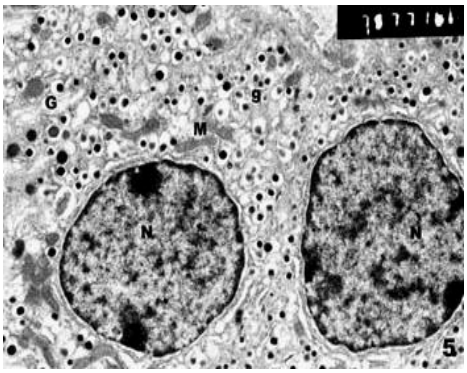


Fig. 5: Electron micrograph of control islet of pancreas of rat (GI) showing parts of two β-cells with slightly oval-shaped nuclei (N); β granules (g) have dark central core surrounded by an electron lucent halo; mitochondria (M) and Golgi area (G). X7500

between these cells in the light microscope, while they were identified and distinguished morphologically by the electron microscope. There were no obvious differences in the nuclear structure between them. The general ultrastructural common feature of both types of cells is the presence of numerous specific dense secretory granules which are differed both numerically and morphologically. Worthy to mention that, we will considered only with studying β-cells of islets in this study, because of its function in secreting the hormone insulin which is correlated with diabetes. Figure 5 showed that β-cells had rounded or slightly oval nuclei of regular contour. The cytoplasm contains many secretory granules with an electron dense core surrounded by an electron lucent halo. The mitochondria are scattered throughout the cytoplasm, they were fine and appeared as rounded or plump filamentous, having dense matrix. The Golgi elements are observed in many cells among the β-granules.

**The diabetic rats (GII)**

**The acinar cells:** Histologically, sections of the diabetic pancreas showed disturbance in the acinar pattern structure. The acinar cells have vesicular nuclei (Fig. 6). In TB-stained sections, the cytoplasm of most acinar cells contains many small vacuoles. Thick septae are observed between these acini (Fig. 7).

The electron micrographs revealed certain irregularities of the nuclear outline of these acinar cells; some nuclei were deeply indented; the heterochromatin formed marked aggregation along their nuclear envelope as well as around the nucleolus (Fig. 8, 9). The zymogen granules had decreased in number in cytoplasm of most

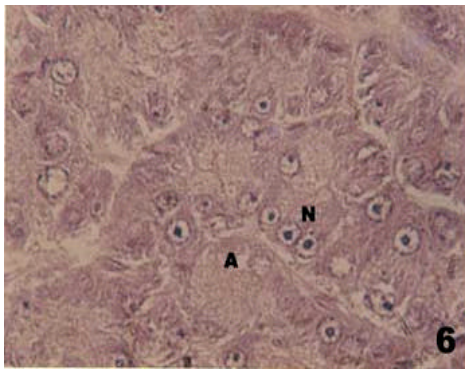


Fig. 6: Light micrograph of diabetic pancreas of rat (GII) showing disturbance of the acinar pattern structure (A); Note: the vesicular nuclei (N) of the acinar cells. X1000

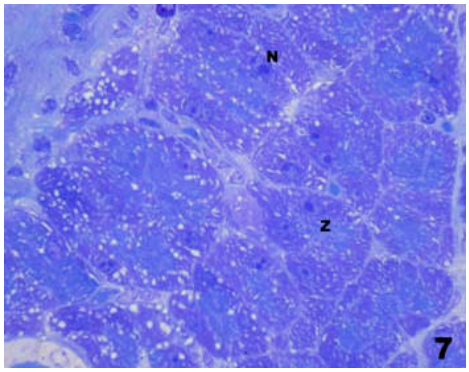


Fig. 7: Semithin section of diabetic pancreas of rat (GII) showing the presence of many vacuoles in cytoplasm of acinar cells; basally located nuclei (N) and zymogen (Z) granules. TB X1000

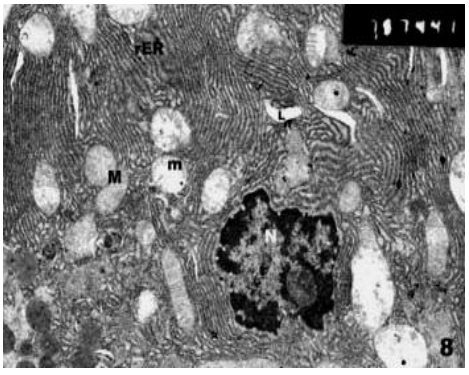


Fig. 8: Electron micrograph of diabetic pancreas of rat (GII) showing the pyknotic nuclei (N) of the acinar cell; normally-shaped mitochondria (M); vacoulized mitochondria (m) and areas of lesions (L) scattered among the rER. X7500

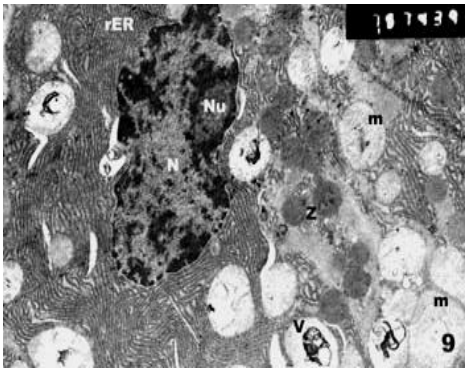


Fig. 9: Electron micrograph of diabetic pancreas of rat (GII) showing the shrinkage nucleus (N) of acinar cell; Note that the nucleolus (Nu) migrates to the outer margin; vacuolized mitochondria (m); extensive arrays of rough endoplasmic reticulum (rER); autophagic vacuoles (V); zymogen (Z) granules and areas of lesions (L). X7500

of these cells (Fig. 8, 9), the mitochondria were increased in number and size. They were enveloped in the lamellar rER, most of them had lost their cristae, some were highly vacuolized and the autophagic vacuoles appeared in its interior (Fig. 9). Other normally-shaped mitochondria are still had their cristae (Fig. 8). Lesions became intensified with an evident increase in the intercellular spaces which exhibited zones of oedema (Fig. 8, 9).

**The endocrine cells:** Islets of the diabetic pancreas were faintly stained and the boundaries of their cells were of ill defined border. There were certain degenerative features in most β-cells of these islets. Their nuclei became packed together in groups and the cytoplasm had lost their granules (Fig. 10). Ultrastructurally, the nuclei of these cells showed variable changes, some appeared pyknotic, others were vesicular, where the euchromatin predominates over the hetrochromatin (Fig. 11). Other nuclei showed marked aggregation of the heterochromatin along their nuclear envelope (Fig. 12). The cytoplasm of many of these β-cells had lost their granules, have small electron dense cores with increased electron lucent halo around them (Fig. 11, 12). The mitochondria were vacuolized and characterized by an extreme loss of matrix, density, some were ruptured so that their criteria were not clearly observed. Obvious dilated and extended Golgi elements were noticed in cytoplasm of many of these β-cells (Fig. 11, 12). These changes indicate the damaging effect of STZ on the pancreatic tissues of the diabetic rats.

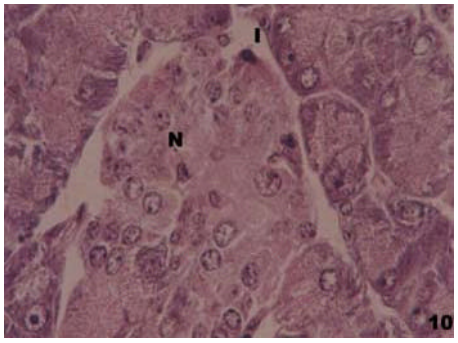


Fig. 10: Light micrograph of diabetic pancreas of rat (G II) showing islet (I) of Langerhans with ill defined border; Note: some fragmented nuclei (N) in islet cells. H and E. X1000

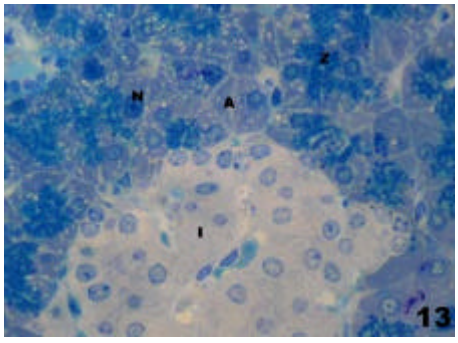


Fig. 13: Semithin section of diabetic pancreas of rat (GIII) showing pale staining islet (I) of Langerhans; pncreatic acini (A); basally located nuclei (N) and zymogen (Z) granules. TB X1000

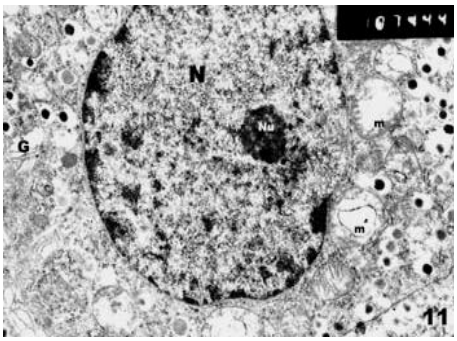


Fig. 11: Electron micrograph of diabetic pancreas of rat (GII), showing an irregularly-shaped nucleus (N) of β-cell of islet; Note the euchromatin predominates over the heterochromatin; nucleolus (Nu); the vacuolized mitochondria (m) lost their cristae and Note: decreased amount of β-granules (g). X10,000

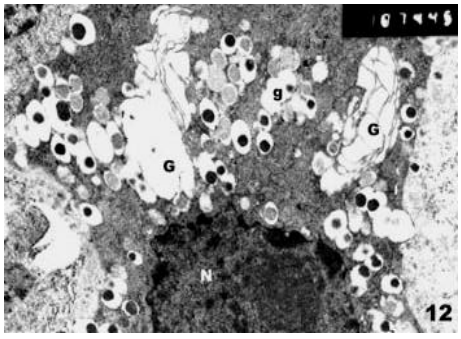


Fig. 12: Electron micrograph of diabetic islet of pancreas of rat (GII) showing part of the irregularly-shaped dark nucleus (N) of β-cell; Note the increase in the halo spaces areas around most of β-granules (g) and extension and vacuolation Golgi areas (G). X 10,000

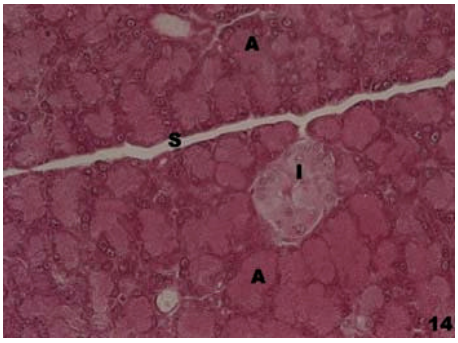


Fig. 14: Light micrograph of pancreas of rat (GIV) showing islet (I) of Langerhans is located within the nearly normal shaped pancreatic acini (A) and Note the thickness of septa (S) between the acini. H and E. X400

**The diabetic rats given β-carotene (GIII and GIV):** The results showed that any of the two doses of β- carotene to the STZ-diabetic rats (GIII and GIV) had protected the pancreatic tissue to a greater extent from the damaging effect of STZ. In addition, GIII provided more protection than GIV.

**The acinar cells:** The light micrographs revealed that the pancreatic acini of both groups (GIII and GIV) had retained their pyramidal- shaped appearance. The acinar cells have basally located nuclei (Fig. 13-15). Mild increase in thickness of pancreatic septae and the moderate decrease in acidophilic cytoplasm were noticed in many sections of GIV (Fig. 14, 15).

In TB-stained sections, numerous zymogen granules were observed in the apical regions of cytoplasm of most acinar cells (Fig. 13, 15). Many sections revealed the presence of islets with their rounded outline as in the

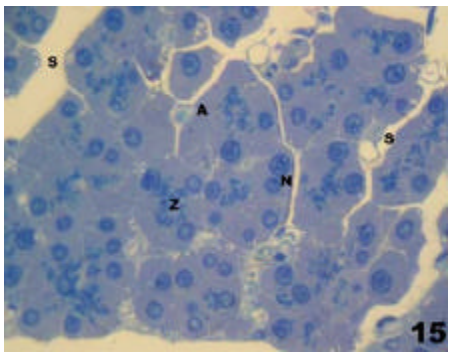


Fig. 15: Semithin section of pancreas of rat (GIV) showing the pyramidal shaped appearance of the pancreatic acini (A); Note: the rounded shaped nuclei (N) of the acinar cells; zymogen (Z) granules and thickness of septae (S) between the acini. TB X1000

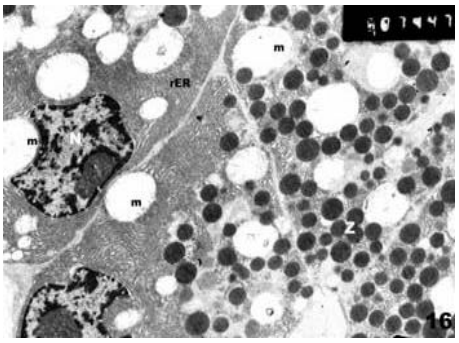


Fig. 16: Electron micrograph of pancreas of rat (GIII) showing parts of three acinar cells (A); basally located and irregularly shaped nuclei (N); zymogen (Z) granules; arrays of rER and Note: the highly vacuolized mitochondria (m) had lost their cristae. X5000

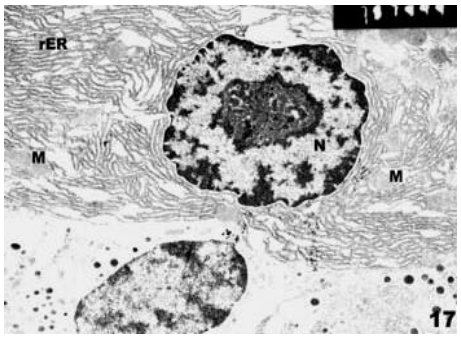


Fig. 17: Electron micrograph of pancreas of rat (GIV) showing the irregular-shaped nucleus (N) of the acinar cell and dilation of rER within which are present mitochondria (M). X7500

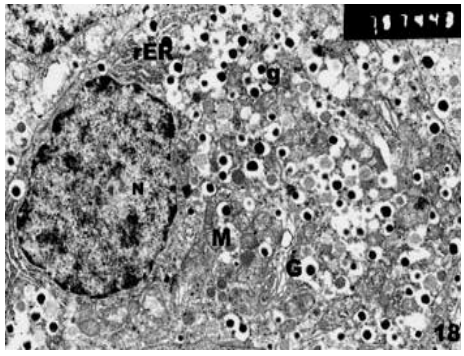


Fig. 18: Electron micrograph of islet of pancreas of rat (GIII) showing normally shaped nucleus (N) of β-cell; Note the presence of many β-granules (g) with dense core and electron lucent halo spaces; mitochondria (M) and Golgi area (G) short profiles of rER. X7500

normal control. They have rounded nuclei and prominent nucleoli (Fig. 13, 14). The electron micrographs of the nuclei of these cells still showed slight irregularities in their nuclear outline (Fig. 16, 17). The mature zymogen granules are nearly filled the apical regions of cytoplasm (Fig. 16). The mitochondria are still had the same previous changes as in the diabetic sections in GII, they had lost their cristae and appeared swollen. Most of them were highly vacuolized, some were pressed on the nuclear envelope of some acinar cells (Fig. 16). Extensive parallel arrays of rER are noticed in the acinar cells of GIII (Fig. 16), however, they were dilated in certain acinar cells of GIV (Fig. 17).

**The endocrine cells:** β-cells of islets of pancreas of both GIII and GIV had retained their cell organelles and many of the previous alterations had returned approximately to the normal. This indicates the protective effect of β-carotene on the morphology of these cells. However, GIII showed an increase in number of β-granules than that in GIV. They were of dense central core surrounded by lucent halo space (Fig. 18-20). This result is correlated with the decrease in the blood glucose levels, indicating the increase in the ability of these cells in secreting hormone insulin. The mitochondria and the Golgi areas in cytoplasm of most of these cells are of the normal shaped appearance as in the control. Short profiles of rough endoplasmic reticulum are present (Fig. 18-20).

**β- carotene-treated rats (GV and GVI)**

**The acinar cells:** The light micrographs revealed the normal pattern arrangement of the pancreatic acini in most sections of both GV and GVI (Fig. 21, 22). The nuclei of

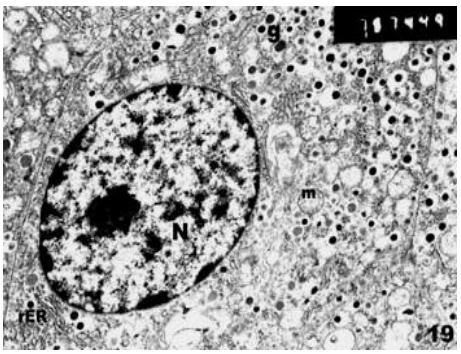


Fig. 19: Electron micrograph of islet of pancreas of rat (GIII) showing the normally shaped nucleus (N) of β-cell; Note the presence of β-granules (g) and vacuolized mitochondria (m); short profiles of rER. H and E., X7500

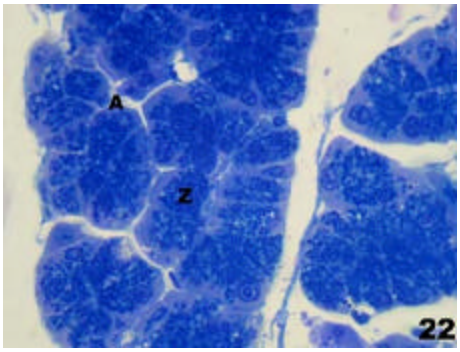


Fig. 22: Semithin section of pancreas of rat (GVI) showing the pyramidal shaped appearance of the pancreatic acini (A) and with an increased amount of zymogen (Z) granules in cytoplasm of most acinar cells. TB. X1000

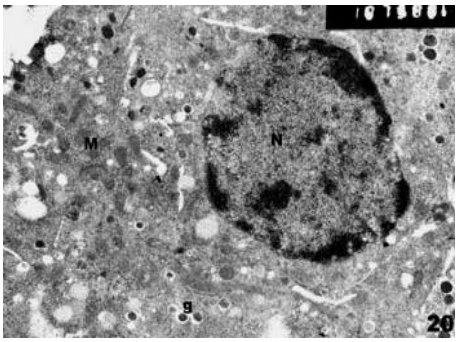


Fig. 20: Electron micrograph of islet of pancreas of rat (GIV) showing the nucleus (N) of β-cell; Note: the presence of moderate amount of β-granules (g) and normally shaped mitochondria (M). X10,000


Fig. 23: Electron micrograph of pancreas of rat (GV) showing parts of three acinar cells with an increased amount of zymogen (Z) granules and Note: the increased vacuolized mitochondria (m). X3000

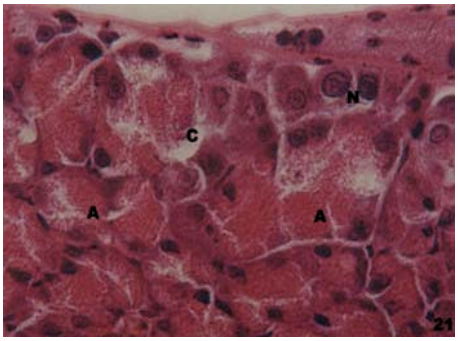


Fig. 21: Light micrograph of pancreas of rat (GV) showing the nearly normal arrangement of the pancreatic acini (A); Note: the basally located nucleus (N) of acinar cells and an increase in acidophilic cytoplasm (C) of most acinar cells. H and E. X1000

the acinar cells have rounded shaped appearance and were basally located. The cytoplasm was deeply acidophilic and the zymogen granules filled most of it (Fig. 21, 22).

The electron micrographs had confirmed the same earlier results shown by the light microscope (Fig. 23, 24). The acinar cells were preserve with the normal structural organelles as in the control. Extensive parallel arrays of rER are present surrounding the nuclei (Fig. 23, 24). GV showed marked increase in number of vacuolized mitochondria than that of GVI, indicating that 0.1 mg β-carotene gives a little side effect of β-carotene than those given 0.3 mg of it.

**The endocrine cells:** In H and E-stained sections, the islets of both GV and GVI had nearly normal shaped structure as in the control, they have many rounded

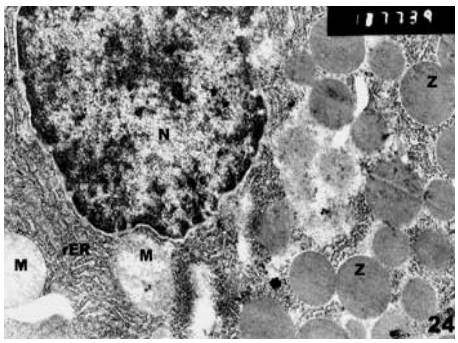


Fig. 24: Electron micrograph of pancreas of rat (GVI) showing part of the normally shaped nucleus (N) of acinar cell; Note the presence of mature zymogen (Z) granules and parallel arrays of rER and mitochondria (M). X10,000

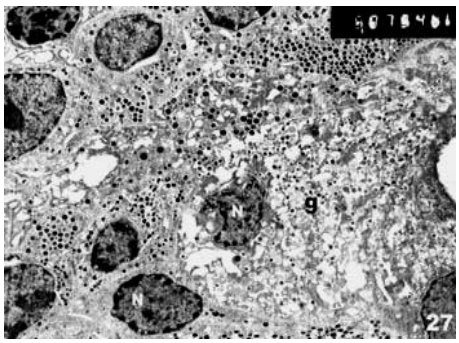


Fig. 27: Electron micrograph of islet of pancreas of rat (GV) showing the nuclei (N) of many β-cells with an increase in amount of β-granules (g). X 5000

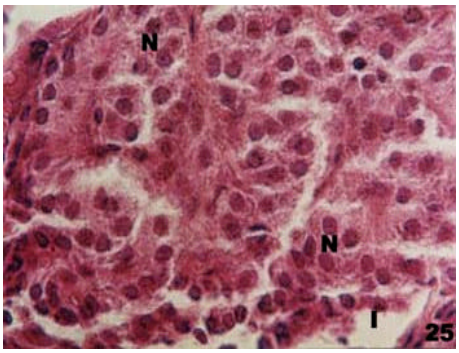


Fig. 25: Light micrograph of pancreas of rat (GV) showing that the islet (I) and of Langerhans contains many rounded shaped nuclei (N). H and E. X1000

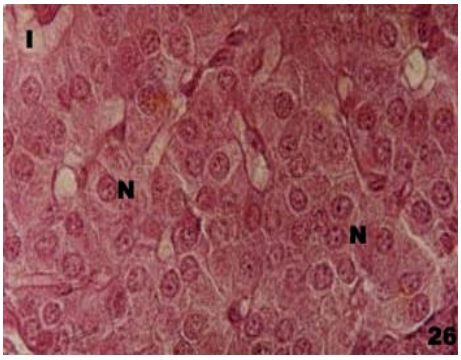


Fig. 26: Light micrograph of pancreas of rat (GVI) showing the nearly normal shaped nuclei (N) and of islet (I) of Langerhans as the control. H. and E. X1000

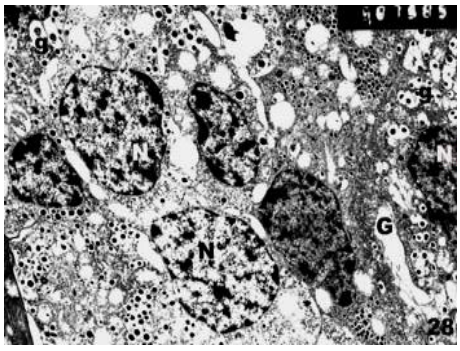


Fig. 28: Electron micrograph of islet of pancreas of rat (GV) showing the nuclei (N) of the nearly normal shaped β-cell; Note: the increase in their β-granules (g) and large extended Golgi (G) element is noticed. X5000

shaped nuclei (Fig. 25, 26). The electron micrographs showed an increase in number of β-cells in GV more than those of GVI (Fig. 27, 28). The nuclei of islet'cells were of normal shaped structures, having small masses of heterochromatin along the inner side of the nuclear envelope as well as in the nucleoplasm (Fig. 27-32). There was an increase in amount of β-granules in most β-cells, they were of normal structure as the control, having dense core surrounded by lucent halo space. Most of the mitochondria were still have certain vacuolation and dissolution in their cristae with an extreme loss of matrix density (Fig. 29, 32). Moreover, the cisternae of rough endoplasmic reticulum appeared dilated in certain areas of islet cells of GVI (Fig. 30).

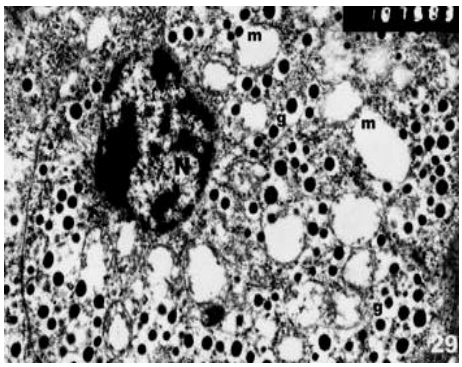


Fig. 29: Electron micrograph of islet of pancreas of rat (GV) showing the nucleus (N) of β-cell; Note that β- granules have dense core surrounded by lucent halo space and vacuolized mitochondria (m). X 10,000.

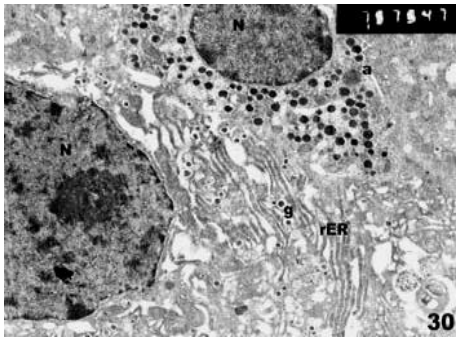


Fig. 30: Electron micrograph of islet of pancreas of rat (GVI) showing the nuclei (N) of β-cell and alpha (α) cell; Note: β-granules of β-cell; dilation of rough endoplasmic reticulum (rER). X7500

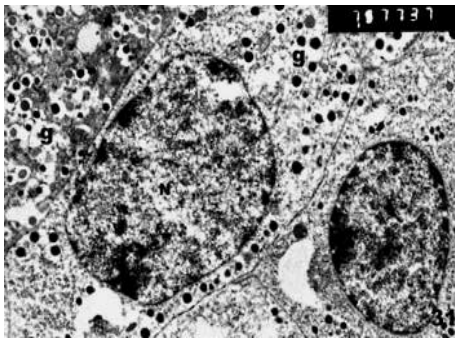


Fig. 31: Electron micrograph of islet of pancreas of rat (GVI) showing the nuclei (N) of α-cell (a) beside a part of β-cell with numerous β-granules (g). X7500

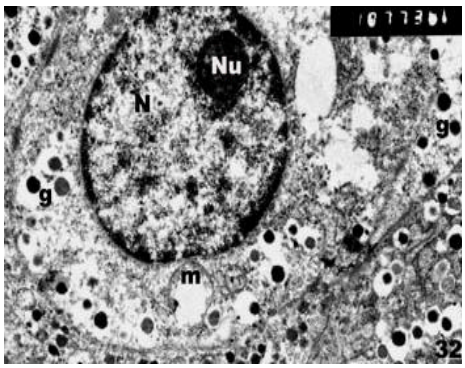


Fig. 32: Electron micrograph of islet of pancreas of rat (GVI) showing parts of β-cells, Note: the rounded shaped nucleus (N) with prominent nucleoli (Nu); β-granules and vacuolized mitochondria (m). X10,000

## DISCUSSION

In this study, we have presented evidence that β-carotene may introduce a prevention role in decreasing incidence of the diabetic rats induced by STZ. It was suggested that β-carotene exerts its protective effect via its antioxidant action.

In the present study, the diabetic rats induced by a single dose of STZ administration showed a significant increase in blood glucose level accompanied by significant decrease in body weight and insulin level. Howrth *et al.* (2000) found that administration of STZ to rats induced β-cell necrosis of the pancreas which gives rise to hypoinsulinaemia and hyperglycaemia. Also, Xianwen and Maeda (2006) declared that the induction of diabetes in mice by STZ administration caused significant reduction in body weight and significant increase in glucose level. Kakkar *et al.* (1998) suggested that oxidative stress started at early onset of diabetes and increased progressively. Ramakrishna and Jailkhani (2007) had reported that prolonged exposure to hyperglycemia causes oxidative stress which increases glycosylation and oxidation of proteins involved in the pathogenesis of the complications of diabetes. Various experimental studies suggested that oxidative stress impaired pancreatic β-cell insulin secretion, thereby accelerating the development and progression of type 2 diabetes (Bast *et al.*, 2002; Evans *et al.*, 2003). The results are in consisting with the suggestion of many authors (Hong *et al.*, 2004; Brownlee, 2005) in that diabetes is usually accompanied by an increased production of free radicals. Paolisso *et al.* (1993) had reported that free radicals have been shown to disrupt insulin action and total body glucose disposal.

Many researchers had explained the mechanism of action of STZ-induced diabetes. Takasu *et al.* (1991) stated that STZ in pancreatic islets β-cells was found to generate Reactive Oxygen Species (ROS) which also contribute to DNA fragmentation and evoke other deleterious changes in these cells. Schnedl *et al.* (1994) suggested that STZ is taken by pancreatic β-cells via glucose transporter. Moreover, Feki *et al.* (1997) had suggested that STZ induces DNA strand breaks in pancreatic β-cells of islets and that stimulates nuclear ADP-ribose synthesis, resulting in depression of insulin synthesis.

Comparing with the diabetic GII, the results showed significant increase in body weights accompanied by significant decrease in blood glucose levels in GIII where the diabetic rats were given 0.3 mg β-carotene. However, GIV where the diabetic rats were given 0.1 mg β-carotene showed no significant differences in blood glucose level. This indicates that 0.3 mg β-carotene revealed more protection for decreasing the diabetic symptoms than 0.1 mg of it. In addition, there were no significant differences in body weights and in the blood glucose levels among other groups (GIV, GV and GVI) and the control.

The results are in confirming with those of Frusho *et al.* (2002), who found that body weight gain in β-carotene +STZ group is significantly higher than that in STZ group and the blood glucose level was significantly lower than those in the STZ group, suggesting that β-carotene may have some beneficial effect on the correction of the biochemical disorders of diabetes because β-cells had retained their ability to synthesize and secrete insulin. They added that β-carotene plays an important role in protecting β-cells against cytotoxicity of STZ.

Similar results were reported by Lee and Park (2000), who found that the dietary soybean prevents induction of experimental hyperglycemia by prevention of β-cell injury by STZ and hence retaining β-cell activity. Also, Lee *et al.* (2003) concluded that β-cells of islet of pancreas of rats treated with PPC extracted from soybean had retained their ability to synthesize and secrete insulin and no alteration of glucose metabolism was detected. Moreover, Arulselvan and Subramanian (2007) found that the level of glucose and insulin in the blood were reverted back to near control levels after the treatment of *M. koenigii* leaves extract.

The results of light and electron micrographs revealed many changes in the pancreatic tissue of the diabetic rats (GII), summarized in disturbance in acinar pattern structure, shrinkage, pyknotic and vesicular nuclei of most acinar cells. The nuclei of β-cells showed aggregation of the heterochromatin along their nuclear envelopes. It was suggested that these changes may be due to condensation and shrinkage of the nuclear material. Buncher and Sreebny (1970) and Edress *et al.* (1987) explained the shrinkage of the nuclei to be as the result of accumulation of secretory granules in the acinar cells that pushed these nuclei to the periphery. The results are in confirming with those of Brosky and Logothetopoulos (1969), who found that the destruction in the pancreatic β-cells induced by STZ results in severe diabetes in experimental animals.

Other authors had reported that STZ at low doses induces apoptosis and at high doses causes necrosis in a murine pancreatic β-cell (Saini *et al.*, 1996; Aughasteen, 2000). In addition, other cytoplasmic changes in the diabetic pancreas of GII such as, the decrease in amount of zymogen granules, presence of many vacuoles, swollen and vacuolized mitochondria, extension and vacuolation of Golgi elements, degranulation of most β-cells and the presence the autophagic vacuoles are observed. These findings are in confirming with the results of Mythili *et al.* (2004) and Flament and Remacle (1987), who found ultrastructural changes in rat pancreatic islets due to STZ such as swelling of nuclear membrane, vesiculation of the Golgi apparatus and mitochondrial destruction. The autophagic vacuoles might reflect an increasing cellular damage, which run parallel to heavy reduction of the protein secretion of the acinar cells. Also, Hruban *et al.* (1965) reported that the autophagic vacuoles were described as cytoplasmic focal degeneration and they interpreted them as a form of cellular reaction to injury and to physiologic stimuli.

Degranulation of β-cells in the present study is most probably due to the decrease in insulin synthesis. This is in consistent with the suggestion of Kloppel *et al.* (1984), who reported that the degree of granulation of endocrine β-cells correlates with their extractable hormone. Also, Cotran *et al.* (1994) stated that β-cell degranulation is usually encountered in insulin dependent diabetes due to depletion of secretory stores of insulin in damaged cells.

In addition, it was of an important in this study to note that administration of β-carotene to these diabetic rats (GIII and GIV) had decreased many of these light and electron microscopic observations. The electron micrographs revealed that β-cells had retained their normal structures with its normal β-granules. The cytoplasm of most β-cells of islets in GIII showed marked increase in β-granules than those observed in GIV, retaining the ability to synthesize and secrete insulin. However, the decrease in β-granules of β-cells in GIV was correlated with the biochemical results, where the blood glucose levels were nearly similar to that in the diabetic (GII).

Appel and Woutersen (1996) had confirmed the chemopreventive effects of dietary β-carotene on the development of acinar pancreatic lesions induced in rats by azaserine. Also the ultrastructural finding of Arulselvan and Subramanian (2007) suggested that *M. keonigii* leaves extract treatment exerts a therapeutic protective nature in diabetes by decreasing oxidative stress and pancreatic β-cell damage, suggesting that to be due to its antioxidant effect.

Earlier data suggested that the protective effect of carotenoids against the development of type 2 DM, possibly via its antioxidant effect that may be attributed either to its molecular structure which stabilizes the unpaired electron of captured free radicals (Appel and Woutersen, 1996) or to its extended system of conjugated double bonds which give it the ability to scavenger peroxyl radicals (Krinsky, 1993; Burton and Ingold, 1984).

Moreover, indirect evidence supporting such a protective role of β-carotene comes from several observational studies relating increased intake of vegetables that are rich in carotenoids with a lower risk of type 2 DM (Colditz *et al*., 1992; Snowdon and Phillips, 1985).

Contrary to present study, Liu *et al.* (1999) had reported that supplementation β-carotene for an average of 12 years for men had no significant effect on the risk of type II DM. They could not exclude the possibility that some carotenoids or other nutrients other than β-carotene are responsible for the observed association. They added that other antioxidants such as vitamin E may play a role in the prevention of type 2 DM, but their efficacy still needs to be evaluated in randomized trials. Kataja-Tuomola *et al.* (2008) concluded that β-carotene supplementation did not prevent type 2 diabetes in male smokers.

In this conclusions, more studies are needed on the effect of β-carotene on carbohydrate metabolism in diabetes is needed and further ultrastructural studies should be taken on the pancreatic β-cells of islets. In addition, it is of an important to know more about the effective dose relative to its action as a preventive factor of DM.

## REFERENCES


Appel, M.J. and R.A. Woutersen, 1996. Effect of dietary β-carotene and selenium on initiation and promotion of pancreatic carcinogenesis in azaserine-treated rats. Carcinogenesis, 17: 1411-1416.

Arulselvan, P. and S.P. Subramanian, 2007. Beneficial effects of *Murraya koenigii* leaves on antioxidant defense system and ultrastructural changes of pancreatic -cells in experimental diabetes in rats. Chem. Biol. Interact., 165: 155-164.

Aughsteen, A.A., 2000. An ultrastructural study on the effect of STZ on the islet of Langerhans in mice. Elect. Microsc., 49: 681-690.

Bast, A., G. Wolf, I. Oberbaumerand and R. Walther, 2002. Oxidative and nitrosative stress induces peroxiredoxins in pancreatic β-cells. Diabetologia, 45: 867-876.

Brosky, G. and J. Logothetopoulos, 1969. Streptozotocin diabetes in mouseand guinea pig. Diabetes, 18: 606-606.

Brownlee, M., 2005. The pathobiology of diabetic complications: A unifying mechanism. Diabetes, 54: 1615-1625.

Buncher, A. and L.M. Sreebny, 1970. Effect of prolonged food reduction on the rat parotid gland and exocrine pancreas. J. Nutr., 100: 655-655.

Burton, G.W. and K.U. Ingold, 1984. β-Carotene: An unusual type of lipid antioxidant. Science, 224: 569-573.

Chakrabarty, A., R.A. Norman and T.J. Phillips, 2002. Cutaneous manifestations of diabetes. Wounds, 14: 267-274.

Colditz, G., J. Manson, M. Stampfer, B. Ronser, W. Willett and F. Speizer, 1992. Diet and risk of clinical diabetesin women. Am. J. Clin. Nutr., 55: 1018-1023.

Cotran, R.S., V. Kumar and S.L. Robbius, 1994. Robbins Pathologic Basisof Disease. 5th Edn., WB Saunders Company, Philadephia, London, Toronto, Montreal, Sydney.

Drury, R.A.B. and E.A. Wallington, 1980. Carleton's Histological Techniques. 5th Edn., Toronto, Oxford, New York.

Edress, Z., I. Zaki, S. El-Kalawyee, S. Fawzy and M. Abd-El-Hafez, 1987. Effect of starvation on certain organs of albino rat. Egypt. J. Histol., 10: 193-193.

El-Feki, M.A., Z.A. Radwan, A.A. Abdel-Monaiem and E. Salah, 1997. Effect of *Nigella sativa* fish oil on alloxan giabetic rats, 2-Immunological studies. J. Egypt. Ger. Soc. Zool., 23: 166-186.

Evans, J.L., I.D., Goldfine, B.A. Maddux and G.M. Grodsky, 2003. Are oxidative stress activated signaling pathways mediators of insulin resistance and ß-cell dysfunction? Diabetes, 52: 1-8.

Flament, P. and C. Remacle, 1987. Ultrastructural aspects of STZ in cytotoxicity on rat pancreatic islets *in vitro*. Test of a protective effect of zinc. Virchows Arch. B Cell Pathol. Zell Pathologie, 53: 107-112.

Florence, T.M., 1995. The role of free radicals in disease. Aust. N. Z. J. Ophthalmol., 23: 3-7.

Ford, E.S., C.W. Julie, A.B. Barbara and K.M. Venkat Narayan, 1999. Diabetes Mellitus and serum carotenoids: Findings from the Third National Health and Nutrition Examination Survey. Am. J. Epidomol., 149: 168-176.

Frusho, T., E. Kataoka, T. Yasuhara, M. Wada and S. Innami, 2002. Administration of beta-carotene suppresses lipid peroxidation in tissues and improves the glucose tolerance ability of streptozotocin-induced diabetic rats. Int. J. Vitam. Nature Res., 72: 71-76.

Ganda, O.P., A.A. Rassini, A.A. Like and C. Boston, 1976. Studies on streptozotocin diabetes. Diabetes, 25: 595-595.

Gandy, S.E., M.G. Buse, J.R. Sorenson and R.K. Crouch, 1983. Attenuation of STZ diabetes with superoxide dismutase like copper II in the rat. Diabetologia, 24: 437-440.

Glauert, A.M. and R.H. Glauert, 1958. Araldite as an embedding medium for electron microscopy. J. Biophys. Biochem. Cytol., 4: 191-191.

Hong, J.H., M.J. Kim, M.R. Park, O.G. Kwag and I.S. Lee *et al*., 2004. Effects of vitamin E on oxidative stress and membrane fluidity in brain of streptozotocin-induced diabetic rats. Clin. Chem. Acta, 340: 107-115.

Howarth, F.C., M.A. Qureshi, P. Lawrence and E. Adeghate, 2000. Chronic effects of STZ induced diabetes on the ultrastructure of rat ventricular and papillary muscle. Acta Diabetol., 37: 119-124.

Hruban, Z., H.D. Swift and D. Lewis, 1965. Effect of B-3-Furylalamine on the ultrastructure of the hepatocytes and pancreatic acinar cells. Lab. Invest., 14: 70-80.

Kakkar, R., S.V. Mantha, J. Radhi, K. Prasad and J. Kalra, 1998. Increased oxidative stress in rat liver and pancreas during progression of STZ-induced diabetes. Clin. Sci., 94: 623-632.

Kataja-Tuomola, M., J.R. Sundell, S. Mannisto, M.J. Virtanen, J. Kontto, D. Albanes and J. Virtamo, 2008. Effect of alpha tocopherol and ß-carotene supplementation on the incidence of type II diabetes. Diabetologia, 51: 47-53.

Kloppel, G., P. Heitz and W. Gepts, 1984. Pancreatic Pathology. Churchill Livingstone, Edinburgh, London, Melbourne, New York.

Kohlmeier, L. and S.D. Hasting, 1995. Epidemiologic evidence of a role of carotenoids in cardiovascular disease protection. Am. J. Clin. Nutr., 62: 13705-13765.

Krinsky, N.I., 1993. Actions of carotenoids in biological systems. Ann. Rev. Nutr., 13: 561-587.

Krishna, M.I. and U.N. Das, 2001. Prevention of chemically induced diabetes mellitus in experimental animals by polyunsaturated fatty acids. Nutrition, 17: 126-151.

Lee, S.H. and I.S. Park, 2000. Effects of soyabean diet on the beta cells in the STZ treated rats for induction of diabetes. Diab. Res. Clin. Pract., 47: 1-13.

Lee, S.H., Y.M. Han, B.H. Min and I.S. Park, 2003. Cytoprotective effects of polyenoylphosphatidyl-choline (PPC) on β-cells during diabetic induction by STZ. J. Histochem. Cytochem., 51: 1005-1015.

Liu, S., U. Ajani, C. Chan, C. Hennekens, J.E. During and J.E. Manson, 1999. Long-term beta-carotene supplementation and risk of type 2 Diabetes Mellitus. J. Am. Med. Assoc., 282: 1073-1075.

Mansford, K.R. and L. Opie, 1968. Comparison of metabolic abnormalities in diabetes mellitus induced by STZ or by alloxan. Lancet, 1: 1-1.

Maritim, A.C., R.A. Sanders and J.B. Watkins, 2003. Diabetes, oxidative stress and antioxidants: A review. J. Biochem. Mol. Toxicol., 17: 24-38.

Mayne, S.T., 1996. Beta-carotene, carotenoids, and disease prevention in humans. FASEB J., 10: 690-701.

Mythili, M.D., R. Vyas, G. Akila and S. Gunasekaran, 2004. Effect of STZ on the ultrastructure of rat pancreatic islets. Microsc. Res. Technique, 63: 274-281.

Olefsky, T.M., 1992. Diabetes Mellitus in Cecil Textbook of Medicine. 19th Edn., WB. Saunders Co., Philadephia, London.

Olson, J.A. and N.I. Krinski, 1995. Introduction: The colorful, fascinating world of the carotenoids: Important physiologic modulators. FASEB J., 9: 1547-1558.

Paolisso, C., A. D'Amore and G. Di Maro, 1993. Evidence for a relationship between free radicals and insulin action in the elderly. Metabolism, 42: 659-663.

Ramakrishna, V. and R. Jailkhani, 2007. Oxidative stress in non-insulin dependent diabetes mellitus patients. Acta Diabetol., 45: 41-46.

Reynolds, E.S., 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol., 17: 208-212.

Saini, K.S., C. Thompson, C.M. Winterford, N.I. Walker and D.P. Cameron, 1996. Streptozotocin at low doses apoptosis and at high doses necrosis in a murine pancreatic beta cell line, INS-I. Biochem. Mol. Iliol. Int., 39: 1229-1236.

Schnedl, W.J., S. Ferber, J.H. Johnson and C.B. Newgard, 1994. STZ transport and cytotoxicity. Specific enhancement in GLUT2-expressing cells. Diabetes, 43: 1326-1333.

Seddon, J.M., U.A. Ajani and R.D. Sperduto, 1994. Dietary carotenoids, vitamins A, C and advanced age-related macular degeneration. J. Am. Med. Assoc., 272: 1413-1420.

Snowdon, D.A. and R.L. Phillips, 1985. Does a vegetarian diet reduce the occurrence of diabetes? Am. J. Public Health, 75: 507-512.

Sylvia, S.M., 1993. Biology. 4th Edn., Wm. C. Brown Publishers, Dubuque, Melbourne, Australia.

Szkudelski, T., 2001. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol. Res., 50: 537-546.

Takasu, N., I. Komiyada, T. Asawa, Y. Nagasawa and T. Yamada, 1991. STZ and alloxan induced $H_2O_2$ generation and DNA fragmentation in pancreatic islets $H_2O_2$ as mediator for DNA fragmentation. Diabetes, 40: 1141-1145.

World Health Organization, 2002. Diabetes mellitus. WHO Fact Sheet No. 138.

Xianwen, Y.I. and N. Maeda, 2006. a-lipoic acid prevents the increase in atherosclerosis induced by diabetes in apolipoprotein E-deficient mice fed high fat/low cholesterol diet. Diabetes, 55: 2238-2244.