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Epidermal Growth Factor Improves the Ultrastructure of Submandibular Salivary Glands of Streptozotocin Induced Diabetic Rats - A Qualitative Study

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Abstract

Diabetes has well known damaging effects on different tissues of the body including salivary glands. Epidermal Growth Factor (EGF) facilitates healing and repair through different mechanisms including the increase in the activation and proliferation of fibroblasts. The aim of this study was to investigate if the effect of EGF was enough to repair the damage caused by diabetes in streptozotocin induced diabetic rats. Sixty adult male albino rats were divided into three groups. A control group, a streptozotocin induced diabetic group, and EGF group that was subjected to a single daily EGF intraperitoneal injection for two months after induction of diabetes. Submandibular salivary glands were dissected and examined using transmission electron microscopy. The diabetic group showed severe signs of atrophy and damage affecting all glandular components. The EGF group showed marked improvement in all elements of the submandibular salivary glands. **Conclusions**: EGF restored the structural integrity of submandibular salivary glands in diabetic rats.

Keywords: Diabetes, Epidermal Growth Factor, Ubmandibular Salivary Gland, Ultrastructure

1. Introduction

Diabetes is a group of metabolic disorders characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Two types of diabetes mellitus are known, type 1 (insulin-dependent) and type 2 (non–insulin-dependent) varieties. Type 1 is caused by damage to the B cells of the pancreas, which are responsible for formation of insulin in the body and is usually diagnosed in children and young adults. While type 2 accounts for the majority of all diagnosed cases of diabetes and is related with increased age, obesity, family history of diabetes, impaired glucose metabolism[1,2].

Epidermal Growth Factor (EGF) is a powerful growth factor that has a vital part in enhancing wound healing by stimulating the proliferation and migration of fibroblasts[3]. EGF is also known to be produced in ducts of human salivary glands[4].

Growth factors derived from salivary glands, including EGF, are thought to have a role in improving wound healing and preserving mucosal health. Oxford et al., 2000 reported for the first time the detection of decreased salivary EGF in humans with diabetes which may eventually contribute to the development of oral and systemic complications of diabetes[5].

The formation of EGF in the submandibular gland and its serum level were studied in diabetic mice. EGF concentrations in the submandibular gland and plasma were found to be noticeably reduced in streptozotocin-treated diabetic mice in comparison to the control after

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five weeks of the drug dose. Moreover, histological examination of the submandibular glands revealed that the size of the granular convoluted tubules, which secretes EGF, was significantly decreased in the diabetic mice. Insulin injection to diabetic mice almost completely counteracted the reduction in EGF level in the submandibular gland and enlarged the granular convoluted tubules. The authors concluded that EGF deficit occurs in diabetes mellitus and that insulin may be important in preserving the normal level of EGF in the submandibular glands[6].

In a previous study that was conducted by our research team, we showed that cytokeratin was highly expressed in STZ induced diabetic rats treated with EGF[7]. In the present study we aim to confirm our previous findings through investigating the ultrastructural changes occurring in the same rat model.

In previous studies, the ultrastructural changes in the submandibular and parotid glands and in the pancreas following diabetes induced by STZ exposure and the effects of fasting and insulin treatment on these changes were analysed. They included 48 Sprague-Dawley type rats divided into 8 groups after the injection of STZ[8]. The authors found that the mucous acini of the gland showed signs of degeneration manifested as accumulation of secretions in the submandibular gland as well as cytoplasmic vacuoles which replaced lipid droplets.

Both the parotid gland and pancreas were affected similarly due to their similar properties. Diabetes-induced granular loss was detected in the serous acini of both glands. Lipid accumulation was clearly diffuse within these cells. Concerning granular content, the authors observed the most noticeable deteriorating alterations in the parotid gland. Apoptosis in the acinar cells was observed although cellular loss was not seen in both the submandibular and the parotid gland. Fasting was found to cause changes within the glandular structure indicating increased activity, while insulin treatment was shown to repair the structure to normal in all of the glands. They concluded that the glands are affected by diabetes and associated fasting, and this effect manifests itself through the granule content. Therefore, our study aims to investigate if Epidermal Growth Factor (EGF) can counteract the effect of STZ induced diabetes on rat submandibular gland without any concurrent treatment with Insulin.

2. Objectives

The aim of the current study is to find out whether EGF can improve the well-known side effects of STZ induced diabetes mellitus on the submandibular salivary gland of adult male Albino rats or not, through ultrastructural examination of all components of salivary glands using transmission electron microscopy.

3. Materials and Methods

This study was reviewed and granted ethical approval by the Suez Canal University Research Ethics Committee (SUEZ-REC 29/2017). Sixty adult male Albino rats, three months old and an average weight of 220 gm were used in this study.

The animals were divided into three groups as follows:

- Control group: consisted of 20 rats.
- Diabetic group: consisted of 20 rats and subjected to STZ (Sigma-Aldrich Co) to induce diabetes mellitus.
- Diabetic + EGF: consisted of 20 rats that were treated with STZ as the diabetic group animals. After verification of their diabetic condition, they were subjected to a single daily dose of intraperitoneal injection of EGF provided by Sigma-Aldrich, Inc. in a dose of 10 µg/Kg body weight[9] for two months.

Diabetes was induced using a single intraperitoneal injection of freshly prepared streptozotocin dissolved in 0.1 M sodium citrate buffer[10].

The specimens were collected and used for Ultrastructural examination in Cairo University -Research Park CURP. Processed specimens where examined by transmission electron microscope JEOL (JEM-1400 TEM) at different magnifications.

4. Results

4.1 Control Group

The serous acini of rat submandibular glands appeared spherical in shape, having a central narrow lumen bordered by large pyramidal cells. Each cell had rounded

basally located nucleus. The nucleus usually showed an electron dense nucleoli (Figure 1A, 1B and 1C). The secretory cells were arranged in a spherical acinus with secretory canaliculi between the acinar cells. Cell membranes of the adjacent cells showed firm intercellular junctions, represented by numerous inter-digitation as well as numerous desmosomes (Figure. 1A, 1B, 1C and 1E).

Myoepithelial cells were associated with secretory acini and intercalated ducts. These cells appeared as flat cell basal to secretory cells between the basal lamina and basal cell membrane of both acini and intercalated duct cells. They had large elongated nuclei (Figure 1C and 1D).

Intralobular ducts had normal histological features. The intercalated ducts appeared small with cuboidal cell lining having centrally placed nuclei and little cytoplasm with rough endoplasmic reticulum, scattered mitochondria and few secretory granules (Figure 1F). The striated ducts were lined by columnar cells with large, rounded and abundant euchromatic nuclei. Their basal cell membrane exhibited numerous infoldings enclosing many radially arranged rod shaped mitochondria with tubular cristae. The supranuclear region contained moderately electron dense granules. The ductal cells contained large rounded, centrally placed nuclei with few rough endoplasmic reticulum and few Golgi complex around them (Figure 1G).

The granular convoluted tubules were lined by tall columnar cells with large rounded, basally located nuclei. A lot of well circumscribed apically located membrane bound granules with various electron densities. The basal part of the cells contained rounded euchromatic nuclei, surrounded by numerous mitochondria (Figure 1H). The interlobular and interlobar spaces were filled with connective tissue fibers, and cells as fibroblast, varying sized blood vessels lined by endothelial cells and filled with electron dense erythrocytes. All embedded in an extracellular matrix of ground substance (Figure 1A, 1C and 1D).

4.2 Diabetic Group

The serous secretory cells showed marked a trophic changes. There was an obvious increase in spacing between acini, the acinar cells were pyramidal in shape with different sizes and irregular basally located nuclei, accumulation of electron-lucent secretory granules pushing some nuclei more basally, a lot of shrunken nuclei were observed. The secretory cells were arranged in irregular acini with narrow lumen. Marked dilation of the rough endoplasmic reticulum (rER) was observed with collection of a lot of cytoplasmic vacuolations. Some acini showed massive degeneration of the cell organelles leaving few strands of rER, and destroyed and ruptured mitochondria which lost their cisternae. A lot of cytoplasmic vacuolations were encountered in most of the parenchymal elements. Widened intercellular junctions, lack of inter-digitation as well as few desmosomes between acinar cells were observed. (Figure 2A, 2B, 2C, 2D and 2E).

The intercalated duct cells showed pleomorphic irregular shrunken centrally located nuclei, with apparent reduction of cytoplasmic organelles and multiple intracytoplasmic vacuoles representing damaged cell organelles (Figure 2D). The striated duct cells showed irregular heterochromatic nuclei and few short basal infoldings with marked decrease in the rER which appeared in the form of a few dilated strands with electron dense material. Mitochondria were numerous, disfigured, distorted or damaged and had marked vacuolization and loss of cristae. Few scattered fine granules and vacuoles were observed stagnation of secretion in their lumena was frequently encountered. (Figure 2E and 2F).

The granular convoluted tubules were severely affected. The duct cells showed multiple cell vacuolization representing damaged and ruptured mitochondria, rough endoplasmic reticulum and other organelles. Their granules showed marked reduction of their number, pleomorphic sizes, shapes and varying electron densities. The lumen appeared wide with stagnant secretion (Figure 2G). The excretory ducts showed moderate deviation from normal. The duct cells were reduced in height, lost their pseudo-stratification showed irregular pyknotic nuclei with intracytoplasmic vacuolization representing damage of cell organelles and wide lumen filled with stagnated secretory material. The connective tissue stroma was seen to have remnants of degenerated cells, dilated blood vessels studded with erythrocytes and vacuolated fibroblast with degenerated cell organelles (Figures 2A, 2B, 2C, 2E and 2G).

4.3 Diabetic + EGF

The ultrastructural examination of submandibular salivary glands of this group revealed marked improvement in comparison to those of the diabetic group with mild

ultrastructural alterations. Almost normal acinar cell shape, less irregular basally located nucleus and in some specimens appeared pyknotic with normal cell organelles including rough endoplasmic reticulum, mitochondria, Golgi complex and other cell organelles few mild dilatation of rough endoplasmic reticulum (rER) and distributed mitochondria were rarely observed. The apical parts of the cytoplasm are packaged with numerous electron lucent secretory granules as seen in normal acinar cells. The secretory cells appeared as large pyramidal cells arranged in a spherical acinus with narrow lumen with secretory canalculi. Myoepithelial cells appeared basally to secretory cells. Numerous desmosomal junction between acinar cells were observed (Figure 3A).

The duct system also showed marked improvement in comparison with diabetic group. Almost normal structure was observed with minimal cytoplasmic vacuolization in the duct cells. The intercalated ducts restored its normal histology with rounded centrally located large nuclei and normal cell organelles. The duct cells showed centrally placed nuclei and few cytoplasm, scattered mitochondria and few secretory granules. Myoepithelial cell located basally to the ductal cell (Figure 3B). The striated ducts showed almost normal cell lining with basal infoldings, moderate number of elongated radially arranged basally located mitochondria with tubular cristae. The supra-nuclear region contains moderately electron dense granules located apically. Numerous desmosomal

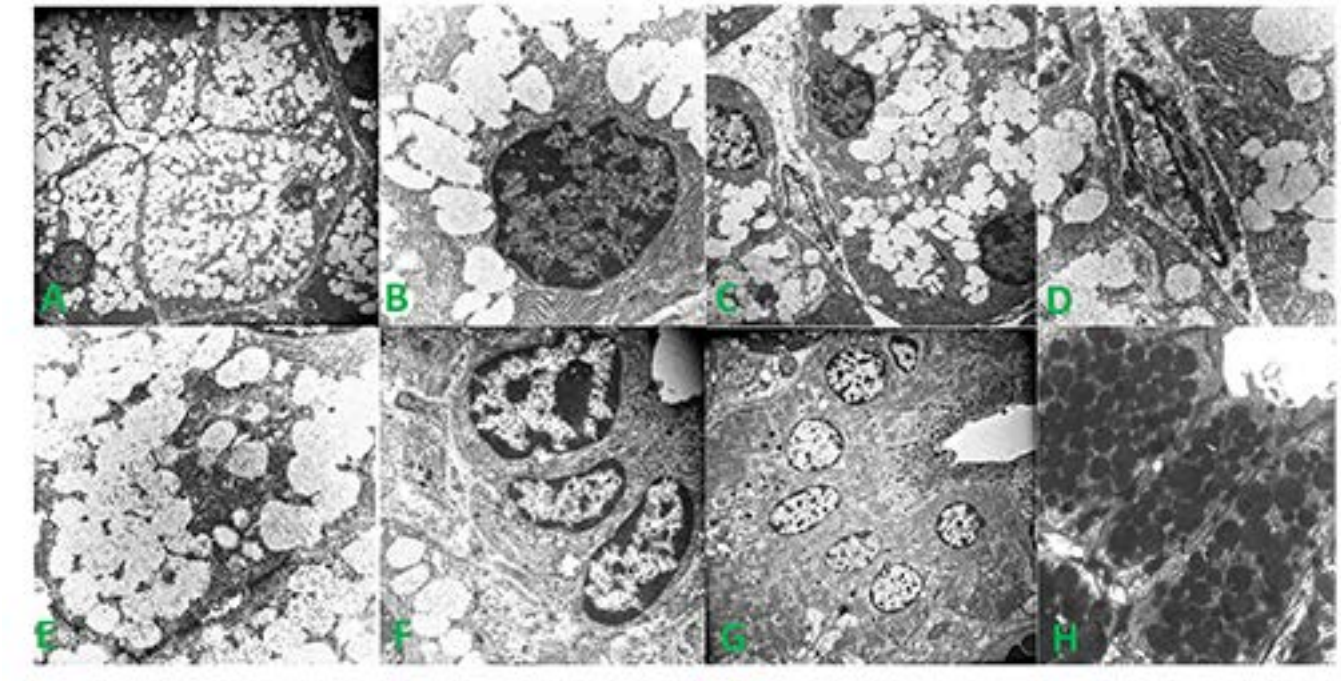

Figure 1. Electron micrograph of a submandibular gland from the control group. (A): showing pyramidal shaped serous acinar cells relatively uniform in size with rounded basally located nuclei (N) and packed with secretory granules (A). The secretory cells are arranged in a spherical acinus with narrow lumen (Lu) and secretory canaliculi between the cells (arrow) (EM 2500X). (B): showing serous secretory cell with rounded basally located nucleus (N) with electron dense nucleolus (Nu). RER condensed basally (RE), Mitochondria (M) and packed secretory granules of varying in densities (A) (EM 10000X). (C): showing serous acinar cells with RER condensed basally (RE), Mitochondria (arrow) and Myoepithelial cell (M) (EM 4000X). (D): showing higher magnification of figure 1C (EM 10000X). (E): showing desmosomal junction between secretory cells, (arrow), secretory canalculi (Ca), rough endoplasmic reticulum (RER) and Mitochondria (M) (EM 10000X). (F): showing intercalated duct with small cuboidal cell lining (EM 8000X). (G): showing striated duct with columnar cell lining with basal infolding, numerous basally located radially arranged mitochondria (M) and moderate electron dense secretory vesicles (arrow) (EM 2500X). (H): showing granular convoluted tubules lined with columnar cells having a lot of electron dense granules and numerous desmosomal junctions (EM 2500X).

junctions between duct cells were observed (Figure. 3C, 3D and 3E).

The granular convoluted tubules showed almost normal appearance with tall columnar cell lining. The ductal cells appeared with large rounded, basally located nuclei with normal cell organelles including mitochondria, rough endoplasmic reticulum, and apically located membrane bounded secretory granules with various electron density (Figure 3F and 3G). The excretory ducts appeared normal and lined by pseudostratified columnar cells. The ductal cells had large nuclei, abundant mitochondria and few rough endoplasmic reticulums. The adjacent cells also showing lateral interdigitation and desmosomal junctions the interlobular and interlobar spaces were filled with almost normal connective tissue fibers, cells and blood vessels (Figure 3A, 3B, 3C and 3G).

5. Discussion

In our study, the ultrastructural examination of the submandibular gland after 2 months of diabetes induction has shown that the secretory cells showed marked

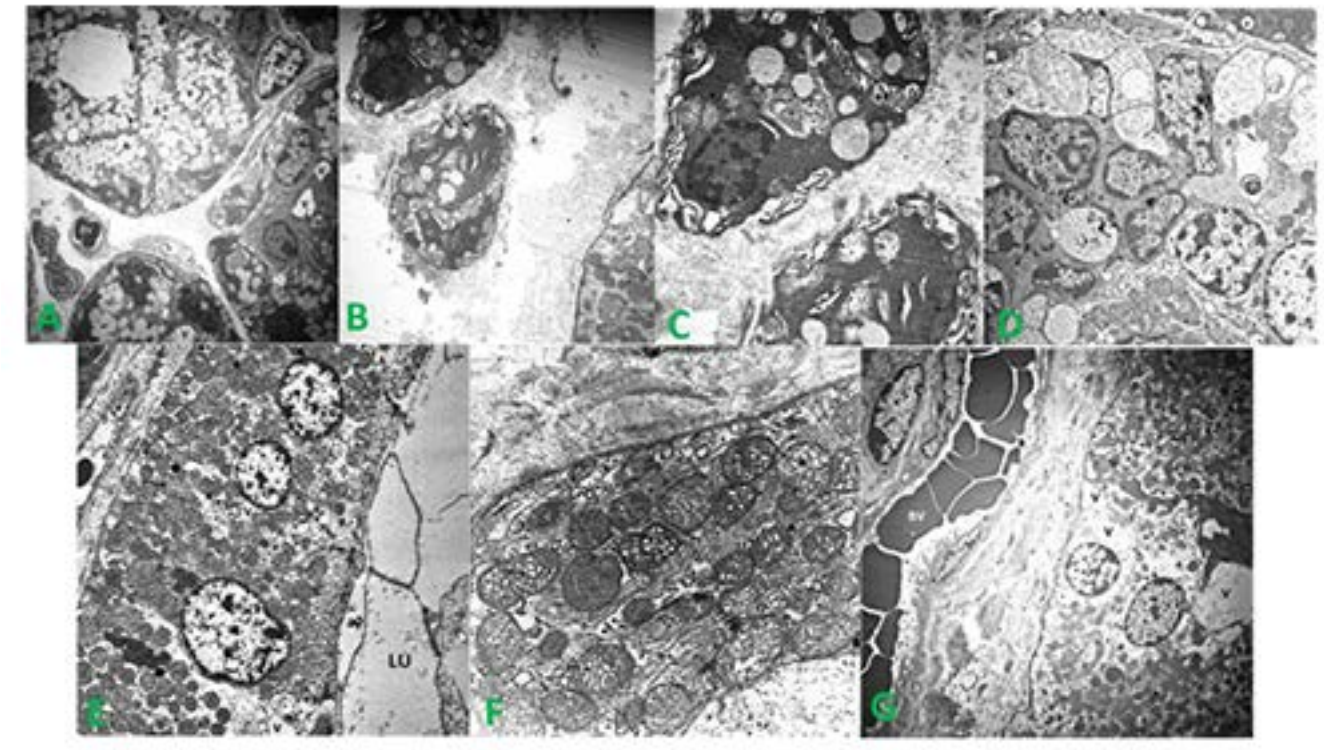

Figure 2. Electron micrograph of a submandibular gland from a diabetic rat. (A): showing an obvious increase in spacing between acini, acinar cells are pyramidal in shape with different sizes and irregular basally located nuclei (N), accumulation of electron-lucent secretory granules (A) pushing some shrunken nuclei more basally(*). The secretory cells are arranged in the irregular acini with narrow lumen with canaliculi (arrow). Large and small cytoplasmic vacuolization were observed in the acinar cells (V). Congested Blood Vessel (BV) is seen in degenerated stromal connective tissue (EM 2500X). (B): showing massive degeneration of acini and acinar cells, marked cell organelles degeneration leaving few strands of rER (R), and destroyed mitochondria (arrows). Notice the extreme widening of the connective tissue septa with degeneration of collagen fibers (EM 4000X). (C): showing higher magnification of figure 2B (EM 8000X). (D): showing intercalated duct with irregular outline and pleomorphic nuclei, cytoplasmic vacuolizations (arrow) (EM 4000X). (E): showing striated duct with stunted cell lining, almost complete absence of basal infolding, cytoplasmic vacuolation (V), severe vacuolated mitochondria (arrows), and absence of secretory vesicles and stagnation of secretion inside lumen (LU) (EM 4000X). (F): showing higher magnification of figure 14, with vacuolated mitochondria of striated duct cells and loss of basal infoldings (arrow) (EM 10000X). (G): showing granular convoluted tubule with marked decrease in size and number of their granular contents, pleomorphism of the granules and great variation in their electron density. Cytoplasmic vacuolization of the duct cells (V) and markedly dilated blood vessel in the connective tissue stroma with electron dense RBCs (BV) (EM 2500X).

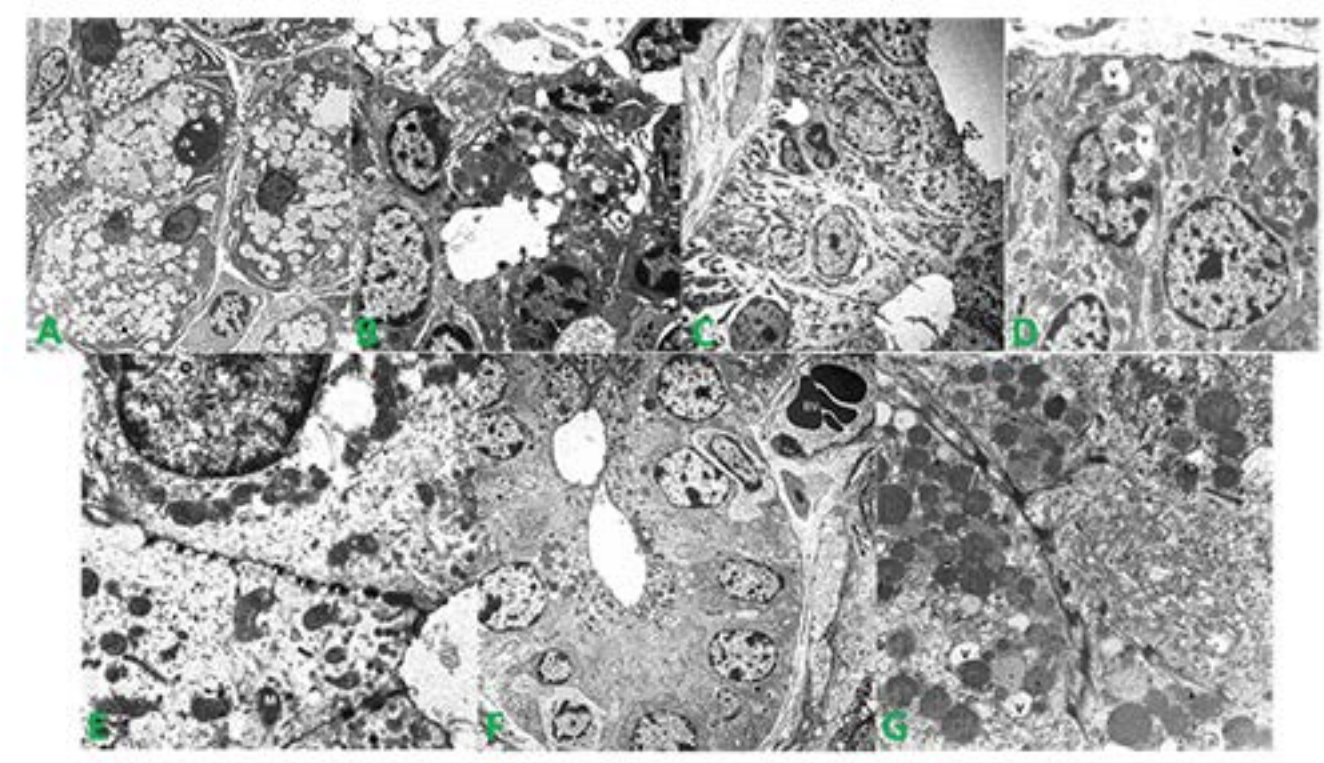

Figure 3. Electron micrograph of a submandibular gland from EGF group. (A): showing normal acinus structure with minimal spacing between acini, almost normal acinar cell shape, basally located nucleus (N), and Myoepithelial cell (M) (EM 3000X). (B): showing intercalated duct with almost normal structure with small cuboidal cell lining having centrally placed nuclei and little cytoplasm, scattered mitochondria (M) and few secretory granules (arrow). Myoepithelial cell located basally to the ductal cell (MY) (EM 5000X). (C): showing striated duct with almost normal basal infolding, moderate number of elongated radially arranged mitochondria (M) and electron dense secretory vesicles (arrow) (EM 2500X). (D): showing striated duct with basal infolding, numerous basally located radially arranged mitochondria (M). Notice the presence of minimal cytoplasmic vacuolization (V) (EM 6000X). (E): showing desmosomal junction between striated duct cell (arrow) and a lot of normal mitochondria (M) (EM 10000X). (F): showing granular convoluted tubules lined by tall columnar cells with large rounded, basally located nuclei (N). A lot of well circumscribed apically located membrane bounded secretory electron dense granules (arrow). Mitochondria (M). Blood vessel with normal endothelial lining (BV) (EM 2500X). (G): showing granular convoluted tubules with desmosomal junction between duct cell (arrow) and a lot of well circumscribed apically located membrane bound secretory granules with various electron density (G). Note that there is still there is a number of cytoplasmic vacuolizations (V) (EM 12000X).

atrophic changes. These results were similar to Mednieks et al. (2009)[11] who stated that "the acinar cells of diabetic rats exhibited variability in the density and structure of secretory granules, increased numbers of lysosomes and autophagic vacuoles, lipid droplets in the basal cytoplasm and folding and redundancy of their basal laminae". These results were also in agreement with those of Hidayat et al. (2013)[12] who concluded that STZ altered the structure of parotid gland by lipid infiltration and degeneration of acini.

In addition to the above, the results of this study are consistent with a previous study performed by High et al. (1985)[13] which revealed that few tiny lipid droplets accumulated in the acinar cells of submandibular gland in diabetic rats. The authors noticed that formation of autophagic vacuoles in the cells coincides with the duration of diabetes. Cutler et al. (1979)[14] studied the changes in the submandibular gland in STZ-induced diabetes in rats at the ultra-structural level during different intervals and reported observing prominent degeneration

in every period of diabetes especially in the acinar cells. They have also found that secretions accumulate in the cytoplasms of the acinar cells and anticipated that the acinar cells being reduced in number resulted in decrease in salivary secretions.

Furthermore, the duct system revealed severe changes in the diabetic group. These results were parallel to those of Lotti and Hand (1988)[15] who found electron dense vacuoles in striated ducts lining cells with degradation of intra cellular protein in streptozotocin induced diabetes. These changes could be attributed to the chronic sodium depletion related to diabetes as was confirmed by High et al. (1985)[13].

The accumulation of cytoplasmic vacuolations and destruction of some acinar and GCTs cells, probably could be related to the lysosomal and mitochondrial disturbance occurred with diabetes. This was in concomitant with Maciejewaski, et al. (1999)[16] who observed mitochondrial damage and swollen lysosomes, together with elevated acid phosphatase activity in submandibular gland. This was correlated to the increased glucose level in rats with alloxan induced diabetes. The connective tissue stroma was seen to have remnants of degenerated cells, dilated blood vessels studded with erythrocytes. This was in agreement with Zeisberg et al. (2000)[17] who reported that activation of fibroblast usually affected by different factors one of those is hyperglycemia.

In a different study conducted by Anderson et al. (1994) [18] the histological, histochemical and ultrastructural effects of STZ-induced diabetes on rat submandibular glands were studied. After induction of diabetes, the gland tissues were examined at different intervals of 3 weeks, and 3 and 6 months and then were compared with glands from controls of the same ages using histological and ultrastructural methods. The results of the light microscope revealed that size and number of granular ducts were decreased in diabetic animals however that of the acinar cells were only affected 6 months after diabetes. Ultrastructurally, there was an accumulation of lipid in the acinar cells. The authors also found that with progressive duration of diabetes, the number of autophagic vacuoles in both the acini and the granular ducts were increased. Therefore, it was concluded that the greatest effect of STZ-induced diabetes was found in the cells of the granular convoluted tubules manifested as hypotrophic changes[18].

Our results showed a marked improvement in the structure of submandibular salivary glands of STZ induced diabetic rats treated with EGF. In adult mice, EGF normally derived from salivary glands seems to play a vital role also in wound healing of skin and soft tissue as well as in maintaining organ homeostasis. Deposition of EGF on wounded skin enhances healing of the wound area. Another mode of action for EGF that enhance wound healing includes their autocrine production and secretion by cells at the site of a wound. It also has been reported that salivary gland–derived EGF promotes the healing of gastric ulcers and tongue lesions[19].

6. Conclusions

Intraperitoneal injection of EGF daily for two months resulted in improvement of the submandibular salivary glands parenchymal and stromal elements of diabetic rats. This was confirmed by ultrastructural examination using transmission electron microscope. Further studies are requiring quantifying the amounts of damage caused by diabetes and the repair process induced by EGF in the submandibular salivary glands of diabetic rats.

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