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Research Article
Effect of Losartan on Islets of Langerhans β-cells in Streptozotocin-induced Diabetes in Adult Male Albino Rats

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Abstract

Background and Objective: Exogenous insulin administration can't achieve the same glycemic control provided by β-cells endogenous insulin. Thus, strategies to improve the structure and function of existing β-cells of diabetic patients would provide a future treatment option. One of new strategies is angiotensin 2 blockers (ARB) such as losartan (LOS). Therefore, this study was designed to investigate the effect of LOS in improving the structure of β-cells in diabetic rats . **Materials and Methods:** Forty healthy adult male albino rats were randomized into four groups, ten animals in each group, (1) Control group, (2) STZ group received an IP injection of 65 mg kg^{-1} b.wt., streptozotocin (STZ), (3) STZ+LOS group received 20 mg kg^{-1} b.wt., LOS orally, daily for 1 week after diabetes induction and (4) LOS group received oral LOS (20 mg kg^{-1} b.wt.) daily for 1 week. Ten days later, animals were sacrificed. Tail of pancreas specimens were processed for Light Microscope (LM) and Transmission Electron Microscope (TEM) examination. Sections were subjected to hematoxylin and eosin stain and anti-insulin immunostaining of β-cells. Histopathological changes in islets were graded from 0 (no changes) to 3 (more than 50% alterations). Mean optical density (MOD) and percentage of β-cells (βp) were calculated. **Results:** In STZ group, most islets were in grade 3, with significant decrease in MOD and βp compared to control group. The STZ+LOS group showed some improvement in islets including significant decrease in grade 3 alterations and significant increase in grade 1. There was significant increase in MOP and βp compared to STZ group. TEM examination confirmed the previous results. **Conclusion:** LOS induced partial cure of β-cells and islets.

Key words: Diabetes mellitus, Losartan, angiotensin 2 blockers (ARB), Mean optical density, β-cells percentages

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The rapidly increasing number of diabetic patients across the world constitutes a great challenge to the side effects of the current traditional therapeutic approaches, thus demanding development of novel strategies[1], one of those new strategies is angiotensin 2 blockers (ARBs) which are found to improve glucose metabolism[2].

The local renin-angiotensin system (RAS) is responsible for the regulation of metabolism, blood flow, inflammation and healing responses. Its existence in the pancreatic tissue has been confirmed in different species such as mice, rats, dogs and humans[3]. The RAS in pancreatic islets was proved to have a pivotal role in regulating islet glucose-stimulated insulin secretion and thus, glucose homeostasis[4].

Several studies have shown that the use of angiotensin pathway blockage therapies could inhibit β-cell-directed immunity in type 1 diabetes[5] and improve islet function and glucose tolerance in type 2 diabetes patients[6].

Losartan (LOS), one of ARBs, is suggested to significantly lower blood glucose levels and lipid peroxidation products and increase endothelial cells' protection and improve the islets' environment against oxidative and nitrosative stress products[5]. Furthermore, it was reported that LOS could increase β-cell mass, pancreatic islet insulin reactivity and accelerate islet β-cell regeneration and proliferation in diabetic rats[5].

There are limited studies showing the role of LOS in improving the β- cells of islets of Langerhans in Streptozotocin (STZ) induced model of diabetes mellitus. Also most of these studies assessed only the biochemical changes. Hence, the current study was designed to demonstrate the possible curative effect of LOS on β-cells of islets of Langerhans in STZ-induced diabetes in adult male albino rats, using light and transmission electron microscopes (LM and TEM).

MATERIALS AND METHODS

This study was conducted in the Histology Department, Faculty of Medicine, Suez Canal University as a Master research project from 2012-2014. The study was conducted on 40 healthy adult male albino rats of the same age and weight. Animals were obtained from the Faculty of Veterinary Medicine, Suez Canal University. Animals were housed in suitable cages and acclimatized for 1 week duration under standardized conditions away from any stressful stimuli, with free access to standard pellet animal diet and tap water. Animals were randomized into four groups, ten animals in each group. Group I (control group) received a single IP injection of 0.1 M sodium citrate buffer (the dissolvent of STZ). Group II (STZ group) received an IP injection of freshly prepared STZ (65 mg kg^{-1} b.wt.) dissolved in 0.1 M sodium citrate buffer, (pH 4.5) and the injected rats were considered diabetics only when exhibiting blood sugar levels, from tail blood samples reach 200 mg dL^{-1} or more within 72 h after STZ injection. Group III (STZ+LOS group) received an IP injection of freshly prepared STZ 65 mg kg^{-1} b.wt., then, 3 days later (on the 4th day), after diabetes confirmation, rats received drinking water containing LOS tablets (20 mg kg^{-1} b.wt.) daily for 1 week. Group IV (LOS group) received drinking water containing LOS tablets (20 mg kg^{-1} b.wt.) daily for 1 week.

At the end of the experiment (after 10 days), all animals were sacrificed by cervical decapitation. Specimens were obtained from the tail of pancreas, some were fixed in 10% neutral-buffered formalin and processed for 5 µm paraffin sections for histological staining (H and E) and immunohistochemical staining of β-cells of islets of Langerhans using the primary antibody, polyclonal Guinea pig anti-insulin antibody (purchased from Abcam {ab7842} and the detection kit {TP-015-HD} was purchased from Thermo Scientific™ Lab Vision™, United States of America, through its supplier in Egypt, Midco Trade Company). Negative controls for immunohistochemistry were obtained by replacing the primary antibody by PBS. Other specimens were fixed in 3% glutaraldehyde and prepared for 50 nm ultra thin sections for transmission electron microscopic examination.

Quantitative and qualitative assessment were done followed by statistical analysis as following:

Quantitative assessment: The following parameters were measured using the immunohistochemical slides at a magnification of 400X with the pro-plus image analyzer computer system in the Histology Department at Faculty of Medicine, Suez Canal University: First, the mean optical density (MOD) of anti-insulin antibody reaction in islets was measured, in five islets from five different microscopic fields from each animal in all groups[7]. Second, the percentage of β-cells (βp) per total islet cells was calculated using the nucleus as the counting base. The nuclei of the stained β-cells (βn) and the nuclei of total islet cells (In) per islet profile were counted. The β-cell percentage per islet cells (βp) was calculated using the following equation:

$$\beta p = \frac{n}{In} \times 100$$

where, βp was calculated in four islets from each animal and 40 islets from each group[7].

Qualitative assessment: The histopathological changes in the form of nuclear changes (pyknosis or karyolysis) and cytoplasmic changes (vacuolations) were graded according to grading system modified by Drachenberg *et al.*[8] as follows:

- **Grade 0: (normal):** Islet is totally free from cytoplasmic or nuclear changes
- **Grade 1:** Cytoplasmic and/or nuclear changes involve <25% of the islet cells
- **Grade 2:** Cytoplasmic and/or nuclear changes involve 25-50% of the islet cells
- **Grade 3:** Cytoplasmic and/or nuclear changes involve >50% of the islet cells with distortion of islet morphology and architecture

All islets in three sections of each animal were graded and percentage of each grade per animal was calculated, then the mean percentage of each grade per group was calculated.

Statistical analysis: The results were expressed as Mean±SD. The significance of difference in values was performed by one-way ANOVA test using the SPSS program. The $p<0.05$ was considered to be statistically significant.

RESULTS

Group I (control group): The H and E stained sections of the control group showed normal appearance of the islets of Langerhans. The islet appeared as pale stained well circumscribed area with regular outline which was surrounded by deeply stained pancreatic acini (Fig. 1). Each islet was formed of cells arranged in cords and separated by blood capillaries in thin connective tissue strands. The cells on periphery were mostly α-cells which appeared large in size while the cells in center were mostly small β-cells (Fig. 2). Almost all islets in this group were in grade 0 (Table 1).

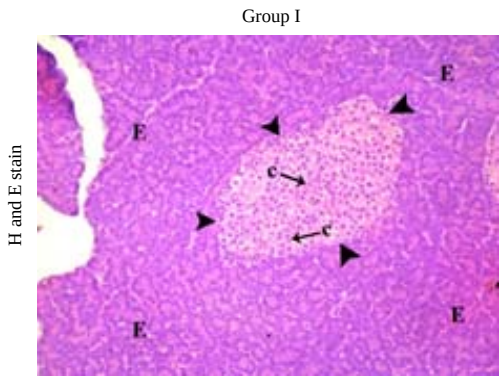

Fig. 1: A photomicrograph of a pancreatic section from control group showing a pale stained area (islet of Langerhans) surrounded by deeply stained exocrine pancreatic acini (E). Islets appeared with regular outline (arrow heads) and formed of cords of cells (C)
H and E 400X

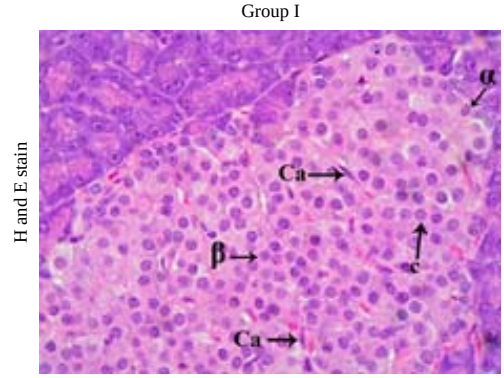

Fig. 2: A high power magnification of the previous figure showing cells of the islet arranged in cords (c) separated by blood capillaries (Ca). The cells on periphery are mostly α-cells (α) which appear large in size while the cells in center are mostly β-cells (β) which appear smaller
H and E 1000X

Table 1: Mean percentage of grade of affection of islets in different studied groups

Grade	Control group	STZ group	STZ+LOS group	LOS group	p-value
Grade 0					
Mean±SD	99±3.2[a]	0[b]	0[b]	96.75±5.4[a]	<0.001*
Range	90-100	0	0	85.71-100	
Grade 1					
Mean±SD	1±3.16[a]	0[a]	19.4±26.99[b]	3.25±5.42[ab]	0.01*
Range	0-10	0	0-63.6	0-14.29	
Grade 2					
Mean±SD	0[a]	19.17±13.2[ab]	32.79±28.8[b]	0[a]	<0.001*
Range	0	0-33.33	0-85	0	
Grade 3					
Mean±SD	0[a]	80.83±13.22[b]	47.81±37.6[c]	0[a]	<0.001*
Range	0	66.67-100	9.1-100	0	

*Statistically significant difference (p-value for "F-statistic" analysis of variance test). [a,b,c]Denoting significant difference within groups (*post hoc* analysis using Bonferroni test)

Table 2: Mean and standard deviation of mean optical density (MOD) and β-cell percentage (βp) of immunostaining of β-cells in different studied groups

	Control group	STZ group	STZ+LOS group	LOS group	p-value
β-cell (%)					
Mean±SD	69.98±2.2[a]	26.63±7.4[b]	39.45±16.76[c]	68.89±4.01[a]	<0.001*
Range	65.25-72.5	17.75-36	0.47-0.27	63-75.75	
MOD					
Mean±SD	1.15±0.08[a]	0.28±0.03[b]	0.47±0.27[c]	1.12±0.09[a]	<0.001*
Range	0.99-1.26	0.23-0.31	0.26-0.88	0.97-1.27	

*Statistically significant difference (p-value for "F-statistic" analysis of variance test). [a,b,c]Denoting significant difference within groups (*post hoc* analysis using Bonferroni test)

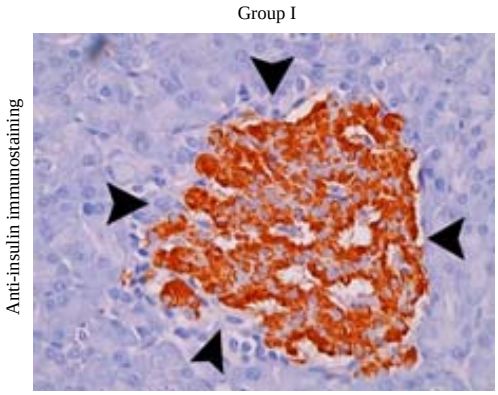

Fig. 3: A photomicrograph of pancreatic section from control group showing islet of Langerhans stained positive for insulin hormone (brown colour). The periphery of the islet shows negative immunoreactivity (arrow heads)
Anti-insulin immunostaining 1000X

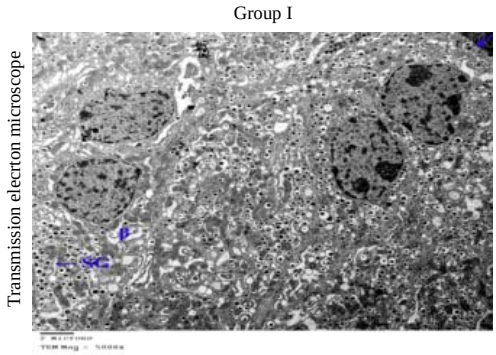

Fig. 4: Electron photomicrograph of a pancreatic section from the control group showing an area of islet of Langerhans containing multiple β-cells which contain numerous secretory granules (SG)
TEM 5000X

Anti-insulin immunostained sections showed diffuse positive brown cytoplasmic immunoreactivity involving mainly cells in the central part of the islet (β-cells), whereas, the peripheral cells exhibited negative immunoreactivity (Fig. 3). The β-cell percentage (βp) was 69.98±2.2 (Table 2)

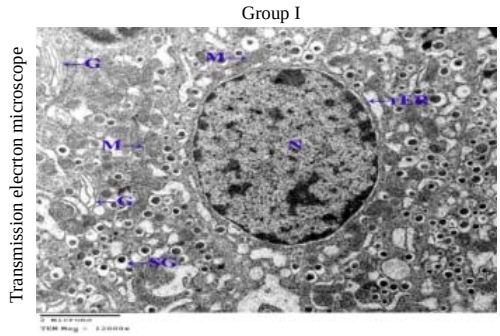

Fig. 5: Electron photomicrograph of a pancreatic section from the control group showing β-cell containing euchromatic nucleus (N) with regular nuclear envelop. Its cytoplasm contains Rough Endoplasmic Reticulum (rER), mitochondria (M) and numerous secretory granules (SG)
TEM 12000X

and the mean optical density (MOD) of immunopositive cells was 1.15±0.08 in this group (Table 2).

The TEM examination showed normal appearance of an islet of Langerhans. The periphery of the islet contained α-cells while the center contained β-cells (Fig. 4). The β-cells had euchromatic nuclei with regular nuclear envelop (Fig. 5). The cytoplasm contained rough endoplasmic reticulum, multiple Golgi complexes, mitochondria with apparent cristae and multiple secretory granules characteristic of β-cell which had crystalloid electron-dense cores with characteristic halos around them (Fig. 5, 6).

Group II (STZ group): The H and E stained sections of STZ group revealed that all islets were affected. The islets were shrunken and had irregular outlines (Fig. 7). The islets showed distorted architecture with cytoplasmic and nuclear changes in the cells of the islets. Cytoplasmic vacuolations were shown, mostly in the central part of the islets. Some cells showed darkly stained pyknotic nuclei and others showed karyolytic nuclei (Fig. 8). Most of islets were in grade 3 and the rest of islets were in grade 2 (Table 1).

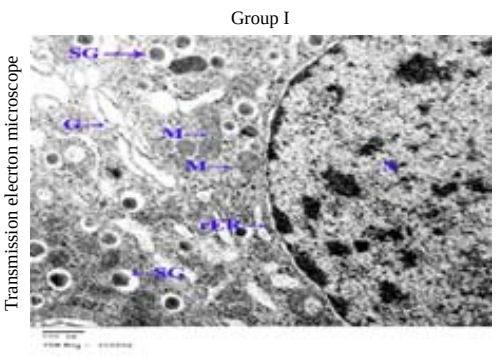

Fig. 6: Electron photomicrograph of the control group showing higher magnification of β-cell. The cytoplasm contains Rough Endoplasmic Reticulum (rER), mitochondria (M) with apparent cristae and is crowded with multiple secretory granules (SG) which have crystalloid electron-dense cores with characteristic halos around them
TEM 25000X

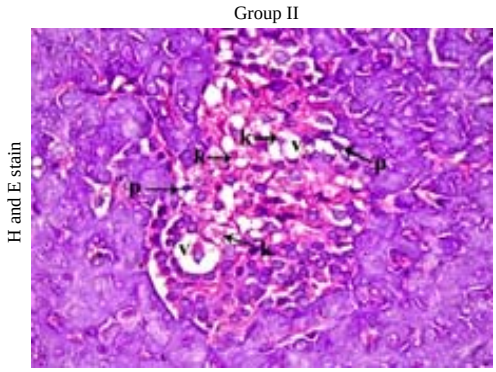

Fig. 8: A high power magnification of the previous figure showing marked distortion of the islet and cytoplasmic vacuolations (v) in the cells of the islet, mostly in the central part of the islet. Some cells show darkly stained pyknotic nuclei (P) and others show karyolytic nuclei (k). There is complete loss of architecture
H and E 1000X

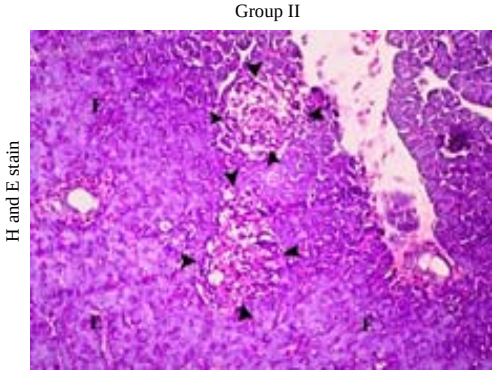

Fig. 7: A photomicrograph of a pancreatic section from STZ group showing two islets surrounded by exocrine pancreatic acini (E). The islets appeared distorted and shrunken with irregular outlines (arrow heads)
H and E 400X

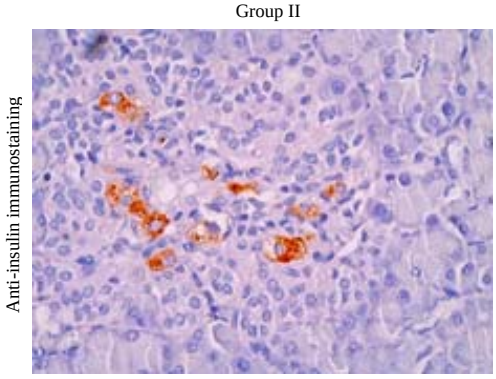

Fig. 9: A photomicrograph of pancreatic section from STZ group showing few insulin positive cells in a pancreatic islet
Anti-insulin immunostaining 1000X

Anti-insulin immunostained sections showed few insulin positive cells compared to control group (Fig. 9). The βp was 26.63±7.4 showing a significant decrease compared to control group (Table 2). The MOD of immunopositive cells was 0.28±0.03 which is significantly less than control group (Table 2).

The TEM examination showed that the islet's area appeared smaller compared to the control group (Fig. 10). The β-cells had dark shrunken nuclei with irregular nuclear envelop, swollen mitochondria and electron-lucent secretory granules. The cytoplasm appeared less crowded with secretory granules compared to control group (Fig. 11). The β-cells showed cytoplasmic vacuolations (Fig. 11). The rough endoplasmic reticulum showed loss of their ribosomes and there was less secretory granules which appeared electron-lucent with loss of their surrounding halos. The mitochondria were swollen with loss of their cristae (Fig. 12).

Group III (STZ+LOS group): The H and E stained pancreatic sections of this group showed evidence of improvement in islets of Langerhans. There was significant decrease in percentage of islets in grade 3 and significant increase in percentage of islets in grade 1, compared to STZ group but

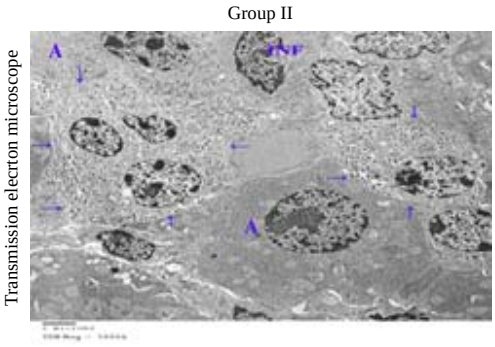

Fig. 10: Electron photomicrograph of a pancreatic section from the STZ group showing an area of islet of Langerhans (arrows) surrounded with acinar cells (A). The islet's area appears smaller compared to the control group. One inflammatory cell (INF) is shown nearby the islet
TEM 5000X

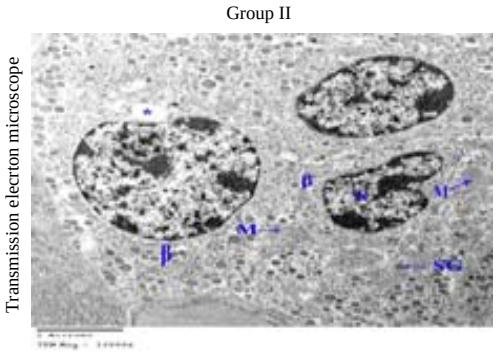

Fig. 11: Electron photomicrograph of higher magnification of the previous figure showing β-cell containing dark shrunken nucleus (N) with irregular nuclear envelop, swollen mitochondria (M) and electron-lucent secretory granules (SG). The cytoplasm appears less crowded with secretory granules (SG) compared to control group. The other β-cell (left one) shows cytoplasmic vacuolations (*)
TEM 12000X

still significantly more than control group (Table 1). More than half of islets showed preserved architecture with regular outline and the islet appeared more crowded with cells compared to the STZ group (Fig. 13). The cells were arranged in cords separated by blood capillaries. Islets showed apparent decrease in cells with cytoplasmic and/or nuclear changes compared to STZ group. There were some dilated capillaries (Fig. 14).

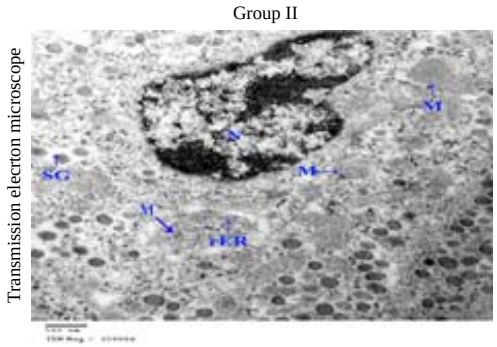

Fig. 12: Electron photomicrograph of higher magnification of the previous figure showing β-cell containing dark shrunken nucleus (N) with irregular nuclear envelop. Its cytoplasm contains Rough Endoplasmic Reticulum (rER) with loss of their ribosomes and swollen mitochondria (M) with loss of their cristae. The cytoplasm appears less crowded with secretory granules compared to control group The secretory granules (SG) appear electron-lucent with loss of their surrounding halos
TEM 25000X

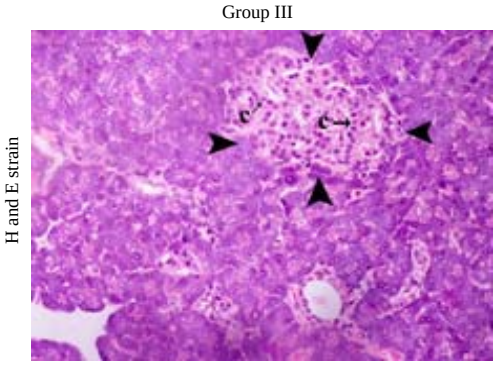

Fig. 13: A photomicrograph of a pancreatic section from STZ+LOS group showing apparently normal architecture of an islet which has regular outline (arrow heads) and surrounded with exocrine pancreatic acini. Its cells are arranged in cords (c). The islet appeared more crowded with cells compared to STZ group
H and E 400X

Anti-insulin immunostained sections showed that the βp was 39.45±16.76 which was significantly more than STZ group but still significantly less than control group (Fig. 15), (Table 2). The MOD of immunopositive cells was 0.47±0.27 showing a significant increase compared to STZ group but was still significantly less than control group (Table 2).

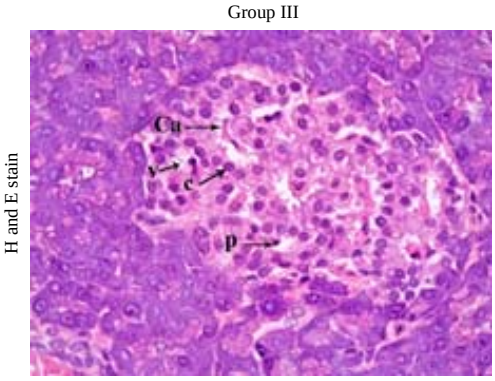

Fig. 14: A high power magnification of the previous figure showing a histological architecture of the islet nearly similar to control group where the cells are arranged in cords (c) separated by blood capillaries (Ca). Some dilated blood capillaries (Ca) are shown and some cytoplasmic vacuolations (v) and pyknotic nuclei (p) are still there
(H and E 1000X)

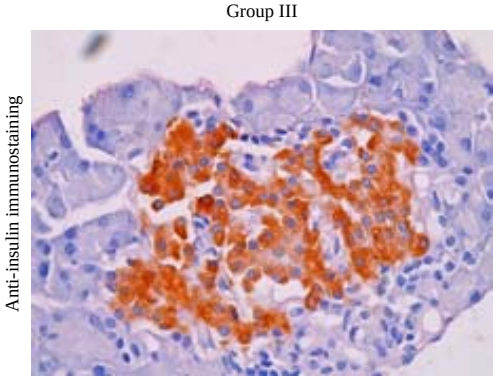

Fig. 15: A photomicrograph of a pancreatic section from STZ+LOS group showing islet of Langerhans stained positive for insulin hormone (brown colour) nearly similar to control group
Anti-insulin immunostaining 1000X

The TEM examination showed that the islet's area appeared larger than that of the STZ group (Fig. 16).

The β-cells contained euchromatic nuclei with regular nuclear envelop. The cytoplasm contained mitochondria, rough endoplasmic reticulum with their ribosomes and showed reappearance of many electron-dense secretory granules nearly similar to control group (Fig. 17).

The mitochondria had apparent cristae and some of them were still swollen. There were numerous electron-dense secretory granules, apparently smaller

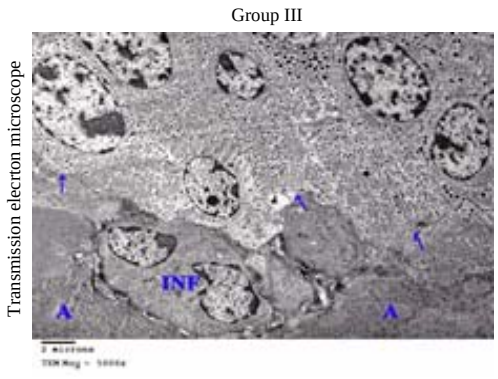

Fig. 16: Electron photomicrograph of a pancreatic section from the STZ+LOS group showing an area of islet of Langerhans (arrows) surrounded by acinar cells (A). The islet's area appears larger than that of the STZ group. One inflammatory cell (INF) is shown nearby the islet
TEM 5000X

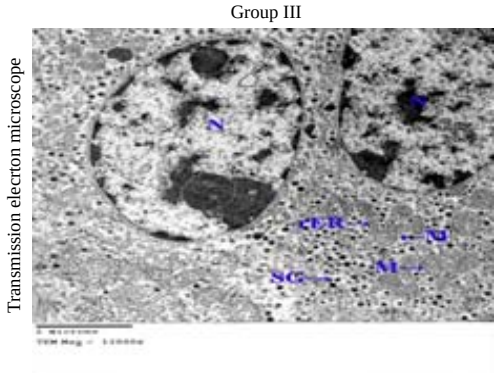

Fig. 17: Electron photomicrograph of a pancreatic section from the STZ+LOS group showing higher magnification of β-cells containing euchromatic nucleus (N) with regular nuclear membrane. The cytoplasm contains mitochondria (M), rough endoplasmic reticulum (rER) and shows reappearance of numerous electon-dense secretory granules (SG) nearly similar to control group
TEM 12000X

compared to control group and their surrounding halos were still not apparent (Fig. 18).

Group IV (LOS group): The H and E stained sections (Fig. 19, 20; Table 1), anti-insulin immunostaining (Fig. 21; Table 2) and transmission electron photomicrographs of the islets of Langerhans of LOS group, were nearly similar to control (Fig. 22, 23).

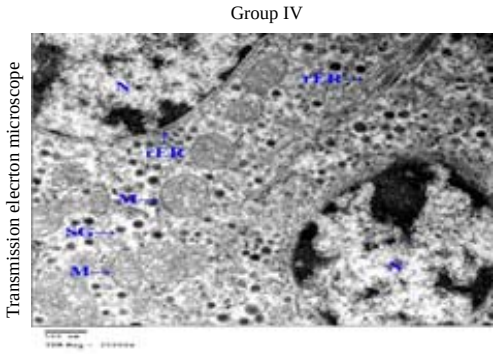

Fig. 18: Electron photomicrograph of higher magnification of the previous figure showing β-cells containing regular nucleus (N) with predominance of euchromatin over heterochromatin. The cytoplasm contains Rough Endoplasmic Reticulum (rER) with their ribosomes, mitochondria (M) with apparent cristae and some of them are still swollen. Reappearance of numerous electron-dense secretory granules (SG) is shown, which are apparently smaller compared to control group and their surrounding halos are still not apparent
TEM 25000X

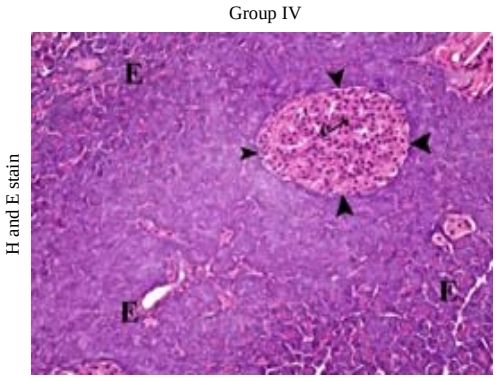

Fig. 19 A photomicrograph of a pancreatic section from LOS group showing a pale stained area (islet of Langerhans) surrounded by deeply stained exocrine pancreatic acini (E). Islets appeared with regular outline (arrow heads) and formed of cords of cells (c)
H and E 400X

DISCUSSION

It was proved that the local pancreatic renin angiotensin system (RAS) could regulate islet function and glycemic control via influences on islet cell mass, inflammation and ion channels[9].

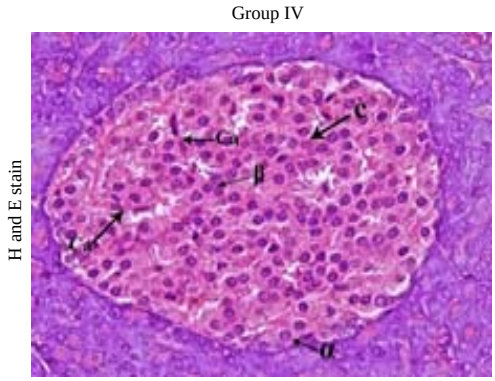

Fig. 20: A high power magnification of an islet of the LOS group showing cells of the islet arranged in cords (c) separated by blood capillaries (Ca). The cells on periphery are mostly α-cells (α) which appear large in size while the cells in center are mostly β-cells (β) which appear smaller
H and E 1000X

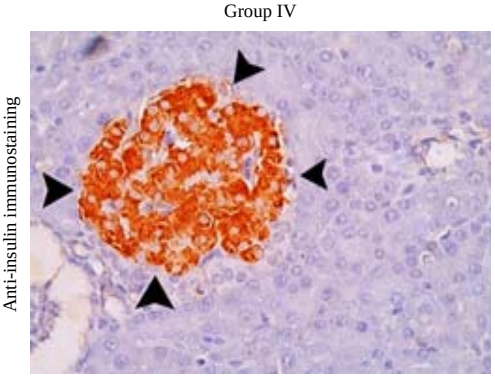

Fig. 21: A photomicrograph of pancreatic section from LOS group showing islet of Langerhans stained positive for insulin hormone (brown colour). The periphery of the islet shows negative immunoreactivity (arrow heads)
Anti-insulin immunostaining 1000X

Therefore, this study was designed to investigate the possible curative effect of angiotensin 2 receptor blocker, LOS, on β-cells of islets of Langerhans in STZ-induced diabetic adult male albino rats.

In this study, H and E stained pancreatic sections of STZ group showed that the islets were shrunken with irregular outline and distorted architecture. Cytoplasmic vacuolations were evident, mostly in the central part of the islet. Some cells were necrotic with either darkly stained pyknotic or karyolytic nuclei. Some islets were markedly shrunken grade 3. These results are consistent with Hmza *et al.*[10] results,

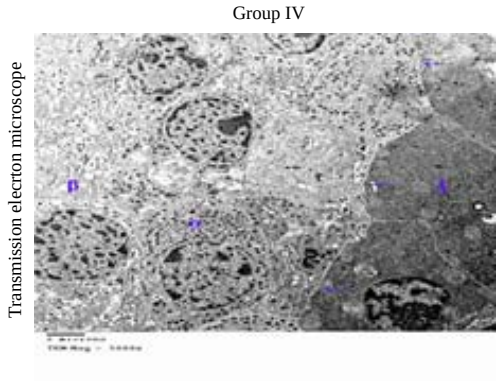

Fig. 22: Electron photomicrograph of a pancreatic section from the LOS group showing an area of islet of Langerhans (arrows) surrounded with acinar cells (A). The periphery of the islet contains α-cells (α) while the center contains β-cells (β)
TEM 5000X

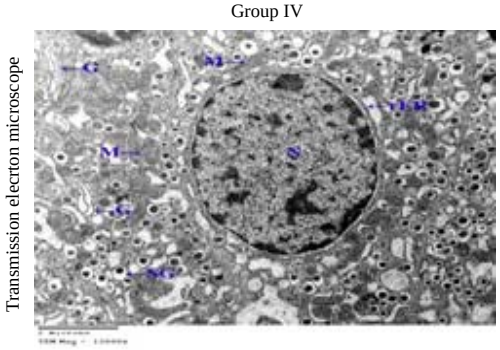

Fig. 23: Electron photomicrograph of a pancreatic section from the control group showing β-cell containing euchromatic nucleus (N) with regular nuclear envelop. Its cytoplasm contains rough endoplasmic reticulum (rER), mitochondria (M), multiple Golgi complexes (G) and numerous secretory granules (SG)
TEM 12000X

who used a similar dose (a single IP injection of STZ in a dose of 65 mg kg^{-1} b.wt.) to induce diabetes in male Sprague Dawley rats aged 12-16 weeks.

It was reported that STZ could cause necrosis of β-cells due to alkylation of DNA and induction of the activation of poly ADP-ribosylation. This poly ADP-ribosylation leads to depletion of cellular NAD^+ and ATP. Enhanced ATP dephosphorylation after STZ treatment supplies a substrate for xanthine oxidase resulting in the formation of free radicals. As a result of STZ action, β-cells undergo destruction by necrosis[11].

The H and E results were confirmed by the TEM examination, which showed that the islet's area appeared smaller compared to control group and β-cells showed dark shrunken nuclei with irregular nuclear envelop and vacuolations of cytoplasm. Similar results were found in a previous study in which diabetes was induced in Sprague Dawley rats with a single IP injection of STZ in a dose of 55 mg kg^{-1} b.wt. and the β-cells of STZ group, in this study, showed electron -translucent areas in the cytoplasm, nuclear pyknosis and indentation of the nuclear membrane[12].

The TEM also showed swollen mitochondria with loss of their cristae, in present study and this was also described by Sellamuthu *et al.*[13], who used a single IP injection of 55 mg kg^{-1} b.wt., of STZ to induce diabetes in Wistar albino male rats. The detected mitochondrial changes could be the result of increased number of electron-dense calcium particles inside the mitochondria and the decrease in the activities of respiratory chain enzymes. This decrease in the activities of respiratory chain enzymes may promote leakage of electrons from the mitochondrial inner-membrane-associated with electron transport complexes and consequently contributes to increase mitochondrial reactive oxygen species which affects mitochondrial membrane permeability[14].

The rough endoplasmic reticulum showed loss of ribosomes which, together, with the dark shrunken nuclei and swollen mitochondria are considered as signs of cell necrosis[15] caused by STZ injury to β-cell.

The cytoplasm appeared less crowded with the secretory granules, compared to control group and that was supported by another study[12]. The secretory granules appeared electron-lucent with loss of their surrounding halos, resembling immature granules. It was reported that secretory vesicles were considered immature if they had a homogeneous light gray appearance similar to the electron density of the cytoplasm or mature if the vesicles contained an electron-dense granule darker than the density of the cytoplasm. Those immature granules reflect impaired insulin processing[16].

Examination of anti-insulin immunostained sections of STZ group showed that there was a significant decrease of βp. This is also found in another study which reported that the islets of male Wistar rats showed scanty number of anti-insulin immunostained cells after induction of diabetes using a single intravenous injection of STZ (40 mg kg^{-1} b.wt.)[17]. The MOD of immunopositive cells in this study, decreased significantly compared to control group. A similar result was found by Adewole and Ojewole[18], who induced diabetes in male Wistar rats using a single intraperitoneal injection of STZ (75 mg kg^{-1} b.wt.) and found weak immunostaining for insulin on day 10 after STZ injection. Their finding means decreased insulin content of β-cells[19].

In addition to the alkylation of β-cell DNA caused by STZ, the deceased insulin in β-cells could be explained by the glucotoxicity, caused by hyperglycemia, which impairs β-cell insulin secretion. This impairment is mediated through angiotensin type 1 receptor activation and an associated enhancement of oxidative stress. Moreover, there is an upregulation of the RAS components in a glucotoxic environment[20].

The H and E stained pancreatic sections of STZ+LOS group showed evidence of improvement in islets of Langerhans. Quantitatively, there was significant decrease in percentage of islets in grade 3 and significant increase in percentage of islets in grade 1. Qualitatively, there was preserved architecture and decreased cytoplasmic and nuclear changes in the islets' cells. These results are confirmed with another angiotensin receptor type 1 blocker (Irbesartan) which showed to improve islet morphology after blockade of the RAS in the Zucker diabetic fatty (ZDF) rat. The Irbesartan was given in a dose of 15 mg kg^{-1} b.wt., daily for 10 weeks and its effect was attributed to reduction of oxidative stress by RAS blocking and the presence of proliferating β-cells[21].

The preserved islet architecture shown in the H and E results were confirmed by the TEM examination where the islet's area appeared larger compared to the STZ group.

The TEM also showed evidence of improvement of β-cell structure compared to STZ group. The nucleus was euchromatic with regular nuclear membrane. The cytoplasm had rough endoplasmic reticulum with preserved ribosomes; and mitochondria with apparent cristae, however, some of them were still swollen. This may be due to blocking of angiotensin receptors type 1 (AT1Rs) by LOS, thus blocking RAS. This hypothesis can be supported by the study of De Cavanagh *et al.*[22], reported that some conditions such as hypertension, diabetes and normal aging show overactivation of RAS, in which A2-dependent oxidant generation becomes a significant contributor to cell oxidation and tissue damage. The A2 stimulates mitochondrial reactive oxygen species (mtROS) production, which in turn depresses mitochondrial energy metabolism. The mtROS contribute to the deleterious effects of A2, which may be mediated by activation of AT1Rs or by direct interaction of A2 with mitochondrial or nuclear components[22].

The improved mitochondrial structure found in this study is supported by a another study which showed that renal mitochondrial dysfunction in spontaneously hypertensive rats was attenuated by LOS and that was attributed to mitochondrial-antioxidant action of LOS that could explain some of the beneficial effects of AT1R antagonists[23].

The cytoplasm of β-cells showed reappearance of many electron-dense secretory granules nearly similar to control group but they are apparently smaller and their surrounding halos were still not apparent. This may be caused by the still present defect in insulin biosynthesis[16]. This defect in insulin biosynthesis could be the result of hyperglycemia. This hypothesis can be supported by Kaneto and Matsuoka[24], who suggested that chronic hyperglycemia suppresses insulin biosynthesis and secretion by increasing oxidative stress, which could be prevented by antioxidant treatment. The LOS was considered as an antioxidant[25]. Therefore, the antioxidant effect of LOS might have protected the nucleus and organelles from damage by STZ or glucotoxicity which might have led to increased insulin synthesis that caused reappearance of multiple electron-dense secretory granules. Besides, LOS could have increased pro-insulin biosynthesis[26]. Moreover, LOS could have improved conversion of pro-insulin to insulin as it was found that LOS selectively inhibited oxidative stress in islets of db/db mice via downregulation of NADPH oxidase which, in turn, suppressed UCP2 expression[27]. The UCP2, an inner mitochondrial membrane protein expressed in many tissues including pancreas, when increases, it impairs ATP generation leading to decreased conversion of pro-insulin to insulin in β-cell granules[28].

In this study, anti-insulin immunostained sections showed that the MOD of anti-insulin immunopositive cells was significantly increased compared to STZ group which means increased insulin content of β-cells compared to STZ group. This is confirmed by the multiple electron-dense secretory granules shown in TEM results. This finding is supported by the strong anti-insulin immune reaction in STZ diabetic rats after treatment with LOS in a similar study[5].

The STZ+LOS group also showed increased βp per total islet cells and this was documented in other studies who found increase in the anti-insulin immunostained area (β-cell area) of islets after treatment with LOS, compared to that of STZ diabetic group[5].

Also a study in which LOS was given for 8 weeks, found to improve β-cell insulin secretion and decrease apoptosis-induced β-cell mass loss in islets of mice. This was explained by selective inhibition of oxidative stress via downregulation of NADPH oxidase, this in turn suppressed UCP2 expression which is a negative regulator of islet function[27].

Another explanation for the increased βp per total islet cells after treatment with LOS is that RAS blockade could stimulate pancreatic β-cell proliferation[29] and thus the increase in βp after LOS blockade of RAS could be the result of increased proliferation rates of the residual β-cells that escaped the STZ toxic effect, β-cell differentiation from exocrine progenitors (neogenesis), a reduction in β-cell death rates or the combined action of the all three mechanisms[5].

CONCLUSION

It is concluded that LOS partially improved the structure of the β-cells of islets of Langerhans and the islets in general.

SIGNIFICANT STATEMENT

This study reveals that LOS has curative effect on the β-cells of islets of Langerhans. Adding to its previously reported effect of lowering blood glucose levels and lipid peroxidation products, its use in diabetics after further research studies can be recommended.

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