Original Article

Effect of Angiotensin II Receptor-Blocker (ARB) Losartan on the Myocardium of Streptozotocin Induced Diabetic Rats.: Histological and Histochemical Study

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ABSTRACT

Introduction: Diabetes Mellitus (DM) is a common endocrine and metabolic condition. It is one of the most rapidly growing diseases worldwide, and is a prime cause of excess cardiovascular morbidity and mortality in populations. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs especially the eyes, kidneys, nerves, heart, and blood vessels. Losartan is an angiotensin II type one (AT1) receptor antagonist authorized for arterial hypertension (HTN) treatment. Losartan is also utilized to avoid renal damage in diabetics and those with decreased ejection fraction (EF) due to heart failure

Objective: This research objects to determine the potential protective impact of Losartan administration on the induced diabetic myocardium and aorta of adult male Sprague-Dawley rats.

Materials and Methods: Sixty adult male Sprague-Dawley rats were utilized in the present research. They were allocated into four groups: group I (control group), group II (Losartan received group) they were received losartan 30 mg/kg/day, group III (diabetic group) induction of DM by a single intraperitoneal injection of 65 mg/kg STZ solution freshly mixed in 0.1 M citrate buffer and group IV (diabetic with Losartan group). After 8 weeks, the animals from all groups were anaesthetized and blood samples were obtained for biochemical study. The heart and aorta were dissected, and histological, immunohistochemical and electron microscopic studies were conducted on the tissues.

Results: Diabetic myocardium revealed histological alterations as fragmented muscle fibers, small darkly stained pyknotic nuclei, cellular infiltration and a significant elevation in collagen fibers percentage area with marked biochemical changes as increased plasma level of MDA. Administration of Losartan to diabetic rats made ameliorating effect on the disturbed structure, decreased the elevated tissue malondialdehyde (MDA) levels and increased the reduced activities of the enzymatic antioxidants superoxide dismutase (SOD), and catalase (CAT) in cardiac tissue. Diabetic group exhibited a significant increase (P<0.001) in collagen fibers mean percentage area, number of Caspase-3 positive cells, and a significant decrease (P<0.001) in the intensity of eNOS immunoreaction in the myocardium tissue of rats than the control group. While diabetic group treated with losartan exhibited a significant decrease (P<0.001) in collagen fibers mean percentage area, the number of Caspase-3 positive cells, and a significant increase (P<0.001) in the intensity of eNOS immunoreaction than diabetic group.

Conclusion: Losartan has a protective effect on the biochemical, histological, and morphometric changes of the myocardium and the aorta of diabetic rats.

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Key Words: Aorta, diabetes, losartan, myocardium.

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INTRODUCTION

Diabetes Mellitus (DM) is among the top endocrinal diseases affect human. It is associated with hyperglycemia and abnormal metabolism of the carbohydrate, lipids, and protein. It resulted from deficiency of insulin or insulin dysfunction or both[1] which contributes to generation of free radicals[2].

DM is a complex and multifarious group of disorders. Today, it is regarded one of the major significant health issues in the globe. There are about 400 million people worldwide were diagnosed with the disease[3].

There are two forms of DM: type 1 (insulin dependent) and type 2 (non-insulin dependent). All forms of DM are described as vascular damage of small blood vessels. Retinopathy, nephropathy, neuropathy, and cardiomyopathy are just a few of the complications that can arise from long-term hyperglycemia, which causes macro- and microvascular injury[4]. Microvascular problems are linked to the emergence of cardiovascular difficulties, such as ischemic heart disease, peripheral vascular disease, and stroke[5]. The development of cardiac fibrosis in diabetic cardiomyopathy would result in cardiac cycle dysfunctions that could lead to heart failure[6]. Diabetes-related cardiomyopathy is also accompanied by additional metabolic conditions, such as decreased nitric oxide levels, inflammation, increased oxidative stress, and increased renin-aldosterone angiotensin system activity[7].

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Advanced glycation end-products (AGEs), O-linked-N acetylglucosamine, changes in microRNA, exosome (O-GlcNAc) pathways, adenosine monophosphate (AMP)-activated protein kinase (AMPK), protein kinase C (PKC), and nuclear factor kappa B (NF-B) formation are among the pathophysiological pathways associated with the progression of diabetic[8]. Toll-like receptor 4 (TL4)/NF-B signaling is triggered by the creation of damage-associated molecular pattern molecules (DAMPs) in diabetes, which increases the production of pro-inflammatory cytokines such interleukin-6 (IL-6) and tumor necrosis factor (TNF-)[9,10]. These inflammatory cytokines cause the diabetic heart's cardiac dysfunction and damage to worsen.

Streptozotocin (STZ), a Streptomyces achromogenes-derived glucosamine–nitrosourea molecule, is utilized to treat pancreatic cell cancer. STZ causes hypoinsulinemia and hyperglycemia by destroying pancreatic cells[11].

Losartan is a treat of a number of medical disorders, including hypertension (HTN)[12]. It belongs to angiotensin II type one (AT1) receptor antagonist[13]. Losartan possesses unique pleiotropic uricosuric and lipid-lowering properties, hence minimizing the risk of cardiovascular problems in subjects with HTN in the future[14].

Although several investigations were conducted to examine AT1 receptor antagonist effects on DM generated by STZ in many animal organs, few research have been conducted to verify the mechanism by which cardiac and aortic manifestations occur in diabetic models. Therefore, this research aimed to assess Losartan administration effect on the myocardium and aorta of rats suffering from DM utilizing histological, histochemical and immunohistochemical studies.

MATERIALS AND METHODS

Chemicals

- Streptozotocin (STZ): A single intraperitoneal injection of STZ solution will cause DM.
- Losartan: Trade name Cozaar, manufactured by Amriya Pharmaceutical Industries Alexandria-Egypt, in form of tablets.

Animals

Sixty adult male Sprague-Dawley rats, each one weighing180-200gm, were utilized. They were kept in stainless steel cages at room temperature. Unrestricted access was granted to laboratory rat food and water. Rigorous cleanliness and attention were done to provide healthy environment for the rats. The general conditions and behavior of the animals were noticed. Animal treatment was according to ethical protocol that was approved by Faculty of Medicine, Menoufia University Ethical Committee with No: 3/2021 HIST 24

Experimental procedure

Rats were allocated at random among four groups:

Group I (negative control group): includes 10 animals. Animals of this group were provided with distilled water orally for 8 weeks.

Group II (positive control group): includes 10 animals. Animals got losartan in form of tablets (each 50 mg losartan potassium), that will be crushed and dissolved in distilled water, 30 mg/kg/day by the oral route through gastric tube. Each rat will receive 0.5ml distilled water containing 6 mg losartan for 8 weeks[15].

Group III (diabetic group): includes 20 animals. A single intraperitoneal injection of 65 mg/kg STZ solution freshly mixed in 0.1 M citrate buffer produced DM (pH 4.5)[16].

Group IV (diabetic with losartan group): includes 20 animals, each was received STZ by the same dose, and route of administration as in group III with losartan 30 mg/kg/day for eight weeks.

Induction of DM

A single intraperitoneal injection of 65 mg/kg STZ solution freshly mixed in 0.1 M citrate buffer (pH 4.5) was administered to rats utilizing a slightly modified version of the standard technique for type I DM development. To avoid early-phase drug-induced hypoglycemia, all animals were given a 5% glucose solution orally. Fasting blood glucose levels were measured daily for 3 days afterwards STZ administration; More than 13.8 mmol/L (250 mg/dL) of blood glucose was termed diabetic[17].

After 8 weeks, ether inhalation was used to anaesthetize the animals of all groups. Blood samples were collected from the tail and drawn into tubes coated with heparin and centrifuged at 2200 g for 10 min. For biochemical investigation, plasma samples were preserved at -20 c. small parts of the myocardium of left ventricle and aorta were excised and cleaned by normal saline. The specimens were subjected to histological, histochemical, immunohistochemical and transmission electron microscopic studies.

I-Biochemical study

All biochemical studies were performed in the central laboratory, Faculty of Medicine, Menoufia University.

Fasting Blood glucose level

Utilizing a commercial Glucose Colorimetric Assay Kit, plasma glucose levels were estimated.

Oxidative stress markers

Tissues from cardiac muscle were excised, immersed in liquid nitrogen to freeze for 1 h at −70 °C. In ice cold phosphate buffer, frozen tissue was homogenized and centrifuged. The supernatant was used as the antioxidant markers to test tissue superoxide dismutase (SOD) and catalase (CAT)[18].

They were identified utilizing commercial kits. Malondialdehyde concentration (MDA) has been calculated as an indicator of peroxidation of lipids and oxidative stress[19].

II-Histological study

Tissue samples of myocardium and aorta were kept in formal saline 10%, washed and converted into paraffin slices. Slices of about 5-6 µm thickness were obtained and stained with hematoxylin and eosin (H&E) to show the histological features. In addition to Masson's trichrome stain to identify collagen fibers in the myocardium[20].

Histochemical study

The specimens were treated and stained with Van Gieson for detection of elastic fibers in aortic tissue[21].

III- Transmission electron microscopic study

For transmission electron microscope (TEM) examination, the tissues of myocardium were chopped into minute pieces, fixed in buffered glutaraldehyde solution and managed for investigation, TEM processing and examination were carried out at the Electron Microscopy Unit, Faculty of medicine, Tanta university.

IV-Immunohistochemical study

1. Caspase-3 protein was detected utilizing a streptavidin-biotin complex immunoperoxidase technique, with lymphoid tissue serving as a positive control[22].
2. Immunohistochemical staining for eNOS antigen using the avidin–biotin peroxidase complex technique, heart sections were used as a standard positive control[23].
3. Detection of TNF-α (index for inflammation). The primary monoclonal antibody utilized was the mouse anti-TNF-α (1:300 with PBS). As a positive control for anti-TNF-, lymph nodes were utilized[24].

The location of the reaction inside the cell was cytoplasmic brown in color.

V-Morphometric study and Statistical analysis

Mean body weight (g) was determined for several experimental groups, Masson-stained slices were used to assess the mean surface area of collagen deposition while the intensity of eNOS immunostaining and Caspase-3 positive cells' mean number were measured in immune-stained sections by x 400 of light microscopy. From each rat, 10 non-overlapping microscopic fields were used. This was performed utilizing the image analyzer's interactive measurement. Image analysis was done in Anatomy and Embryology Department, Menoufia University.

Utilizing the student's t-test, the biochemical and morphometric findings were evaluated and compared. The P-value was utilized to determine if each variable in the experimental animals differed significantly from the control group. The acquired data were tabulated as mean and standard deviation (SD) and evaluated utilizing statistical software for the social sciences (SPSS) (version 17.0 on an IBM compatible computer; SPSS Inc., Chicago, Illinois, USA). *P value* ≤ 0.05 was deemed significant[25].

RESULTS

General Results

Control group (group I) and losartan (group II) treated groups: Animals of these groups were noticed in a good general condition and showed normal behavior, activity and appetite. However, animals of the diabetic group (group III) showed polyphagia, polydipsia, polyuria and decreased activity when compared with the control group. On the other hand, animals of the diabetic group treated with losartan (group IV) showed improvement of their activities and appetite.

Biochemical results

Fasting blood glucose level

Statistical analysis for blood glucose level, showed a non-significant decrease in the mean value between group 1 and II and a significant increase ($p<0.001$) in group III than group I. However, there was a significant decrease ($p<0.001$) in the blood glucose level on administration of losartan in group IV than group III (Table 1, Histogram 1).

Lipid peroxidation and antioxidant enzymes

Data in (Table 2) exhibited a non-significant decrease in the mean value of plasma level of MDA (the marker of lipid peroxidation) between group 1 and II. Group III exhibited a significant increase ($p<0.001$) in the plasma level of MDA than group I. Significant decrease($p<0.001$) in the activities of SOD and CAT was noticed in the rats of this group. On the other hand, group IV demonstrated a decrease in MDA and a dramatic increase in the antioxidant status (SOD, CAT) (Histogram 2).

Histological results

In H& E sections, Longitudinal sections of the myocardium of the left ventricle of group I showed normal histological architecture of the cardiac muscle. The cardiac muscle fibers were surrounded by delicate connective tissue (endomysium) and had anastomosing and branching striated muscle fibers with acidophilic cytoplasm and centrally placed oval vesicular basophilic nuclei (Figures 1,2). Group II showed no structural changes and the microscopic pictures were similar to control group (Figures 3,4).

Group III exhibited disturbed muscle structure with sever interstitial hemorrhage, widened endomysium, and cellular infiltration also seen (Figure 5). Nuclei of cardiomyocyte appeared with small darkly stained nuclei. There were areas of disintegrated fibers, with vacuolation of their cytoplasm and loss of normal architecture (Figure 6)

On the other side, examination of group IV revealed marked reduction of the pathological changes of the myocardium, which became more or less like control group as exhibited regular anastomosing and branching of heart muscle fibers with oval nuclei and acidophilic cytoplasm (Figures 7,8).

Regarding transverse sections of the aorta wall of control group demonstrated normal histological architecture. The aorta wall consisted of tunica intima made up of thin flattened endothelial cells with flattened nuclei, tunica medium with regular parallel elastic lamellae, tiny gaps between and regularly distributed spindle smooth muscle cells, and tunica adventitia (Figures 9a,10a). Regarding group II was like control group (Figures 9b,10b). However, group III showed focal desquamation of the endothelial cells of tunica intima, destruction of the elastic lamellae of tunica media, fat deposition with appearance of foam cells and degenerated adventitia (Figures 9c,10c). While examination of group IV showed obvious improvement of the histological picture (Figures 9d,10d).

Regarding to the Masson's Trichrome stained myocardium sections of groups I&II, showed presence of negligible number of collagen fibers that stained blue in the connective tissue endomysium (Figures 11 a,b), group III exhibited a significant increase in collagen fibers (blue color) around blood vessels and in the endomysium (Figure 11c). Group IV showed moderate amount of collagen fibers (Figure 11d).

Histochemical study

Van Gieson-stained aortic sections of groups I&II, showed regularly arranged parallel elastic lamellae that stained orange -red in the tunica media (Figures 12 a,b), in group III, the elastic fibers became thinner, fragmented and disorganized with widening of interlamellar spaces (Figure 12c). Group IV showed nearly normal distribution of elastic fibers (Figure 12d).

Transmission electron microscopic results

Cardiomyocytes in the left ventricle of the group I exhibited a euchromatic nucleus with a noticeable nucleolus and were packed with well-organized myofibrils, with mitochondria rows and sarcoplasmic reticulum positioned among the myofibrils (Figure 13). Intercalated disc and glycogen particles also were seen (Figure 14). Group II showed no pathological changes as control group (Figure 15). Cardiomyocytes in group III had irregularly indented nuclei with peripherally condensed margined chromatin and the presence of vacuoles (Figure 16), In addition disorganized intercalated disc, abundant glycogen granules in- between the degenerated myofibrils were seen (Figure 17). Furthermore, there were severely destructed and degenerated myofibrils, degenerated mitochondria and dilatation of T- tubule with areas of interrupted Z-lines (Figure 18).

Group IV exhibited bundles of myofibrils with alternating dark A and light I bands, Z lines emerged bisecting I bands, regularly ordered myofibrils, with mitochondria rows among and undamaged intercalated discs, similar to the control group (Figures 19,20).

Immunohistochemical study

Caspase-3 immunohistochemistry: sections of the myocardium of group I and group II revealed week positive cytoplasmic immunoreaction to caspase-3 (Figures 21 a,b). Group III showed strong positive cytoplasmic immunoreaction to caspase-3 (Figure 21c). Group IV showed moderate positive cytoplasmic immunoreaction to caspase-3 (Figure 21d). Regarding to aortic sections of group I and group II revealed weak positive cytoplasmic immunoreactivity for caspase-3 (Figures 22 a,b). Group III exhibited strong positive cytoplasmic immunoreactivity for caspase-3 (Figure 22c). Group IV exhibited moderate positive cytoplasmic immunoreactivity for caspase-3 (Figure 22d).

Endothelial nitric oxide synthase (eNOS): immunoreaction of eNOS was strong positive in myocardium from control group and Losartan treated group (Figures 23 a,b). However, myocardium sections from diabetic group revealed weak positive cytoplasmic immmunostaining for eNOS (Figure 23c). While group IV showed strong positive cytoplasmic immune reaction (Figure 23d). Regarding to aortic sections of control group and Losartan treated group showed strong positive endothelial immmunostaining for eNOS (Figures 24 a,b). However, aortic sections from diabetic group revealed weak positive endothelial immmunostaining for eNOS (Figure 24c). Group IV revealed strong positive endothelial immmunostaining for eNOS (Figure 24d).

Tumor necrosis factor-α(TNF-α): Immunoreaction of TNF-α showed weakly expressed immunoreactions in myocardium from control group and Losartan treated group (Figures 25 a,b). However, myocardial sections from diabetic group revealed a strong positive immunoreaction for TNF-α (Figure 25c). While moderate positive immune reaction for TNF-α in the myocardium sections of group IV (Figure 25d). Regarding to aortic sections of control group and Losartan treated group showed weak positive immunoreactions for TNF-α (Figures 26 a,b). However, aortic sections from diabetic group revealed strong positive cytoplasmic immunoreactions for TNF-α (Figure 26c). While mild positive cytoplasmic immunoreactions for TNF-α in the aortic sections of group IV was found (Figure 26d).

Morphometric and Statistical analysis results

Data in (Table 3) showed that group III exhibited a significant increase (P<0.001) in collagen fibers mean percentage area than group I. Group IV exhibited a significant decrease (P<0.001) than group III (Histogram 3).

Data in (Table 4) demonstrated that group III exhibited a significant decrease (P<0.001) in the intensity of eNOS immunereaction in the myocardium tissue of rats than the control group. While, group IV exhibited a significant increase (P<0.001) in the intensity of eNOS immunereaction than group III (Histogram 4).

In addition, (Table 4) also showed that group III exhibited a significant increase (P<0.001) in the number of Caspase-3 positive cells in the myocardium tissue of rats than the control group. Group IV showed a significant decrease(P<0.001) in the number of Caspase-3 positive cells than group III (Histogram 5).

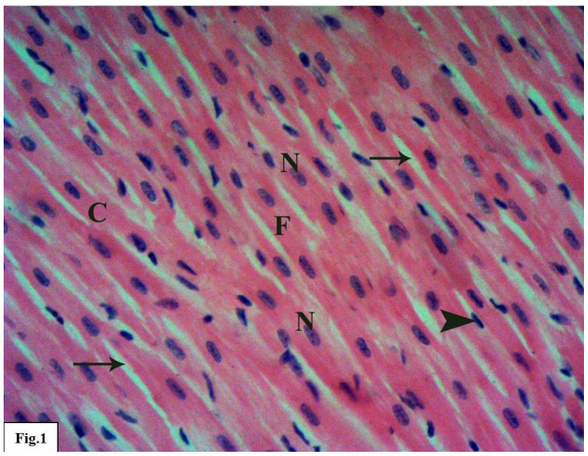


Fig. 1: A photomicrograph of a longitudinal section of the myocardium of the left ventricle of a control rat showing normal structure of the myocardium formed of branching and anastomosing cardiac muscle fibers(F) with centrally located oval nuclei (N) and acidophilic cytoplasm (C).Delicate connective tissue endomysium (arrows)with dark oval nuclei of fibroblast (arrow heads) , surrounds the cardiac muscle fibers. (Hx & E × 200)

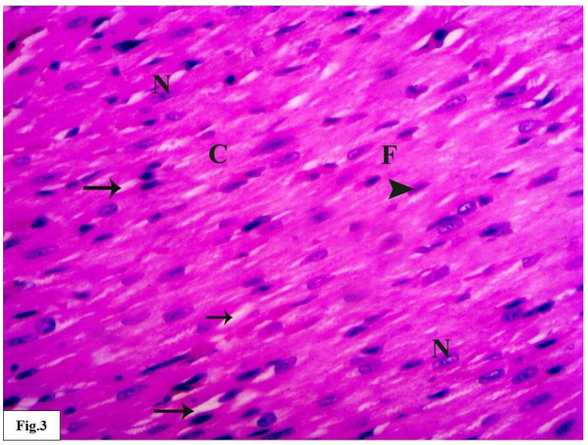


Fig. 3: A photomicrograph of longitudinal section of the myocardium of the left ventricle of group II (losartan treated group)showing that the cardiac muscle fibers appeared normal within different directions formed of branching and anastomosing cardiac muscle fibers (F) with centrally located oval nuclei (N) and acidophilic cytoplasm (C).Delicate connective tissue endomysium (arrows)with dark oval nuclei of fibroblast (arrow heads) , surrounds the cardiac muscle fibers. (Hx & E × 200)

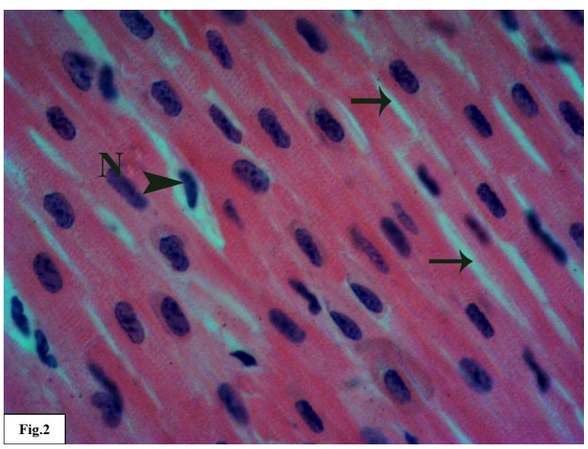


Fig. 2: A photomicrograph of a longitudinal section of the myocardium of the left ventricle of a control rat showing cardiomyocytes with centrally located oval vesicular nuclei (N). and separated by the connective tissue endomysium (arrows) Note:fibroblast with dark oval nuclei (arrowheads). (Hx & E × 400)

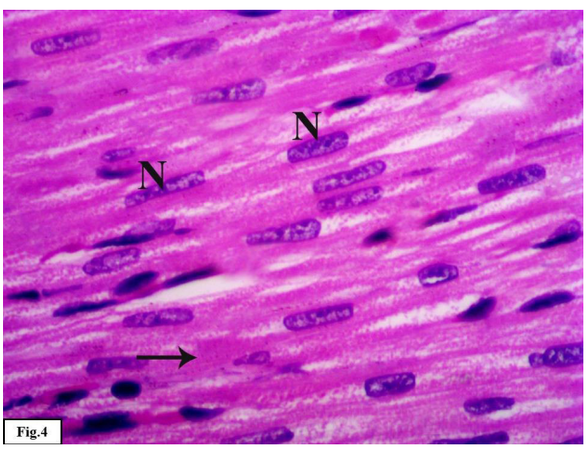


Fig. 4: A photomicrograph of a longitudinal section of the myocardium of the left ventricle of group II (losartan treated group) showing normal appearance of the cardiac muscle fibers with centrally located oval vesicular nuclei (N). Note: The normal appearance of transverse striation of the cardiac muscle fibers (arrow). (Hx & E × 400)

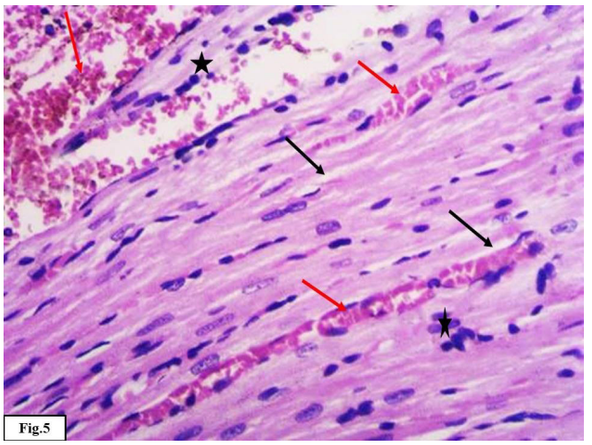


Fig. 5: A photomicrograph of a longitudinal section of the myocardium of the left ventricle from group III (diabetic group) showing disturbed muscle structure with sever interstitial hemorrhage (red arrows) and widened endomysium (black arrows) Note: cellular infiltration also seen (star) . (Hx & E × 200)

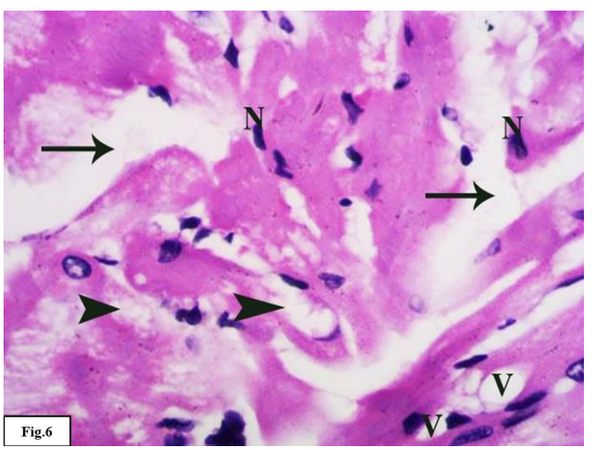


Fig. 6: A photomicrograph of a longitudinal section of the myocardium of the left ventricle from group III (diabetic group) showing widening of the endomysium (arrow) and areas of disintegrated fibers (arrow head). Nuclei of cardiomyocyte (N) appear with small darkly stained pyknotic nuclei. Note: vacuolation of the cytoplasm(V). (Hx & E × 400)

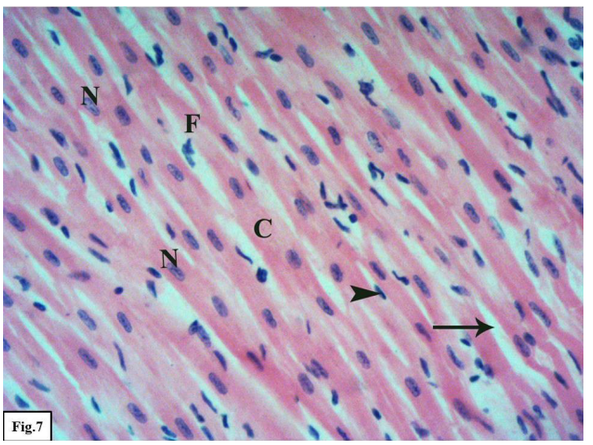


Fig. 7: A photomicrograph of a longitudinal section of the myocardium of the left ventricle from a diabetic rat treated with losartan (group IV) showing branching and anastomosing cardiac muscle fibers(F) with centrally located oval nuclei (N) and acidophilic cytoplasm (C).Delicate connective tissue endomysium (arrows)with dark oval nuclei of fibroblast (arrow heads) , surrounds the cardiac muscle fibers. (Hx & E × 200)

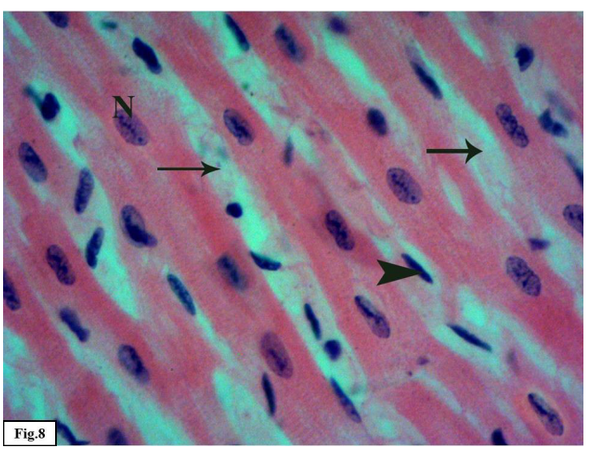


Fig. 8: A photomicrograph of a longitudinal section of the myocardium of the left ventricle from a diabetic rat treated with losartan (group IV) showing fibroblast with oval dark nuclei (arrowheads) ,and widening of the endomysium (arrows).Note: oval vesicular nuclei of cardiomyocyte (N). (Hx & E × 400)

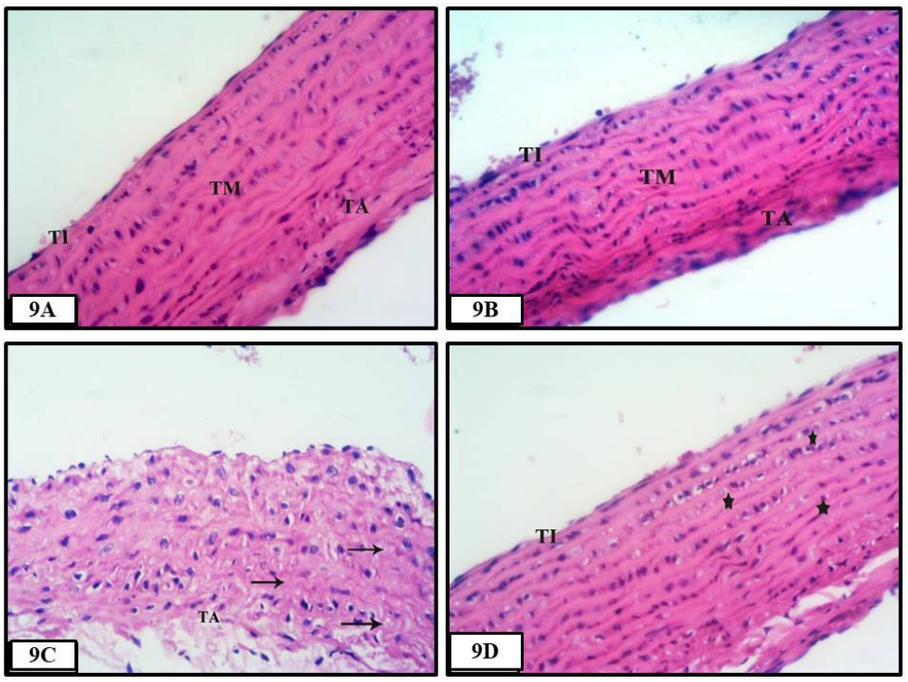


Fig. 9: Photomicrographs of a transverse staining section of Hematoxylin and Eosin (Hx &E) of the wall of the rat aorta showing: (a) a normal alignment of the three tunics; Tunica intima (TI), Tunica media (TM), and Tunica adventitia (TA) in a control group. (b) normal shaped, as in control, of the three tunics; Tunica intima (TI), Tunica media (TM) and Tunica adventitia (TA) in group II (losartan treated group) (c) thin fragmented and disorganized distribution of elastic fibers of tunica media (arrows) in group III (diabetic group). Note: The degeneration in the tunica adventitia (TA). (d) almost normal structure and distribution of elastic lamellae (stars) in a diabetic rat treated with losartan (group IV). Note: the tunica intima appears almost normal like control (TI). (Hx & E × 200)

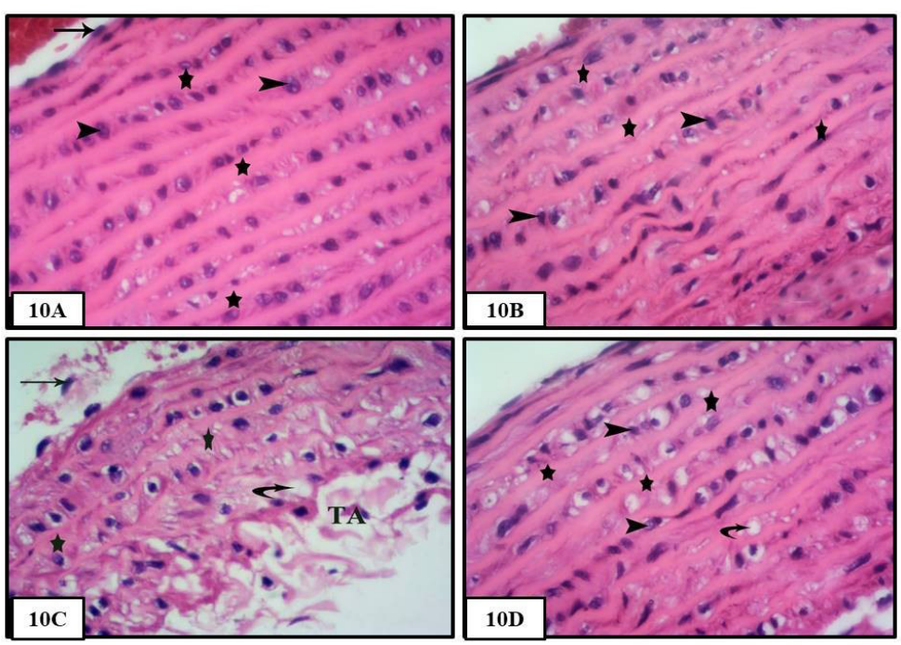


Fig. 10: Photomicrographs of a transverse staining section of Hematoxylin and Eosin (Hx &E) of the wall of the rat aorta showing: (a) tunica intima formed of endothelial cells(arrow), tunica media shows regularly arranged spindle smooth muscle cells (arrowheads) in between normal pattern of distribution of elastic fibers that appears as regularly arranged parallel lamellae (stars) in a control group.(b) normal shaped, as in control, of pattern of distribution of elastic fibers that appears as regularly arranged parallel lamellae in the tunica media (stars) in group II (losartan treated group).Note: Regularly arranged spindle smooth muscle cells (arrowheads) in between the elastic lamellae.(c) focal desquamation of the endothelial cells(arrows) of tunica intima. Tunica media showing destruction of the elastic lamellae (stars),fat deposition with appearance of foam cells (curvedarrow). Tunica adventitia also containing degeneration (TA) in group III (diabetic group).(d) tunica media, having regularly arranged spindle smooth muscle cells (arrowheads) in between normal pattern of distribution of elastic fibers that appears as regularly arranged parallel lamellae (stars) in a diabetic rat treated with losartan (group IV). Note: some foam cells are still present (curvedarrow).(Hx & E × 400)

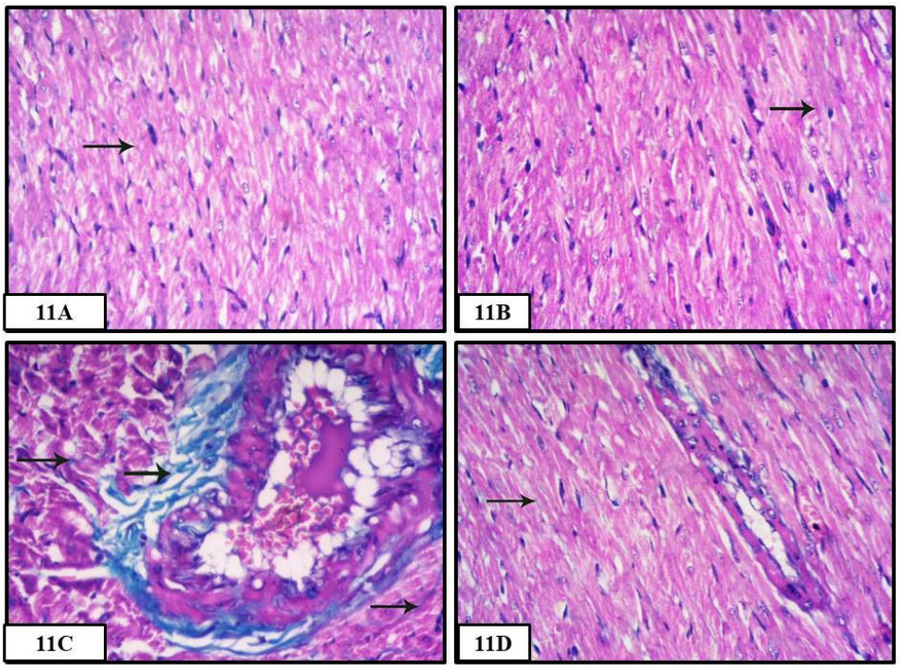


Fig. 11: Masson trichrome staining of rat myocardium showing : (a)A photomicrograph of a longitudinal section of the myocardium of the left ventricle of a control rat (group I) showing normal distribution of collagen fibers (small amount) in the endomysium (arrows). (b) A photomicrograph of a longitudinal section of the myocardium of the left ventricle from losartan treated rat (group II) showing small amount of collagen fibers in the endomysium (arrows). (c) A photomicrograph of a longitudinal section of the myocardium of the left ventricle from a diabetic rat (group III) showing marked amount of the collagen fibers around the blood vessel and in the endomysium (arrows). (d) A photomicrograph of a longitudinal section of the myocardium of the left ventricle from a diabetic rat treated with losartan group (group IV)showing moderate amount of collagen fibers in the endomysium (arrows). (Masson trichrome x 200)

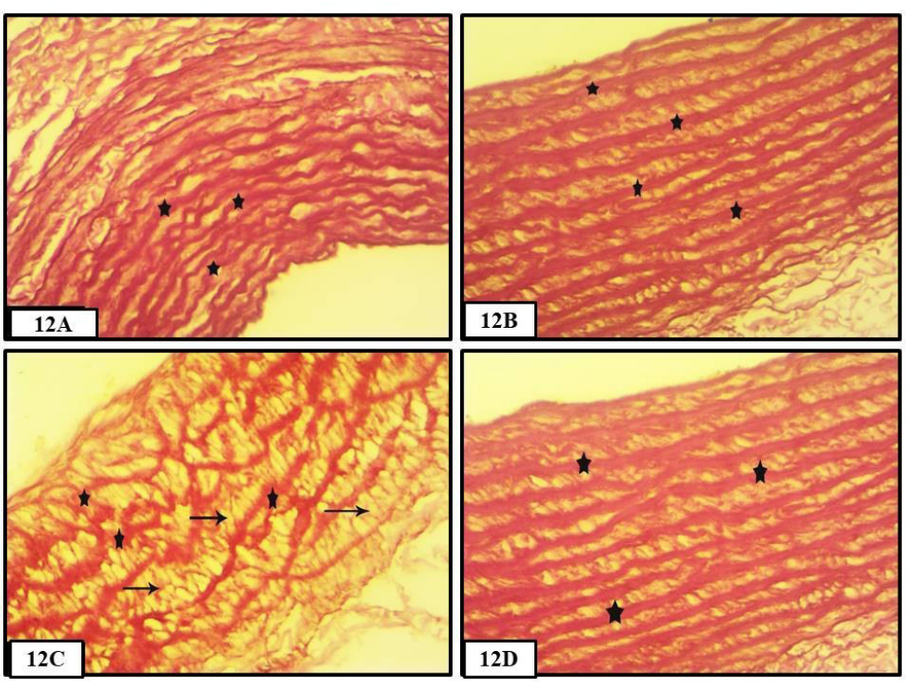


Fig. 12: Van Gieson staining of rat aorta showing :(a) A photomicrograph of a transverse sections of the wall of the aorta of a control rat showing normal pattern of distribution of elastic fibers that appears as regularly arranged parallel lamellae in the tunica media (stars). (b)A photomicrograph of a transverse sections of the wall of the aorta of group II (losartan treated group) showing normal pattern of distribution of elastic fibers as in control group that appears as regularly arranged parallel lamellae in the tunica media (stars). (c) A photomicrograph of a transverse section of the wall of the aorta from group III (diabetic group) showing thin fragmented and disorganized distribution of elastic fibers (stars). Note:Widening of interlamellar spaces (arrows). (d) A photomicrograph of a transverse section of the wall of the aorta from a diabetic rat treated with losartan (group IV)showing almost normal structure and distribution of elastic lamellae in protected rat (stars).(Van Gieson × 400).

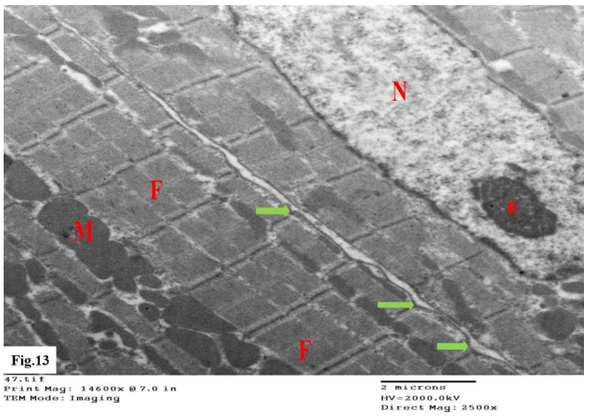


Fig. 13: An electron micrograph of cardiomyocyte of control group (group I) having an euchromatic nucleus (N) with prominent nucleolus (n) and filled with well organized myofibrils(F). Rows of mitochondria (M) and sarcoplasmic reticulum (green arrow) are situated between the myofibrils. (TEM× 2500)

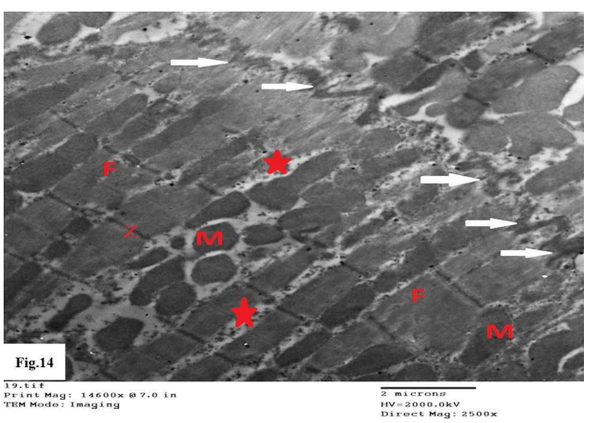


Fig. 14: An electron micrograph of the myocardium of control rat showing cardiomyocyte filled with myofibrils (F) with prominent Z lines (Z). Rows of mitochondria (M) in-between myofibrils are present. Apparently normal intercalated disc is also seen (white arrows). Notice, glycogen particles (star) are seen in-between myofibrils. (TEM× 2500)

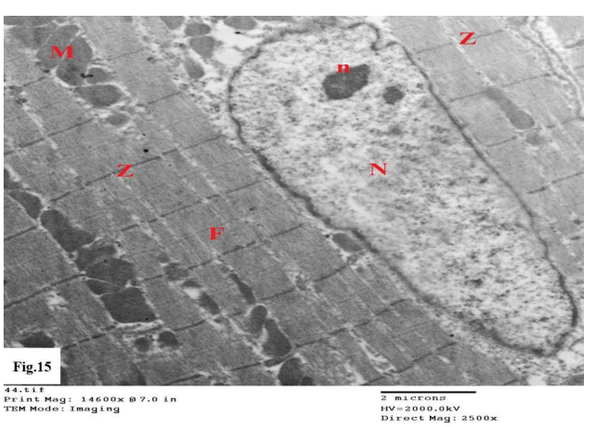


Fig. 15: An electron micrograph of cardiomyocyte of losartan treated group (group II) showing an oval euchromatic nucleus (N) with prominent nucleolus (n), regularly arranged myofibrils (F) and rows of mitochondria (M) in-between. Z lines (Z) are easily seen like control group. (TEM × 2500)

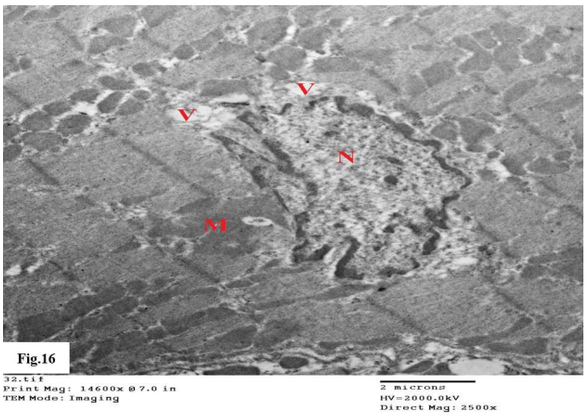


Fig. 16: An electron micrograph of cardiomyocyte of diabetic group (group III) showing irregular indented nucleus (N) with peripherally condensed marginated chromatin with presence of vacuoles(V).(TEM × 2500)

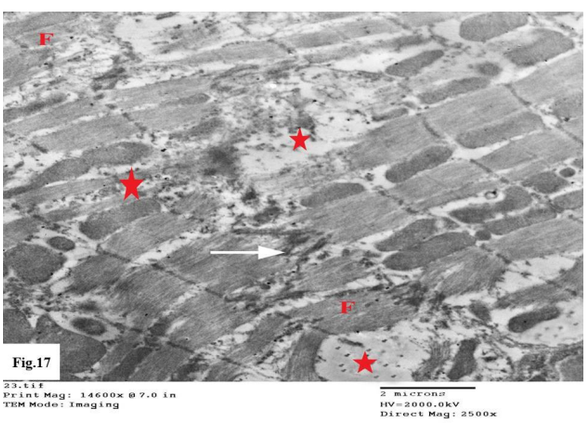


Fig. 17: An electron micrograph of cardiomyocyte of diabetic group (group III) showing severe disorganization of the intercalated disc (white arrows) with degenerated myofibrils (F) and abundant glycogen granules (star) in- between the degenerated myofibrils.(TEM × 2500)

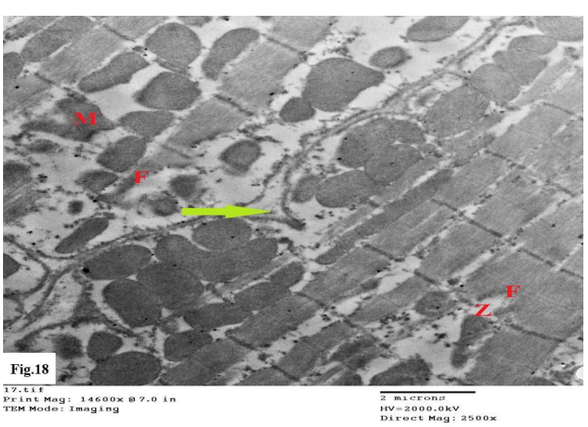


Fig. 18: An electron micrograph of cardiomyocyte of diabetic group (group III) showed severely destructed and degenerated myofibrils (F), degenerated mitochondria (M) and dilatation of T- tubule (greenarrow) with areas of interrupted Z-lines (Z).(TEM × 2500)

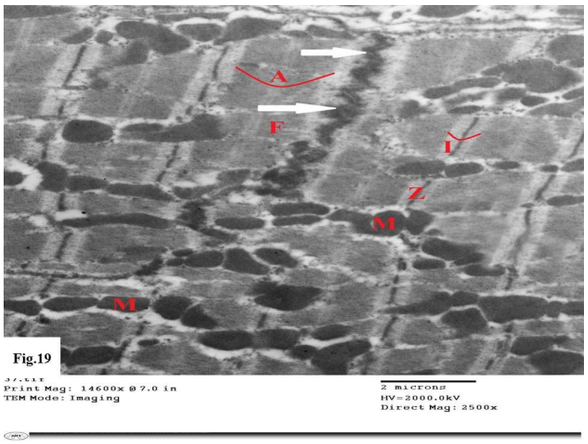


Fig. 19: An electron micrograph of cardiomyocyte of diabetes and losartan treated group (group IV) showing regularly arranged myofibrils (F) and rows of mitochondria (M) in-between. Bundles of myofibrils are seen with alternating dark A (A) and light I (I) bands . Z lines (Z) appear bisecting I bands. Intact intercalated discs also seen (white arrow). (TEM × 2500)

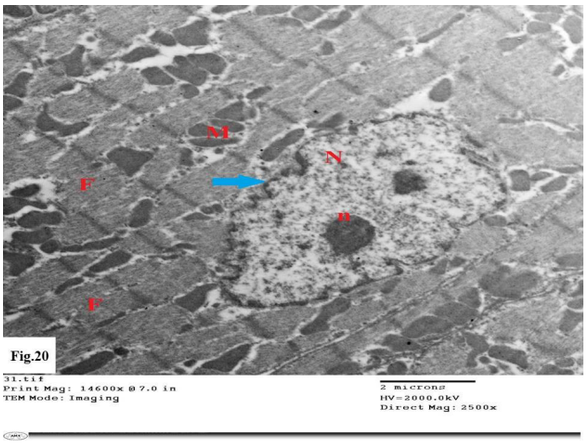


Fig. 20: An electron micrograph of cardiomyocyte of diabetes and losartan treated group (group IV) showing an oval nucleus (N) with prominent nucleolus (n) with slight irregular nuclear membrane (blue arrow), nearly regularly arranged myofibrils (F) and rows of mitochondria (M) in-between. (TEM × 2500)

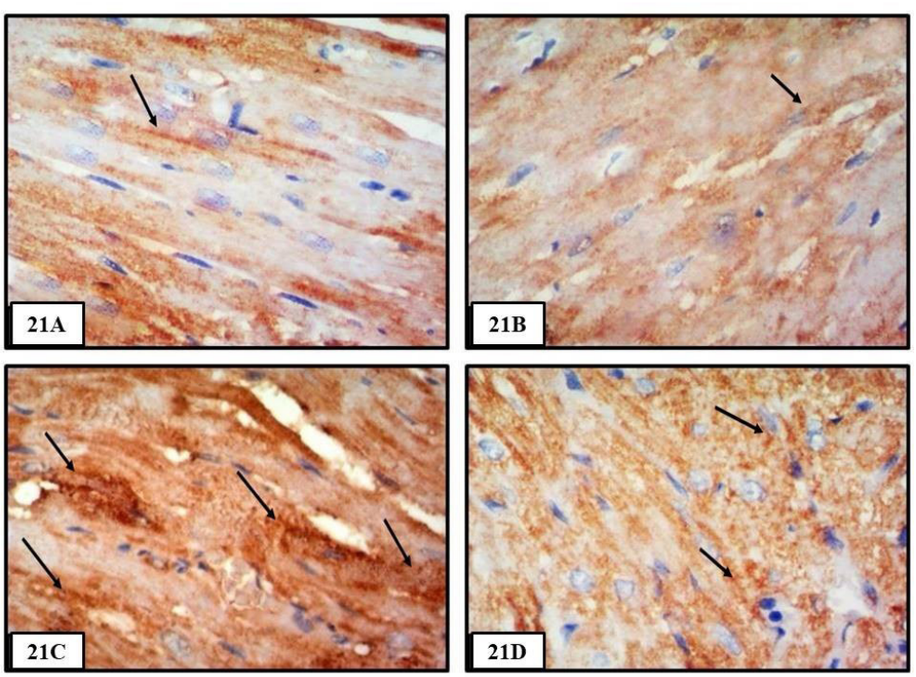


Fig. 21: caspase-3 immunohistochemical staining of rat myocardium showing: **(a)**A photomicrograph of a longitudinal section of the myocardium of the left ventricle from a control rat (group I) showing weakly expressed cytoplasmic immunoreactivity for caspase-3 .**(b)** A photomicrograph of a longitudinal section of the myocardium of the left ventricle from losartan treated rat (group II) showing mild positive cytoplasmic immunoreactivity for caspase-3. **(c)** A photomicrograph of a longitudinal section of the myocardium of the left ventricle from a diabetic rat (group III) showing a strong positive cytoplasmic immunoreactivity for caspase-3. **(d)**A photomicrograph of a longitudinal section of the myocardium of the left ventricle from a diabetic rat treated with losartan group (group IV) showing moderate positive cytoplasmic immunoreactivity for caspase-3. (Caspase-3 X400 immunostaining

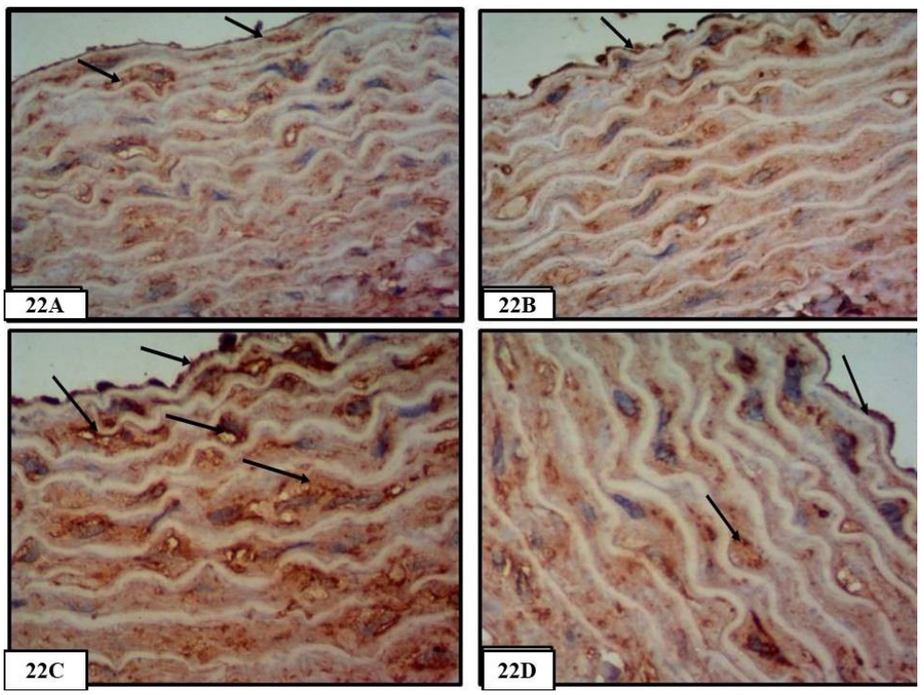


Fig. 22: caspase-3 immunohistochemical staining of rat aorta showing : **(a)**A photomicrograph of a transverse sections of the wall of the aorta of a control rat(group I) showing weak positive cytoplasmic immunoreactivity for caspase-3 . **(b)**A photomicrograph of a transverse sections of the wall of the aorta of group II (losartan treated group) showing weak positive cytoplasmic immunoreactivity for caspase-3 . **(c)**A photomicrograph of a transverse sections of the wall of the aorta from group III (diabetic group)showing a strong positive cytoplasmic immunoreactivity for caspase-3. **(d)**A photomicrograph of a transverse sections of the wall of the aorta from a diabetic rat treated with losartan (group IV) showing moderate positive cytoplasmic immunoreactivity for caspase-3. (Caspase-3 X400 immunostaining)

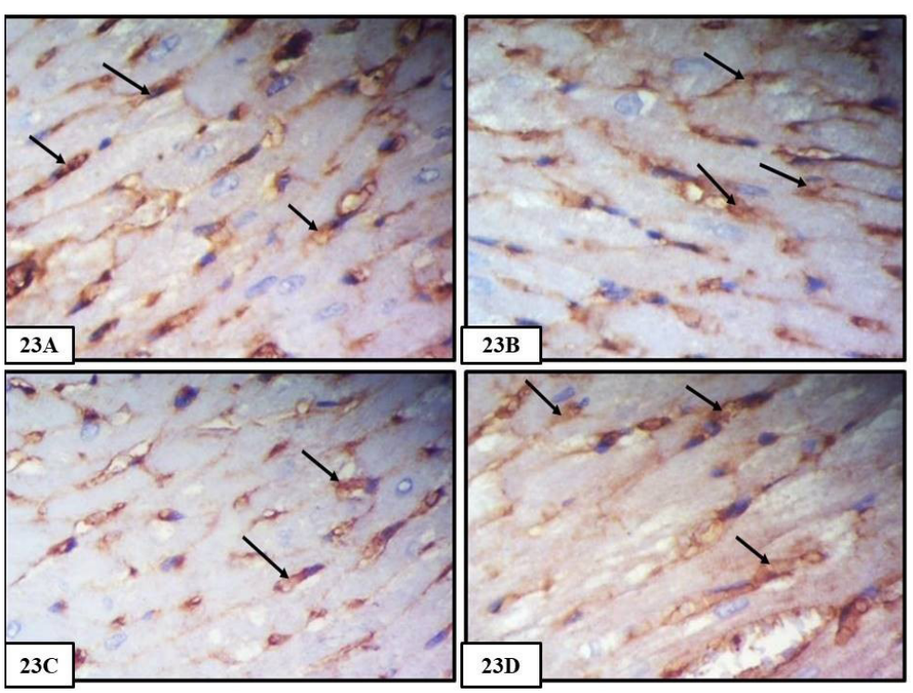


Fig. 23: eNOS immunohistochemical staining of rat myocardium showing :**(a)**A photomicrograph of a longitudinal section of the myocardium of the left ventricle of a control rat (group I) showing strong positive cytoplasmic immunoreactions for eNOS .**(b)** A photomicrograph of a longitudinal section of the myocardium of the left ventricle from losartan treated rat (group II) showing strong positive cytoplasmic immunreaction for eNOS **(c)** A photomicrograph of a longitudinal section of the myocardium of the left ventricle from a diabetic rat (group III) showing a weak positive cytoplasmic immunoreaction for eNOS .**(d)** A photomicrograph of a longitudinal section of the myocardium of the left ventricle from a diabetic rat treated with losartan group (group IV) showing strong positive cytoplasmic immmunoreaction for eNOS (eNOS x 400 immunostaining)

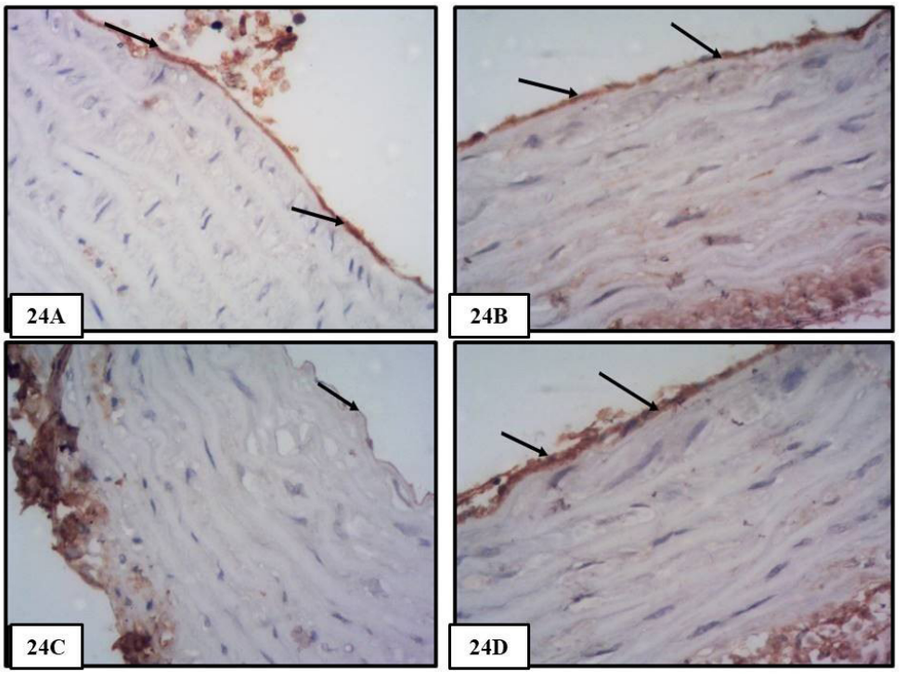


Fig. 24: eNOS immunohistochemical staining of rat aorta showing : **(a)** A photomicrograph of a transverse sections of the wall of the aorta of a control rat showing strong positive endothelial immunoreactions for eNOS . **(b)** A photomicrograph of a transverse sections of the wall of the aorta of group II (losartan treated group) showing strong positive endothelial immunreaction for eNOS **(c)** A photomicrograph of a transverse sections of the wall of the aorta from group III (diabetic group) showing weak positive endothelial immunoreaction for eNOS **(d)** A photomicrograph of a transverse sections of the wall of the aorta from a diabetic rat treated with losartan (group IV)showing strong positive endothelial immunreaction for eNOS. (eNOS x 400 immunostaining)

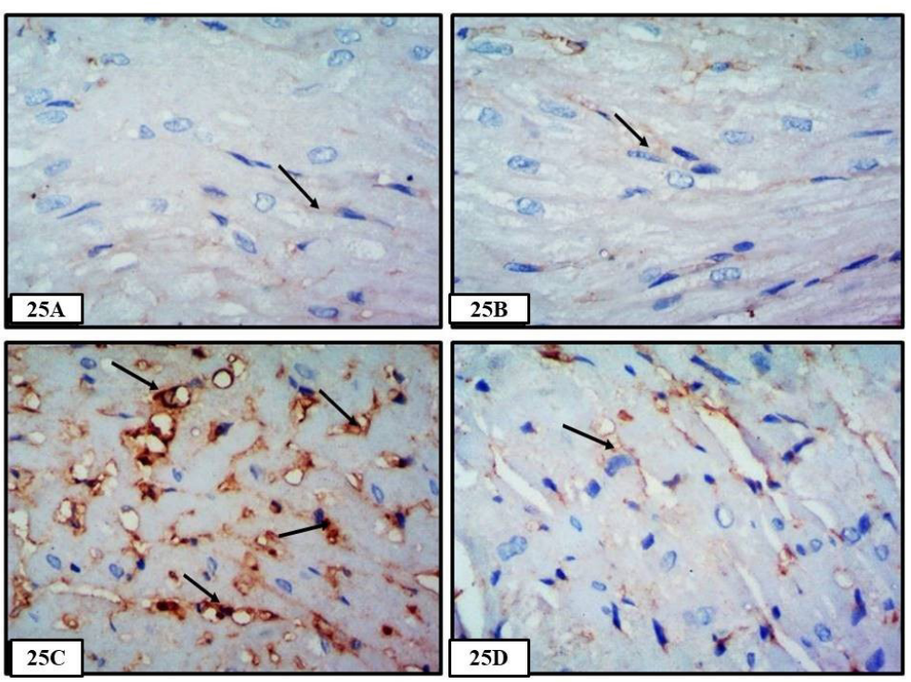


Fig. 25: TNF immunohistochemical staining of rat myocardium showing : **(a)** A photomicrograph of a longitudinal section of the myocardium of the left ventricle from a control rat (group I) showing weakly expressed immunoreactions for TNF **(b)** A photomicrograph of a longitudinal section of the myocardium of the left ventricle from losrtan treated rat (group II) showing weak positive immunreaction for TNF . **(c)** A photomicrograph of a longitudinal section of the myocardium of the left ventricle from a diabetic rat (group III) showing a strong positive immunoreaction for TNF **(d)** A photomicrograph of a longitudinal section of the myocardium of the left ventricle from a diabetic rat treated with losartan group (group IV) showing moderate positive immmunoreaction for TNF. (TNF x 400 immunostaining)

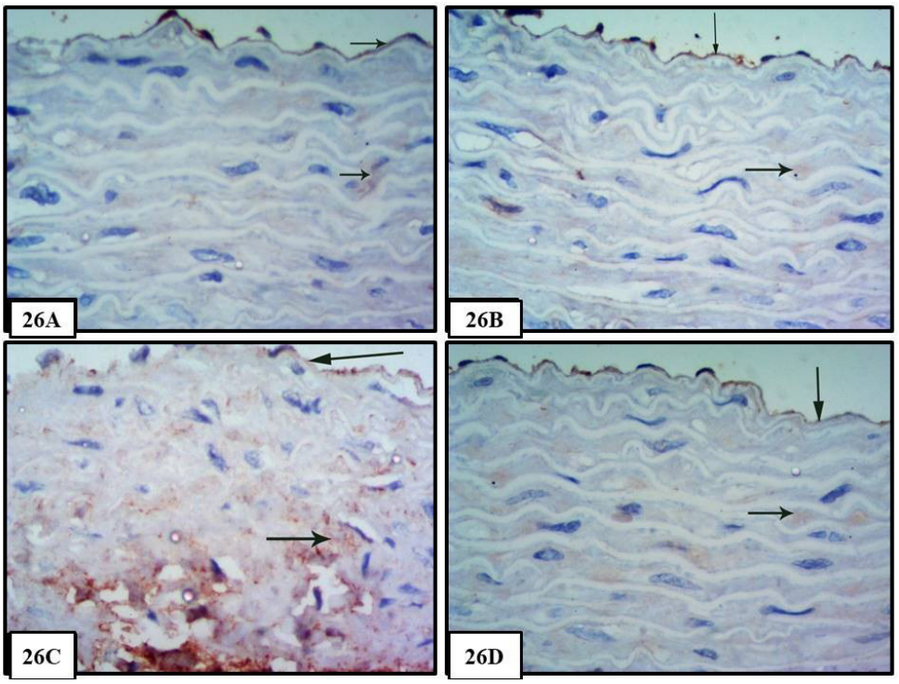


Fig. 26: TNF immunohistochemical staining of rat aorta showing : **(a)** A photomicrograph of a transverse sections of the wall of the aorta of a control rat showing weakly expressed positive cytoplasmic and endothelial immunoreactions for TNF(arrows). **(b)**A photomicrograph of a transverse sections of the wall of the aorta of group II (losartan treated group) showing weak positive cytoplasmic immunoreactions in tunica media and weak positive endothelial immunoreactions for TNF(arrows) . **(c)** A photomicrograph of a transverse sections of the wall of the aorta from group III (diabetic group) showing a strong positive cytoplasmic immunoreactions in tunica media and strong endothelial immunoreactions for TNF(arrows). **(d)**A photomicrograph of a transverse sections of the wall of the aorta from a diabetic rat treated with losartan (group IV) showing mild positive cytoplasmic immunoreactions in tunica media and mild positive endothelial immunoreactions for TNF(arrows). (TNF x 400 immunostaining)

Table 1: Means of fasting blood glucose level (mg /dl) of various experimental groups

Experimental groups	Blood glucose level (mg/dl) $\bar{x}$±SD
Control group (Group I)	120.9 ± 7.2
Losartan group (group II)	118.1± 7.00
Diabetic group (group III)	233.8 ± 17.8**
Diabetic + losartan Group (IV)	133.5 ± 6.1♦♦

$\bar{x}$= the mean value. SD= the standard deviation.
** Highly significant from the control group (p<0.001).
♦♦Highly significant from the diabetic group (p<0.001).

Table 2: Effect of diabetes on the antioxidant enzymes activities and the level of lipid peroxidation in the plasma of all groups of rats

Group	MDA (micromoles/ml) $\bar{x}$±SD	SOD(u/ml) $\bar{x}$±SD	CAT(u/ml) $\bar{x}$±SD
Control (group I)	3.5 ± 0.4	33.6 ± 1.2	27.6 ± 1.00
Losartan (group II)	3.8 ± 0.6	32.4 ± 1.7	26.2 ± 1.9
Diabetic (group III)	18.5 ± 3.5**	17.2 ± 1.12**	14.9 ± 1.8**
Protective (group IV)	5.34 ±1.2	28.9 ± 0.8	22.1 ± 1.4

$\bar{x}$= the mean value. SD= the standard deviation.
* Significant at P<0.05 compared with control group
** Highly significant from the control group (p<0.001).

Table 3: Means and standard deviations of percentage area of collagen fibers of various experimental groups

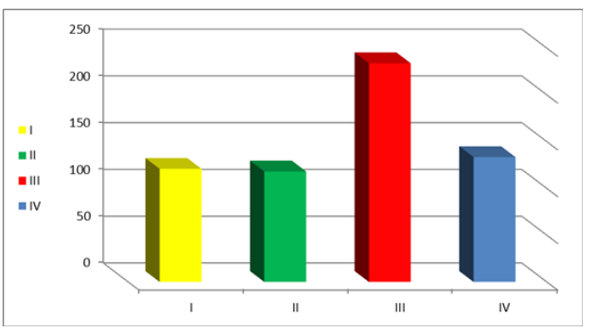
Experimental groups	% area of collagen fibers
Control group (group I)	12.50 ± 0.98
Losartan group (group II)	13.50 ± 0.98
Diabetic group (group III)	25.75 ± 1.98**
Diabetic + Losartan Group (IV)	14.43 ± 2.65##

Values are means ± SD= the standard deviation.
** Highly significant from the control group (p<0.001).
Highly significant from the diabetic group (p<0.001).

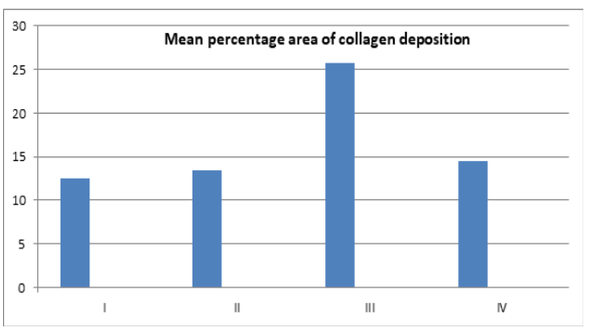
Table 4: Means and standard deviations (SD) of the intensity of eNOS immune reaction & The number of Caspase-3 positive cells of various experimental groups

Experimental groups	Intensity of eNOS immune reaction $\bar{x}$±SD	The number of Caspase-3 positive cells $\bar{x}$±SD
Control group (group I)	14.96± 1.012	5.42±.17
Losartan group(group II)	13.91± 1.347	6.42±.17
Diabetic group (group III)	5.591± 0.824**	32.48±.31**
Diabetic + Losartan Group (IV)	14.35± 1.025##	17.75±.38##

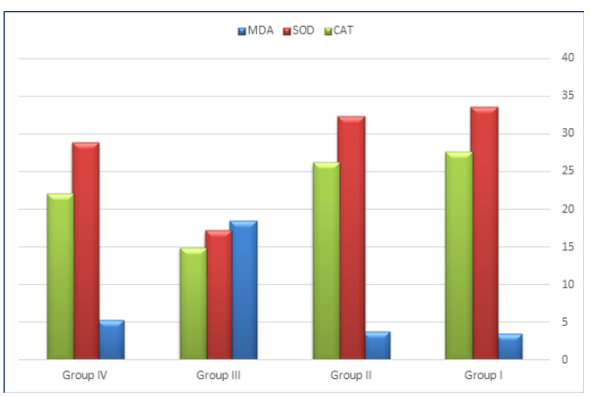
$\bar{x}$= the mean value. SD= the standard deviation.
** Highly significant from the control group (p<0.001).
Highly significant from the diabetic group (p<0.001).



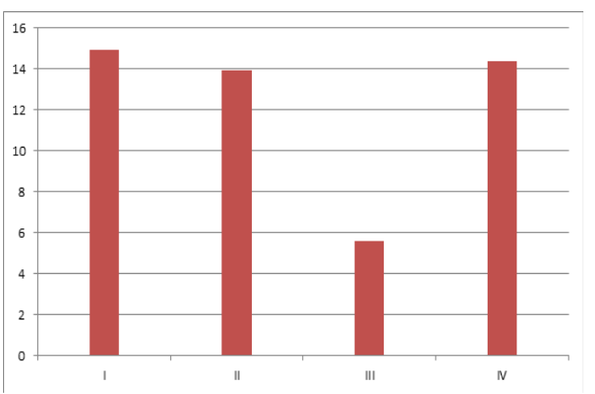
Histogram 1: The Mean of fasting blood glucose level in different studied groups



Histogram 3: The mean of the percentage area of collagen fibers in different groups



Histogram 2: The Mean MDA, SOD and CAT levels in experimental different groups



Histogram 4: Means of the intensity of eNOS immune reaction in the different experimental groups

DISCUSSION

In this study, experimental DM was induced effectively with STZ, proved by hyperglycemia. In this result the typical features of DM like polydipsia, polyphagia and polyuria were noticed in the behavior of diabetic animals. Compared to the diabetic group, diabetic case supplemented with Losartan had a considerable drop in blood glucose levels. This indicates a positive impact of this drug on blood glucose levels. This was in accordance with Murthy *et al*,[26] who found that Losartan exhibited an antihyperglycemic impact through enhancing insulin sensitivity and homeostasis. In addition, Kitamura *et al*,[27] explained that AT II blockers may enhance glucose metabolism by inhibiting AT II's influence on insulin signal transmission.

In the current study, plasma MDA levels significantly increased in diabetic rats which indicated elevation of lipid peroxidation and oxidative stress. Also, DM decreased SOD and CAT significantly which are enzymes with the most important defense mechanism against oxidative stress induced by free radicals. The elevation in lipid peroxidation in the heart was previously recorded by other studies on different tissues Kakkar *et al*,[28], Suryawanshi *et al*,[29] & Marjani,[30]. The administration of Losartan to diabetic rats dramatically decreased MDA levels and increased antioxidant enzyme levels. This was in accordance with Mohamed *et al*,[31] who verified that Losartan reduced oxidative stress, enhanced endothelial function, and served as a potent antioxidant. Also, Ivanov *et al*,[32] explained that Losartan caused a substantial rise in CAT activity.

In the current research, optical and electron microscopy revealed fragmented, deformed, and severely deteriorated muscle fibres in the heart muscle of STZ-treated rats. Also, muscle fibers with intramuscular hemorrhagic regions and vacuolation were exist. Numerous darkly stained pyknotic nuclei widened endomysium with cellular infiltration were also detected in addition to a pronounced variation in the mitochondrial size. These findings supported and confirmed those of Tamás Radovits *et al*,[33] who evaluated the features of diabetic cardiomyopathy identified in LV sections of diabetic groups with H&E staining, revealed disorganization and degradation of myofibers in diabetic rat LV myocardium.

In this current research, diabetic myocardium showed the intercalated disc appeared fragmented in some areas and disorganized with separation of the cardiomyocytes in others. This was in agreement with Hanan *et al*,[34] who verified that disruption of intercalated disc and cardiac apoptosis had been described as adverse changes complicating DM.

In this research, T- tubule dilation was seen by electron microscopy in the heart muscle of rats treated with STZ. These findings confirmed and supported those of Ibrahim Michael *et al*,[35] who studied heart failure in animal models and reported sections of myocytes showed disarray and dilatation of T-tubule. This was also in agreement with Guo *et al*,[36] who identified that In the hearts of cases with end-stage dilated or ischemic cardiomyopathy, irregularly shaped or dilated T-tubules were seen. Multiple myocardial insults, including persistent tachycardia and myocardial infarction, seemed to have produced this Balijepalli *et al*,[37] Heinzel *et al*,[38].

In this work, diabetic myocardium showed widening of the endomysium with dilated congested blood vessels and obvious infiltration. This was in accordance with, Jia G *et al* 2016[39], who proved that A maladaptive proinflammatory response has been linked to diabetic cardiomyopathy development. Neutrophils, mast cells, dendritic cells, macrophages, and eosinophils of the innate immune system were engaged. The degenerative changes in our study were suggested as a result of an elevated lipid peroxidation with a reduction of antioxidants that were confirmed by the biochemical results of this work. Bonnefont,[40] suggested that hyperglycemia is the major factor in production of free radicals, development of oxidative stress and decrease of endogenous antioxidant enzymes. Consequently, excess free radicals damage the cell membrane phospholipids and other cell components through lipid peroxidation Shirpoor *et al*,[41]. Administration of losartan improved the histological changes in the diabetic animals. This improvement can be explained by the Losartan antioxidant effect.

In the present work, aorta of diabetic rats showed the feathers of atheroma in the form of focal desquamation of the endothelial cells and appearance of foam cells. This was in line with Patel *et al*,[42], Sharma *et al*,[43], Kapoor *et al*,[44] and Castillo *et al*,[45] who ascribed these pathological alterations to the buildup of oxidized lipids from low-density lipoprotein particles in the endothelial wall of arteries. They added, the particles induce focal desquamation of the endothelial cells, and appearance of foam cells. Foam cell is monocytes that infiltrate the arterial wall and differentiate into macrophages accumulating oxidized lipids inside it. In addition, it considered as significant event in atherogenesis Deepa and Varalakshmi,[46].

On the other side, Komolafe *et al*,[47] attributed these pathological changes to the accumulation of glycogen deposits resulting from diabetic deranged carbohydrate metabolism. Although, the hyperglycemia detected in the diabetic group in this study could reflect the carbohydrate metabolism disorder, but it has come that lipid disorder may be the initiator of diabetic arterial atherosclerosis due to detection of sub-intimal lipid deposition. However, this could be considered limitation of this work for further study.

However, we could assure the marked prevention of atherosclerotic picture detected in Losartan protected group, through histological, and immunohistochemical examination, Lee *et al*,[48] supported this result, who found that the administration of losartan to mice considerably lowered atherosclerotic lesion areas in the whole aorta.

In the present study, sections stained with Masson trichrome revealed an apparent increase in collagen fibres in diabetic rats than non-diabetic control rats. There were collagen fibres in the endomysium and around the blood vessel. These outcomes were considerably in accordance with the outcomes of Wang *et al*,[49] and Mytas *et al*,[50] who reported that in diabetic cardiomyopathy, the development of myocardial fibrosis includes stiff collagen deposition and its linkage, gradual abolition of muscle fibrils, cardiac interstitial fibrosis, and perivascular fibrosis. Consistent with what is known about fibrosis as a frequent pathological reaction to tissue insults such as hyperglycemia, HTN, and dyslipidemia, the results were consistent with what is known about fibrosis. Chronic hyperglycemia may induce tissue damage, resulting in fibrosis with major extracellular matrix (ECM) deposition. This ECM accumulation may result from an increase in matrix protein production or an impairment of ECM breakdown. It was also shown that hyperglycemia may alter ECM turnover through both hemodynamic and metabolic routes[51]. In addition, hyperglycemia causes the existence of large quantities of non-enzymatically generated advanced glycation end-products that may directly induce the formation of ECM[52]. Furthermore, Unal *et al*,[53] observed abnormal changes in the metabolism of ECM in diabetic cases with diarrhea. Such defects in the metabolism of extracellular matrix may be also the cause of the abnormal changes noticed in the endomysium in this study.

However, administration of Losartan to diabetic rats showed remarkable reduction in the collagen content in the endomysium of heart. This may be due to antioxidant properties of Losartan, confirmed by a study done by Mohamed *et al*,[54] who reported that Losartan reduced oxidative stress, enhanced endothelial function, and served as a potent antioxidant.

In this research, the elastic lamellae in the tunica medium of the aorta in the diabetic group were destroyed, as were the internal and external elastic laminae. These outcomes were in accordance with Ishikawa *et al*,[55] Park *et al*,[56]. They said that it might be due to discomfort from fatty deposits. Aguila and Mandarim,[57] also suggested that fragmentation of elastic lamellae may be caused by a combination of two causes. The first component is the progressive buildup of lipids and calcium salts in the elastic fibres. The second element is an elastase activity rise. These two concurrent processes rob elastic fibres of their elasticity and make them more susceptible to elastolytic enzymes.

In the current study, myocardium of diabetic rats indicated a significant elevation in the expression level of caspase-3. Liu *et al*,[58] supported this result, who found that in the STZ-induced diabetes group, caspase-3 expression was considerably higher than in the control group.

However, administration of Losartan to diabetic animals exhibted a marked reduction in caspase-3. This may be due to suppressing properties of Losartan for the Akt/Bad/Bax/caspase-3 and Nrf2/Keap-1 pathways, this is confirmed by a study done by Wang, *et al*[59] who reported that Losartan effectively inhibited cellular apoptosis and oxidative stress in erectile dysfunction associated with ageing.

Cardiac sections from diabetic group revealed weak positive immmunostaining for eNOS. This was in agreement with a research by Mario Felaco *et al*,[60] on rats, who showed that diabetic hearts showed less intensity of the eNOS immunoprecipitate, compared to control hearts,and another research by Oltulu *et al*,[61] reported that rat liver histochemical analyses revealed that eNOS protein expression was decreased in the DM group than the control and Losartan-treated groups.

However, treatment with Losartan increased eNOS activity in diabetic rats. These results were supported by the previous works of Tetsu Watanabe *et al*,[62] who showed that Losartan metabolite EXP3179 stimulates eNOS phosphorylation, also demonstrated that Losartan may boost eNOS activity via stimulating eNOS phosphorylation by activating the VEGFR2/PI3K/Akt pathway.

Myocardial sections from diabetic group revealed a strong positive immmunostaining for TNFα . This was in accordance with a research by Westermann *et al*,[63] on animal models, who showed that the levels of cytokines such as TNF- and IL-6 were elevated in diabetic myocardium. Also in agreement with, Liu *et al*,[64] who reported that, TNF- and NF-B expression levels were considerably elevated in the STZ group. Chronic hyperglycemia leads in diminished antioxidant capacity and elevated oxidative stress, which contribute to DM development and its consequences, as Sun *et al*,[65] explained in their study.

However, treatment with Losartan reduced TNFα in diabetic animals. These results were supported by the previous works of Abdel *et al*,[66] who showed that when provided to rats with arthritis, Losartan significantly decreased blood levels of TNF and IL-6, hence reducing inflammation. Wang *et al*.,[67] showed a comparable drop in TNF and IL-6 following Losartan medication, confirming our results. Refaat *et al*.[68] found that The downregulation of proinflammatory cytokines such as TNF and IL-6 was caused by the downregulation of AT1 by losartan. Kim *et al*.,[69] showed that Losartan decreased inflammatory cytokines including IL-1 and TNF- in the Dorsal Root Ganglia, which was consistent with our results.

CONCLUSION

From all the mentioned data and results, it was concluded that administration of Losartan in rats with induced DM could enhance the histological, biochemical and morphometric abnormalities of the myocardium. It also has a protective effect on the aortic tissue. Therefore, it was recommended that cases with DM might be administered losartan to improve cardiac and aortic symptoms.

Further investigations will be done to elucidate the potential usefulness of this drug as a source of protective agent against the diabetic myocardium and aortic damage in clinical trials.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

تأثير حاصرات الأنجيوتنسين (II اللوسارتان) على عضلة القلب لدى الجرذان المستحثه بداء السكري الناجم عن الستربتوزوتوسين: دراسة هستولوجية وهستوكيميائية

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مقدمه: الداء السكري هو حدث شائع في الغدد الصماء والتمثيل الغذائي. إنه أحد أسرع الأمراض نموًا في جميع أنحاء العالم وهو السبب الرئيسي لزيادة معدلات الإصابة بالأمراض القلبية الوعائية والوفيات بين السكان. ويرتبط ارتفاع السكر في الدم الناتج عن مرض السكري المزمن بأضرار طويلة الأمد، وخلل وظيفي، وفشل في أعضاء مختلفة، وخاصة العينين والكليتين والأعصاب والقلب والأوعية الدموية. اللوسارتان هو أحد حاصرات مستقبلات الأنجيوتنسين من النوع الأول وهو مصرح به لعلاج ارتفاع ضغط الدم الشرياني. يستخدم اللوسارتان أيضًا لتجنب تلف الكلى لدى مرضى السكر والذين يعانون من انخفاض الكسر القذفي بسبب قصور القلب.

الهدف: يهدف هذا البحث إلى تحديد التأثير الوقائي المحتمل لإعطاء اللوسارتان على عضلة القلب والشريان الأورطي الناجم عن مرض السكري لدى ذكور فئران سبراغ داولي البالغة.

المواد والطرق: تم استخدام ستين ذكراً بالغاً من فئران سبراغ داولي في هذا البحث. تم تقسيمهم إلى أربع مجموعات: المجموعة الأولى (المجموعة الضابطة)، المجموعة الثانية (المجموعة المتلقية اللوسارتان) تم إعطاؤهم اللوسارتان ٣٠ ملغم / كغم / يوم، المجموعة الثالثة (مجموعة مرضى السكري) لإحداث السكر عن طريق حقنة واحدة داخل البريتون قدرها ٦٥ ملجم / كجم / يوم من محلول الستربتوزوتوسين مخلوط حديثًا في ٠,١ م من محلول السترات والمجموعة الرابعة (مرض السكري مع مجموعة اللوسارتان). بعد ٨ أسابيع، تم تخدير الحيوانات من جميع المجموعات وتم الحصول على عينات الدم للدراسة البيوكيميائية. تم تشريح القلب والشريان الأورطي ، وأجريت الدراسات النسيجية والكيميائية المناعية والإلكترونية المجهرية على الأنسجة.

النتائج: كشفت عضلة القلب السكرية عن تغيرات نسيجية مثل ألياف عضلية مجزأة، نواة تشنجية صغيرة ملطخة باللون الداكن، وارتشاح خلوي، وارتفاع كبير في نسبة ألياف الكولاجين مع تغيرات كيميائية حيوية ملحوظة مثل زيادة مستوى البلازما من المالونديالدهيد. أدى إعطاء اللوسارتان إلى الجرذان المصابة بداء السكري إلى تحسين البنية المضطربة، وخفض مستويات المالونديالدهيد المرتفعة في الأنسجة، وزيادة الأنشطة المنخفضة لمضادات الأكسدة الأنزيمية فوق أكسيد ديسموتاز، والكاتلاز في أنسجة القلب. أظهرت مجموعة مرضى السكري زيادة كبيرة في متوسط مساحة ألياف الكولاجين، وعدد الخلايا الإيجابية لـ للكاسبس ٣، وانخفاض كبير في شدة الاستجابة المناعية لـ eNOS في أنسجة عضلة القلب لدى الجرذان مقارنة بمجموعة مرضى السكري مقارنة بالمجموعة الضابطة. في حين أظهرت مجموعة مرضى السكري الذين عولجوا باللوسارتان انخفاضًا ملحوظًا في متوسط ألياف الكولاجين، وعدد الخلايا الإيجابية لـ لكاسبس ٣، وزيادة كبيرة في شدة الاستجابة المناعية لـ eNOS مقارنة بمجموعة مرضى السكري.

الاستنتاج: اللوسارتان له تأثير وقائي على التغيرات البيوكيميائية والنسيجية والمورفومترية لعضلة القلب والشريان الأورطي في الجرذان المصابة بالسكري.