Comparative histological study of the potential protective effect of Acarbose, Linagliptin and Quercetin on the aorta of type 2 diabetic rat

Original Article

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ABSTRACT

Background: Diabetes mellitus (DM) is a worldwide health problem affecting a population of different ages, which inflicts a large economic load on the worldwide health-care system. This disease is mostly characterized by hyperglycemia which causes deleterious effects on both the macro- and microvasculature. Pharmacological treatment includes many drugs with different mechanisms of action. These drugs aim to control the hyperglycemic state and subsequently may protect the vessels from its deleterious effect.

Aim of the Work: The aim of our study is to compare the potential protective effect of three of these antidiabetic drugs; Acarbose, Linagliptin and Quercetin, on the aorta of type 2 diabetes mellitus rat model.

Materials and Methods: Forty male albino rats were divided into 5 groups. Two control groups; normal control group (normal group) and diabetic control group (DM group). Diabetes was induced by feeding rats with high-fat diet (HFD) for 2 weeks followed by a single dose Streptozotocin (STZ) injection. Three diabetic groups received different treatments; diabetic group treated with Acarbose (DM+AC group), diabetic group treated with Linagliptin (DM+LN group) and diabetic group treated with Quercetin (DM+QR group). Blood samples were taken for biochemical evaluation and specimens of the aorta were taken for histological and morphometric analysis.

Results: Diabetic group showed histological findings recorded in atherosclerotic aorta. The three tested antidiabetic drugs Acarbose, Linagliptin and Quercetin, showed an ameliorative effect on the structure of the aorta but no noticeable differences were recorded.

Conclusion: Our study revealed that the changes produced in the structure of the aorta of the diabetic group were ameliorated in the groups received the three drugs with no noticeable differences between them.

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Key Words: Acarbose, aorta, diabetes type 2, linagliptin, quercetin.

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INTRODUCTION

Diabetes mellitus (DM) is a worldwide health problem affecting a population of different ages, which inflicts a large economic load on the worldwide health-care system[1]. According to the WHO, there was a 5% increase in premature mortality from diabetes between 2000 and 2016[2]. This disease is mostly characterized by hyperglycemia which causes deleterious effects on both the macro- and microvasculature. Thus, DM is considered a key risk factor for cardiovascular diseases (CVDs)[3]. It is reported that vascular stiffening is accelerated, in type 2 diabetes, this is attributed to endothelial dysfunction, changes in the vascular tone and extracellular matrix remodeling[4]. In addition, prolonged hyperglycemia causes premature atherosclerotic lesions[5].

Pharmacological treatment of DM includes many drugs with different mechanisms of action, which may be in a form of a single or combined treatment. These drugs aim to control the hyperglycemic state, subsequently they may protect the vessels from this hyperglycemic deleterious effect[6].

Acarbose (AC), an inhibitor of intestinal alpha-glucosidase reduces hyperglycemia by lowering glucose absorption from the intestine[7]. It is reported that it reduces the incidence of CVDs in diabetic patients[8]. This is attributed to its ameliorative effect on the endothelial dysfunction and the arterial stiffness[7,9].

Linagliptin (LN) is another oral antidiabetic drug, which reduce hyperglycemia by another mechanism. It is a dipeptidyl peptidase-4 (DPP-4) inhibitor. Dipeptidyl peptidase-4 is an enzyme that degrades the incretin hormones; glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). Incretins increase glucose-induced insulin release and suppress glucagon secretion, which subsequently will reduce glucose output in the liver[10]. Latest experimental studies

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have hinted to its beneficial vascular effects, but clinical evidence is limited[11]. Preclinical evidence suggests that DPP-4 inhibitors may have cardiovascular benefits independent of their glycemic lowering effects[4].

Quercetin (QR) is one of the flavonoids. It is present in different vegetables, fruits, and many other dietary sources such as tea, olive oil, leafy vegetables, apples, onions, and cereal grains[12]. It acts as an anti-diabetic drug by different mechanisms including, inhibition of intestinal glucose absorption, adjustment of insulin secretion and insulin-sensitizing activities, besides it improves glucose consumption in peripheral tissues[13]. In addition to its hypoglycemic effect, latest studies showed that QR have a protective effect on the cardiovascular system[12].

AIM OF STUDY

The aim of our study is to compare the potential protective effect of Acarbose, Linagliptin and Quercetin on the aorta of type 2 diabetes mellitus rat model.

MATERIALS AND METHOD

Experimental animals

Forty male albino rats, weighing 150–180 grams were purchased from the animal house of Faculty of Medicine, Alexandria University. Animal care and management was in accordance with ARRIVE guidelines of Animal Care. This study was approved by the Ethics Committee of the Faculty of Medicine, Alexandria University, Egypt. Serial number of the approved protocol is (0104331).

Rats were divided into 5 groups (8 rats/group). Two control groups; normal control group (normal group) and diabetic control group (DM group). In addition to three diabetic groups received different treatments; diabetic group treated with Acarbose (DM+AC group), diabetic group treated with Linagliptin (DM+LN group) and diabetic group treated with Quercetin (DM+QR group).

Diabetes was induced by feeding rats with a high-fat diet (HFD); 58% fat, 25% protein and 17% carbohydrate, as a percentage of total kcal, and after 2 weeks, rats received a single intraperitoneal injection of Streptozotocin (STZ) (35 mg/kg)[14–16]. Induction of diabetes was confirmed by measuring serum glucose level one week after STZ injection, rats with serum glucose level > 200 mg/dl were included in the study[17–20]. The normal control rats were fed normal rat chow throughout the study.

Diabetic group treated with acarbose (DM+AC group) received (30 mg/kg) acarbose[21], diabetic group treated with Linagliptin (DM+LN group) received (3 mg/kg) Linagliptin[22] and diabetic group treated with Quercetin (DM+QR group) received (50mg/kg) Quercetin[23]. All the treatments were introduced by oral gavage, daily for 6 weeks following induction of diabetes.

Drugs and chemicals

Streptozotocin powder (STZ, purity ≥ 98%) and Quercetin powder (purity ≥ 95%) were purchased from (Sigma-Aldrich St Louis, MO, USA). Linagliptin (Trajenta®) was purchased from (Boehringer Ingelheim limited) while Acarbose (Glucobay®) was purchased from (Bayer Pharmaceuticals Pvt Ltd). All other chemicals used in the study were obtained from local commercial sources.

Biochemical parameters

The insulin levels were measured using ELISA kit (Sigma-Aldrich) according to the manufacturer's protocol[24]. Insulin resistance and β-cell function were calculated by the homeostasis model assessment of insulin resistance (HOMA-IR) and of β-cell function (HOMA-β), respectively, using HOMA calculator 2. Fasting blood glucose (FBG) was evaluated according to the glucose oxidase peroxidase method using a colorimetric kit for glucose (BioSystems) [25]. Serum triglycerides (TGs) and serum cholesterol were measured using enzymatic colorimetric methods (N.S. BIOTEC, Wellkang Ltd, UK)[26,27]. The serum high density lipoprotein (HDL) was done using precipitating reagent (BioSystems, Spain),[28] and low density lipoprotein (LDL) was calculated by Friedewald formula, utilizing the values of TGs, cholesterol and HDL[29].

Histological and Morphometric Analysis

Specimens of the aorta were taken for histological and morphometric studies. For the light microscopic examination, specimens were excised and fixed in 10% formol saline and processed to get 3–5 μm thick paraffin sections. Sections of the aorta were stained with haematoxylin and eosin (H&E) stain for histological evaluation, Verhoeff-Van Gienson (VVG) stain to observe the elastic fibers and Trichrome stain to detect smooth muscle fibers. Images were taken at magnification (400X) to measure the thickness of tunica media (TM) in H&E stained sections, the area percentage of elastic fibers in TM in the VVG stained aortic sections and the area percentage of smooth muscle fibers in TM of Trichrome stained sections. Measurements were expressed using NIH Image J (v1.49) software[5,30]. Additionally, aortic specimens were taken and cut into small pieces (1/2-1 mm3), fixed in 3% glutaraldehyde solution, and processed to get ultrathin sections for transmission electron microscope (TEM) examination.

Statistical Analysis of the Data

Data were supplied to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). The Kolmogorov- Smirnov was used to verify the normality of distribution of variables, ANOVA was used for comparing the different studied groups and followed by Post Hoc test (Tukey) for pairwise comparison. Significance of the obtained results was judged at the 0.05% level.

RESULTS

Biochemical results

Results of fasting blood glucose, plasma insulin, insulin resistance (HOMA-IR) beta cell function (HOMA- β %)

Fasting blood glucose (FBG) and plasma insulin levels were significantly increased in diabetic group when compared to the normal group. The groups received the three drugs showed a significant improvement in these levels compared to the diabetic group, but the plasma insulin levels were still higher than the normal group with a significant difference. Homeostasis model assessment (HOMA) calculation showed that, insulin resistance significantly increased in the diabetic group with a significant decrease in β-cell function when compared to the normal control group. The groups received the three drugs showed a significant decrease and increase in these (HOMA-IR) and (HOMA- β %) levels respectively, compared to the diabetic group, but the (HOMA-IR) levels were still significantly higher than the normal group. No significant differences were revealed between the groups treated with the three drugs in any of these parameters ($p \leq 0.05$; Table 1).

Lipid profile results Serum triglycerides, cholesterol, HDL and LDL

The diabetic group showed a significant rise in the serum triglycerides (TGs), cholesterol and low-density lipoprotein (LDL) levels when compared to the normal control group. However, groups received the three drugs showed a significant improvement in these parameters. For high density lipoprotein (HDL), the diabetic group showed a significant lower level compared to the normal group. The groups received AC and LN showed a significant amelioration to HDL level, while QR group showed no improvement ($p \leq 0.05$; Table 1).

Histological results

Light microscopic results

Light microscopic examination of aortic sections from normal control group stained by H&E stain showed, normal aortic structure. Where, tunica intima (TI) is lined by regular endothelial cells, tunica media (TM) contains concentric parallel layers of elastic laminae interposed with smooth muscle fibers, tunica adventitia (TA) appeared as a thin external layer. While aortic sections of diabetic group stained by H&E stain showed, thickened TM with proliferation of smooth muscle fibers. Elastic laminae showed disrupted parallel arrangement. Moreover, they appear relatively thicker when compared to other groups, where some of them are split and ruptured. On the other hand, the aorta from diabetic groups treated with the three drugs, revealed better structure as compared to the diabetic group, as TM thickness decreased, and smooth muscle fibers are not proliferated. Nevertheless, still some disturbance in elastic laminae arrangement, split and ruptured elastic laminae were noticed in some areas. The elastic and smooth muscle fibers distribution is also revealed in VVG and Trichrome stained sections, respectively (Figures 1,2).

Electron microscopic results

Electron microscopic examination of the aortic sections of normal groups showed, endothelial cells lining the luminal surface of the aorta. The smooth muscle cells were obliquely oriented in between the concentrically arranged elastic laminae. Collagen fibrils were seen forming sheets around elastic laminae (Figure 3).

Conversely, aorta of diabetic group revealed disturbance in its structure. Tunica intima showed, endothelial cells with contracted cytoplasm and nucleus and increased collagen fibrils deposition in the subendothelial layer. In addition, disturbed and ruptured internal elastic lamina was revealed. Some smooth muscle fibers were seen invading towards the subendothelial layer. Tunica media showed disturbed distribution of smooth muscle fibers, losing their oblique orientation to the elastic laminae. Collagen fibrils deposition is markedly increased in between smooth muscle fibers and around elastic laminae. In addition, many smooth muscle fibers showed increased vacuolation of the cytoplasm (Figure 4).

On the other hand, aorta of the three groups received treatments showed improvement in the aortic structure with different extents (Figures 5-7).

Morphometric results

Morphometric analysis for the aortic sections revealed that the mean TM thickness measured in H&E stained sections photomicrographs, is significantly increased in the diabetic group when compared to the normal one. On the other hand, TM thickness of the groups treated with the three drugs is significantly decreased when compared to diabetic group, but still significantly higher than normal control group, except for QR group which revealed results equivalent to those of the normal group. In trichrome stained sections photomicrographs, the measurement of area percentage of smooth muscle fibers in the TM of the aorta showed, significant increase in diabetic group when compared to the normal control group. While groups treated with the three drugs showed results equivalent to the normal control group and significantly lower than diabetic one. In VVG stained sections photomicrographs, the measurement of area percentage of elastic fibers in the TM of the aorta showed, significant decrease in the mean area percentage of elastic fibers in diabetic group when compared to normal control group. On the contrary, groups received the three drugs showed an increase in the area percentage of elastic fibers when compared to diabetic group but still significantly lower than the normal group ($p \leq 0.05$; Table-2)

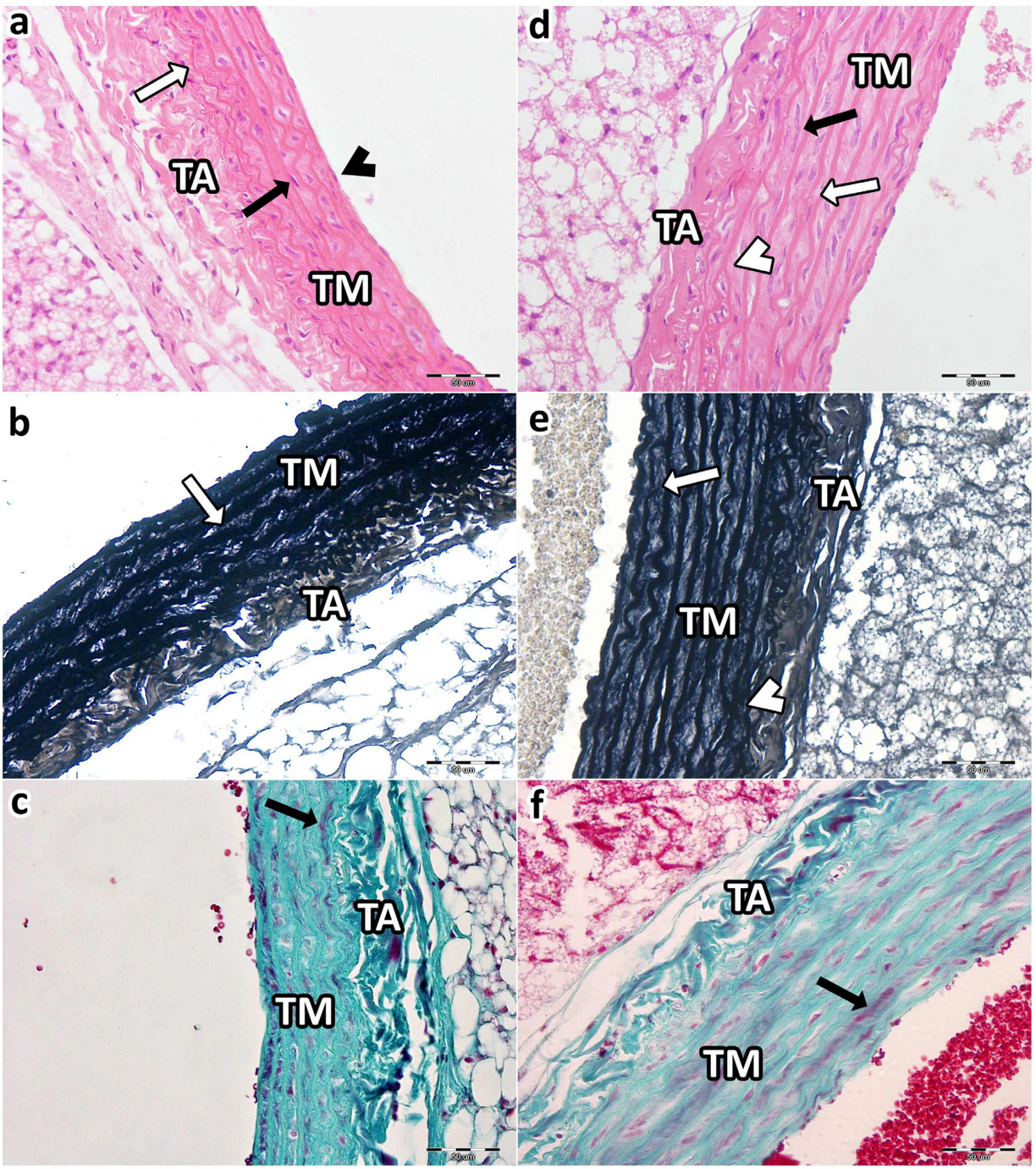

Fig. 1: Photomicrographs of aortic sections (a-c): Photomicrographs of aortic sections from normal control group showing, normal aortic structure, tunica intima is lined by regular endothelial cells (black arrowhead), tunica media (TM) contains concentric parallel layers of elastic laminae (white arrow) interposed with smooth muscle fibers (black arrow), tunica adventitia (TA) appeared as a thin external layer. (d-f): Photomicrographs of aortic section of DM group showing, thickened tunica media (TM) with proliferation of smooth muscle fibers (black arrow). Elastic laminae showed disrupted parallel arrangement, appearing relatively thick and some of them are split (white arrowhead) and others are ruptured (white arrow). TA; tunica adventitia. (a,d): H&E-stained sections. (b,e): VVG stained sections. (c,f): Trichrome stained sections. (Magnification X400).

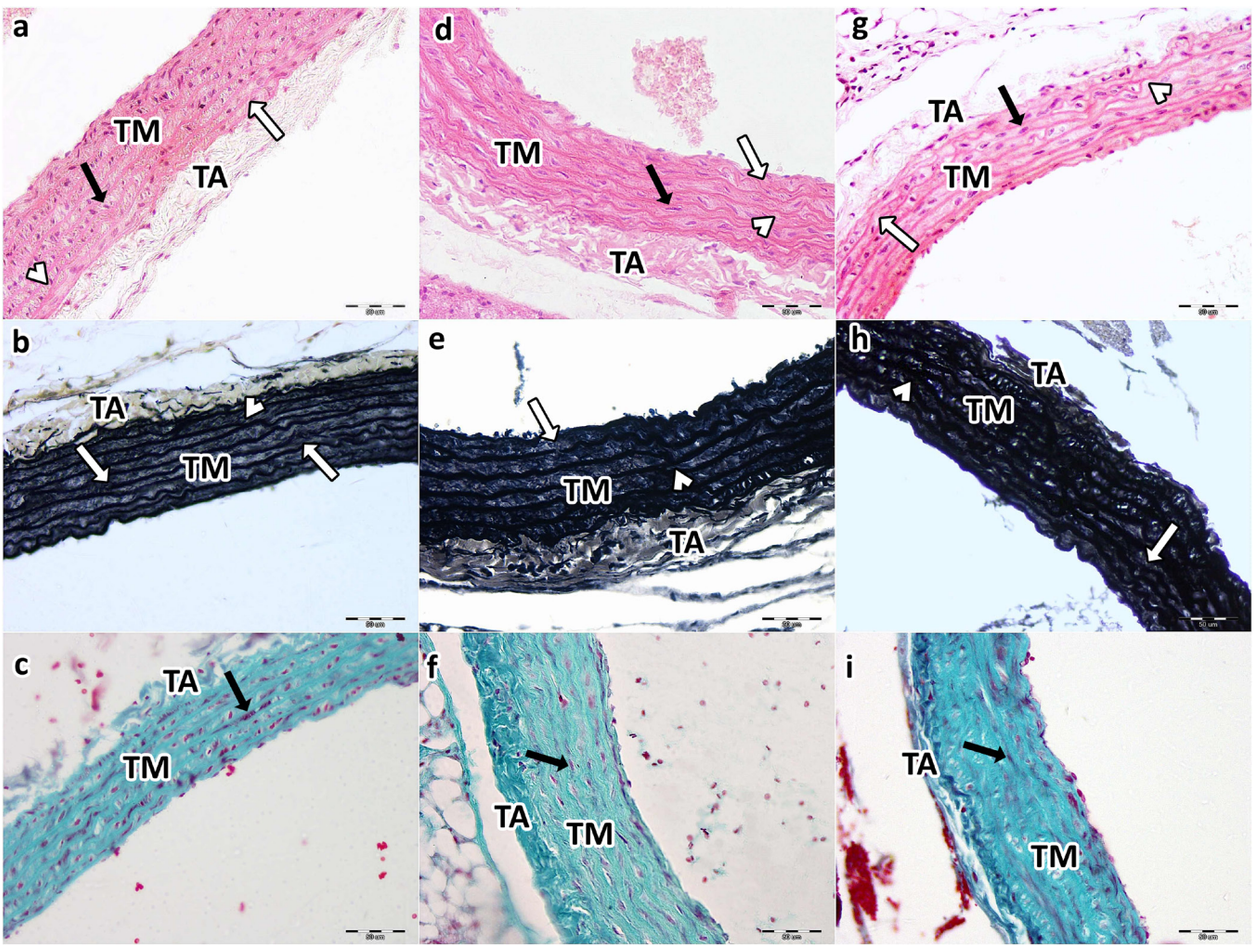

Fig. 2: Photomicrographs of aortic sections of the treated groups showing tunica media (TM) with relative disturbance in the parallel arrangement of the elastic laminae. Notice split (white arrowhead) and ruptured (white arrow) laminae. TA; tunica adventitia, Black arrow; smooth muscles. (a-c); (DM+AC), (d-f); (DM+LN), (g-i); (DM+QR). (a,d,g): H&E-stained sections. (b,e,h): VVG stained sections. (c,f,i): Trichrome stained sections. (Magnification X400).

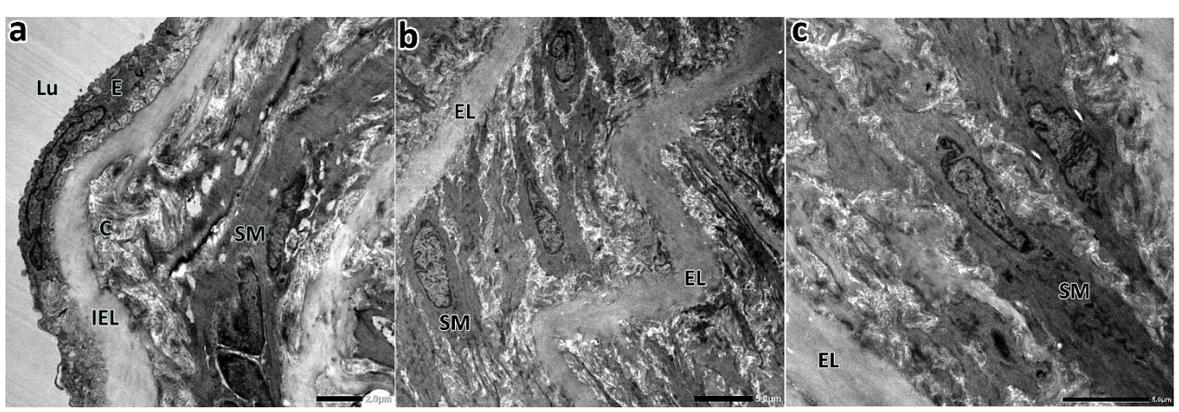

Fig. 3: Electron micrographs of the aortic sections of (normal control) group (a): Showing, endothelial cell (E) lining the luminal surface of the aorta (Lu). Internal elastic lamina (IEL) is separating the tunica intima from the tunica media. C; Collagen fibrils. (b,c): Showing tunica media, the smooth muscle cells (SM) are obliquely oriented in between the concentrically arranged elastic laminae (EL). (Uranyl acetate/lead citrate stain) (a; Magnification X2000. b; Magnification X1000. Magnification X1500.)

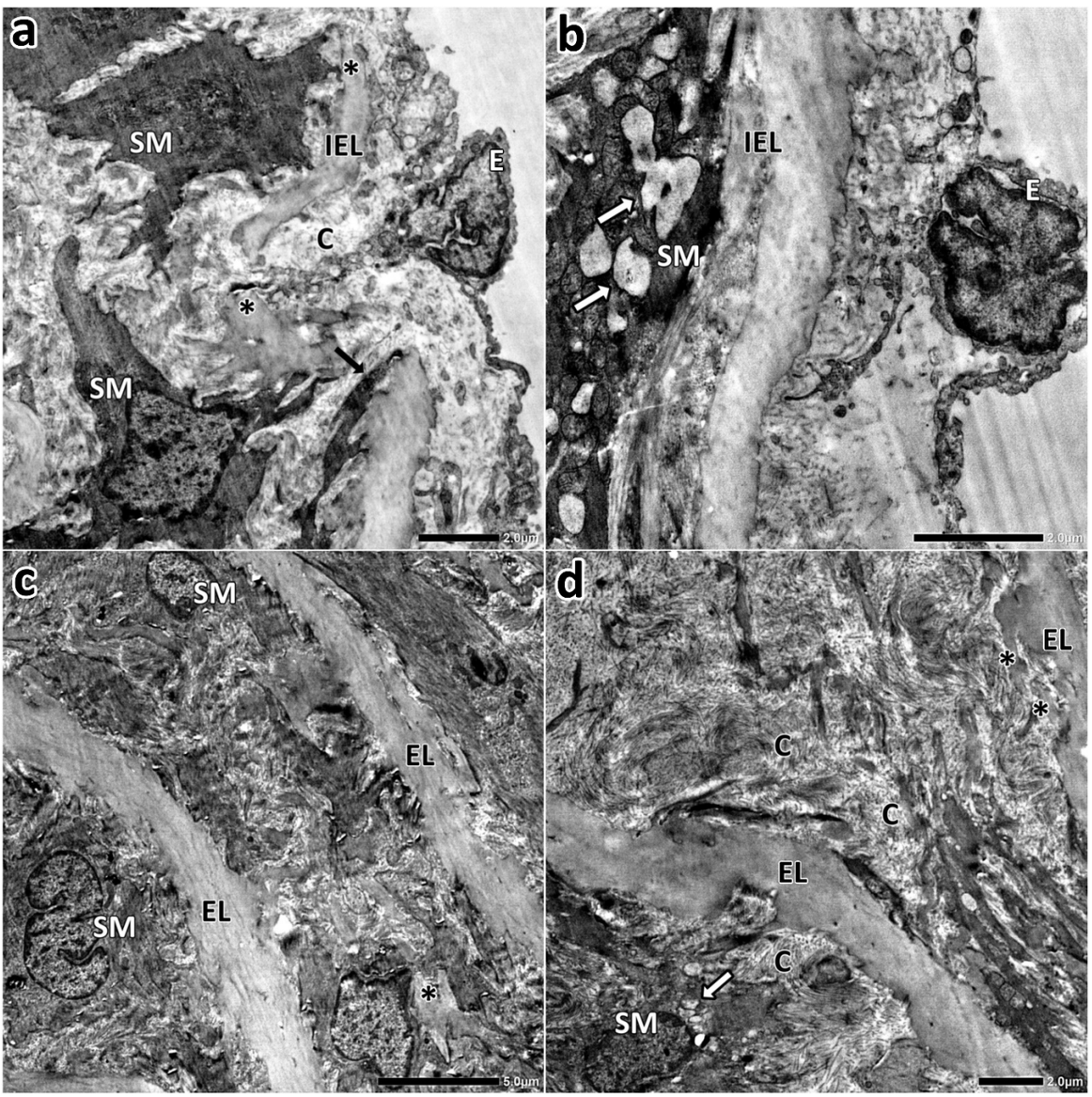

Fig. 4: Electron micrographs of the aortic sections of (DM) group. (a): showing ruptured (*) internal elastic lamina (IEL). Notice disorganized smooth muscle fibers (SM) with fibers invading towards the subendothelial layer (black arrow). Collagen fibrils (C) deposition is markedly increased in subendothelial layer. Contracted cytoplasm and nucleus of the endothelial cell (E) are revealed. (b): Electron micrograph showing, disturbed internal elastic lamina (IEL), smooth muscles fibers (SM) with many vacuoles (white arrow). Contracted cytoplasm and nucleus of the endothelial cell (E). (c,d): Tunica media showing, smooth muscle fibers (SM) with vacuoles (white arrow) and disturbed distribution, losing the oblique orientation to the elastic laminae (EL). Notice ruptured (*) elastic laminae and increased collaged deposition (C) in the extracellular matrix. (Uranyl acetate/lead citrate stain) (a; Magnification X2500. b; Magnification X4000. c; Magnification X1500. d; Magnification X2000.)

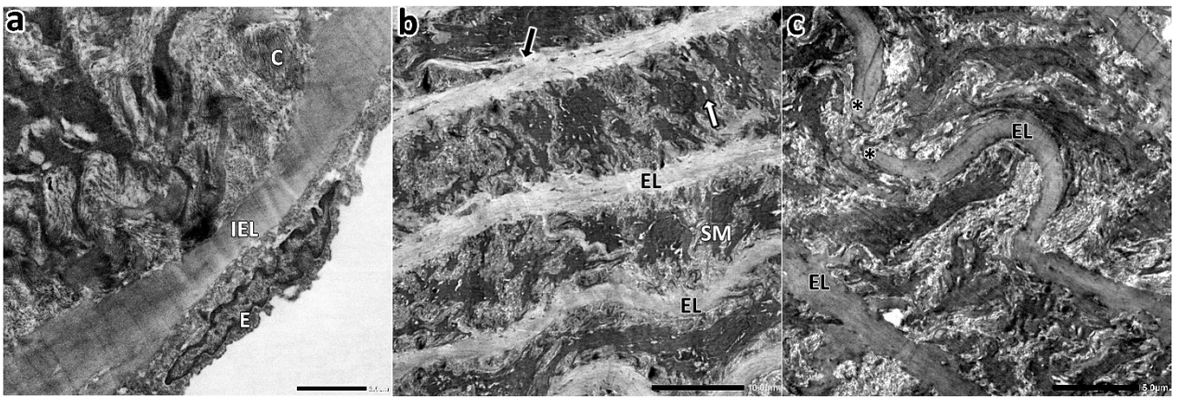

Fig. 5: Electron micrographs of the aortic sections of (DM+AC) group. (a): Electron micrograph showing, endothelial cell (E) lining the luminal surface of the aorta, attached to an intact internal elastic lamina (IEL). C; collagen fibrils. (b,c): Tunica media showing, the smooth muscle fibers (SM) obliquely oriented in between the concentrically arranged elastic laminae (EL) with areas of disturbed distribution. Some muscle fibers are vacuolated (white arrow). Notice split (black arrow) and rupture (*) in elastic laminae. (Uranyl acetate/lead citrate stain) (a; Magnification X3000. b; Magnification X800. c; Magnification X1500.)

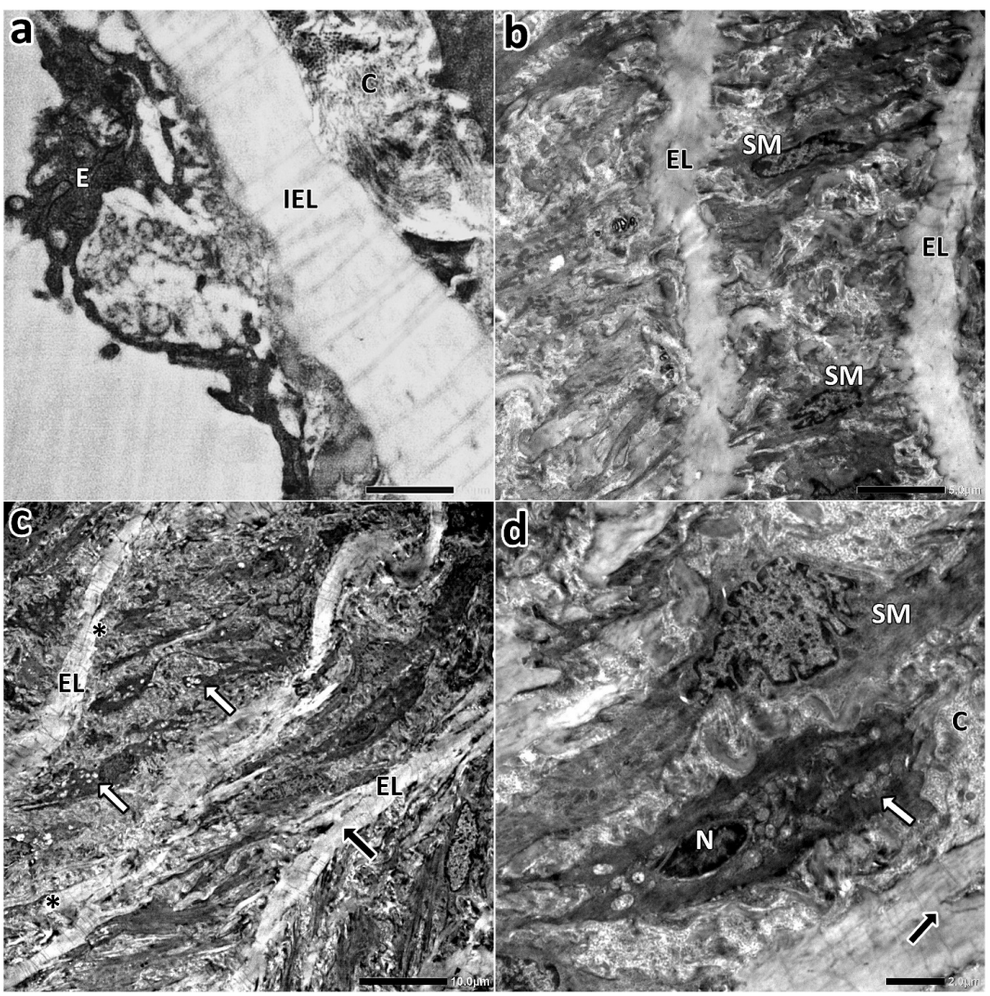

Fig. 6: Electron micrographs of the aortic sections of (DM+LN) group. (a): Electron micrograph showing, endothelial cell (E), attached to intact internal elastic lamina (IEL). (b): Tunica media showing, the smooth muscle fibers (SM) obliquely oriented in between the concentrically arranged elastic laminae (EL). (c): Smooth muscle fibers (SM) with disturbed distribution and vacuolated cytoplasm (white arrow). Notice disturbed organization of elastic laminae (EL) with split (black arrow) and rupture in some parts (*). (d): Electron micrograph showing smooth muscle fiber with vacuolated cytoplasm (white arrow) and pyknotic nucleus (N). C; collagen fibrils. SM; smooth muscle fibers. (Uranyl acetate/lead citrate stain) (a; Magnification X6000. b; Magnification X1200. c; Magnification X600. d; Magnification X2000.)

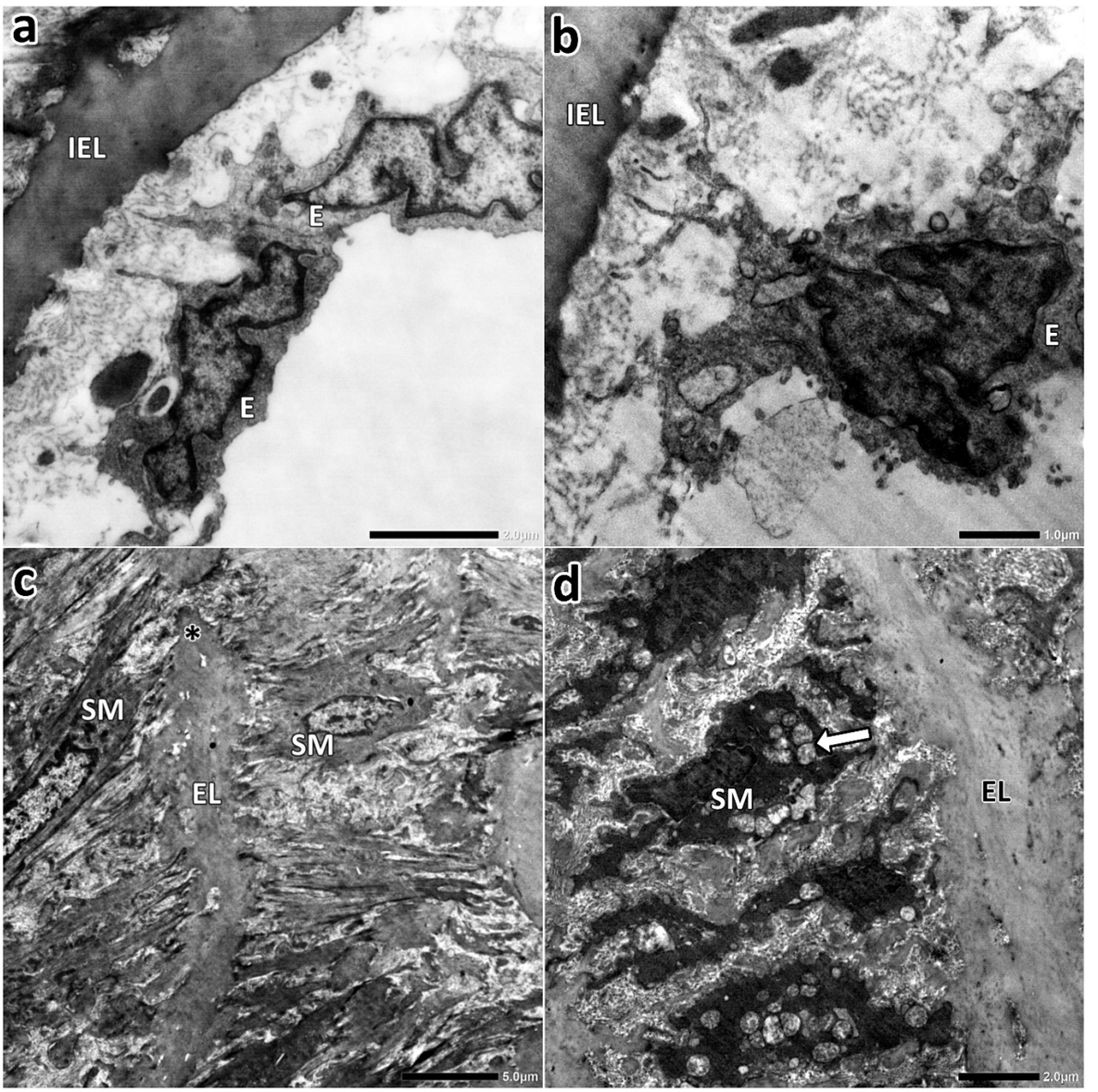

Fig. 7: Electron micrographs of the aortic sections of (DM+QR) group (a): Showing non contracted endothelial cell (E) and intact internal elastic lamina (IEL). (b): Contracted cytoplasm and nucleus of the endothelial cell (E) are revealed. (c): Smooth muscle fibers (SM) obliquely oriented in between the concentrically arranged elastic laminae (EL). Notice rupture in elastic lamina (*). (d): Smooth muscle fibers (SM) with vacuoles (white arrow). EL; elastic laminae. (Uranyl acetate/lead citrate stain) (a; Magnification X4000. b; Magnification X5000. c; Magnification X1200. d; Magnification X2500.)

Table 1: Comparison between the different studied groups according to different biochemical parameters

	Normal (n = 6)	DM (n = 6)	DM+AC (n = 6)	DM+LN (n = 6)	DM+QR (n = 6)
Serum glucose(mg/dL)					
Mean ± SD.	98.5 ± 15.2	322.8$^{\#}$ ± 35.2	119.3$^{@}$ ± 6.4	117.7$^{@}$ ± 14.7	118.8$^{@}$ ± 14.6
Serum insulin (IU/L)					
Mean ± SD.	16.8 ± 2.5	29.1$^{\#}$ ± 2.1	22.2$^{\#@}$ ± 1.9	22.5$^{\#@}$ ± 2.1	22.1$^{\#@}$ ± 2.8
HOMA IR					
Mean ± SD.	2 ± 0.3	6.3$^{\#}$ ± 0.9	3$^{\#@}$ ± 0.2	3$^{\#@}$ ± 0.3	3$^{\#@}$ ± 0.4
HOMA- β %					
Mean ± SD.	135.4 ± 40	29.9$^{\#}$ ± 2.8	113. 3$^{@}$ ± 15.8	107.6$^{@}$ ± 10.5	117.8$^{@}$ ± 19.4
Serum triglycerides (mg/dL)					
Mean ± SD.	29.5 ± 6.2	460.7$^{\#}$ ± 102.2	50.5$^{@}$ ± 6.5	41.7$^{@}$ ± 14	47.3$^{@}$ ± 7.9
Serum cholesterol (mg/dL)					
Mean ± SD.	64 ± 14.3	277$^{\#}$ ± 66.8	54.8$^{@}$ ± 17.1	55.8$^{@}$ ± 15.1	56$^{@}$ ± 13.9
High density lipoprotein (mg/dL)					
Mean ± SD.	42.5 ± 3	27.3$^{\#}$ ± 7	39.5$^{@}$ ± 7.7	38.2$^{@}$ ± 2.5	27.3$^{\#\blacklozenge\$}$ ± 3.7
Low density lipoprotein (mg/dL)					
Mean ± SD.	29.2 ± 4.6	159$^{\#}$ ± 46.9	25.8$^{@}$ ± 3.5	28.2$^{@}$ ± 5.7	24$^{@}$ ± 6

#: Significant with Normal @: Significant with DM ♦: Significant with DM+AC
$: Significant with DM+LN Statistically significant at $p \leq 0.05$

Table 2: Morphometric comparison between the different studied groups

	Normal	DM	DM+AC	DM+LN	DM+QR
TM Thickness	(n = 12)	(n = 12)	(n = 12)	(n = 12)	(n = 12)
Mean ± SD.	62.5 ±3.4	114.5$^{\#}$ ± 6.9	71.7$^{\#@}$ ± 4.4	77.1$^{\#@}$ ± 15.3	62.7$^{@\$}$ ± 2.8
Area% of smooth muscle fibers	(n = 4)	(n = 4)	(n = 4)	(n = 4)	(n = 4)
Mean ± SD.	15.6 ±0.5	26.2$^{\#}$ ± 8.3	14.6$^{@}$ ± 1.6	13.1$^{@}$ ± 4.6	15$^{@}$ ± 1.7
Area% of elastic fibers	(n = 4)	(n = 4)	(n = 4)	(n = 4)	(n = 4)
Mean ± SD.	54.8 ±3.5	38.2$^{\#}$ ± 2.8	47.5$^{\#@}$ ± 2.9	47.4$^{\#@}$ ± 1.3	48.8$^{\#@}$ ± 1.9

TM thickness: Tunica media thickness #: Significant with Normal @: Significant with DM
$: Significant with DM+LN Statistically significant at $p \leq 0.05$

DISCUSSION

Diabetes mellites is a chronic metabolic disease, characterized by hyperglycemia which causes deleterious effects on both the macro- and microvasculature[1]. In our study we examined the potential protective effect of Acarbose, Linagliptin and Quercetin on the aorta of type 2 diabetes mellitus rat model. Diabetes was induced by feeding the rats by HFD for two weeks followed by a single STZ dose. The biochemical parameters showed the metabolic imbalances observed in type 2 DM including, increased serum blood glucose level >200 mg/dL, increased insulin resistance where HOMA-IR was significantly increased in the diabetic group, in addition to the compensatory hyperinsulinemia noticed in such group[31,32]. Moreover, a significant decrease in HOMA-β % when compared to the normal group was noticed. Furthermore, lipid profiles showed a significant rise in TGs, cholesterol and LDL and a decrease in HDL levels when compared to the normal group. These results verified the standard characteristics associated with type 2 DM in such rat model[18,19,31,33,34].

Although diabetic groups received the three drugs showed a significant improvement in these parameters, but the plasma insulin levels, and HOMA-IR were still significantly higher than the normal group. Interesting, the HDL level improved in groups received AC and LN, while the group received QR showed no improvement. No significant differences were revealed between the groups treated with the three drugs in any of these parameters except for HDL level in (DM+QR) group was significantly lower than the two other treated groups. Results of (DM+AC) group were in agreement with Salemi *et al*. 2016 who reported that AC improved the fasting blood glucose and lipid profiles in STZ induced type 2 DM rat model[35]. For the results showed by (DM+LN) group, it was in accordance with Aboulmagd *et al*. 2020 results[36]. While Porras *et al*. 2017 showed results similar to our results for the (DM+QR) group in some parameters[37]. Other studies conducted on mice, showed different results as they showed that QR improved all the lipid profiles including HDL[38].

Light microscopic examination of aortic sections from DM groups showed, thickened TM with proliferation of smooth muscle fibers. This result was confirmed by the morphometric analysis as TM thickness and the area percentage of the smooth muscle fibers, were significantly increased in the DM group when compared to the normal group. Elastic laminae showed disrupted parallel arrangement. Moreover, they appeared relatively thick and some of them are split and ruptured. Morphometric analysis showed a significant decrease in the area percentage of elastic fibers in comparison to normal group. Our results were in accordance with Thent *et al* 2012 who recorded, an increased TM thickness and disordered elastic fibers in the aorta of STZ-induced diabetic rats[5]. Besides, Salum *et al*. 2012 noticed irregular arrangement of elastic laminae in aorta of diabetic rats[39]. These results are considered as premature atherosclerotic lesions, which may be induced by the hyperglycemic state in DM[5].

These results were confirmed by the electron microscopic examination. The aorta of diabetic group revealed disturbance in its structure. Tunica intima showed, endothelial cells with contracted cytoplasm and nucleus and increased collagen deposition in the subendothelial layer. Disturbed and ruptured internal elastic lamina was revealed. Some smooth muscle fibers were seen invading towards the subendothelial layer. Tunica media showed disturbed smooth muscle fibers distribution, losing their oblique orientation to the elastic laminae, with increased vacuolation of their cytoplasm. Collagen fibrils deposition is markedly increased in between smooth muscle fibers and around elastic laminae. Other studies revealed results similar to these changes in diabetic animals[40]. In addition, these findings are present in atherosclerotic aorta and in the aorta with increased vascular stiffness[4,41].

Hyperglycemia is accused to be the main factor in the pathogenesis of diabetic complications. Pathological changes observed in the vasculature of diabetic animals and humans occurs through different mechanisms as hyperglycemia induces oxidative stress which promotes the formation of advanced glycosylation end products (AGEs) and protein kinase C (PKC) activation[42]. It is also reported that endothelial dysfunction precedes the development of micro- and macrovascular complications associated with Type 2 diabetes. Endothelial dysfunction is caused by the increased AGEs formation, activation of protein kinase C and increased pro-inflammatory signaling pathways. Interestingly, hyperglycemia and insulin resistance are acknowledged to be of the factors causing diabetes-associated atherosclerosis, due to the pro-inflammatory environment created by them[43–45].

On the other hand, the aorta from diabetic rats treated with the three drugs, revealed better structure as compared to the DM group. Comparing the results revealed by the three drugs, no noticeable differences were recorded except that (DM+QR) group showed better TM thickness results.

Each of these drugs act by a different mechanism. Acarbose, an inhibitor of intestinal alpha-glucosidase reduces hyperglycemia by lowering intestinal glucose absorption[7]. It is reported that the improvement of hyperglycemia is associated with a decrease in the levels of inflammatory markers, amelioration of the endothelial dysfunction and the arterial stiffness[7,9]. Chan *et al*. 2016 reported results similar to our results, as they stated that AC decrease smooth muscle proliferation in aortic arch of high cholesterol fed rabbits[46].

Linagliptin improves hyperglycemia by DPP-4 inhibition, causing reduction of glucose output in the liver [10]. In addition, preclinical indication suggests that DPP-4 inhibitors have cardiovascular benefits independent of glycemic lowering effects[4]. Our results were in accordance with what Manrique *et al*. 2016, who stated that DPP-4 inhibition prevented aortic fibrosis and the increased medial thickness induced in the aorta of mice fed by western diet; which is a diet rich in fat and simple sugars[4].

The flavonoid Quercetin is present in many natural sources, making it a good choice as antidiabetic drug[12]. It is stated that it modulates the hyperglycemic state in different ways including, inhibition of intestinal glucose absorption, modifying insulin secretion and improving glucose consumption in peripheral tissues[13]. In addition, other studies reported that Quercetin has a protective effect on the cardiovascular system, which is assumed to be due to its antioxidative effect causing endothelium protection and anti-inflammatory impact[12,47]. Similar to our results, Kondo *et al*. 2020 reported the protective effect of Quercetin on elastin degradation in mice with an induced aortic disease[47].

CONCLUSION

Our study revealed that HFD-STZ induced type 2 diabetes mellitus rat model, showed alteration in the structure of the aorta. These histological findings were also recorded in atherosclerotic aorta. The three tested antidiabetic drugs; Acarbose, Linagliptin and Quercetin, showed an ameliorative effect on the structure of the aorta but no noticeable differences were recorded between them.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

دراسة نسيجية لمقارنة التأثير الوقائي المحتمل للأكاربوز ولیناجلیبتین وكيرسيتين على الشريان الأبهر للجرذان المصابة بداء السكري من النوع ٢

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المقدمة: مرض السكري (DM) هو مشكلة صحية عالمية تؤثر على السكان من مختلف الأعمار، مما يلقي بعبء اقتصادي كبير على نظام الرعاية الصحية في جميع أنحاء العالم. يتميز هذا المرض في الغالب بفرط سكر الدم الذي يسبب آثارًا ضارة على كل من الأوعية الدموية الكلية والميكروية. يشمل العلاج الدوائي العديد من الأدوية بآليات عمل مختلفة. تهدف هذه الأدوية إلى السيطرة على حالة ارتفاع السكر في الدم وبالتالي قد تحمي الأوعية من تأثيرها الضار.
الهدف من العمل: الهدف من دراستنا هو مقارنة التأثير الوقائي المحتمل لثلاثة من الأدوية المضادة لمرض السكر. أكاربوز ، ليناجليبتين وكيرسيتين ، على الشريان الأبهر لجرذان مصابة بداء السكري من النوع ٢.
الطريقة وخطة العمل: تم تقسيم أربعين من الجرذان الذكور البيضاء إلى ٥ مجموعات. مجموعتان ضابطة ؛ مجموعة الضابطة الطبيعية (المجموعة الطبيعية) والمجموعة الضابطة بمرض السكري (مجموعة DM). تم تحفيز مرض السكري عن طريق تغذية الفئران بنظام غذائي عالي الدهون (HFD) لمدة أسبوعين متبوعًا بجرعة واحدة من الستربتوزوتوسين (STZ). تلقت ثلاث مجموعات من مرضى السكري علاجات مختلفة. مجموعة مرضى السكر عولجت بالأكاربوزمجموعة (DM + AC) ، مجموعة مرضى السكر عولجت بليناجليبتين مجموعة (DM + LN) ومجموعة مرضى السكري عولجت بالكيرسيتين مجموع (DM + QR) . تم أخذ عينات الدم للتقييم البيوكيميائي وأخذت عينات من الشريان الأبهر للتحليل النسيجي والمورفومتري.
النتائج: أظهرت المجموعة المصابة بمرض السكري نتائج نسيجية مماثلة لنتائج لوحظت في تصلب الشريان الأبهر. و قد أظهرت الأدوية الثلاثة المضادة لمرض السكر المختبرة أكاربوز وليناجليبتين وكيرسيتين تأثيرًا محسنًا على نسيج الشريان الأبهر ولكن لم يتم تسجيل اختلافات ملحوظة.
الأستنتاج: كشفت دراستنا أن التغيرات التي طرأت على نسيج الشريان الأبهر للمجموعة المصابة بداء السكري قد تحسنت في المجموعات التي تلقت الأدوية الثلاثة مع عدم وجود اختلافات ملحوظة بينها.