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Original Article

The Protective Role of Pentoxifylline on Diabetes-Induced Vasculopathy in the Male Albino Rat

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ABSTRACT

Background: Diabetes mellitus is one of the chronic metabolic diseases, which affects about 450 million people in the world with one of them dies every 6 seconds from diabetic related macro and micro-vascular complications as cerebrovascular disease and nephropathy.

Aim of the work: This study was designed to investigate the protective effects of pentoxifylline (PTX) on macro-vascular complications as in aorta and renal artery in a streptozotocin induced diabetic rat model.

Material and Methods: Forty male albino rats randomly allocated into four groups ten rats each; group I(control group), group II (PTX group), group III (Diabetic group) and group IV (Diabetic + PTX group). Experimental diabetes was induced by single dose of streptozotocin (50 mg/kg) intraperitoneally. PTX and Diabetic + PTX groups received PTX in a single daily dose of (100 mg/kg) in the drinking water. At the end of the study, rats were anaesthetized and aorta and renal artery were extracted for H&E, Orecin and immunohistochemical stains to be examined by light microscope.

Results: The main histological alterations in diabetic rat aorta and renal arteries were high significant thickening in the tunica media, smooth muscle proliferation, vacuolation, disruption of elastic fibers in aorta, strong positive reaction of tunica media by anti-NF-κb, and increased thickness of smooth muscle fibers by anti-α-SMA. The Diabetic group treated with pentoxifylline showed significant decrease in these findings.

Conclusion: Pentoxifylline can ameliorate the structural changes in blood vessels resulting from diabetes mellitus.

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Key Words: α-SMA, diabetes, NF-κb, pentoxifylline.

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INTRODUCTION

Diabetes mellitus (DM) is a long-lasting metabolic disease that tends to spread throughout the world[1]. The disease leads to development of multisystem complications as macro and micro-vascular changes[2], which include vascular retinopathy, nephropathy and neuropathy. Also macro-vascular complications may have a role in ischemic heart disease, cerebrovascular disease and peripheral vascular disease[3].

The pathophysiological effects of diabetes on blood vessels include an impaired vasodilatation due to decreased nitric oxide (NO), smooth muscle cells (SMCs) proliferation, chronic inflammation, atherosclerosis, hemodynamic dysregulation, increased platelet aggregation and impaired fibrinolytic ability[3,4].

The underlying mechanisms of these vascular complications are oxidative stress and overproduction of reactive oxygen species (ROS)[5].

Pentoxifylline (PTX) is a synthetic methylxanthine, prescribed for cerebrovascular and peripheral vascular diseases[6]. PTX improves the effectiveness of microcirculation, decreases platelet aggregation and lowers plasma viscosity[7]. It inhibits the phosphodiesterase and can cause vasodilatation of blood vessels by endothelium- dependent and independent mechanisms[8].

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Pentoxifylline decreases oxidative stress and inhibits lipid peroxidation[9]. It also reduces the adhesion of neutrophils to endothelial cells and lowers the free radicals production[10].

AIM OF THE WORK

The aim of this study was to demonstrate possible protective effect of pentoxifylline on diabetic vasculopathy in the rat model.

MATERIAL AND METHODS

Animals:

Forty adult male albino rats were used weighing 150-200 gm each. The rats were bred in Pharmacology Department, Ain Shams University. They were housed in medium sized cages (4 animals per cage) and left 1 week for acclimatization. They had a free access to water, the standard diet, and kept under regular dark and light cycles. Blood glucose test was done for all the animals in order to exclude any possibility of diabetes.

Ethical considerations:

The Experiment was done in accordance with guidelines approved by the Committee of Animal Research Ethics, Ain Shams University College of Medicine.

Drugs:

1- Streptozotocin (STZ): From Sigma–Aldrich Co., STZ is the most common drug which is used to induce type I or II DM in experimental animals. It leads to emergence of diabetes clinically within 3-4 days[11]. A single dose of 50 mg/kg/bw (body weight; bw) of STZ in 0.1 mol/L of citrate buffer (pH 4.5) was injected intraperitoneally into each rat. The rats were kept on glucose 5% for the next 24 h to prevent hypoglycaemia. The blood glucose level was measured three days after the administration of STZ using a digital glucometer. The rats with a blood sugar level of 270 mg/ml or greater were accepted as diabetic[12,13].

2- Pentoxifylline: From Sigma–Aldrich Co., it was given in oral daily dose of 100 mg/kg/bw in the drinking water[14].

Animal groups:

The animals were randomly allocated into 4 groups (10 rats \ each).

Group I: (Control group): They received no medications.

Group II: (PTX group): They received pentoxifylline in a single daily dose throughout the duration of experiment.

Group III: (Diabetic group): The diabetic rats left without any further medication for a period of 7 weeks.

Group IV: (Diabetic + PTX group): The diabetic rats received pentoxifylline as a protective treatment starting from one week after induction of diabetes and lasting for further 7 weeks.

The experimental period was 8 weeks, which was sufficient for induction of diabetic vasculopathy in the rats[15].

At the end of the experimental period, the animals were anaesthetized by 7 mg/ kg/bw thiopental sodium intraperitoneal[16], the anterior abdominal wall was incised and specimens of the abdominal aorta (above renal artery level) and the distal renal artery were carefully dissected out and obtained.

Histological studies:

1- Tissue processing for light microscopic study:

The fixation of the collected specimens was done by 10% neutral formalin over one night. After that; the tissues were dehydrated in ethanol (ascending grades), cleared in xylol and then embedded in blocks of paraffin. Serial sections of 5 micrometer thickness were cut and stained with Hematoxylin and Eosin (H&E) for routine histological examination[17]. Other sections were stained with Orecin stain for demonstration of elastic laminae[18].

2- Immunohistochemical study:

Some sections from all the specimens were picked upon positive slides for immunohistochemical staining using:

A- Immunohistochemistry for Nuclear factor kappa (Anti-NF-κb):

The tissue sections were deparaffinized and antigen retrieval was done. Endogenous peroxidase was clenched by 3% hydrogen peroxide solution for 10 min and washed by phosphate puffer, followed by incubation of sections with rabbit polyclonal antibody (Cat. No. RB-1638-R7, Ready to use, Lab Vision), then washing. The sections were covered by 4-5 drops of secondary antibody (Ultra Vision biotinylated goat anti-polyvalent antibody). The technique used in staining was a standard avidin-biotin complex staining procedure. Then, the slides were counter stained with hematoxylin[19].

B- Anti-Alpha smooth muscle actin antibody (anti-α-SMA):

After deparaffinization of sections, antigen retrieval was done. The sections were incubated in hydrogen peroxide (3%) for 5 minutes to eliminate the endogenous peroxidase. Then they were incubated in the primary antibody (anti-actin antibody; actin, smooth muscle Ab-1 in dilution of 1:200) at room temperature. The secondary antibody used was En Vision + System HRP anti rabbit. After that, counter staining was done using Hematoxylin[20].

Morphometric Study:

Image Analysis

Morphometric study was done by measuring the thickness of the tunica intima and the tunica media in the wall of both abdominal aorta and distal renal artery. Measuring was done from at least three H & E stained slides for each animal. For each slide, measuring was done from 3-5 points along the aortic and renal artery sections. The measured tunica intima was the area that extends from the surface of endothelium to the first elastic lamina detected[20]. Regarding the measuring of the thickness of tunica media, it was done from the first elastic lamina to the last elastic lamina[21]. Measures were done using Leica EZ4D stereomicroscope with an internal digital camera using ×40 objective lens. The digital camera is programmed with a software image analyzer[20].

Statistical Analysis

The mean values and standard deviation (SD) were calculated by the SPSS statistical program version 20 (IBM corporation, New York, USA). Analysis of variance (one way ANOVA) was done followed by Tukey Post Hoc test to compare the groups. Significance of data was determined by the probability value (*P. value*). The difference was non-significant at $P > 0.05$, significant ≤ 0.05 and highly significant ≤ 0.001.

RESULTS

Light microscopic examination of sections of the abdominal aorta stained with H&E. Group I (control group) showed that the aorta consisted of three layers; namely tunica intima, tunica media and tunica adventitia. The tunica intima appeared as a thin continuous layer of squamous endothelial cells with flattened nuclei resting on the internal elastic lamella. The tunica media was the thickest one and consisted of SMCs intermingled with elastic fibers. The SMCs appeared spindle shaped cells with single oval nuclei .The tunica adventitia appeared as the outermost layer and consisted of dense irregularly arranged connective tissue (Fig. 1A). Group II (PTX group) showed the same histological picture as control group (Fig. 1B). While, the diabetic group (group III) revealed irregular endothelial lining of tunica intima, the internal elastic lamella was disrupted in some areas and showed SMCs migration through it. The tunica media became apparently thickened disorganized and showed SMCs proliferation with bizarre shaped nuclei; some became rounded and dark. Some SMCs showed perinuclear halo and cytoplasmic vacuolation. Tunica adventitia consisted of sparse, widely separated connective tissue (Fig.1C). The group IV (Diabetic+ PTX) showed marked reduction in histological alternation; tunica intima became almost normal, tunica media showed marked decrease in SMCs proliferation and minimal vacuolation and tunica adventitia became less separated (Fig.1D).

Orecin stained sections of rat abdominal aorta showed that the elastic fibers in tunica media of the control group (group I) and PTX group (group II) consisted of regularly distributed, parallel wavy lamellae (Fig. 2A, B). Sections in group III (diabetic group) showed that the elastic fibers became flattened and showed areas of

disruption and fragmentation with network of thin fibrils (Fig. 2C). The diabetic group treated with PTX showed regaining of the normal organization with a minimal flattening of elastic fibers in aortic tunica media (Fig. 2D).

Examination of sections of the distal renal artery stained with H&E. Group I (control group) showed that the distal renal artery consisted of the three characteristic layers as aorta. The tunica media consisted of SMCs mainly and few elastic fibers (Fig.3A). Group II (PTX group) showed the same histological picture as the control group (Fig.3B). Group III (diabetic group) showed that the tunica media became apparently thickened and disorganized, some SMCs showed perinuclear halo and cytoplasmic vacuolation and the tunica adventitia became widely separated (Fig.3C). The degenerative changes became markedly decreased in group IV (Diabetic+ PTX) compared to the diabetic group (Fig.3D).

Examination of sections of the aorta and the distal renal artery Immunohistochemical stained with anti NF-κB control group showed a negative reaction in the tunica intima and tunica media which appeared in the form of a negative nuclear staining and a faint cytoplasmic staining of the endothelial cells and SMCs (Figs. 4A, 5A). In PTX group showed negative reaction as the control group (Figs. 4B, 5B). While the diabetic group showed a strong positive nuclear reaction and a massive cytoplasmic staining (Figs. 4C, 5C). The diabetic group treated with PTX showed a decreased reaction as compared to the diabetic group (Figs. 4D, 5D).

Immunohistochemical sections of the aorta and the distal renal artery stained with anti-α-SMA, the control group revealed positive reaction of smooth muscle fibers in tunica media (Figs. 6A, 7A). The group II (PTX group) showed the same reaction as control group (Figs. 6B, 7B). The diabetic group III showed positive smooth muscle fibers in the tunica media which became longitudinally arranged instead of parallel, and some reaction was present in the tunica intima denoting SMCs migration to it (Figs. 6C, 7C). In diabetic group treated with PTX their thickness were decreased compared to the diabetic group and restored the normal arrangement parallel to the tunica intima (Figs. 6D, 7 D).

Morphometeric results:

A- Measuring thickness of the tunica media of the aorta:

The mean thickness of the tunica media of the abdominal aorta was 57.43±5.15 µm in control group, 56.31 ± 3.74µm in PTX group, 80.65 ± 6.95µm in diabetic group and 73.40± 3.74µm in DM + PTX group. There was a highly statistical significant increase in the mean thickness of the diabetic group as compared to the control group (*P value <0.001*), highly significant increase DM+PTX group as compared to the control group (*P value <0.001*) and a highly significant decrease DM+PTX group as compared to the diabetic group (*P value <0.001*) (Table1).

B- Measuring thickness of the tunica media of the distal renal artery:

The mean thickness of the tunica media of the distal renal artery was 27.40± 4.02µm in the control group, 27.93± 4.19µm in PTX group, 31.19 ± 2.4µm in the diabetic group and 28.56 ± 2.84µm in DM+PTX group. There was highly statistical significant increase in the mean thickness of the diabetic group as compared to the control group (*P value <0.001*), non-significant increase DM+PTX group as compared to the control group (*P value= 0.555*) and significant decrease DM+PTX group as compared to the diabetic group (*P value=0.018*) (Table 2).

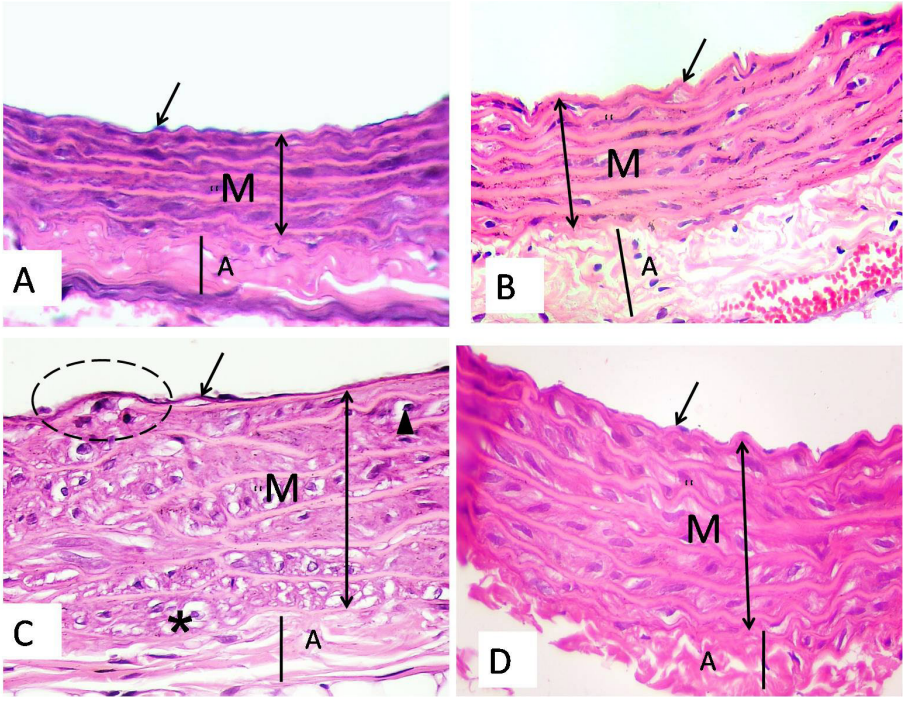

Fig. 1{A-D}: Photomicrographs of cross sections in the rat abdominal aortae. 1A; Control group showing the normal histological picture of aorta; formed of 3 layers; tunica intima, tunica media & tunica adventitia.1B; PTX group showing a similar histological picture as the control group. 1C; Diabetic group showing an irregular endothelial lining of the tunica intima with an area rupture of the internal elastic lamella and a smooth muscle migration through it (dotted circle), an apparent thickening of the tunica media with SMCs vacuolation (*), and a widely separated tunica adventitia. Notice some SMCs nuclei became dark pyknotic (arrow head).1D; Diabetic + PTX group showing a reduction of degenerative changes, the tunica intima and the tunica adventitia became almost normal and tunica media showed a minimal vacuolation. Note: Tunica intima (black arrow), Tunica media (M), Tunica Adventitia (A) (H&E X400).

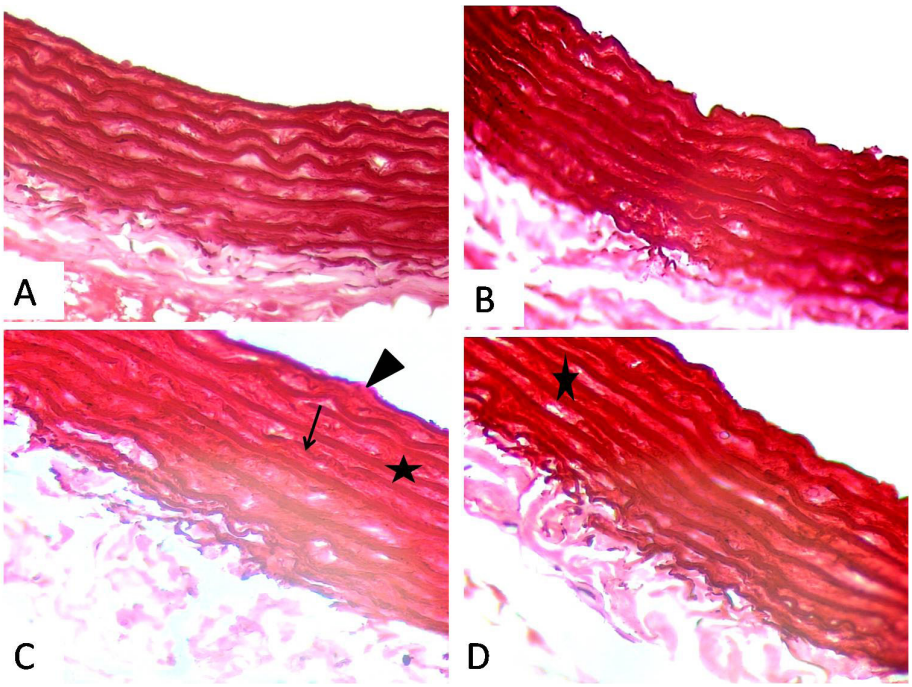

Fig. 2 {A-D}: Photomicrographs of cross sections in rat abdominal aortae. 2A; Control group showing thick parallel wavy elastic fibers. 2B; PTX group showing the same histological picture as the control group.2C; Diabetic group showing an area of disruption of the internal elastic lamina(arrow head), flattening (star) and fragmentation of the elastic fibers which made of thin fibrils(black arrow). 2D; Diabetic + PTX group showing a minimal flattening of the elastic fibers (star). (Orecin X400).

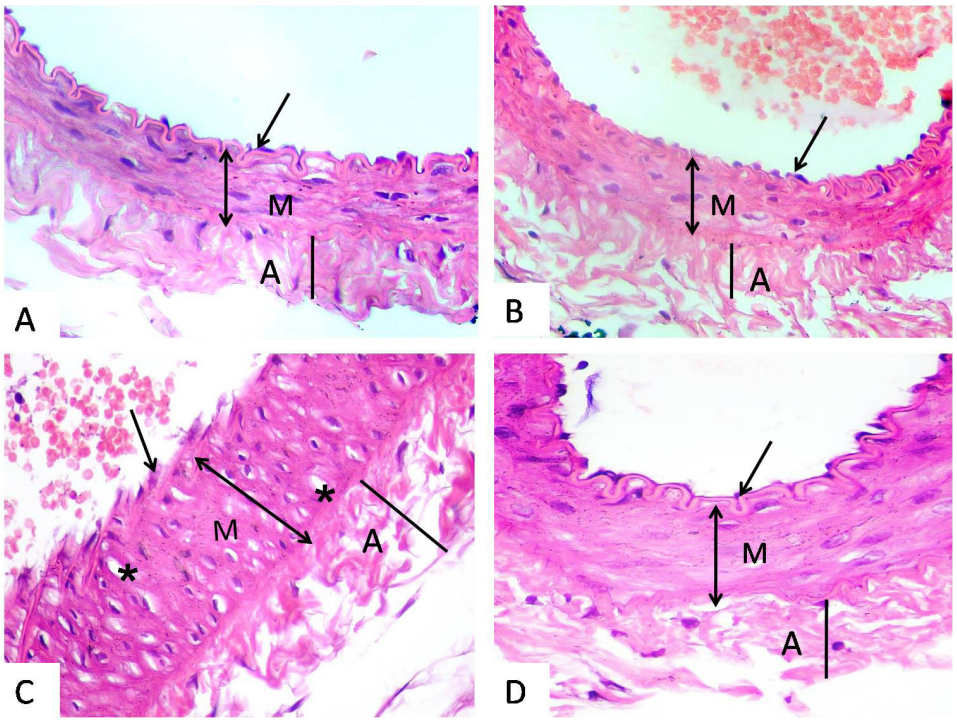

Fig. 3 {A-D}: Photomicrographs of cross sections in the rat distal renal arteries. 3A; Control group showing a normal histological picture of the renal artery formed of 3 layers; tunica intima, tunica media and tunica adventitia. 3B; PTX group showing a similar histological picture as control group. 3C; Diabetic group showing an apparent thickening of the tunica media and vacuolation of SMCs (*). 3D; Diabetic+PTX group showing a restoration of the normal histological architecture. Note; Tunica intima (black arrow), Tunica media (M), Tunica Adventitia (A) (H&EX400).

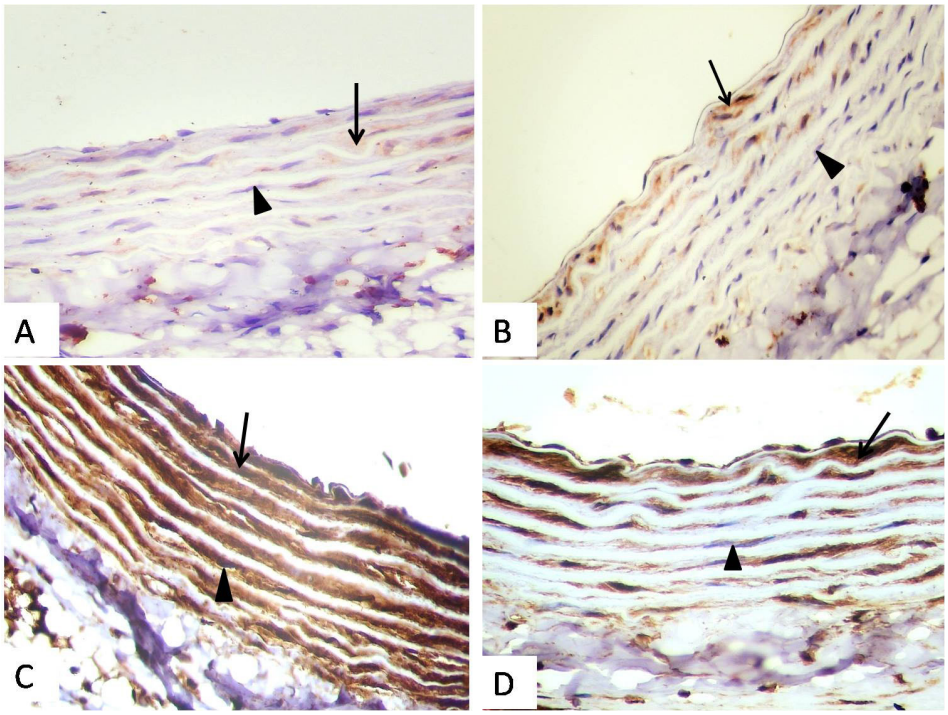

Fig. 4 {A-D}: Photomicrographs of cross sections of the abdominal aortae of the adult male albino rats stained immunohistochemical with anti NF-κB.4A; Control group showing negative nuclear reaction and scanty cytoplasmic staining of endothelial and SMCs. 4B; PTX group showing similar reaction as control group. 4C; Diabetic group showing a strong positive nuclear and cytoplasmic reaction. 4D; Diabetic + PTX group showing a decreased intensity of the nuclear and cytoplasmic staining compared to the diabetic group. Note; Nuclear reaction (arrow head), cytoplasmic reaction (black arrow) (Immunohistochemistry with Anti-NF-κB X400).

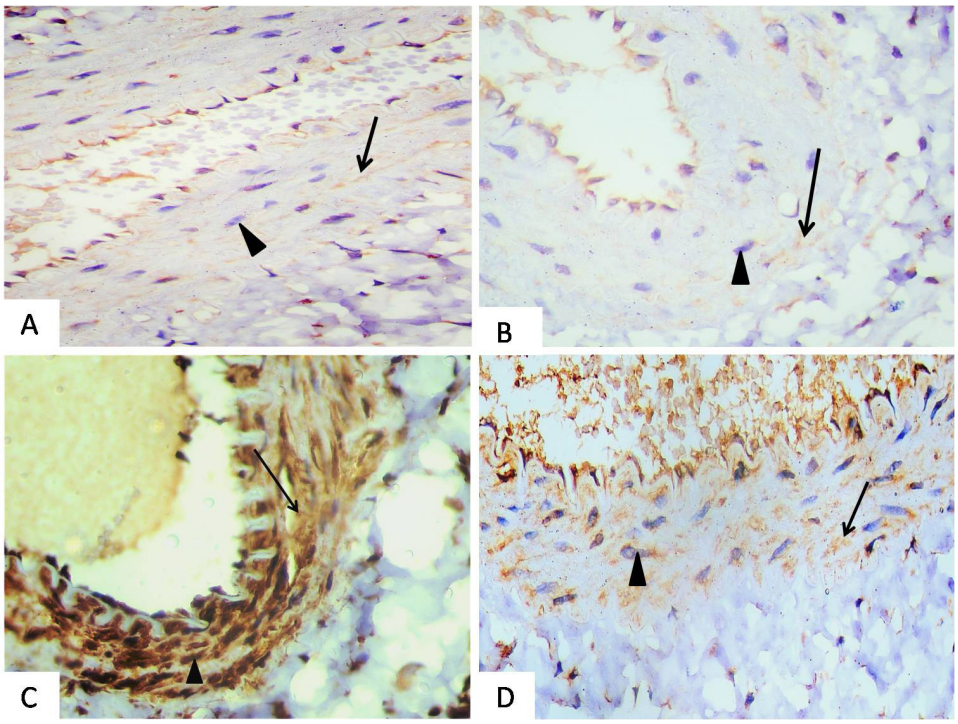

Fig. 5 {A-D}: Photomicrographs of cross sections in the distal renal arteries of the adult male albino rats. Control group showing a negative nuclear reaction and scanty cytoplasmic staining.5B; PTX group showing a similar reaction as the control group. 5C; Diabetic group showing a strong positive nuclear and cytoplasmic staining. 5D; Diabetic + PTX group showing a mild nuclear reaction and cytoplasmic reaction. Note: Nuclear reaction (arrow head), cytoplasmic reaction (black arrow) (Immunohistochemistry with Anti-NF-κB X400).

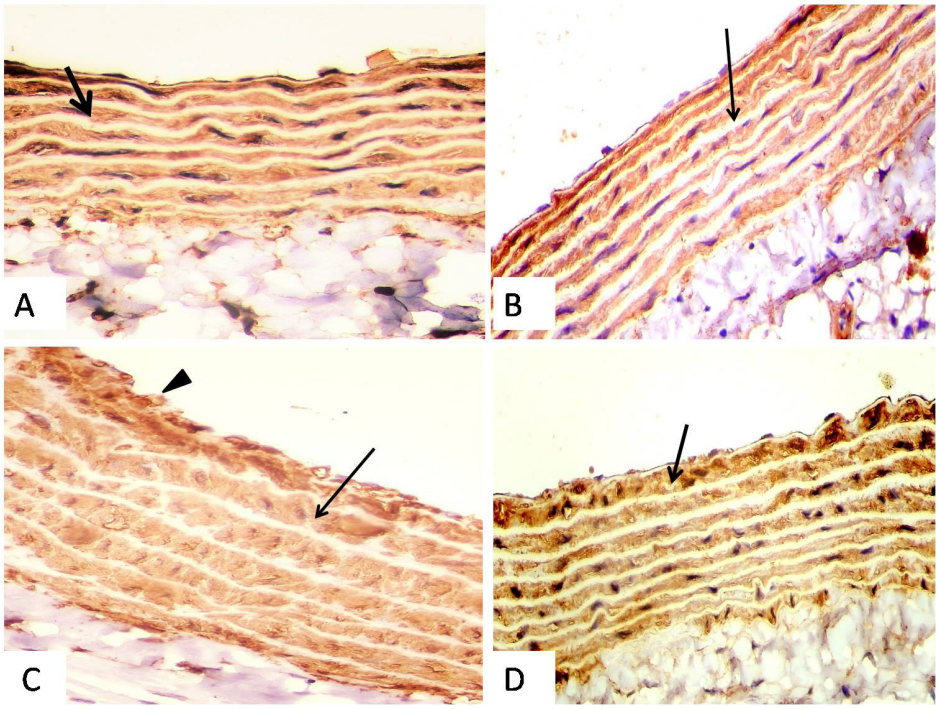

Fig. 6 {A-D}: Photomicrographs ofcross sections in the rat abdominal aortae. 6A; Control group showing apositive reaction of smooth muscle fibers present in the tunica media (black arrow). 6B; PTX group showing the same reaction as thecontrol (black arrow). 6C; Diabetic group showing proliferation and hypertrophy of individual muscle fiber laminae present in-between the negative reacted elastic fibers in thetunica media (black arrow) and smooth muscle migration to the tunica intima (arrow head). 6D; Diabetic + PTX showinga decreased thickness of smooth muscle fibers than that of thediabetic group (black arrow). (Immunohistochemistry with Anti -α-SMA X400).

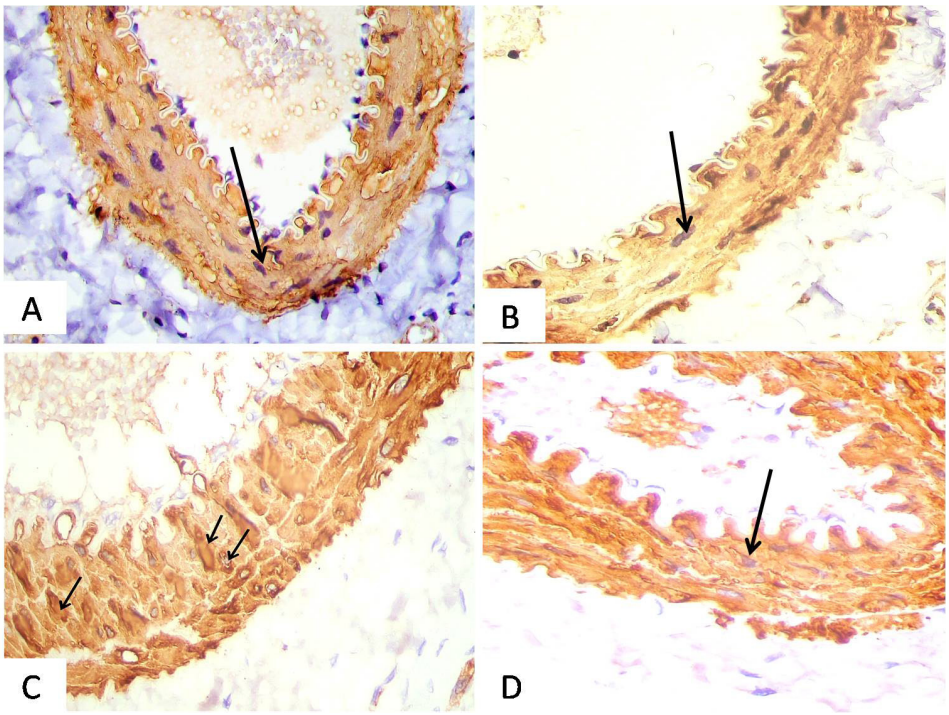

Fig. 7 {7A-7D}: Photomicrographs of cross sections in the rat distal renal arteries. 7A; Control group showing a positive reaction of the smooth muscle fibers in the tunica media arranged parallel to the tunica intima(black arrow). 7B; PTX group showing the same reaction as the control group (black arrow). 7C; Diabetic group showing a thickened tunica media induced by proliferation of smooth muscle cells (discussion) which become perpendicularly arranged to the tunica intima (black arrow). 7D; Diabetic + PTX group showing a decreased thickness of the tunica media than that of the diabetic group. The smooth muscle cells regain the normal parallel arrangement (black arrow). (Immunohistochemistry with Anti-α-SMA X400).

Table 1: Mean thickness of the tunica media of the aorta in µm for all the groups.

Groups	Mean	±	SD	*P value*
Control	57.43	±	5.15	
PTX	56.31	±	3.74	0.817
DM	80.65	±	6.95	<0.001**
PTX+DM	73.40	±	3.74	<0.001**a <0.001**b

Non-significant difference $P > 0.05$
* Significant difference $P \leq 0.05$
** High significant difference $P \leq 0.001$
a: Compared with the control group
b: Compared with the diabetic group.

Table 2: Mean thickness of the tunica media of the renal artery inµm for all the groups.

Groups	Mean	±	SD	*P value*
Control	27.40	±	4.02	
PTX	27.93	±	4.19	0.935
DM	31.19	±	2.4	<0.001**
PTX+DM	28.56	±	2.84	0.555a 0.018*b

Non-significant difference $P > 0.05$
* Significant difference $P \leq 0.05$
** High significant difference $P \leq 0.001$
a: Compared with the control group
b: Compared with the diabetic group

DISCUSSION

The aim of this study was to investigate the possible protective effect of pentoxifylline on diabetic vascular complications. The pathophysiological mechanism of the diabetic vasculopathy was due to formation of advanced glycation end products (AGEs) which form non enzymatic reaction of glucose with proteins, lipids and nucleic acids. In addition to high flux of glucose in the endothelial cells leads to generation of reactive oxygen species[22].

Nitric oxide (NO), which is an important vasodilator with anti-inflammatory, anti-thrombotic and anti-oxidant effect, was inactivated by ROS leading to endothelial dysfunction[23,24]. Endothelial dysfunction means an impairment of the ability of the endothelium to properly maintain vascular homeostasis due to loss of NO bioavailability, and it precedes atherosclerotic vascular degeneration[25].

According to the present findings, the diabetic aorta showed an irregular endothelial lining of the tunica intima with areas of disruption of the internal elastic lamella and SMCs migration through them. The tunica media became high significantly increased in thickness, disorganized, showed proliferation and vacuolation of SMCs with dark pyknotic nuclei, and the elastic fibers became irregularly arranged, flattened and disrupted. Tunica adventitia became widely separated. These findings are in accordance with the previous studies who explained that the intimal changes and endothelial damage in diabetes were due to over production of ROS[26]. The biochemically damaged endothelium became permeable, immunogenic and thrombogenic leading to the adherence of platelets to it and the release of platelet derived growth factor (PDGF) which is a powerful mitogen and attractant for vascular SMCs[27]. While, the previous studies explained that increased thickness of tunica media of diabetic blood vessels was due to proliferation of SMCs and increased production of extracellular matrix[28]. The proliferation of SMCs might be due to an activation of protein kinase C as a result of oxidative stress in diabetes mellitus. Also, PDGF might stimulate proliferation of SMCs[29].

The vacuolated cells seen in the tunica media of diabetic group were foam cells. These cells are the SMCs and macrophages which absorbed lipid and their cytoplasm became swollen[30]. Foam cells are able to oxidize lipoproteins of low density in the presence of high glucose concentration which plays a significant role in the development of atherosclerosis[31]. Also some SMCs had pyknotic nuclei indicating apoptosis which occurred as a result of generation of ROS[32].

The tunica adventitia became widely separated in diabetes due to inflammation with subsequent edema as the vasa vasorum, small blood vessels present in the tunica adventitia, is a source of inflammatory cells[26].

In the present study, the renal arteries of diabetic rats also showed a highly significant increase in thickness of the tunica media with SMCs proliferation and vacuolation. These results are in an accordance with a previous study who explained that the increased tunica media thickness of the renal artery may be due to an increased angiotensin II in diabetes mellitus which is the most important cause of hypertrophy of the arterial wall and its atherosclerosis due to stimulation of SMCs proliferation[33]. However, two previous studies demonstrated that the renal arteries of diabetic rats showed a minimal histological picture by examination with the light microscope[34,35].

By immune staining with anti-NF-κB, the diabetic group of the aorta and the renal artery showed strong positive immune reaction. A previous study stated that the positive reaction of NF-κB was constitutive NF-κB activation[19]. The activation of the NF-κB is implicated in the diabetic vasculopathy as it is involved in the inflammatory response and in the control of smooth muscle proliferation which plays an important role in the initiation and progression of atherosclerosis[22].

While by immune staining with anti-α-SMA, the thickness of smooth muscle fibers in the tunica media of the diabetic rat aorta and renal arteries was increased when compared to the control group, demonstrating the smooth muscle proliferation, as a previous study had used α-SMA protein to identify SMCs development in response to the disease[28].

In the current study, the diabetic group treated with PTX showed a marked decrease in the degenerative changes; the thickness of

tunica media of both aorta and renal artery was significantly decreased, smooth muscle cells proliferation and vacuolation were markedly decreased, the elastic fibers in aortic tunica media restored the normal architecture with minimal flattening and the tunica adventitia became less separated. These degenerative changes might be decreased by PTX. PTX has an antioxidant effect by decreasing the oxidative stress and free oxygen radicals which are implicated in the pathogenesis of diabetic vasculopathy[28,36].

Moreover, a previous study added that PTX might inhibit the production of PDGF which then prevented the vascular SMCs proliferation[7]. Also, PTX reduces NO destruction by free radicals, thus it can improve endothelial dysfunction that contribute to pathogenesis of atherosclerosis in diabetes mellitus[37,38].

In the present study, immunohistochemical sections of diabetic aorta and renal artery treated with PTX stained with anti-NF-κb showed a marked decrease reaction compared to diabetic group, asPTX inhibits activation and down-regulates the activated NF-κb, also it lowers the plasma level of pro-inflammatory cytokines as TNF-α , IL1, IL6[39].

Immunohistochemical sections of the aorta and the renal artery of the diabetic group treated with PTX stained with anti-α-SMA showed a decreased thickness of smooth muscle fibers in the tunica media compared with the diabetic group and regaining of their normal arrangement parallel to the tunica intima due to decreased SMCs proliferation[28].

CONCLUSIONS AND RECOMMENDATION

Pentoxifylline was found to have a great effect on improving the histological changes of diabetic vasculopathy. So, it is recommended to be used as an adjuvant therapy in diabetes mellitus.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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دور البنتوكسفيلين الوقائى فى اعتلال الأوعيه الدموية الناتج عن داء السكرى فى ذكورالفئران البيضاء

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ملخص البحث

المقدمة: يعتبر مرض السكر من الامراض المزمنة الاكثر انتشارا في جميع أنحاء العالم، و قد توقعت الدراسات إزدياد أعداد المرضي المصابين به في الأعوام المقبلة و يعد اعتلال الأوعية الدموية إحدي المضاعفات الخطيرة لمرض داء السكري، تؤدي بدورها الي المضاعفات المتعددة الأخري والتى تؤدى الى زيادة عدد الوفيات.

الهدف من البحث: اثبات الدور الوقائي للبنتوكسيفيلين في اعتلال الأوعية الدموية الناتج عن داء السكري لدي الفئران.

المادة وطرق البحث: تم استخدام 40 من ذكور الفئران البيضاء في هذه الدراسة حيث تم تقسيمهم عشوائيا إلى 4 مجموعات (10 فئران لكل منها):

المجموعة الأولى (مجموعة ضابطة)، المجموعة الثانية (مجموعة تلقت البنتوكسيفيلين فقط) ،المجموعة الثالثة (المجموعة المصابة بمرض السكر)، المجموعة الرابعة (مجموعة مصابة بمرض السكرى وتم علاجها بالبنتوكسيفيلين). المجموعة الثانية و الرابعة تلقيا البنتوكسيفيلين كجرعة يومية واحدة 100 مجم / كجم من وزن الجسم بالفم طوال مدة التجربة، وتم اعطاء المجموعة الثالثة والرابعة استربتوتوسن بجرعة 50 مجم / كجم من وزن الجسم داخل البريتوني كجرعة وحيدة من أجل اصابتهما بمرض السكرى، في نهاية التجربة تم تخدير الفئران وأخذ عينات من الشريان الأورطي والشريان الكلوي الابعد وتم تمريرها و معالجتها ثم فحصها بواسطة المجهر الضوئي، ايضا تم صباغة بعض الشرائح بالصبغات الهستوكيميائية المناعية لإظهارالخلايا العضلية الملساء واظهارمضادات الاجسام.

النتائج: لقد أظهر الفحص الهستولوجى للانسجة ان المعالجة المسبقة باعطاء البنتوكسيفيلين قد ادى الى حماية الاوعية الدموية من الاعتلال المصاحب لداء السكرى وقد اتضح ذلك من خلال التحسن الظاهر فى التركيب النسيجى للشريان الاورطى و الشريان الكلوى حيث ان البنتوكسيفيلين ادى الى تقليل سمك الطبقة الوسطى بشكل ملحوظ وانتظام شكلها وقد قل نمو العضلات الملساء المتزايد بها ،وأظهرت الألياف المرنة الموجودة بالطبقه الوسطى للشريان الأورطي تحسنا كبيراو انتظام ،و قد اظهرت النتائج ايضا قوه تأثير للبنتوكسيفيلين كمضاد للاجسام.

الاستنتاج والتوصيات: أثبتت هذه الدراسة أن العلاج الوقائي باستخدام البنتوكسيفيلين قد حسن بشكل كبيرمن التغيرات المرضية المصاحبة للاعتلال السكري للاوعية الدموية ،ويوصى باستخدامه كعلاج مساعد في داء السكري.