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Sildenafil citrate (Viagra) does not cause structural changes in the arterial wall but modulates renal artery vascular tone in atherosclerosis-induced male rabbits

Akeel AM Zwain[1, *], Najah R. Hadi[2], Nada Alharis[3], Karrar H. Kemmona[2], Ahmed Almodhaffar[2], Premanand Ponoth[4]

[1]Department of Non-invasive Cardiovascular Studies, Najaf Cardiac Center, AlSadr Teaching Hospital, Kufa College of Medicine, Iraq
[2]Department of Pharmacology and Therapeutics, College of Medicine, University of Kufa, Kufa Iraq
[3]Department of Radiology, College of Medicine, University of Kufa, Kufa Iraq
[4]Kamakshi Memorial Hospital, chief of cardiothoracic surgery Channai, India

Email address:

drzwain@zwayn.com (A. A. Zwain), drnajahhadi@yahoo.com (N. R. Hadi), nadaalharis@yahoo.com (N. Alharis), pponoth@hotmail.com (P. Ponoth)

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Abstract

Background: Phosphodistrase type 5 (PDE-5) inhibitors are shown to improve vasomotor aspect of endothelial dysfunction through modulatory effects on nitric oxide NO-cyclic guanosine monophosphate (NO-cGMP) mediated responses. However, the effects of PDE-5 inhibitors on the progression of atherosclerosis and blood flow alteration need further evaluation. *Aim:* To investigate in atherosclerosis-induced male rabbits, whether a selective PDE-5 inhibitor (sildenafil), can prevent atherosclerosis progression or modulate blood flow in the aorta and renal arteries. *Materials and Methods:* Eighteen local domestic healthy male rabbits were randomly divided into three groups of six each. Group I, were fed a normal chow (oxiod) diet, for 12 weeks and served as a control. Group II, were fed with an atherogenic diet, for 12 weeks. Group III, rabbits that were fed with cholesterol enriched diet for six weeks, were treated with oral sildenafil 5mg/kg/day, for a further six weeks. All rabbits were subjected to ultrasound/Doppler study of the abdominal aorta, renal artery and intrarenal arteries, along with oxidative stress measurements. Autopsy of the aortic arch sectioning for histopathology was done at the end of the study. *Results:* Treatment with sildenafil resulted in non-significant reduction ($P>0.05$) in aortic diameter and intima-media thickness (IMT); sildenafil elicited an atherosclerotic effect that did not reach the significant level ($P>0.05$). There was no significant change ($p>0.05$) in aortic flow peaked systolic velocity (PSV), end diastolic velocity (EDV), pulsatility index (PI) , and resistive index (RI). Sildenafil caused significant reductions ($P<0.05$) in renal artery and intra-renal artery PSV, EDV, PI and RI. *Conclusion:* Sildenafil neither resulted in significant structural changes in the atherosclerotic aortic wall, nor caused alteration in the arterial blood flow. Sildenafil exerted a significant relaxant effect on the atherosclerotic renal artery and intra -renal arteries. These findings may have a clinical implication favoring the use of PDE-5 inhibitors (Sildenafil) in the treatment of patients with atherosclerosis and renal hypertension.

Keywords

Sildenafil, PDE-5, Atherosclerosis, Hypercholestrolemic Rabbits, Doppler, Renal Artery, Intra-Renal Arteries Blood Flow

1. Introduction

Sildenafil is a potent inhibitor of PDE-5 which is the predominant isozyme that metabolizes cGMP the second messenger of NO and a principal mediator of smooth muscle relaxation and vasodilatation in the corpora cavernosa of the penis.[1] Sildenafil possesses direct muscle relaxant potential, possibly via inhibiting Ca^{2+} influx through both receptor-operated and voltage dependent Ca^{2+}channels.[2] In a dose-dependent manner, sildenafil induces vaso relaxation as a result of increased in the phosphorylation of heat shock-related protein 20.[3]

The data from hemodynamic studies have shown that sildenafil possesses a modest vasodilator effect on the venous and arterial trees and improves microcirculation in patients with Raynaud's phenomenon, without significant decreases in blood pressure (BP) or heart rate (HR). [1 - 6] During exercise and recovery, sildenafil did not cause clinically significant alterations in the hemodynamic parameters in men with coronary artery disease (CAD). [7] At a dose of 2 mg/kg of sildenafil, systemic BP did not change, but the blood flow in a normal coronary artery increased.[8] Small, yet significant decreases in BP in diabetic hypertensive patients with erectile dysfunction (ED), have been observed with regular use of sildenafil.[9] Recent evidence suggests that based upon their modulatory effect on the preload and Afterload, PDE-5 inhibitors such as sildenafil possess cardioprotective and anti ischemic effects and may be useful in treating patients with heart failure (HF).[10] These agents, reduce cardiovascular remodeling associated with hypertensive cardiomyopathy and inhibits platelet activation in patients with CAD.[11-13] Care should be taken when using these agents in combination with any agent acting as a NO donor because of the possibility of life-threatening hypotension and an increase in vulnerability to ventricular tachycardia-ventricular fibrillation.[14]

Few detailed studies are available regarding the antioxidant and modulatory effects of PDE-5 inhibitors on the atherosclerotic arterial wall and their effects on blood flow alteration, particularly in the aorta and renal arteries, which are the key contributors to renal vascular resistance and renal hypertension.

1.1. Aim

This study aimed to investigate whether a selective PDE-5 inhibitor such as sildenafil, can halt atherosclerotic progression by inducing structural changes in the aortic wall in atherosclerosis-induced male rabbits. A second aim was to investigate whether these agents can modulate blood flow in the aorta, renal, and intra-renal arteries.

2. Materials and Methods

This study was conducted on 18 healthy domestic male rabbits weighing between 1400-1700 grams, after having approval from the animal ethical committee at Kufa College of Medicine, Iraq. The animals were randomly divided into three groups of six. The group I rabbits were fed a normal (Oxiod) diet for 12 weeks and served as a control. The group II rabbits were used as a model for experimental induction of atherosclerosis [15] and were fed a 2% cholesterol-enriched diet of cholesterol powder mixed with Oxiod pellets for 12 weeks. The group III rabbits were fed the cholesterol- enriched diet for 6 weeks and were continued on this diet and treated with oral sildenafil (Pfizer, USA, Batch No 89R001E) at 5mg/kg/day for an additional 6 weeks.

Each rabbit was subjected to ultrasound/Doppler study of the abdominal aorta, renal artery, and intra-renal arteries.

The rabbits were examined with a colored Doppler ultrasound machine at the beginning of the study, at 6weeks, and at the end of the study, using the Siemens Versa Sonoline equipment (230 PAL versions, Germany) with color flow and PW Doppler capabilities. The Doppler examination was performed in the morning after overnight fasting to avoid the occurrence of intestinal gases that could avert optimal visualization of the arteries under study. The rabbits were sedated by a 5mg/kg body weight intra-peritoneal dose of diazepam. [16] For technical reasons, the left lateral side was used for insonating the aorta and renal arteries, using a 7.5-10 MHz probe. The aorta was visualized and identified by its characteristic pulsation using color flow Doppler imaging. The aortic diameter and intima- media thickness (at the origin of the renal artery) were measured. Care was taken to insonate all the vessels under study at an angle of 0° and to avoid excessive pressure by the transducer on the flank or abdomen.

The aortic diameter, aortic intima-media thickness, and aortic blood flow parameters, including PSV, EDV,RI, and PI, [17] were measured electronically by the Doppler software. The renal artery and intra-renal arteries (the blood flow changes in the inter-lobar artery at mid pole were utilized in this study) of the left kidney were visualized and identified using color flow mapping, and their blood flow parameters were also measured.

Doppler parameters including RI and PI, were calculated according to the formulae presented elsewhere. [18]A post mortem histopathological examination of the aortic sectioning was performed at the end of the study. The histological changes were determined according to the American Heart Association classification of atherosclerosis phases, [19, and 20] which distinguishes atherosclerotic lesions by degree and phases.

Blood sampling was performed at the beginning of the study, at six weeks in the induction period and weekly during the treatment course, to measure the fasting serum lipoprotein components, including total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL-C), low density lipoprotein (LDL-C) and very low density (VLDL) lipoprotein levels. Oxidative stress parameters, such as serum malondialdehyde (MDA) and reduced glutathione (GSH) levels, were also measured. [21]

2.1. Histopathological Procedure

At the end of the protocol (12 weeks on the diets), the rabbits were sacrificed with a high intravenous dose of phenobarbital sodium (200mg). The rabbits were dissected through the chest wall to make the aorta accessible for resection. The aortic arch was exteriorized and cleaned of adherent fat, and connective tissue was excised. The specimens were immediately fixed in a 10% formaldehyde solution. After fixation, the samples were processed in the usual manner. The sections were examined by microscope under magnification power of (×10 and ×40), and the histological changes were determined according to the American Heart Association classification of atherosclerosis phases, [18] which divides atherosclerotic lesions into 6 types as follows.

Type I (initial) lesions have increased numbers of macrophages and the appearance of foam cells distributed at random. Type II lesions consist primarily of layers of macrophage foam cells, lipid-laden smooth muscle cells and the accumulation of fatty streaks. Type III (intermediate) lesions have intimal thickening and are pre-atheromatous. Type IV (atheroma) lesions show type II changes, a necrotic fatty core and a well-formed cellular cap. Type V (fibro-atheroma) lesions have thick layers of fibrous connective tissue (thick cellular caps) overlying a largely necrotic fatty mass. Type VI (complicated fibro-atheroma) lesions show plaque and surface defects, and/or hematoma/hemorrhage and/or thrombotic deposit.

2.2. Statistical Analysis

The statistical analyses were performed using SPSS 12.0 for Windows, lnc. The data were expressed as the mean ± SEM; the paired t-test was used to compare the mean values within each group at different times. The Analysis of Variance (ANOVA) text was used for the multiple comparisons among all of the groups, followed by the post-hoc tests using LSD method. The Pearson correlation coefficient was used to assess the associations between the two continuous variables of the study parameter. The Chi-squared test was used to compare the histopathological changes in the groups. In all the tests, $p < 0.05$ was considered to be statistically significant. [22]

3. Results

Feeding the rabbits a high cholesterol diet resulted in a significant ($p<0.05$) increase in the values of serum lipid components, including serum TC, TG, HDL, LDL, and VLDL, compared with the normal control group. Treatment with sildenafil did not result in significant ($P>0.05$) change in the TC, TG, HDL-C, LDL-C, and VLDL-C values compared with the induced, untreated high cholesterol diet group (group II)

As shown in figure 1, the serum MDA levels of the sildenafil treated rabbits (group III) were significantly lower ($p<0.05$) than those of the atherosclerosis- induced and untreated rabbits (group II). The serum GSH levels of sildenafil treated rabbits (group III) were significantly higher ($p<0.05$) than those of the atherosclerosis-induced and untreated rabbits (group II). In the atherosclerosis-induced untreated rabbits, the increase in MDA was reversed by sildenafil, with a concomitant decrease in GSH compared to the control group ($p<0.05$ for both).

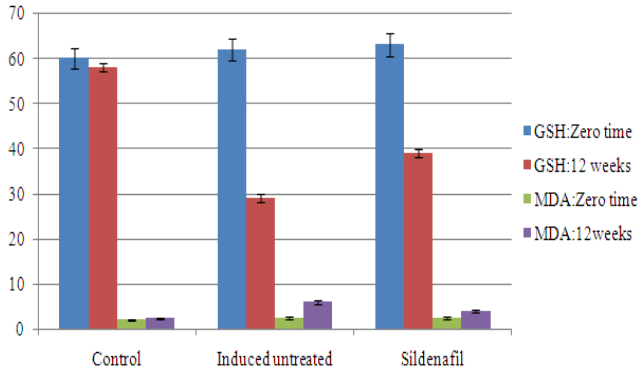

Figure 1. *Sequential changes of serum MDA and GSH levels mmol/l, of the four experimental groups. Data were expressed as Mean ±SEM (N=6 in each group)*

A 2%cholesterol enriched diet resulted in the development of various atherosclerotic stages (Fig. 2), with a significant increase ($p<0.05$) in aortic diameter, intima-media thickness, PI, RI, without a significant change ($p>0.05$) in the aortic PSV and EDV (Fig.3, A and B; Fig. 4 and 5). Significant increases ($p<0.05$) in renal artery PSV, PI, RI, intra-renal artery PSV, PI, and RI were observed in the induced untreated group compared to the normal group (Fig. 5 and 6). The Doppler waveforms of the aortic blood flow, renal artery and intra- renal arteries are shown in Fig.7.

The treatment of the rabbits with sildenafil resulted in a non-significant ($p>0.05$) reduction in the aortic diameter and intima-media thickness (Fig. 4). As demonstrated in figures 5 and 6, there was no significant change ($p>0.05$) in the aortic PSV, EDV, PI and RI in the rabbits in the treated group compared with the rabbits in the induced, untreated group. Treatment with sildenafil at the renal level resulted in a significant reduction ($p<0.05$) in the renal artery and intra-renal artery PSV, EDV, PI and RI.

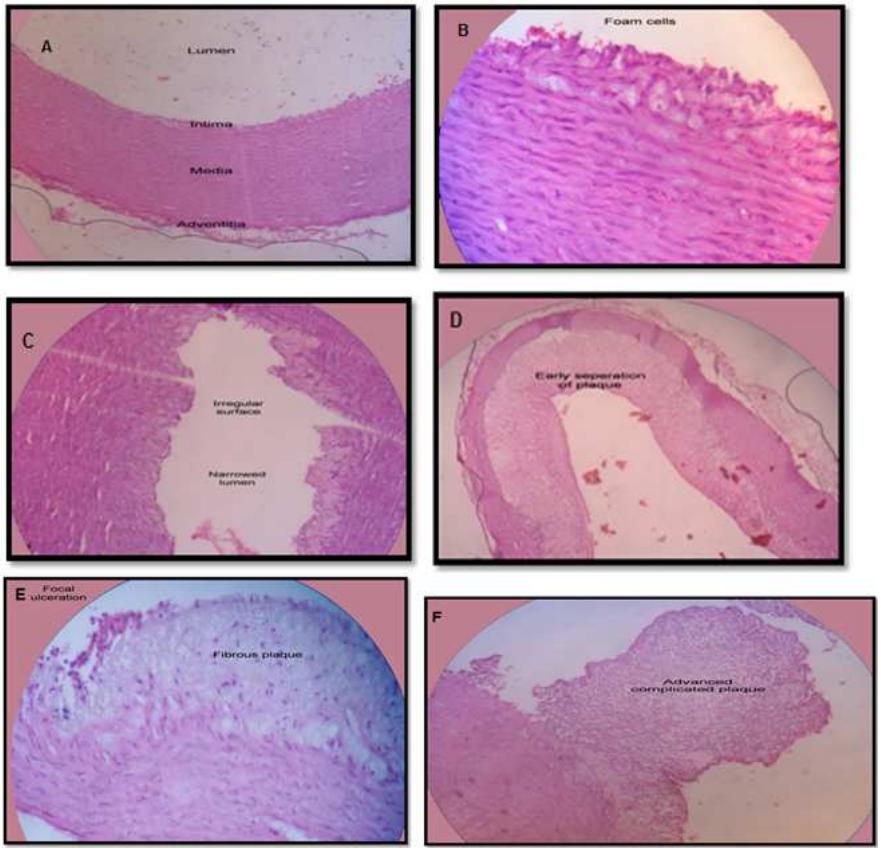

Figure 2. *Cross section aortas demonstrating (A): normal appearance of arterial wall layers of a healthy rabbit: intact continuous endothelium (intima), and regularly arranged smooth muscle fibers (media) and adventitia . (B): lipid laden macrophages (Foam cells) which represent an early atherogenic event (fatty streak) with fatty streaks affecting mainly the intima (Type II atherosclerosis). (C): wide spread fatty streaks with irregular surface (diffuse intimal thickening and narrowing of lumen) (Type III atherosclerosis). (D): advanced wide spread atheromatous plaques involving both intima and media with early separation of the media (Type IV atherosclerosis). (E): fibrous atheromatous plaques with focal ulceration (Type V atherosclerosis). (F): advanced complicated atheromatous plaques with marked organization and sloughing (Type VI atherosclerosis). Sections were stained with haematoxylin and eosin (×10).*

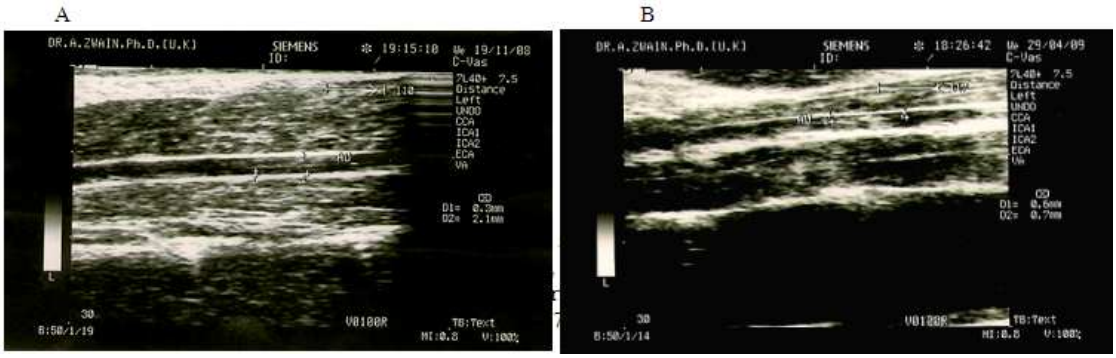

Figure 3. *B-mode ultrasound image illustrating (A): abdominal aorta of a healthy rabbit with normal aortic wall thickness, D1 is the intima-media thickness = 0.3 mm, D2 is the aortic diameter = 2.1 mm. (B): abdominal aorta of an atherosclerosis-induced rabbit showing increased aortic thickness (sclerotic wall), with dissemination of plaques inside the aorta , D1 is the intima media thickness = 0.7 mm.*

With respect to the histopathological results, all the rabbits fed the cholesterol enriched diet developed different phases of atherosclerosis (Fig. 2), with a significant difference ($p<0.05$) between the induced untreated group and the normal control group.

Table 1 shows that the high cholesterol diet in the untreated rabbits resulted in all the animals having involvement of the aorta, with atherosclerosis with varying degrees of severity. Of this group, 16.7% had initial atherosclerotic lesions as phase II, 33.33% had intermediate phase III atherosclerotic lesions and 50% had advanced atherosclerotic lesions (33.33% of phase IV, and 16.7% of phase V).

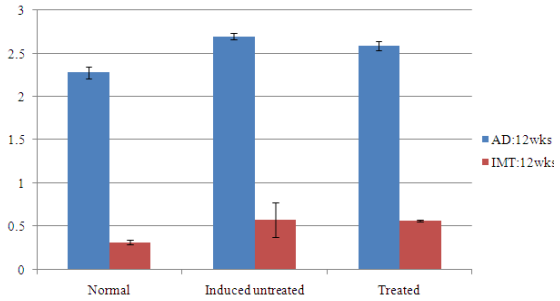

Figure 4. *Effect of sildenafil at a dose of 5mg/kg/day treatment on aortic intima-media thickness (IMT) and aortic diameter(AD) measured in (mm), of the atherosclerosis-induced rabbits compared to the two control groups (normal and induced untreated).*

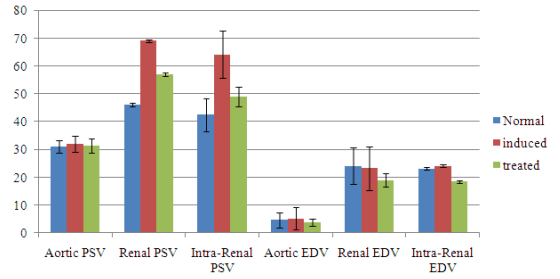

Figure 5. *Demonstrating the effect of sildenafil at a dose of 5mg/kg/day treatment on aortic, renal, and intra-renal arteries peak systolic velocity (PSV) and end diastolic velocity (EDV), measured in (cm/s), of the atherosclerosis-induced rabbits compared to the two control groups (normal and induced untreated).*

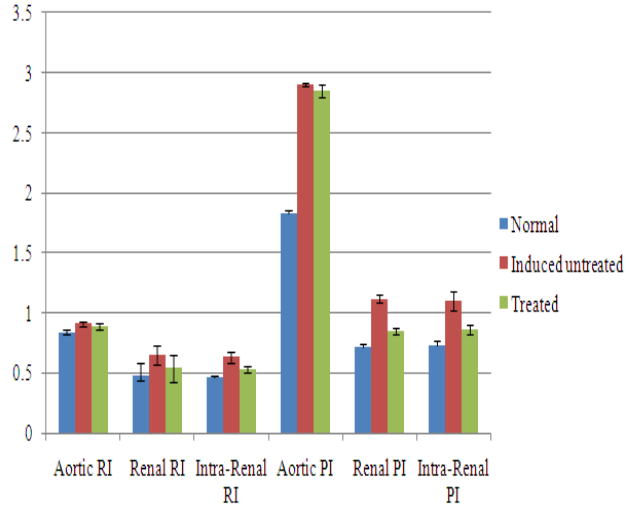

Figure 6. *Showing the effect of sildenafil at a dose of 5mg/kg/day treatment on aortic, renal, and intra-renal arteries resistive index (RI) and pulsatility index (PI), of the atherosclerosis-induced rabbits compared to the two control groups (normal and induced untreated).,*

The treatment of atherosclerotic rabbits with sildenafil did not have a significant effect ($p>0.05$), compared with the induced untreated group. The sildenafil treated rabbits did not develop complicated atherosclerotic lesions (Table 1).

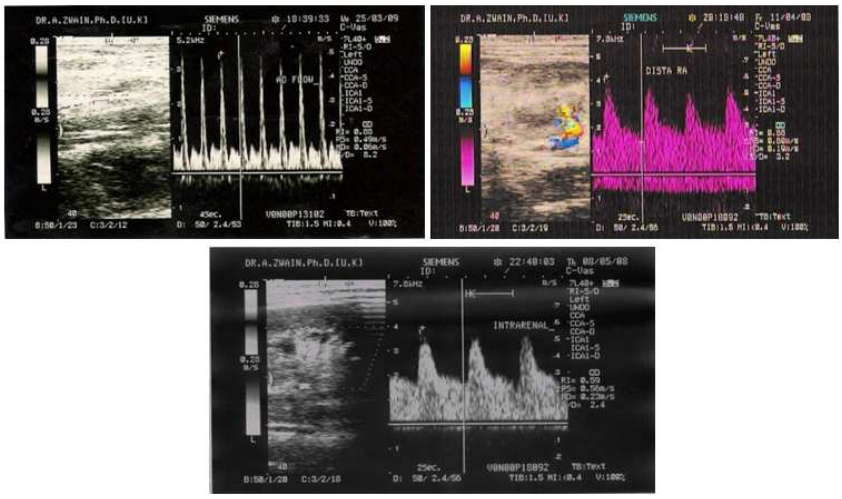

Figure 7. *A Doppler spectral wave form in a healthy rabbit, showing (A): aortic blood flow, (B): renal and (C): intra-renal artery blood flow velocities: PSV, EDV (m/s), RI and S/D ratio.*

Table 1. *Demonstrating different aortic atherosclerotic lesions in different groups: dietary induced versus sildenafil treated rabbits at the end of 12 weeks of the study.*

		Normal	Initial lesion	Intermediate lesion	Advance lesion	Complicated lesion
Normal control NO= 6	NO.	6	0	0	0	0
	%	100	0	0	0	0
Dietary induced untreated NO= 6	NO.	0	1	2	2	1
	%	0	16.7	33.33	33.33	16.7
Sildenafil 5mg/kg/day	NO.	0	1	3	2	0
	%	0	16.7	50	33.33	0

4. Discussion

During the last two decades, concern about the vascular modulatory potential resulting from PDE-5 inhibition has increased. It has been demonstrated that PDE-5 isozymes are expressed in the arterial smooth muscle and endothelium, with high expression levels in the corpus cavernosum and pulmonary vessels. The magnitude of the NO-mediated vasodilation reaction to sildenafil is dependent on the PDE-5 expression level and sufficient generation of cGMP in that artery.[23] As a selective inhibitor of PDE5, sildenafil increases NO-mediated vasodilation and smooth muscle relaxation and was recently shown to lower pulmonary vascular resistance. [24]

In this study, we intended to research the use of selective PDE-5 inhibitors, such as sildenafil, in therapies that go beyond their frequent use as vasodilators in pulmonary hypertension or ED. We investigated the efficacy of using sildenafil in the prevention of atherosclerotic progression and in the modulation of the blood flow in arteries of different diameters.

In this study, a high cholesterol diet elicited different stages of atherosclerosis (Fig. 2), with an increase in aortic diameter and IMT (Fig. 3 and 4). The significant increase in aortic diameter compared to baseline values in cholesterol-enriched diet fed rabbits, can be explained by progressive plaque formation and arterial remodeling. [25, 26] These findings are in accordance with the observation of Armstrong et al [27] who reported that the radial enlargement of vessels that occurred in macaque monkeys fed a cholesterol enriched diet was due to progressive plaque growth.

In this study, examination of the aortas of the rabbits showed that administration of sildenafil did not elicit structural changes in the aorta in terms of lumen dilation or a reduction in the IMT. These findings corroborate other studies performed on arteries of different diameters, in which the administration of a NO donor alone or in combination with sildenafil did not result in a beneficial effect on the geometry of the carotid and coronary arteries. [28] In normal subjects, sildenafil caused an increase in retinal blood flow but had no statistically significant effect on the retinal arterial diameters. [29]

The efficacy of sildenafil observed in the present study can be validated by that it interfered with oxidative stress markers, caused significant reduction in MDA and increased in glutathione levels despite the ongoing atherosclerotic process (Fig.1). In the atherosclerosis-induced rabbits, sidenafil elicited an atherolytic effect, and no animal in the sildenafil treated group developed phase 5 (severe lesions) atherosclerosis. Of the untreated animals, 16.7% developed severe atherosclerotic lesion (Table 1). However, the difference between the two groups did not reach significance. The failure to demonstrate a significant effect by sildenafil on atherosclerosis progression or alteration in the IMT of the aorta may be attributed to the short duration of treatment, an ineffective dose, and a small sample size.

Recent studies have indicated that PDE-5 inhibitors may be able to improve endothelial function through an antioxident – atherolytic effect. In a double-blind placebo-controlled study that used Doppler technique and monocyte oxidative activity, to mark endothelial dysfunction, It was shown that treatment with sildenafil plus propionyl L-carnitine reduced monocyte oxidative activity and endothelial dysfunction markers in patients with diabetes with ED.[30] Moreover, In an invitro study, it is theorized that PDE-5 inhibitors generate an anti proliferative effect on human endothelial cells that may decrease the vulnerability of atherosclerotic plaques to rupture.[31] The antiproliferative effect of sildenafil was explained as being NO- mediated. This concept was refuted by other authors who concluded that the antiproliferative effect of nitric oxide was independent of cGMP. [32]

The finding that the aortic PSV did not increase significantly can be explained by the fact that the aorta is too large for the plaques to cause significant stenosis that would alter blood flow velocity. A study in men showed that administration of oral sildenafil did not have a significant effect on aortic and superior mesenteric artery (SMA) blood flow pattern and that this drug, induced only mild changes in the carotid artery circulation. [33]

The increased RI and PI in aorta and renal arteries associated with arterial stiffness found in atherosclerosis-induced rabbits, are consistent with previous studies that have demonstrated a significant link between hypercholesterolemia and atherosclerosis. [34]

In the present study, treating hypercholesterolemic rabbits with sildenafil caused a significant reduction in the renal artery and intra-renal artery peak systolic and diastolic velocities, with decreases in RI and PI. This finding could indicate that sildenafil exhibits a positive relaxant effect by acting directly on the dysfunctional renal endothelium and improving its function, thereby improving renal artery blood flow via increasing cGMP levels. It has been proposed that sildenafil has a relaxant effect on the vascular smooth muscle. The relaxant effect may involve one or more of the following mechanisms. Treatment with the PDE-5 inhibitor sildenafil may exert an indirect NO-cGMP dependent effect and a direct effect by lowering the intracellular free Ca^{2+} concentration. This later effect may be mediated via the activating of calcium pumps, the inhibition of voltage-gated Ca^{2+} channels and the inhibition of Ca^{2+}-induced G protein–coupled receptor activation.[23] It is possible that the vasodilation action of sildenafil could potentially release endogenous mediators of reconditioning, such as adenosine or bradykinin, from endothelial cells. Such mediators could trigger a signaling cascade through kinase action and result in NO synthase phosphorylation and NO release.[10] PDE-5 inhibitors have been shown to improve vascular tone in syndromes in which NO synthase activity is compromised, including diabetes, and sildenafil

has been shown to enhance impaired cerebral vasoreactivity in patients with type 2 diabetes mellitus.[19] There is an implication that these agents improve blood flow and increase the diameter of the vertebral artery in patients with vertebrobasilar ischemia. [35]

A few studies using renal Doppler ultrasound, reported that a single 50 mg-dose of sildenafil did not cause a significant effect on the renal artery hemodynamic in 12 healthy individuals one hour after drug administration, [36] or in 6 healthy dogs 75 min or 15 days after drug administration. [37] The authors reported a decrease in the peak systolic maximum-velocity of the left renal segmental artery in the dogs, after sildenafil administration. This discrepancy with our findings may be explained in the following way. First, there was a difference in the experimental protocol design. A longer period (6 weeks) of sildenafil administration in the rabbits would contribute to the larger vascular relaxant response observed in the present study. Second, in our experimental model, we tested the effect of sildenafil on atherosclerotic dysfunctional vascular endothelium. It has been proposed endothelial dysfunction can be caused by a variety of causes, [38] including decreased sensitivity to NO, decreased expression and activity of endothelial nitric oxide synthase, increased destruction of NO by free radicals, increased endogenous vasoconstrictors such as endothelin, and increased levels of endogenous NO antagonists, such as asymmetric dimethylarginine. Moreover, the improvement of endothelial dysfunction due to treatment with PDE-5 inhibitors in some certain conditions and not others is to be expected.

Data from more recent studies corroborate the present findings. In miniature pigs with renal autotransplantation, It was shown that 100mg oral sildenafil significantly increased renal vascular flow and significantly decreased renal vascular resistance and improved endothelial cell structure.[39] In rats underwent renal artery clamping to induce renal ischemia, it was proposed that sildenafil improved renal haemodynemic and had a protective effect in renal ischemia/reperfusion injury. [40]

5. Conclusion

Sildenafil has no significant effect on aortas affected by atherosclerosis; further study with a larger sample size is therefore necessary to unequivocally prove the hypothesis that sildenafil has an anti-atherosclerotic effect. Sildenafil dilates atherosclerotic renal and segmental intra-renal arteries. These results may have an implication in the long term management of type 2 diabetics with hypertension with or without associated erectile dysfunction.

6. Strength and weakness of the study

The strength of our study lies in the fact that it demonstrates a dilatory effect of a selective PDE-5 inhibitor such as sildenafil on renal arteries after induction of generalized severe atherosclerotic changes in the same animal. In the present word of increasing atherosclerosis the result of this study could be utilized for further evaluation to see the clinical use of this in renal artery hypertension. This may be another effect of sildenafil which causes reduction of pulmonary artery pressure, even though the drug was primarily used for erectile dysfunction. However our study suffered from some limitations, including the small sample size studied, needs further evaluation before we make it use in human subjects in clinical trials.

Authors' Contributions

AZ conceived of the study, performed the Doppler examination and wrote the manuscript. NR participated in the study protocol, sequence alignment, experimental design and coordination. NA assisted in the Doppler examination. KK conducted most of the animal research work, lab measurements and statistical calculations. AA did some statistical issues, PP revised the manuscript. All of the authors read and approved the final manuscript.

Competing Interests

The authors declare that they have no competing interests.

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