ORIGINAL ARTICLE

HISTOLOGICAL CHANGES FOLLOWING PROLONGED ORAL ADMINISTRATION OF SILDENAFIL CITRATE IN DIABETICS RATS

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ABSTRACT

INTRODUCTION

Background: Diabetes is a cluster of metabolic disorders reflected by abnormal hyperglycemia that causes chronic microvascular, macrovascular, and neuropathic diseases.Sildenafil citrate is widely used to dilate penile arteries, particularly in patient with erectile dysfunction which suggest that it may also dilate pulmonary arteries in patients with pulmonary hypertension. Objectives: The purpose of this study is to evaluate the effect of sildenafil citrate on heart and lung tissues of diabetic rats. Method: The study was performed using forty-two rats (42) weighing between 150-200 grams. The rats were grouped into 7 groups (A-G) of six (6) rats per group. Wound area of 1.5 by 1.5 cm^2 was created at the dorsal surface of each rat under sedation with ketamine and lignocaine. Type I diabetes was induced using a single dose of Alloxan monohydrate at dose rate of 130 mg/kg. Sildenafil citrate was administered at a dose rate of 50 mg/kg orally daily for 21 days and 10 international units of insulin was administered intra-peritoneal to the control group once. At the end of 21 days lung and heart tissues were collected for histological studies. Results: The results revealed moderate thickening of interstitial demonstrated by congestion of blood vessels and oedema of the lungs. The heart muscles were swollen with loss of striation in myocardial fibers. Conclusion: Prolonged oral administration of sildenafil citrate caused appreciable pulmonary and cardiovascular damage to diabetic rats. Therefore, precautionary measures are needed when treating diabetic patients with pulmonary and cardiovascular diseases.

Keywords: Diabetic, Alloxan, Histology, Sildenafil citrate, Rats

INTRODUCTION

Diabetes mellitus is a heterogeneous metabolic disorder characterized by the presence of hyperglycemia due to impairment of insulin secretion, defective insulin action or both.[1] The chronic hyperglycemia in diabetics is associated with relatively specific long-term microvascular complications affecting the eyes, kidneys and nerves, as well as an increased risk for cardiovascular diseases.[2] Consequently, insulin resistance develops in target organs and peripheral sites, which is associated with endothelial dysfunction and by number of mechanisms including interruption of the subcellular signaling pathways of both insulin and nitric oxide (NO) production.[1]

Sildenafil citrate is a powerful and selective inhibitor of cGMP specifically phosphodiesterase type 5 (PDE5) which is the dominant isoenzyme metabolizing cGMP.[3] The Phosphodiesterase type

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5 enzyme is present in the penile tissue, skeletal muscle, visceral, vascular smooth muscle and platelets. 4PDEs are classified into 11 distinct families, based on their different substrate specificities, mode of regulation, and sequence homology. 5 Several PDEs including PDE1, PDE2, PDE3, PDE4, PDE5, and PDE7 are expressed in vascular endothelial cells(ECs) of different origin and affect their migration, proliferation, as well as cells apoptosis. An in vivo study reported[6] showed that, sildenafil citrate causes the dilation of peripheral arteries and veins which prevent thrombi formation by platelets.

Sildenafil also increases NO release by stimulating inducible nitric oxide synthase(iNOS) and endothelial derived nitric oxide synthase(eNOS) at the mRNA and protein level.[3] Sildenafil citrate which is used for different indications such as erectile dysfunction (ED) by enhancing NO / cGMP-mediated signaling in cavernous smooth muscle and also used for alleviation of pulmonary hypertension.[1] Although, sildenafil citrate has been shown to have powerful preconditioning like cardioprotective effects in animal models of ischemia-reperfusion injury, the precise cellular mechanism underlying these effects and the long-term hemodynamic effects and safety of the drug in pulmonary hypertension are not clear.

Sildenafil are commonly prescribed to treat pulmonary arterial hypertension and erectile dysfunction in diabetic and / or obese patients Since the approval of sildenafil citrate usage, several reports of sudden death among patients treated with this drug have raised some concerns regarding its safety in patients with coronary artery disease.[7] Therefore there is need for follow up studies to re-evaluate the drug safety. Although in the past few years, the cardiovascular effects of sildenafil citrate have been investigated, there is need for further extensive investigation in a large cohort population to validate some of these claims.

The use of sildenafil citrate by many individuals is basically for sexual arousal. However, there is a growing apprehension that sildenafil citrate abuse may have harmful effects on cardiovascular and pulmonary system.

In the present study we intended to investigate the cardiovascular and pulmonary effects of sildenafil citrate on diabetic rats and whether sildenafil citrate treatment could also alleviate some of the complications experienced by diabetic patients.

MATERIALS AND METHODS

Experimental Animals

Wister albino rats of both sexes were used for the experiments. They were kept in plastic cages and allowed to adjust to the laboratory environment for a period of two weeks before the commencement of the experiments. They were fed with growers' pellets (vital feed grower) and water provided *ad libitum*. The dosage of sildenafil citrate 50 mg / kg was earlier adopted from[8].

Experimental procedure

A total of 42 Wister albino rats (10-12 weeks) of both sexes were obtained from the Sanda Kyarimi Zoo, Maiduguri. The rats were housed and acclimatized in an individual cage for two weeks prior to the experiment. The rats were fed standard pellets (vital feed grower) and water *ad-libidum*. The rats were then weighed and randomly divided into seven (7) treatment groups with each group having six rats. The animals were handled according to the International Guiding Principles for Biomedical Research Council for International Organizations of Medical Sciences[9].

Definition of treatment groupsA= Diabetes + wound + Sildenafil.
B= Diabetes + wound + Sildenafil + insulin.
C= Diabetes + wound + insulin.
D= Diabetes + wound
E= Wound + Sildenafil.
F= Wound + injection water.
G= Sildenafil citrate.

Induction of diabetes

Type I diabetes was induced by a single intra-peritoneal injection of prepared alloxan mono-hydrate at dose rate of 130 mg / kg as described by[11] with slight modifications. Diabetes was confirmed three (3) days following administration of alloxan and by measuring the fasting blood glucose concentration using glucometer. Only rats with blood glucose level of 180 mg / dl and above were used for the experiment.

Tissue preparation for Histology

From each group, 5 rats were randomly picked and humanely sacrificed and samples of the heart and lungs were collected for histological examination. This was done prior to (day 0) and after the administration of the sildenafil citrate for 21 days. The tissue samples of heart and lung obtained were fixed in 10% formalin. The tissues were dehydrated through graded concentration of ethanol (70%, 95% and absolute), cleared in xylene and embedded in paraffin wax. The embedded tissues were stained with hematoxylene and eosin (H & E) for light microscopic examination. The processed slides were viewed and investigated under light microscope using Vanox T Olympus light microscope[11].

RESULTS

Figure 1 showed a normal section of the heart in the control group showing normal architecture and striation of the heart muscle fiber (white arrow) (×100; H&E). The results for the histology revealed swollen heart and loss of muscle fibers striation (Figure 2) in diabetic group, the intensity of swelling and loss of striation increased by 21-day in the diabetic group (Figure 3) which also indicate that prolonged administration of sildenafil citrate has progressively and adversely affected the heart tissues in diabetic rats.

Figures 4, 5 and 6 showed section of the lungs showing marked thickening of the interstitium by congestion of blood vessels, oedema and inflammatory cells (arrow).

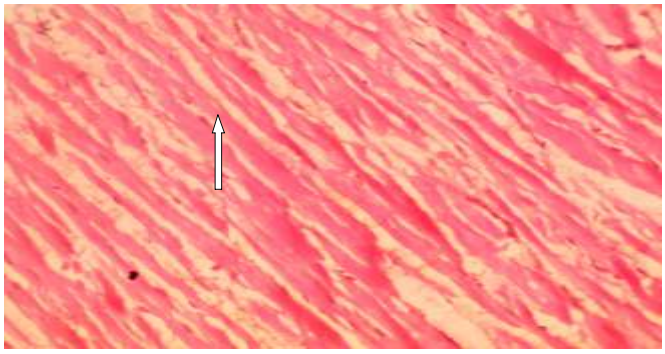

Figure 1: Section of the heart in the control group showing normal architecture and striation of the heart muscle fiber (white arrow)(×100; H&E).

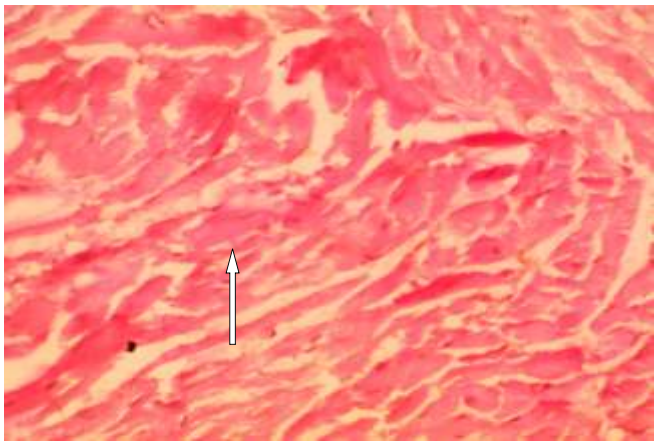

Figure 2: Section of the heart showing swelling and loss of striation of the muscle fibers (x400; H&E)

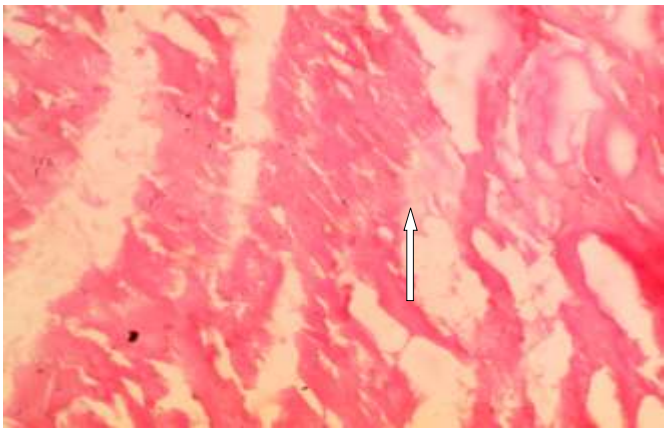

Figure 3: Section of the heart showing marked swelling and loss of striation of the muscle fibers (x400; H&E)

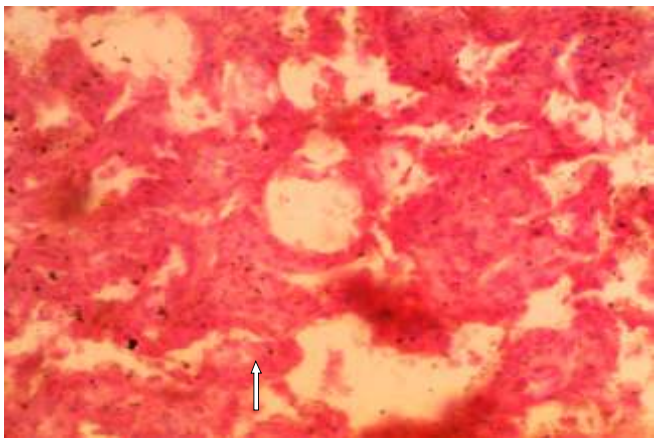

Figure 4: Section of the lung showing marked thickening of the interstitium with congestion of blood vessels, oedema and inflammatory cells (arrow)

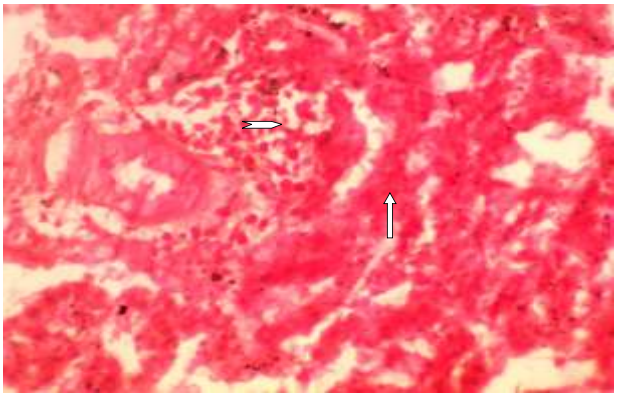

Figure 5: Section of the lung showing marked thickening of the interstitium by congestion of blood vessels (arrow), oedema and inflammatory cells (arrow head) (x400; H&E)

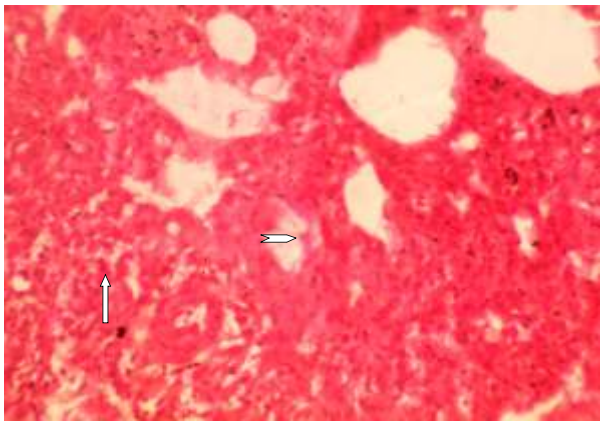

Figure 6: Section of the lung showing marked thickening of the interstitium by congestion of blood vessels (white arrow), oedema and inflammatory cells (arrow head) (x400; H&E)

DISCUSSION

The present study revealed that sildenafil citrate has markedly caused swelling and distortion of striated cardiac muscle fibers in diabetic rats at 21-day of the treatment. These findings indicate that sildenafil failed to protect the lung from the injury at vascular beds. As a selective type 5 phosphodiesterase (PDE5) inhibitor, by blocking PDE5, sildenafil may reduce the catabolism of cyclic guanosine monophosphase (GMP) through a cascade of intracellular events, for example cyclic GMP may promotes smooth muscle relaxation and widening of blood vessels thus,diminishes cellular proliferation and migration. These may interfere with pre-existing vascular beds formation. Although previously reported,[12] sildenafil has shown to prevent and reversed pulmonary hypertension in monocrotaline treated rats, these was achieved via improved function of endothelin system in pulmonary artery and to a lesser degree dilation in systemic circulation.[8] Has demonstrated that PDE5 inhibitors have powerful protective effect against myocardial ischemia/reperfusion (I/R) injury, ischemic and diabetic cardiomyopathy. The discrepancy is that the later used 100 mg/day of sildenafil in an acute study for only three days, which was not sufficient enough to scientifically validate the findings, unlike in our case the administration was prolonged to 21 day. It is important to note that PDE5 inhibition promotes antioxidants like effect in diabetic heart, which exposed myocardium to intense oxidative stress eventually lead to myocardial tissue injury and dysfunction. Generally, oxidant and antioxidant imbalances in the cardiomyocytes, constitutes oxidative stress, may favor the accumulation of oxidant species, leading to cellular damage.[14]

In a similar way the histological findings from the lung tissues showed marked thickening of interstitials with congestion. Previous studies by[8, 13]indicated that sildenafil widely used to dilate penile arteries, suggesting that it may also dilate pulmonary arteries in patients with pulmonary hypertension. Sildenafil has been reported to have antifibrotic activity in patients with lung disease[15]and may decreased the activity of PDE5, so that more cyclic GMP will be available for the blood vessels inside the lungs to relax the smooth muscles of the arterioles via nitric oxide (NO) – dependent mechanism.[16] In this study sildenafil failed to inhibit the pro-inflammatory cytokine responses and ROS generation thereby causing more damage to the lung tissues of diabetic rats. In nutshell, the pro-inflammatory effects of ROS which includes endothelial damage, formation of chemotactic factors, neutrophil reinforcement, cytokine release and mitochondrial injury, all may contribute to free radical overload and to oxidant–anti-oxidant imbalance which results in lung tissue damage.

It was concluded that prolonged treatment with sildenafil citrate failed to protect the heart and lung tissues of diabetic rats. Hence, precautionary measures should be taken particularly in diabetic patient with erectile dysfunction taking medication with specific PDE5 inhibitors. There is

also need to enlighten the public on the risk associated with prolonged medication with PDE5 inhibitor for sexual arousal. Tadalafil and Vardenafil are newer phosphodiesterase type 5 inhibitors which have been launched recently to compete with sildenafil in a highly lucrative market but adverse effects and drug interactions of the new compounds appear to be similar to that of sildenafil. Hence, it is deduced that similar precautionary measures should be taken using such compounds.

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