ORIGINAL ARTICLE

EFFECT OF SILDENAFIL CITRATE ON THE BODY WEIGHT, BLOOD GLUCOSE AND WHITE BLOOD CELL COUNT DURING WOUND HEALING PROCESS IN DIABETIC RATS

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ABSTRACT

Background: Diabetes mellitus is a chronic metabolic disorder with major complication of delayed wound healing.

Objectives: The purpose of this study was to evaluate the effect of sildenafil on blood glucose level, body weight and leucocyte count during wound healing in diabetic rats.

Method: Forty-two Albino rats randomly divided into 7 groups of 6 rats per group were treated orally with sildenafil (50 mg/kg) for 21 days and/or single dose of intraperitoneal injection of insulin (10 I.U.). Before treatments, diabetes was induced in groups A, B, C and D by a single intraperitoneal injection of alloxan (130mg/kg) and a square-shaped wound measuring $1.5cm^2$ was created under anesthesia on the dorsum of rats in all groups except group G. The rats in group A were treated with sildenafil citrate orally at the dose rate of 50 mg/kg body weight for 21 days and the rats in group B were treated with insulin injection of 10 international units once and sildenafil citrate as in Group A. The rats in group C were treated with only insulin injection of 10 international units once and the rats in group D were treated with only distilled water. Groups E and F were non diabetic rats with wounds similar to those of diabetic rats in the previous groups and each rat in group E was also treated with sildenafil citrate as in Group A and those in group F were treated with only distilled water. The rats in Group G were normal rats without wound treated with sildenafil citrate as in Group A. Data collected were analysed using SPSS version 20.

Results: The results indicated that sildenafil causes a non significant (P>0.05) decrease in the blood glucose of diabetic rats, a significant (P<0.05) increase in the leucocyte count and a significant decrease (p<0.05) in the rate of percentage decrease in body weight.

Conclusion: Sildenafil may have the potential of reducing the rate of body weight loss in diabetic rats (with or without wound) receiving insulin treatment.

Keywords: Sildenafil, Diabetic Rats, Wound, Body weight, Leucocytes, Glucose

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder that constitutes a major public health problem throughout the world. Current estimates indicate that approximately 4% of the global population suffers from DM, a percentage which is expected to reach 5.4% in 2025.[1] This disease is a multifactor disorder associated with chronic hyperglycemia and troublesome disruptions in carbohydrate, fat, and protein metabolisms emanating from deficiencies or disruptions in insulin secretion, defects in reactive oxygen species scavenging enzymes, and high oxidative stress impairing pancreatic beta cells. Hyperglycemia leads to long-term tissue damages and complications, such as liver-kidney dysfunctions, often associated with serious diseases.[2]

The classical symptoms of untreated diabetes are weight loss, polyuria (increased urination), polydipsia (increased thirst) and polyphagia (increased hunger). The overall risk of dying among people with diabetes is at least double the risk of their peers without diabetes.[3]

Diabetes is projected to raise from 171 million to 366 million in 2030 worldwide.[3] This major increase in morbidity and mortality of diabetes is due to the development of both macro- and micro-vascular complications, including failure of the wound healing process. Wound healing is a very orderly and highly controlled process characterized by four distinct but overlapping phases of hemostasis, inflammation, proliferation and remodeling.[4] The repair process needs the coordination of various cells, growth factors and cytokines. Currently, the approved growth factor and cell therapies for diabetic foot ulcers are not routinely used during treatment. Improper wound healing control may result in diabetic foot ulcer or even amputation.[5] Impaired wound healing is a serious challenge in diabetes, although the exact nature of the pathogenesis of the poor wound healing in diabetes is not completely understood. Evidences from studies involving both human and animal models of diabetes reveal several abnormalities in the various phases of wound healing process including defects in inflammatory response, angiogenesis, fibroplasia, collagen deposition and differentiation of extracellular matrix.[6]

Sildenafil is a selective inhibitor of phosphodiesterase enzyme type 5 (PDE-5) that effectively inactivates cyclic guanosine monophosphate (cGMP) and enhances the effect of nitric oxide.[7] Phosphodiesterase-5 is primarily distributed within the arterial wall, smooth muscles of the lungs and penis. Thus making sildenafil citrate to act selectively in these areas without inducing vasodilatation in other areas of the body.[8] It was used as an antianginal drug in the 1980's and due to an unexpected side effect caused erection in males. It increases the nitric oxide level and nitric oxide in turn increases the cGMP level within the cell. The accumulation of cGMP allows for an enhanced smooth muscle relaxation and increased blood flow in target tissues.[9] Thus, sildenafil has been recognized as being effective for the treatment of erectile dysfunction. Inhibiting cGMP degradation by sildenafil might be a rational approach to treat patients with diabetes, coronary artery disease or heart failure.[10] Sildenafil dilates epicardial coronary arteries, improves endothelial dysfunction and inhibits platelet activation in patients with coronary artery disease[11] and acutely enhances flow-mediated vasodilatation in patients with heart failure.[12] Similarly, it was used as an antihypertensive agent.[13]

The roles for nitric oxide (NO) in the regulation of vasodilation, control of the cell cycling and apoptosis, cell proliferation and differentiation, enhancement of oxygen delivery, and even antimicrobial activity have been described in previous studies.[14,15,16] In the present studies, we hypothesized that such angiogenic and endothelial cell proliferative effect of sildenafil via nitric oxide enhancement could potentiate body weight changes, blood glucose level and leucocyte count during wound healing process of diabetic rats.

MATERIALS AND METHOD

Experimental animals: Forty two (42) Wister albino rats of both sexes obtained from Sanda Kyarimi Park (Zoo) Maiduguri were used for this study. The rats were housed and acclimatized in individual cages for three (3) weeks prior to the experiment. The rats were fed standard pellets growers (vital) feed and water *ad libitum*. The rats were then weighed and randomly divided into seven (7) treatment groups with each group having six (6) rats (three males and three females). The animals were handled according to the International Guiding Principles for Biomedical.[17]

Diabetes induction: Diabetes was induced in groups A, B, C and D by a single intra-peritoneal injection of freshly prepared alloxan monohydrate (130 mg/kg) after the rats were deprived feed for 18 hours. Diabetes was confirmed 3 days after administration of alloxan monohydrate through tail tipping using a glucometer.[18] Only rats with blood glucose level of 180 mg/dl and above were considered diabetic and used for the experiment.

Wound creation: A square shaped wound measuring 1.5 cm by 1.5 cm was created on the dorsum of the anaesthetized (ketamine 50mg/kg intraperitoneal injection) rat by excising the full thickness of the

skin using scalpel blade and scissors. Wound was created on all rats (except those in group G) and dressed with antiseptic (Septol) on day zero and day one while diclofenac 20 mg/kg was administered intraperitoneally daily during the first 3 days of the experiment.

Experimental design and treatment: Rats in the first four groups A-D were diabetic and wound created (TABLE 1). The rats in group A were treated with sildenafil citrate orally at the dose rate of 50 mg/kg body weight for 21 days and the rats in group B were treated with insulin injection of 10

international units once and sildenafil citrate as in Group A. The rats in group C were treated with only insulin injection of 10 international units once and the rats in group D were treated with only distilled water. Groups E and F were non diabetic rats with wounds similar to those of diabetic rats in the previous groups and each rat in group E was also treated with sildenafil citrate as in Group A and those in group F were treated with only distilled water. The rats in Group G were normal rats non diabetic without wound treated with sildenafil citrate as in Group A.

TABLE 1: Grouping and rat treatment

Treatment group	Diabetes induction	Wound Creation	Sildenafil Administration	Insulin Injection	Water Injection
A	Yes	Yes	Yes	No	No
B	Yes	Yes	Yes	Yes	No
C	Yes	Yes	No	Yes	No
D	No	Yes	No	No	Yes
E	No	Yes	Yes	No	No
F	No	Yes	No	No	Yes
G	No	No	Yes	No	No

Determination of blood glucose: Blood glucose was determined using blood glucose meter (glucometer) and testing strips as described by.[18]

Determination of body weight: The rats in all treatment groups were weighed using Ada digital balance on day zero just before wound creation and then weekly for up to 21 days. Weighing was performed in the morning before feeding the rats.

Determination of White Blood Cell (WBC) Count: Standard method[19] was used for the determination of WBC count. The white blood cell diluting blood pipette was used to draw blood to 0.5 mark from the tail vein of the rat. After wiping the tip of the pipette, it was used to draw WBC diluting fluid to 11 mark above the bulb. The pipette was shaken thoroughly to mix its content and then allowed to stand for 3 minutes. After discarding few drops from the pipette, the counting chamber was

charged with fluid. One minute later the cells were counted using ×40 objective of light microscope (Leitz, Wetzler, Germany). The cells from the four squares at the corners were counted and multiplied by one thousand (1,000) to get the total number of white cells in thousand per cubic millimeter ($\times 10^3/mm^3$).

Statistical analysis: Differences between group means were assessed by one way analysis of variance (ANOVA) and Dunnett post test using SPSS version 20 Computer programme.[20] Results with $P < 0.05$ were considered statistically significant.

RESULTS

Effect of sildenafil citrate on body weight of normal and diabetic rats: The effect of sildenafil citrate on body weight is presented in Table 2. There was a general non significant ($p > 0.05$) decrease in body weight across all groups at day 7 and 14. At day 21, there was non significant ($p > 0.05$) decrease in body weight in diabetic groups (A, B, C and D) and non significant ($p > 0.05$) increase in non diabetic groups (E, F and G) compared to day zero. However, a significant difference exist ($p < 0.05$) between the percentage differences of body weight of groups A (diabetes+wound+sildenafil), E (wound+sildenafil) and F (wound only) compared to group C (diabetes+wound+insulin) at day 7 as well as groups A, E, F and G (sildenafil only) at day 14.

Effect of sildenafil citrate on blood glucose level of diabetic rats: The effect of sildenafil citrate on blood glucose level of diabetic rats is presented in Table 3. The result shows significant ($p < 0.05$) increase in the blood glucose levels at day zero in groups A (diabetes+wound+sildenafil), C (diabetes+wound+insulin) & D (wound+diabetes+water) compared to prediabetic values. That is, there was increase in the blood glucose level in all groups treated with alloxan. At day 7, there was significant ($p < 0.05$) increase in groups A and D while at day 14 only group A remained significant ($p < 0.05$).

Effect of sildenafil citrate on white blood cell count of diabetic and non diabetic rats: The result of the effects of sildenafil citrate on white blood cell count as presented in Table 4 indicated a significant increase ($p < 0.05$) in the white blood cell count of the diabetic groups A (diabetes+wound+sildenafil), B (diabetes+wound+sildenafil+insulin), C (diabetes+wound+insulin) and D (wound+diabetes+water) at day 0 when compared with the prediabetic values; a significant decrease at day 7 and 21 when compared with day 0, and a significant decrease only exist between groups A and B at day 14 when compared to day 0 but significant increase in groups C and D compared to the prediabetic values.

In the non diabetic groups E (wound+sildenafil+water), F (wound) and G (sildenafil) there is a significant increase of WBC count at day 14 and 21 in group E, day 21 in group F as well as significant decrease at day 7 in groups F and G when compared to prediabetic values. Similarly, a significant decrease exist at day 7 in groups F and G and at day 14 in group G when compared to day 0.

TABLE 2: Effect of sildenafil citrate on body weight (g) of normal and diabetic rats

Group	Days post treatment			
	0	7	14	21
A	170.83±12.68	153.67±11.68 (3.03±1.49*)	149.67±8.20 (6.07±1.34)*	166.40±20.37 (17.23±3.27)
B	183.17±13.75	147.00±17.79 (14.80±2.34)	146.67±17.70 (14.87±3.30)	178.30±19.90 (6.77±1.08)
C	182.67±10.84	145.33±9.68 (21.13±4.44)	142.67±12.12 (23.57±4.83)	180.90±13.16 (8.90±3.93)
D	177.00±11.17	150.75±12.50 (14.78±1.31)	150.00±13.42 (15.00±4.51)	176.83±9.34 (6.53±1.68)
E	165.67±10.49	152.83±8.57 (7.50±1.61*)	161.00±9.01 (5.63±1.76*)	187.37±6.33 (14.27±3.98)
F	181.00±12.01	170.83±10.78 (5.47±1.20*)	174.00±9.50 (3.90±1.33*)	210.70±10.13 (17.20±2.68)
G	190.17±15.60	168.50±16.85 (11.75±3.68)	174.50±16.31 (8.45±3.64*)	201.82±17.40 (8.80±2.95)

Values are for Mean ± SEM (percentages)

*=signifantly ($p < 0.05$) different from group C along the column for the same treatment day. A- diabetes+wound+sildenafil; B- diabetes+wound+sildenafil+insulin; C- diabetes+wound+insulin; D- wound+diabetes+water; E- wound+sildenafil+water; F- wound and G- Sildenafil

TABLE 3: Effect of sildenafil citrate on blood glucose (mg/dl) of diabetic rats

Group	Prediabetes	Days of treatment				P value
		0	7	14	21	
A	96.00±3.04	301.25±45.76*	492.33±53.30*	451.33±98.10*	151.67±13.62	0.000
B	86.6±4.23	189.00±77.46	298.67±150.95	327.33±124.29	186.33±71.05	0.135
C	97.17±6.23	496.33±38.33*	423.33±149.16	423.33±176.67	218.67±60.41	0.019
D	83.00±3.13	42300±80.77*	309.25±63.39*	263.50±106.57	215.25±34.25	0.010

Values are for Mean ± SEM

*= signifantly ($p < 0.05$) higher than prediabetic value along the same row within the same group.
A- diabetes+wound+sildenafil; B- diabetes+wound+sildenafil+insulin; C- diabetes+wound+insulin;
D- wound+diabetes+water

TABLE 4: Effect of sildenafil citrate on white blood cell count (x 10^3/l) of normal and diabetic rats

Group	Prediabetes	Days of treatment			
		0	7	14	21
A	7833.3±294.0[b]	21966.7±1615.7[a]	5850±960.9[b]	6833.3±1592[b]	14333.3±1666.7[ab]
B	7441.7±360.0[b]	24716.7±2952.1[a]	9333.3±1682.6[b]	11693.3±2968.3[b]	9333.3±2034.2[b]
C	7533.3±203.3[b]	28200±1584.9[a]	4000±1836[b]	24100±2811.3[a]	16900±1193[ab]
D	7791.7±336.8[b]	22250±3412.4[a]	4187.5±1558.9[b]	19837.5±991.9[a]	13305±2002.7[b]
E	7666.7±744.2	7541.7±222.3	4125±871.56	12408.3±1366[ab]	11766.7±1672.9[ab]
F	6033.3±744.2	8950±1594.1	3600±964.8[ab]	9191.7±249.1	13216.7±2287.6[a]
G	7333.3±366.7	6608.3±708.8	3233.3±351.8[ab]	10475±1245.6[b]	8870±912.9

Values are for Mean ± SEM

[a] =signifantly (p<0.05) different from prediabetic value along the same row

[b] =signifantly (p<0.05) different from day zero value along the same row

A- diabetes+wound+sildenafil; B- diabetes+wound+sildenafil+insulin; C- diabetes+wound+insulin; D- wound+diabetes+water; E- wound+sildenafil+water; F- wound and G- Sildenafil.

DISCUSSION

Sildenafil citrate is typically known for treating erectile dysfunction in male and it works by inhibiting the enzyme (phosphodiesterase 5) that break down the natural blood vessel relaxing chemical, cyclic guanosine monophosphate (cGMP). Several studies have been conducted to explore other potential benefit of sildenafil citrate. In this experiment, the effects of oral administration of sildenafil citrate at the dose of 50 mg/kg for twenty-one days was studied in relation to the body weight and white blood cell count (WBC) of diabetic and non diabetic rats as well as the blood glucose level of diabetic rats. The result of the effect of sildenafil citrate on the body weight of both diabetic and non diabetic rats revealed a non significant difference in the mean body weight of diabetic and non diabetic rats. However, the percentage difference at day 7 and 14 of treatment in groups A (diabetes+wound+sildenafil), E (wound+sildenafil+water) and F (wound) was significantly lower when compared to group C (diabetes+wound+insulin). Also, at day 14 there was a significant decrease in the percentage

difference in group G (sildenafil only) when compared to group C. The significantly lower percentage difference observed in groups A, E and G (all treated with sildenafil) is an indication that sildenafil citrate has effect in reducing the rate of body weight loss in diabetic and non diabetic rats. Earlier work has shown that sildenafil citrate improve fetal weight in mouse model of fetal growth restriction.[21]

The result on the effect of sildenafil citrate on the blood glucose level of diabetic rats showed a significant increase in the blood glucose level of group A (sildenafil treated) similar to that of negative control group D that was neither treated with insulin nor sildenafil. This is an indication that sildenafil citrate has a subtle effect in reducing the blood glucose level in diabetic rats. However the non significant increase observed in group B suggests that sildenafil citrate might have increase the production or utilization of insulin. To explain this, it should be noted that increasing cGMP is a fundamental mechanism of action of sildenafil citrate. A study on rat hepatocytes cGMP indicated

that sildenafil citrate increases hepatocytes in vitro level but does not significantly alter glycogenolysis and gluconeogenesis.[22] That is partly in agreement with the present findings when considering that blood glucose does not significantly change in sildenafil treated group.

Nitric oxide has been suggested as a second messenger molecule for the stimulatory effect of insulin in carbohydrate metabolism.[23] Similarly, another work revealed that only high dose of sildenafil citrate affected blood glucose[24] which is also in agreement with this finding. It is pertinent to note that because sildenafil citrate showed insignificant effect in reducing the blood glucose level of diabetic rats, the resultant net effect of diabetes which include poor protein synthesis, dehydration, muscle wasting and metabolic derangement such as glycogenolysis, gluconeogenesis and acidosis[25]could be a justified reason for the decreasing body weight observed in this experiment.

The result on the effect of sildenafil citrate on the white blood cell (WBC) count of diabetic and non diabetic rats showed a significant increase of the WBC count in the diabetic groups compared to their prediabetic values indicating that diabetes increases the WBC counts of the rats. Furthermore, in the sildenafil a treated diabetic group A and B, there was a significant increase in the WBC count

throughout the days of the experiment unlike in groups C and D that showed a non significant increase at day 14 compared to values at day zero. Similarly, in the non diabetic groups (E, F and G), there was a significant increase in the sildenafil treated non diabetic groups E and G while a non significant increase in group F which was not treated with sildenafil citrate. This result can be interpreted that sildenafil citrate increases the WBC count of both diabetic and non diabetic rats which confers favourably with previous findings who also reported a significant increase of WBCs in sildenafil treated domestic rabbits.[26]

The significant increase in the WBC count indicated there was activation of defense mechanism of the immune system of rats. This induction of WBCs is a positive response for survival due to cell mediated immune response in the rats.

CONCLUSION
Sildenafil citrate may have the potential of reducing the rate of body weight loss in diabetic rats receiving insulin treatment whether the rats are having wound or not. The study also found out that sildenafil citrate at 50mg/kg (orally) does not reduce the blood glucose level in diabetic rats but increases the white blood cell count. Further study using graded dose of sildenafil may reveal a better relationship between the effect of sildenafil and the measured parameters in this study.

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