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Haematological changes and wound healing effects of sildenafil citrate in diabetic albino rats

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Abstract

This study presents baseline data on the effect of sildenafil citrate on some of the haematological and biochemical parameters in wistar rats with diabetes and wound formation. Forty two albino rats weighing between 139 and 225 g were separated at random into seven groups (A, B, C, D, E, F and G) of six rats per group. Type 1 diabetes mellitus was induced in groups A, B, C and D by a single intra-peritoneal injection of 130 mg/kg Alloxan. Rats in group A were treated with orally dose of 50 mg/kg sildenafil citrate for 21 days and rats in group B were administered 10 IU of insulin intramuscularly once and sildenafil citrate as in Group A. The rats in group C were treated with a single dose of 10 IU of insulin intramuscularly, and rats in group D with only distilled water. Groups E and F were normal 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. Group G were normal rats without wound treated with sildenafil citrate as in Group A. Blood samples were taken before (day 0) and after the administration of the sildenafil citrate on day 7, 14 and 21. The results of the study showed significant decreased in red blood cells count from $6.28 \pm 0.46 \times 10^6$ to $2.81 \pm 0.46 \times 10^6$ in diabetic rats during the 14 days of treatment with sildenafil citrate, however, there was an indication that continues treatment for up to 21 days reduced the blood glucose and increased the red blood cells count and this may be an indication that sildenafil citrate improves insulin mediated glucose pathways.

Keywords: Diabetes, Haematological parameters, Rats, Red blood cells, *Sildenafil citrate*

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Introduction

Diabetes mellitus is a chronic hyperglycemic disorder associated with disruption 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 (Kim *et al.,* 2006).

The classical symptoms of diabetes are weight loss, polyuria (increased urination), polydipsia and polyphagia (Das *et al.,* 2012). There are three types of diabetes; Type 1 diabetes is characterized by a lack of insulin production. Without daily administration of insulin, type 1 diabetes is rapidly fatal. Type 2 diabetes results from the body's ineffective use of insulin. About 90% of people with diabetes around the world have type 2. It is largely the result of excess body weight and physical inactivity (Das *et al.,* 2012). Gestational diabetes is another form of diabetes that occurs when pregnant women without a previous history of diabetes develop high blood glucose level.

Sildenafil citrate is a selective inhibitor of phosphodiesterase enzyme type 5 (PDE5) that effectively inactivates cyclic guanosine monophosphate (cGMP) and enhances the effect of nitric oxide (Jung *et al.,* 2011), Phosphodiesterase-5 is primarily distributed within the arterial wall, smooth muscles of the lungs and penis (Shadi *et al.,* 2012). It was used as an antianginal drug in 1980's and showed an unexpected side effect of causing 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 tissue (Ayyildiz *et al.,* 2006). 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 (Gross*,* 2005). Sildenafil dilates epicardial coronary arteries, improves endothelial dysfunction and inhibits platelet activation in patients with coronary artery disease (Halcox *et al.,* 2002) and acutely enhances flow-mediated vasodilation in patient with heart failure (Hryniewicz *et al.,* 2005).

Wound healing constitutes a great challenge in the diabetic patients (Fahey *et al.,* 1991). This challenge is seen both at the cellular and humoral levels involving white blood cells and their significant roles in the wound healing of diabetic rats administered sildenafil citrate (Tas *et al.,* 2011).

This study which involves analysis of red blood cells count in diabetic rats with wound formation following treatment with sildenafil citrate is of significance because wound healing also depend on oxygenation which is supplied to the bodily parts via hemoglobin in the circulating red blood cells (Girasole *et al.,* 2007; Hamed *et al.,* 2010).

The roles of nitric oxide in the regulation of vasodilation (Furchgott, 1996), control of the cell cycling and apoptosis, cell proliferation and differentiation (Weller *et al.,* 1998) have been described in previous studies. In the present study, we hypothesized that such angiogenic and endothelial cell proliferative effect of sildenafil citrate could potentiate wound healing process in diabetic rat by reducing the blood glucose and increasing the red blood cells count thus increasing the oxygen supply to the wound site. The objectives of this study therefore are to observe the effect of sildenafil citrate on some of the haematological and biochemical parameters in the early phase of wound healing in diabetic albino rats.

Materials and Methods

Experimental animals and methods

Forty two (42) Wistar albino rats of both sexes were obtained from Sanda Kyarimi Zoo Park, Maiduguri. The rats were housed in individual cages and fed standard feed (grower's mash) and water provided *ad libitum*. They were acclimatized for three (3) weeks prior to the experiment. The rats were weighed and randomly divided into seven (7) treatment groups with each group having six (6) rats (three males and three females kept in two separate cages to ensure that there was no mating). The animals were handled according to the International Guiding Principles for Biomedical Research (CIOMS, 1985).

Induction of diabetes

Type – 1 diabetes mellitus 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. After three days, rats with blood glucose level of 180 mg/dl and above were considered diabetic and used for the experiment (Meiton, 2006).

Determination of blood glucose

Blood glucose was determined using Accu - chek® blood glucose meter and testing strips as described by (Meiton, 2006).

Wound creation

Wound was made on the back of each rat by excision according to the method of Abu-Al-Basal (2001). Briefly, the back of each animal was shaved with hair clipper and sterilized with 70 % ethanol before a square sized wound (0.5 × 0.5cm) was made by lancet knife on a pre-determined area under ketamine anesthesia. The wound was left undressed, and no local or systemic antimicrobial agent was administered.

Experimental design

The rats in group A were treated with sildenafil citrate orally at a dose of 50 mg/kg once daily for 21 days and the rats in group B were treated with insulin injection of 10 IU once and sildenafil citrate orally at a dose of 50 mg/kg once daily for 21 days. The rats in group C were administered a single dose of 10 IU of insulin intramuscularly while the rats in group D were treated with distilled water to serve as control. Groups E and F rats were not diabetic and have a square sized wound (0.5×0.5cm) created on

their back. Each rat in group E was also treated with sildenafil citrate orally at a dose of 50 mg/kg once normal rats without wound but treated with sildenafil citrate orally at a dose rate of 50 mg/kg once daily for 21 days. The precise dosage of 50 mg / kg sildenafil citrate was controlled because: Dose = Dosage × Body Weight / Concentration. A concentration of 20 mg / ml was chosen for calculating our dose.

Haematology

One milliliter of blood was obtained from the tail vein of each rat at days 0, 7, 14 and 21 into commercially prepared bottles with ethylene diamine tetra-acetic acid as anticoagulant (1 mg/ml) and used for the determination of haematological parameters such as packed cell volume (PCV); haemoglobin (Hb) concentration and red blood cells count (RBC). The PCV was determined by microhaematocrit method; The Hb concentration was measured calorimetrically by cyanmethaemoglobin method; The RBC count was done by haemocytometry. The mean corpuscular volume (MCV) and the mean corpuscular haemoglobin concentration (MCHC) were calculated using standard formulae (Schalm *et al*., 1975).

Statistical analysis

Data were analyzed using statistical software IBM SPSS Statistic 22. Data were expressed as mean ± standard deviation. Differences between group means were analyzed by one way analysis of variance (ANOVA). Differences were statistically significant when $P < 0.05$.

daily for 21 days and those in group F were treated with distilled water. The rats in Group G were

Results

Induction of diabetes

A single high dose of alloxan monohydrate (130 mg/kg given intraperitoneally) led to 80 % incidence of type 1 diabetes mellitus and only 10 % mortality.

Effects of Sildenafil citrate on blood glucose of diabetic rats

Significant decrease ($P < 0.05$) in the blood glucose was observed in diabetic rats with wound treated with sildenafil citrate orally at a dose of 50 mg/kg once daily for 21 days (Figure 1). The decrease in blood glucose (mg/dl) of diabetic rats with wound treated with distilled water was not significant ($P > 0.05$) (Figure 2).

Effect of sildenafil citrate on some haematological parameters

The results of the study showed that diabetic rats with wound treated with sildenafil citrate orally at a dose of 50 mg/kg had decreased red blood cells count during the 14 days of treatment (Figure 3; Figure 4), however, there was an indication that prolonged treatment for up to 21 days increased the red blood cells count (Figure 3; Figure 4). Red blood cells count of diabetic rats with wound treated with distilled water is shown in Figure 5 and the red

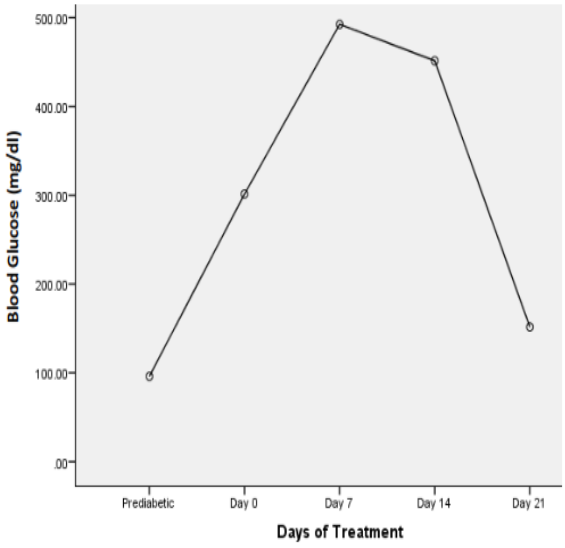

Figure 1: Blood glucose (mg/dl) of diabetic rats treated with sildenafil citrate

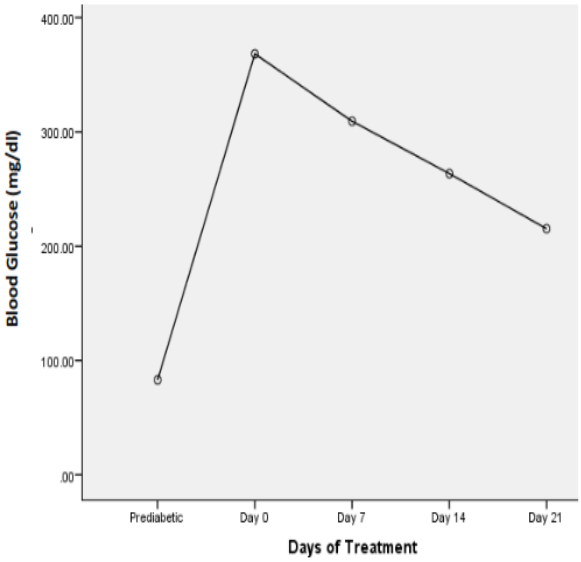

Figure 2: Blood glucose (mg/dl) of diabetic rats treated with distilled water

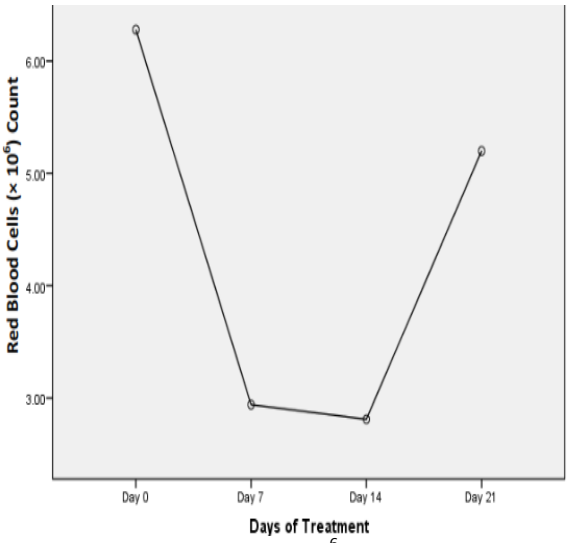

Figure 3: Red blood cells (×10^6) count of diabetic rats treated with sildenafil citrate

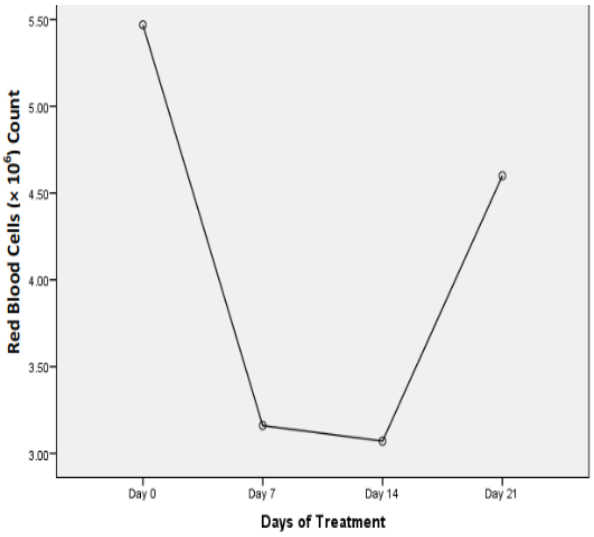

Figure 4: Red blood cells (×10^6) count of diabetic rats treated with sildenafil citrate and insulin

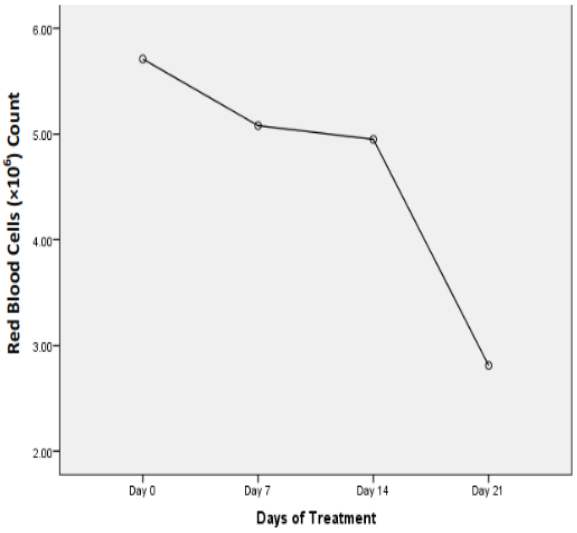

Figure 5: Red blood cells (×10^6) count of diabetic rats treated with distilled water

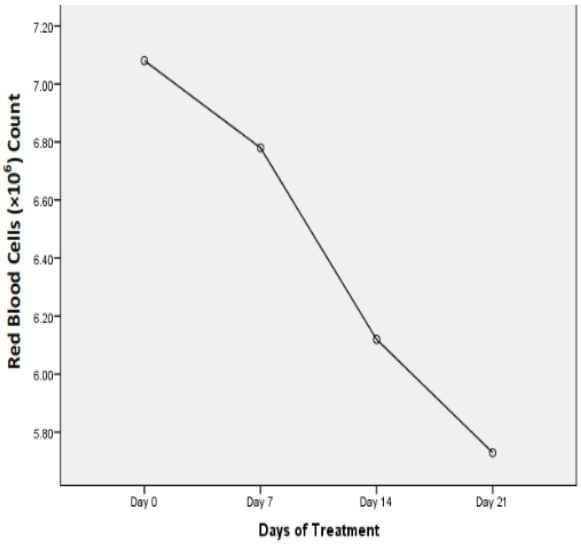

Figure 6: Red blood cells (×10^6) count of normal rats treated with distilled water

Table 1: Effects of sildenafil citrate on haemoglobin (g/dl) concentration of diabetic and normal rats

Group	Days of Treatment			
	0	7	14	21
A	11.5±0.41	11.07±0.73	12.50±1.22	9.97±1.58
B	10.13±0.65	11.13±1.03	9.93±0.87	10.57±1.91
C	10.32±0.41	10.70±1.10	10.07±1.45	8.30±1.73
D	12.08±0.54	10.80±0.34	11.65±1.47	9.93±0.64
E	12.80±0.54	12.10±0.51	10.93±0.54	10.93±0.82
F	12.53±0.65	10.28±0.71	8.40±0.48	11.18±0.80
G	13.28±0.49	11.40±0.27	9.88±0.57	11.62±0.82

Table 2: Effects of sildenafil citrate on packed cell volume (%) of diabetic and normal rats

Group	Days of Treatment			
	0	7	14	21
A	41.33±1.20	37.67±1.76	39.67±2.33	34.33±1.45
B	42.67±0.67	32.67±4.37*	36.67±1.20*	34.67±2.33
C	40.50±0.42	35.33±5.51	36.67±1.86	37.00±0.88
D	41.00±1.46	40.50±0.96	39.25±±1.70	37.75±1.80
E	42.33±1.84	41.33±0.42	44.17±1.82	36.83±1.38
F	43.50±1.36	38.33±0.92	41.33±0.92	35.83±1.66
G	42.67±1.31	40.33±0.88	41.83±1.01	40.40±0.51

Table 3: Effects of sildenafil citrate on mean corpuscular haemoglobin concentration (MCHC, g/dl) of diabetic and normal rats

Group	Days of Treatment			
	0	7	14	21
A	27.02±0.90	29.48±2.26	31.85±4.27	28.85±3.61
B	23.69±1.30	34.56±1.93	27.20±4.27	30.02±3.48
C	25.49±1.04	30.48±2.61	27.28±2.90	22.32±4.25
D	31.25±0.99	26.66±0.35	30.18±4.66	26.34±1.16
E	30.29±1.02	28.23±0.81	24.79±0.87	29.44±1.65
F	28.87±1.42	26.88±1.82	20.29±0.89	31.27±2.07
G	30.59±1.09	28.27±0.36	23.74±1.65	28.66±2.11

Table 4: Effects of sildenafil citrate on mean corpuscular volume (MCV, fl) of diabetic and normal rats

Group	Days of Treatment			
	0	7	14	21
A	67.09±3.53	181.21±62.18	210.17±86.31	66.29±4.61
B	79.75±5.88	99.54±31.27	121.20±7.90	81.36±16.96
C	84.35±12.22	99.99±14.31	87.17±12.85	91.36±22.84
D	101.41±14.91	85.56±12.95	84.95±14.83	66.61±4.46
E	98.09±17.75	70.45±5.85	68.53±5.34	72.19±8.39
F	63.62±5.12	59.04±5.23	67.62±1.67	69.18±12.73
G	69.60±5.60	67.72±4.44	62.72±2.53	76.59±3.52

Group identification**:**
A: diabetes + wound + sildenafil citrate
B: diabetic + wound + sildenafil citrate + insulin
C: diabetic + wound + insulin
D: diabetic + wound + H_2O
E: wound + sildenafil citrate
F: wound + H_2O
G: sildenafil citrate

blood cells count of normal rats without diabetes and wound is shown in Figure 6. Treatment with sildenafil citrate orally at a dose of 50 mg/kg has no effect on hemoglobin concentration (Table 1), packed cell volume (Table 2), mean corpuscular haemoglobin concentration (Table 3) and mean corpuscular volume (Table 4).

Discussion

The dosage of sildenafil citrate at 50 mg/kg was adapted from previous studies (Etuk *et al.,* 2010) and our pilot study using rat models. The results of the present study have provided a baseline data on the effects of sildenafil citrate on some of the haematological parameters in the early phase of

wound healing in diabetic rats. The present study observed the involvement of the red blood cells and their significant roles in the wound healing of diabetic rats treated with sildenafil citrate orally at a dose of 50 mg/kg for 21 days. The results of the study showed that the administration of sildenafil citrate orally at a dose of 50 mg/kg decreased the red blood cells count of the experimental rats during the 14 days of the treatment. The decreased in the red blood cells may be due to hyperglycemia which appears to induce oxidative stress on the red blood cells and this can cause an increase in the production of free radicals, the result of glycosylation product in the blood (Brownlee, 1992; Jin *et al.,* 2010). Treatment with sildenafil citrate orally at the dose rate of 50 mg/kg has no effect on hemoglobin concentration, packed cell volume, mean corpuscular hemoglobin concentration and mean corpuscular volume. Sildenafil citrate, a specific inhibitor of cyclic guanylate monophosphate phosphodiesterase type 5 (PDE5) known to stimulate release of nitric oxide, that plays an important role in wound healing involving oxidative stress by reactive oxygen species (Derici *et al.,* 2010). However, at high concentration, sildenafil citrate adversely affects the red blood cells membrane architecture and cellular mechanical behavior (Jin *et al.,* 2010).

Treatment with sildenafil citrate orally at the dose rate of 50 mg/kg for 21 days significantly decreased blood glucose and increase the red blood cells count of diabetic rats. The decreased blood glucose and increased red blood cells count observed during the 21 days treatment may be due to the action of sildenafil citrate in the inhibition of cGMP degradation (Gross, 2005; Hamed *et al.,* 2010) and may be an indication that sildenafil citrate improves insulin mediated glucose pathways and reduce blood glucose and increase red blood cells count (Hamed *et al.,* 2010; Das *et al.,* 2012). This supports the findings of Derici *et al.* (2010) which states that, sildenafil citrate, a specific inhibitor of cyclic guanylate monophosphate phosphodiesterase type 5 (PDE5) is known to stimulate the release of nitric oxide, that plays an important role in wound healing involving oxidative stress by reactive oxygen species. However, it is recommended that further studies should be carried out in this field of research using lower dosages of sildenafil citrate administered through the systemic route for a longer period of time.

In conclusion, the results of the study showed that the administration of sildenafil citrate orally at a dose of 50 mg/kg decreased red blood cells count of the experimental rats during the 14 days of the treatment, however, there was an indication that prolonged treatment for up to 21 days reasonably reduced the blood glucose and increased the red blood cells count thus increasing the oxygen supply to the wound site as seen in the healing of diabetic rats in this research work.

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