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THE POTENTIAL EFFECT OF SILDENAFIL CITRATE ON SOME HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN HYPERGLYCEMIC RATS

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ABSTRACT

Sildenafil citrate (SC) is a phosphodiesterase type-5 inhibitor, which acts to increase cyclic guanosine monophosphate (cGMP). This study aims to assess the effects of Sildenafil citrate (SC) on glycemic control and hematological parameters of diabetic rats induced with streptozotocin (STZ). Fifty male Wistar rats were divided into four groups (n=10/non-diabetic group; n=15/diabetic group): i. control (I), ii. SC-treated control (II), iii. diabetic (III), and iv. SC-treated diabetic (IV). Diabetes was induced by an intraperitoneal injection of STZ (50 mg/kg). SC (20 mg/kg body weight) was administered orally for six weeks. Blood specimens were taken to measure levels of serum glucose (FBG), insulin, glycated hemoglobin (HbA1c), aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, creatinine, prothrombin time (PT), partial thromboplastin time (aPTT), fibrinogen, protein C, and protein S. Levels of FBG, HbA1c, AST, ALT, urea, creatinine, and fibrinogen were significantly elevated in the rats with diabetes compared to the controls. Conversely, insulin, aPTT, protein C and S were significantly lower in the diabetic groups. No significant difference was seen in the PT of control and diabetic rats. No effect was found in the administration of SC on healthy control rats. Upon administration of SC to the diabetic rats, these parameters returned to normal ($P<0.05$). SC was found to improve glycemic control as well as microcirculatory perfusions in diabetic rats, warranting its consideration as a way to lessen diabetic complications.

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Introduction

Diabetes mellitus (DM) is a serious chronic health condition affecting a considerable proportion of the population globally, in both developed and developing countries. Almost 400 million people were diagnosed with DM in 2013, and this number is expected to rise to around 600 million by 2035 [1]. One of the primary conditions associated with DM is chronic hyperglycemia, which can cause glucose to react non-enzymatically with peptides, proteins, and lipids to form advanced glycation end-products (AGEs). These AGEs are associated with chronic inflammation, which in turn leads to microvascular complications, especially in the kidneys, eyes, and nerves [2].

Sildenafil citrate (SC) is a phosphodiesterase type 5 (PDE-5) inhibitor, so its role in vasodilation is widely known [3] and is commonly used to treat erectile dysfunction. It has also been investigated for the treatment of several other conditions, including lupus rheumatoid arthritis, and pulmonary hypertension, and its clinical effects were approved for treating several cardiovascular and obstructive pulmonary diseases in men [2, 4]. The effect of sildenafil is to raise cyclic guanosine monophosphate (cGMP) levels by rendering cGMP-metabolizing PDEs inactive [3]. At the intracellular level, cGMP mediates the nitric oxide (NO) pathway, increasing its synthesis. NO relaxes the vascular smooth muscle, promotes vasodilation and blood flow, and inhibits platelet aggregation and microcirculation [5]. NO has also become associated with the regulation of blood glucose and insulin [6].

Both SC and insulin promote NO synthesis in tissues (e.g., liver, brain, skeletal muscle, endothelium, and beta cells) that play a role in maintaining glucose homeostasis, with evidence of improved glucose metabolism in diabetics given SC [7, 8].

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Although the use of SC in patients with DM is increasing, research on the effects of continuous use of SC on glycemic control is lacking. The present study, then, aims to evaluate the impacts of daily oral sildenafil (20 mg/kg) on glycemic control and to investigate if SC can improve the coagulation abnormalities that lead to hypercoagulability in streptozotocin-induced diabetic male rats.

Materials and Methods

Experimental Animals

Fifty adult male Wistar albino rats weighing between 25–30 g were obtained as an animal models for the present study. The animals were obtained from the animal housing unit of the Faculty of Medicine of King Abdulaziz University (KAU) in Jeddah, Saudi Arabia. The rats were acclimatized to lab conditions (held at controlled room temperature with a 12-hour light-dark cycle) for at least a week. They had free access to drinking water and were given a balanced diet consisting of commercial pellets.

The rats were cared for following KAU's policy and the International Ethical Guidelines on the Care and Use of Laboratory Animals. The Research Ethics Committee at the Faculty of Medicine at KAU approved the experimental study.

Induction of Diabetes

To induce diabetes, the animals in the diabetic groups were given one intraperitoneal (IP) injection of streptozotocin (STZ) (50 mg/kg) dissolved in a fresh citrate buffer (0.1 M, pH 4.5). STZ was obtained from Sigma (Sigma-Aldrich, St. Louis, MO, USA). For the following 48 hours, the rats were given an oral 10% glucose solution in addition to their regular food to prevent the initial drug-induced hypoglycemia. To confirm diabetes in these rats, their fasting blood glucose levels were measured three days after the STZ injection using a fine test glucometer. Rats with blood glucose readings ≥ 250 mg/dl were regarded as diabetic and included in the study.

Sildenafil Citrate Application

SC (Viagra, Pfizer; St Louis, MO, USA) was crushed and mixed with 2–3 ml of drinking water until a homogenous suspension was obtained. The resulting solution was given by mouth using a gavage injector. A daily dose of 20 mg/kg of SC was administered by gavage for six successive weeks.

Animal Grouping

Each of the 50 experimental rats was randomly assigned to one of four groups:
Group I: Normal control (n=10); received a single IP injection of citrate buffer (pH=4.5).
Group II: Control SC (n=10); received the same IP injection + oral SC.
Group III: Control diabetic (n=15); received a single IP injection of STZ (50 mg/kg).
Group IV: Diabetic SC (n=15); received the same IP injection of STZ + oral SC.

Collection of Samples for Analysis

After the 6^{th} week, blood samples were obtained from the retro-orbital veins of each rat under light ether anesthesia after 12-hour overnight fasting (access to water ad libitum). Blood samples were centrifuged at 2500 rpm for 15 minutes, and the serum was used for the measurement of the studied biochemical parameters.

Biochemical Studies

Glycosylated hemoglobin (HbA1c) concentration was estimated using a VITROS 5,1 FS chemistry auto-analyzer (Ortho-Clinical Diagnostics Inc., Rochester, NY, USA).

Fasting glucose in serum was measured employing a colorimetric method, using a VITROS 250 Clinical Chemistry Auto-analyzer (Ortho-Clinical Diagnostics Inc., Rochester, NY, USA).

Insulin levels were estimated in serum by a sandwich CLIA using a LIAISON autoanalyzer (DiaSorin Inc, Stillwater, MN, USA).

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, and creatinine were estimated in serum using the colorimetric method using a VITROS 250 Clinical Chemistry Auto-analyzer (Ortho-Clinical Diagnostics Inc., Rochester, NY, USA).

Prothrombin time (PT), partial thromboplastin time (aPTT), fibrinogen, protein C, and protein S were estimated using Biomed diagnostic kits (Biomed, Cairo, Egypt) and performed on an automated coagulation analyzer according to manufacturer instructions.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Science (SPSS 17 for Windows; SPSS Inc, Chicago, IL, version 21.2001). Results were expressed as mean ± standard error of the mean (SEM). One-way ANOVA was used followed by post hoc Tukey's test for comparing the mean. Differences were considered significant at $P < 0.05$.

Results and Discussion

Single IP injection of STZ (50 mg/kg body weight (BW)) in male rats resulted in hyperglycemia in all cases with no deaths occurring during the experiment. There were no significant differences in laboratory results in the subgroups of the control group (I and II).

The concentrations of serum glucose, insulin, and HbA1c in normal (control) and STZ-induced diabetic rats are presented in **Table 1**. The mean blood glucose level of the diabetic groups was significantly higher (P<0.05) than that of the control rats. Administration of SC produced significantly lower blood glucose levels compared to diabetic rats not treated with SC (P<0.05). Induction with diabetes resulted in decreased insulin levels compared to normal control rats. There was a significant (P<0.05) rise in serum insulin concentration in the group of SC-treated diabetic rats compared to that of the diabetic control rats. Induction with diabetes raised HbA1c levels compared to normal control rats (6.91± 0.04% vs. 4.20 ± 0.06%). However, treatment with SC significantly (P<0.05) lowered the level of HbA1c when compared to the untreated diabetic rats (4.80 ± 0.03% vs. 6.91± 0.04%).

Table 1. Serum glucose, insulin, and % HbA1c in the studied groups

	Group I	Group II	Group III	Group IV
Glucose (mg/dl)	73.21 ± 0.74	74.0 ± 0.63	265.5 ± 0.78*	105.60 ± 0.72£※
Insulin (IU/dl)	11.7 ± 1.2	12.0 ± 1.1	4.18 ± 0.3*	9.2 ± 1.1£※
HbA1c %	4.20 ± 0.06	4.23 ± 0.09	6.91 ± 0.04*	4.80 ± 0.03£※

Data are expressed as mean ± SE, *: P<0.05 as compared to the controls (I and II), £: P<0.05 as compared to the diabetic rats' III, ※: P<0.05 as compared to the controls (I and II).

Liver function tests after 6 weeks showed a significant difference between the normal control groups of rats and the diabetic groups (P<0.05). A significant increase in the AST and ALT measurements was seen in the diabetic rats compared to the non-diabetic controls (P<0.05). However, treatment with SC in diabetic rats produced a marked lowering of the elevated serum AST and ALT. No significant difference was found in AST or ALT levels in the SC-treated and non-SC control groups.

Serum creatinine and urea levels, as markers of renal function, are summarized in **Table 2**. Rats in the diabetic groups had significantly higher (P<0.05) serum creatinine and urea concentrations. However, diabetic rats that were given SC had significantly lower creatinine and urea levels than the diabetic rats that were not given SC. No significant difference was found between the control groups.

Table 2. Effect of sildenafil on serum AST, ALT, and kidney function biomarkers in the different groups

	Group I	Group II	Group III	Group IV
AST (U/L)	94.7 ± 2.7	95.3 ± 2.2	179.6 ± 6.4*	114.8 ± 6.1£※
ALT (U/L)	24.63 ± 1.81	23.16 ± 0.89	57.63 ± 5.70*	32.67 ± 2.3£※
Urea (mg/dl)	28.20 ± 0.24	26.81 ± 0.17	93.85 ± 1.45*	56.9 ± 1.02£※
Creatinine (mg/dl)	0.56 ± 0.08	0.60 ± 0.01	2.42 ± 0.34*	0.93 ± 0.01£※

Data are expressed as mean ± SE, *: P<0.05 as compared to the controls (I and II), £: P<0.05 as compared to the diabetic rats' III, ※: P<0.05 as compared to the controls (I and II).

The effect of SC on the coagulation parameters in the studied rats is shown in **Table 3**. No significant difference was found between the control groups (I and II) in all measured parameters. The PT of rats given STZ (III) did not differ significantly from that of the control rats. Compared with the controls, the diabetic group had significantly shorter aPTT values. Similarly, anticoagulation factors such as fibrinogen, protein C, and protein S were significantly different in the diabetic control group, with higher levels of fibrinogen and lower levels of proteins C and S. Sildenafil treatment in diabetic rats (IV) significantly lowered the elevation of fibrinogen and raised aPTT, protein C, and protein S levels in comparison to the diabetic group not given SC (III).

Table 3. Effect of sildenafil on coagulation parameters in rats

	Group I	Group II	Group III	Group IV
PT (sec)	20.52 ± 0.23	19.95 ± 0.27	20.55 ± 0.50	20.41 ± 0.39
aPTT (sec)	41.46 ± 2.5	41.67 ± 2.7	37.11 ± 2.1*	40.25 ± 2.5£※
Fibrinogen (mg/dl)	230 ± 0.94	229.42 ± 1.50	298.3 ± 0.96*	251.06 ± 0.78£※
Protein C (%)	56.93 ± 0.81	56.60 ± 1.23	36.82 ± 0.93*	55.57 ± 2.07£※
Protein S (%)	62.34 ± 0.92	62.42 ± 1.4	41.03 ± 0.85*	56.01 ± 0.87£※

Data are expressed as mean ± SE, *: P<0.05 as compared to the controls (I and II), £: P<0.05 as compared to the diabetic rats' III, ※: P<0.05 as compared to the controls (I and II).

This study investigated the effect of SC (20 mg/kg BW) administered orally for six weeks on serum glucose level and biochemical and hematological parameters in STZ-induced diabetic rats. STZ is routinely used to produce diabetic animals for research purposes. On a molecular level, it is similar to glucose, having a cytotoxic effect on pancreatic beta cells [9]. In the current study, STZ was used successfully to reach and maintain hyperglycemic status in rats for the duration of the experiment. These results are in line with other studies reporting success in using a single dose of STZ (50–60 mg/kg BW) to reproduce a hyperglycemic model in animals [10].

The present investigation demonstrated that SC significantly improved almost all measured parameters in the treated diabetic group compared to those of the diabetic rats that were not treated with sildenafil. These findings are in agreement with several previous studies indicating that SC is beneficial in the management of diabetes [11]. Meky *et al*. found that oral administration of SC (10 mg/kg BW) to diabetic rats improved most diabetic complications including hyperglycemia, hemoglycation, and hyperfibrinogenemia. Although these effects were partial, they were significant and more noticeable when SC was given for 14 days compared to just 7 days [11]. In their study with prediabetic patients, Ramirez *et al*. found that daily administration of SC (25 mg, 3x/day) for three months raised insulin sensitivity, glucose uptake through the muscle, and glucose-stimulated insulin release [7]. Zimmermann *et al*. concluded that SC may improve glycemic control in diabetic patients [8].

The results of the current study of SC in diabetic rats showed that after six weeks of treatment, the group of diabetic rats treated with SC had significantly lower blood glucose levels and significantly higher serum insulin concentrations than the diabetic rats that did not receive SC. These findings agree with earlier studies, demonstrating the part sildenafil plays in reducing blood glucose concentration at higher doses [4, 12, 13].

Enhanced insulin action in rats has been associated with chronic PDE-5 inhibition [4]. PDE-5 inhibitors may raise insulin sensitivity and thereby improve endothelial function [14]. Some research suggests that endothelial dysfunction may cause insulin resistance and type 2 diabetes [15] and may also impair glucose uptake into the muscles by reducing NO levels and cGMP production [16]. Therefore, preventing a drop in cGMP levels will lead to enhanced insulin action and glucose uptake into the muscles. The central nervous system seems to be involved in this pathway as SC has been shown to cross the blood-brain barrier, and PDE-5 has been found in brain tissue [17], so cGMP signaling in the central nervous system may be instrumental in regulating insulin action and energy homeostasis [14].

El Sayed *et al*. (2014) found that giving diabetic rats SC at doses ranging from 5–20 mg/kg BW) decreased their blood glucose level and raised their serum insulin to an extent proportional to the SC dose given. They suggested that when cGMP hydrolysis is inhibited, cGMP-dependent protein kinase G is activated, which results in the phosphorylation of hormone-sensitive lipase, thereby boosting free fatty acids and energy expenditure [12]. This is also in line with research by Taha *et al*. (2021), who reported a significant (48%) reduction in serum blood glucose and a significant rise (17%) in serum insulin in diabetic albino rats treated with SC (10 mg/kg BW) for four weeks compared to their counterparts [13]. The results of the current study can be taken as further indication that SC replicates the effects of insulin, including that of lowering glucose levels in the blood.

However, the findings of the present study are at odds with those of Milani *et al*., who reported that 15-day administration of SC (1 mg/kg BW) did not result in lowering elevated serum glucose in diabetic rats [18], a finding which may reflect the low dosage compared to other studies of SC in rats. Further research on SC's effects on blood glucose levels should focus on determining the optimal dose and duration of treatment needed.

Hyperglycemia—with its associated oxidative stress—is implicated in most diabetic complications [2, 19]. With their role in oxygen transportation, red blood cells are more at risk of oxidative damage. In the present study, significantly higher glycated hemoglobin levels were seen in the diabetic rats than in the non-diabetic rats, a finding in line with prior research [19]. However, daily intake of SC for 6 weeks reduced the glycated hemoglobin level in diabetic rats, thereby protecting red blood cells against oxidative damage. Glycation in diabetes affects not only hemoglobin but also other red blood cells and serum proteins. Over time, advanced glycated end-products accumulate, which negatively impacts the structural and functional properties of protein and eventually leads to diabetic complications [20].

The liver, with its insulin-dependent tissue, plays a key role in glucose homeostasis [21], and hepatocellular damage is frequently found in patients with DM. This can include abnormal levels of liver enzymes, necrosis, inflammation, and acute liver failure [22]. In the present study, a significant rise in AST and ALT, markers of hepatic injury, signals hepatocytic damage in diabetic rats, results that support those of previous studies [23]. Fluctuating levels of AST and ALT occur primarily when these enzymes leak from the cytosol of liver cells into the bloodstream, a phenomenon frequently found in diabetic patients. Phosphodiesterase-5 enzyme inhibitors have been found to affect the hemodynamics of the liver [24]. The current investigation found that treatment with SC improved serum levels of AST and ALT, suggesting a hepatoprotective effect. This supports the results of prior research suggesting that prolonged use of SC works to improve liver function impacted by hyperglycemia [25]. Hameed and Farooq [26] also found significant improvement in histological parameters of the liver in mice treated with SC for 21 days.

In the current study, serum creatinine and urea concentration were measured as indicators of kidney function. Significant variation was found in the level of these markers in the diabetic rats compared to the control rats, in line with previous studies. Nogueria *et al*. reported a significant rise in blood urea and creatinine levels in diabetic rats given STZ (45 mg/kg) [27]. Khan and Ola reported significantly higher serum urea and creatinine in rats that had been induced with diabetes seven days earlier [28]. Comparable to what happens in humans with diabetes, rats induced with diabetes undergo renal pathological changes in the kidneys and impaired kidney function [29]. In the current investigation, impaired renal function in diabetic rats was assumed, based on the significant increase in serum creatinine and urea values versus the controls. These higher levels reflect

the severity of the clinical renal damage and injury to functioning nephrons associated with diabetic kidney disease [30]. The increase in serum creatinine and urea could also be due to hyperglycemia, which promotes the generation of free radicals through glucose auto-oxidation, and this rise in free radicals may result in damage to kidney cells. However, treatment with SC significantly reversed the impaired renal function as shown by closer-to-normal serum urea and creatinine values. This indicates that SC may act to protect the kidneys of diabetic rats, which is documented in previous studies. For example, eight-week administration of SC lowered the raised serum creatinine levels in rats with 5/6 nephrectomy [31]. In another study, Rizk *et al*. observed a protective role of SC in cisplatin-induced nephrotoxicity in rats through improved renal function tests and reversal of histological renal changes [32]. These findings highlight the positive impact of SC on impaired renal function. The action of SC may involve an improvement in the function of nephrons more than the prevention of direct renal injury due to hyperglycemia.

Although the administration of STZ in the current study did not affect PT, a significant difference in aPTT was seen in the diabetic rats compared to the non-diabetic controls. PT evaluates the extrinsic coagulation pathway, and aPTT evaluates the intrinsic coagulation pathway, making these effective tests of the risk of clotting or excessive bleeding. Zhao *et al*. reported statistically significant variances in aPTT between diabetic and normal groups [33]. Tripodi *et al*. (2004) found an independent association between hypercoagulability determined by shortened aPTT values and venous thromboembolism and suggested that shortened aPTT could be a risk factor for venous thromboembolism [34].

In the current study, the diabetic rats had significantly higher fibrinogen values than the controls. Elevated plasma fibrinogen levels have also been demonstrated in cases of STZ-induced diabetes [28]. Plasma fibrinogen plays an important role in determining the flow of blood and its viscosity. Fibrinogen has been linked to cardiovascular conditions in the general population, and elevated plasma fibrinogen values have been found in patients with type 2 diabetes [35]. The mechanism for this may be that when fibrinogen undergoes glycation, a denser fibrin clot forms, one with finer fibers that increase its resistance to fibrinolysis [35].

In the present study, administration of SC significantly prolonged aPTT and decreased fibrinogen in diabetic rats, but PT values did not differ significantly. The observed prolongation of aPTT in diabetic rats after treatment with SC may be explained by reduced activity or inhibition of factors in the intrinsic pathway as well as factors II, V, and X, of the common coagulation pathways. The underlying mechanism may be that SC acts to suppress thrombin generation and leads to direct or indirect inhibition of factor Xa and its cofactor Va.

Besides their recognized anticoagulant properties, proteins C and S also play a role in regulating inflammation and stabilizing endothelial barrier protection. The function of these anticoagulant proteins may be impaired when they become glycated due to hyperglycemia [36]. When these natural anticoagulants become impaired, clotting factors are activated, leading to hypercoagulability in patients with diabetes mellitus. Research on plasma antigen levels of protein C and protein S suggests that these values may be elevated or reduced depending on the type of diabetes. In the present study, protein C and S activity was significantly lower in the diabetic rats than in the controls. It is thought that these proteins undergo structural changes caused by non-enzymatic glycation, which results in dysfunction [37]. However, in the present study, these deficiencies in plasma protein C and S in diabetic rats were significantly reversed after they received sildenafil.

Conclusion

The findings of this study indicate that 20 mg/kg of SC given orally for 42 days in STZ-induced diabetic rats may mitigate the functional biochemical and hematological changes associated with diabetes. No deleterious effects linked to SC administration were found on all parameters assessed in the non-diabetic rats. However, significant beneficial effects were seen in the diabetic rats given SC for six weeks. SC reduces kidney damage in diabetic rats by bringing serum urea and creatinine levels closer to normal values. Although the published literature suggests a possible link between structural and functional changes in the liver and the use of SC, the mechanism underlying liver toxicity is still unknown. The few cases reported to date suggest that SC-associated liver damage is a rare but not impossible event. In this study, SC corrected liver damage in diabetic rats by improving their serum ALT and AST levels. In addition, SC administration may result in the improvement or even prevention of the coagulopathy and vascular complications linked to diabetes, as this PDE-5 inhibitor normalizes the values of aPTT and fibrinogen and raises the levels of the anticoagulant proteins C and S. Further research is needed to further investigate the clinical impact of these findings.

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