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Research Article

IMPROVEMENT IN RENAL BIOCHEMICAL AND HEMATOLOGICAL ALTERATIONS AND OXIDATIVE STRESS FOLLOWING USE OF SILDENAFIL IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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A B S T R A C T

Introduction: Diabetes mellitus is a chronic metabolic disease marked by persistent hyperglycemia. It is associated with oxidative stress and the dysfunction, long-term damage, and failure of different organs. The aim of the present study is to evaluate the effects of sildenafil (Sild) on serum biochemical and hematological alterations and oxidative stress status in the kidneys of rats with streptozotocin (STZ)-induced diabetes.

Materials and methods: In this experimental study, fifty male Wistar rats were separated into the following four groups (n=10/group): i. control (Ia), ii. Sild-treated control (Ib), (n=15/group): iii. diabetic (IIa), and iv. Sild-treated diabetic (IIb). Diabetes induction was carried out through a single intraperitoneal (IP) injection of STZ (50 mg/kg). Sild (20 mg/kg body weight [BW]) was given orally for six weeks. Blood samples were used to determine levels of fasting blood glucose (FBG), glycated hemoglobin (HbA1c), alanine aminotransferase (ALT), urea, creatinine, prothrombin time (PT), partial thromboplastin time (APTT), fibrinogen, protein C, and protein S. The antioxidant status was evaluated by determining the levels of malondialdehyde (MDA) and catalase (CAT) in the kidneys. Data were analyzed by one-way analysis of variance (ANOVA) with $P<0.05$ as the significance level.

Results: FBG, HbA1c, AST, urea, creatinine, and MDA were significantly higher in the diabetic groups compared to the controls. No significant difference was seen in PT of control and diabetic rats, whereas APTT was significantly lower in diabetic rats than control animals. On the other hand, fibrinogen was significantly higher in diabetic rats than in normal controls. Treating the diabetic rats with Sild brought the changes in the above parameters back to normal levels ($P<0.05$). In addition, Sild significantly improved low levels of CAT as well as high levels of MDA in the kidney ($P<0.05$).

Conclusion: Sild showed beneficial effects and improved oxidative damage induced by STZ in the kidneys of rats; therefore, it may be considered as an effective treatment for minimizing diabetic complications.

INTRODUCTION

An elevated fasting blood glucose (FBG) level is a typical feature of diabetes mellitus (DM), due to the loss or damage of insulin-producing pancreatic β-cells (Type I diabetes) or through loss of insulin sensitivity in target tissues like muscle and adipose (Type II diabetes) or both [1]. Having high FBG levels causes dysfunction in various organs, particularly the eyes, kidneys, liver, heart and blood vessels[2]. Significantly elevated FBG levels in diabetic rats could be due to the destruction of β-cells in the pancreatic islets by streptozotocin (STZ) [3]. Experimental and clinical research has shown that oxidative stress is implicated in the pathogenesis and/or complications of DM [4, 5].

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Oxidative damage may contribute to the development of diabetes and its complications [6]. Malondialdehyde (MDA), produced by polyunsaturated fatty acid peroxidation, is a highly toxic aldehyde and is widely considered to be a biomarker of lipid peroxidation [7]. Tissue damage from lipid peroxide has been reported in the development of type I and type II DM [8]. Increased oxidative stress may intensify the susceptibility of cells to injury from free radicals through the lowering of plasma antioxidants and heightened lipid peroxidation [7].

Antioxidant enzymes such as catalase (CAT) neutralize free radicals by donating electrons. These enzymes react with the reactive oxygen species (ROS) to form harmless products and inactivate the free radicals. In this way, antioxidants shield cells from oxidative damage [9]. In diabetes mellitus, these antioxidant enzymes are impaired [10].Poor glycemic control may also result in an impaired antioxidant defense system,

with ensuing injury to cellular organelles and enzymes, raised levels of lipid peroxidation, and the development of diabetic complications [5].

Research on people with diabetes mellitus has highlighted changes in certain hematological indices [11]. Various DM–related abnormalities in hemostasis and thrombosis have been reported [12]. The RBCs in patients with DM have high glucose concentrations, leading to the glycation of hemoglobin, prothrombin, fibrinogen, and other proteins that play a role in the clotting mechanism [13]. The activation and function of the clotting cascade is incomplete because of this glycation [14]. When glycation of intrinsic and extrinsic clotting proteins occurs, the availability of these proteins decreases, thereby impairing the clotting capacity [15]

Despite the advancement in coagulation diagnostic tools, routine tests such as activated partial thromboplastin time (APTT) and prothrombin time (PT) remain effective screeningtools forcoagulation. Shorter APTT values may signal a hypercoagulable state, which is often linked to higher risk of thrombosis and cardiovascular disease [15,16]. When circulating activated coagulation factors accumulate in plasma (due to heightened in vivo coagulation activation), lower APTT values may result [15, 17]. APTT, therefore, is an effective method of assessing the risk of thromboembolic events in DM patients [15,18].

Fibrinolytic dysfunction is commonly seen in DM patients because fibrin structure, clot generation and fibrinolytic resistance are affected by persistent hyperglycemia and tissue glycation [19]. In diabetic patients, the rate of fibrinogen clearance is increased, and the half-life of circulating fibrinogenis shortened [20]. Oxidative stress may be a possible connection between diabetes and hyperfibrinogenemia as free radicals trigger thrombin formation in diabetics [21]. Increased levels of fibrinogen in plasma may shorten APTT, indicating cardiovascular disease since it signals heightened thrombin formation and thus a greater likelihood of thrombosis. Known for their anticoagulant properties, proteins C and S also help regulate inflammation and stabilize the endothelial barrier protection [22, 23]. Hyperglycemia may impair the function of proteins C and S by inducing the glycation of these anticoagulants [22, 24].

Sildenafil citrate is a selective inhibitor of cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase (PDE5), which acts as a catalyst of cGMP hydrolysis and boosts the effect of nitric oxide (NO) [25]. In addition to its use in the treatment of erectile dysfunction[26],other potential benefits to sildenafil have been explored in various clinical and experimental studies [27-29].Therefore, the aim of the present study was to explore the effectiveness of sildenafil in improving biochemical alterations, oxidative stress status, and coagulation parameters of streptozotocin-induced diabetic rats.

MATERIALS AND METHODS

Chemicals

Streptozotocin (STZ) was purchased from Sigma (Sigma-Aldrich, St. Louis, MO, USA). Sildenafil citrate was purchased from Pfizer, USA (Viagra). All chemicals used in this study were of analytical grade, obtained from Sigma-Aldrich (St Louis, MO, USA).

Experimental animals

Fifty adult male Wistaralbino rats weighing between 250–300 gm were selected for the present study. The animals were obtained from King Fahad Research Center at King Abdulaziz University and kept in suitable cages in well-ventilated conditions with a natural light regimen (25± 2°C temperature and 12-hour light/dark cycle). They were givenas much standard laboratory food and water as they wanted throughout the study. The rats were left to acclimatize to the environment at least one week before use. The rats were treated according to the guidelines of the KAU ethical committee for the control and supervision of experimental animals.

Animal Groups and Diabetes Induction

We divided the 50 male rats into two groups randomly: group I (n=20) and group II (n=30). Group I (control) was subdivided into two groups. Group Ia (n=10) received a single intraperitoneal (IP) injection of citrate buffer (pH=4.5), and group Ib (n=10) received the same IP injection in addition to oral sildenafil (a single daily dose of 20 mg/kg body weight [BW]) for six weeks. Diabetes was induced in the other 30 rats after 12-hour overnight fasting by a single IP injection of streptozotocin (STZ) at the dose of 50 mg/kg BW in 0.1 ml citrate buffer (pH=4.5) [30]. On the day of the STZ injection, a 10% oral glucose solution was given to the rats to overcome the initial drug-induced hypoglycemia[31]. Hyperglycemic status was verified by testing fasting blood sugarconcentration 72 hours after the injection. The rats with fasting blood sugar above 200 mg/dl were considered diabetic and chosen for the study. The diabetic rats were put randomly into two groups: group IIa (n=15) was the diabetic control; group IIb (n=15) was diabetic and received sildenafil (single daily 20 mg/kg BW/6 weeks).

Measurement of biochemical and coagulation assays

Blood samples were taken from each rat from retro-orbital veins under light ether anesthesia after 12-hour overnight fasting. The blood sample was spun in a clinical centrifuge at 2000 rpm for 10 minutes, and the serum obtained was used to measure the following biochemical parameters, using spectrophotometric assay: fasting glucose, AST, urea, and creatinine. Vitro diagnostic kits were used to determine concentrations. Whole blood was collected separately in ethylenediaminetetraacetic acid-preserved collection tubes for analysis of glycated hemoglobin (HbA1c). The percentage of HbA1c was measured by spectrophotometric assay (Glycated Hemoglobin Kit, Sigma Diagnostics, USA).

Plasma was separated in sterile sodium citrate tubes to measure PT, APTT, fibrinogen, protein C and S, using Biomed diagnostic kits (Biomed, Cairo, Egypt) and performed on an automated coagulation analyzer according to manufacturer instructions.

Tissue preparation for measurement of oxidative stress parameters

After the rats were sacrificed, the kidney tissue was excised and rinsed with isotonic saline. The renal tissue was then minced, and a homogenate was made with 10% (w/v) phosphate-buffered saline (PBS, 0.1 mol/L, pH=7.4). The homogenate underwent centrifugation (Sigma, Germany) at

10,000 rpm for 5 minutes at -4˚C. The supernatant was directly used to determine MDA and CAT levels.

Malondialdehyde assay

MDA levels were determined spectrophotometrically according to the methods of Rajasekaran and Kalaivani[32]. About 0.1 ml of the supernatant was combined with 2 ml of thiobarbituric acid (TBA) trichloroacetic acid (TCA)-HCl reagent (1:1:1 ratio, 0.37% TBA, 15% TCA and 0.25 N HCl, Sigma, USA). After cooling, a pink color appeared due to the MDA–TBA reaction. The absorbance was measured by spectrophotometry (Pharmacia, Novaspec II, Biochrom, England) at 535 nm, with results expressed in nmol/mg of protein.

Catalase assay

The catalase activity in the renal homogenate was assessed following the procedure reported by Aebi[33]. Briefly, 0.2 ml of tissue homogenate and 1.2 ml of PBS were combined, but the reaction was triggered by adding 1.0 ml of 30 mM H_2O_2 solution. The absorbance was measured by means of spectrophotometry (Pharmacia, Novaspec II, Biochrom, England) at 240 nm at a 30-second interval, with results expressed as units per mg of tissue.

Statistical analysis

Data analysis was carried out using the Statistical package for Social Science (SPSS 17. for Windows; SPSS Inc, Chicago, IL, version 21.2001). Results were expressed as mean ± SEM. Differences were assessed by one-way analysis of variance (ANOVA) with Tukey post-hoc test. Statistical significance was set at $P < 0.05$.

RESULTS

A single IP injection of STZ (50 mg/kg BW) in fasting rats produced hyperglycemia in all cases with no deaths occurring during the experiment. There were no significant differences in laboratory and histological results in the subgroups of the control group (Ia and Ib).

Table 1 shows the concentration of serum glucose and HbA1c in the normal (control) and STZ-induced diabetic rats. After the 6-week treatment period, the serum glucose levels of group IIbwere significantly lower than those of the negative control group IIa (P<0.05). The average estimated HbA1c concentration was also significantly higher in the diabetic rats (IIa) (*P*<0.05; 6.91 ± 0.7%) compared to that of the Sild-treated group IIb(4.80 ± 0.07%). Diabetic rats who received sildenafil had glucose and glycosylated hemoglobin levels significantly closer to normal levels.

Table 1 Serum glucose and % HbA1c in the studied groups

	Ia	Ib	IIa	IIb
Glucose (mg/dl)	73.21 ± 0.74	74.0 ± 0.63	265.5 ± 0.78*	105.60 ± 0.72£※
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 (Ia and Ib), £: P<0.05 as compared to the diabetic ratsIIa, ※: P<0.05 as compared to the controls (Ia and Ib).

Table 2 shows the level of serum aspartate aminotransferase (AST), kidney function biomarkers (urea and creatinine), and oxidative stress biomarkers (MDA and CAT) in the studied groups. The rats who had STZ-induced diabetes had highly significant (P<0.05) elevation of serum AST, urea, and creatinine compared to the control rats. Treating the diabetic group IIb with Sild produced a marked lowering of the elevated serum AST, urea, and creatinine levels. No significant difference was found between Iaand Ib groups.

MDA was used to indicate oxidative stress [7]. The MDA concentration in the kidney tissues of the diabetic (IIa) group were significantly (P<0.05) higher than those of the control groups. These MDA levels in the IIb group were significantly lower than those of the IIa group. No significant variance was seen in MDA levels in the kidney tissues of groups Ia and Ib. The mean CAT activities in the kidney tissue of the IIa group were significantly (P<0.05) lower than those of groupsIa and Ib. The renal CAT activities of the IIb group were also lower than those of the control animals, but not to as great an extent. No significant difference was seen in the CAT activities of the kidney tissues of groups Ia and Ib.

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

	Ia	Ib	IIa	IIb
AST (U/L)	94.7 ± 2.7	95.3 ± 2.2	179.6 ± 6.4*	114.8 ± 6.1£※
Urea (mg/dl)	28.20 ± 5.32	27.85 ± 4.03	56.85 ± 9.52*	38.38 ± 5.45£※
Creatinine (mg/dl)	0.56 ± 0.08	0.60 ± 0.10	2.42 ± 0.34*	0.93 ± 0.01£※
MDA (µmol/dl)	2.30 ± 0.04	2.33 ± 0.02	6.87 ± 0.01*	3.70 ± 0.02£※
Catalase (U/g)	19.45 ± 1.69	19.56 ± 1.54	9.24 ± 1.41*	16.33 ± 1.81£※

Data are expressed as mean ± SE, *: P<0.05 as compared to the controls (Ia and Ib), £: P<0.05 as compared to the diabetic ratsIIa, ※: P<0.05 as compared to the controls (Ia and Ib).

The effect of sildenafil on the coagulation parameters in the studied rats is summarized in Table 3. There was no significant difference between the control groups (Ia&Ib) in all measured parameters. The PT of rats given STZ (IIa) was not significantly different 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 group, with higher levels of fibrinogen and lower levels of proteins C and S.Sildenafil treatment in diabetic rats (IIb) significantly lowered the elevation of fibrinogen and raised APTT, protein C, and protein S levels in comparison to the diabetic group (IIa).

Table 3 Effect of sildenafil on coagulation parameters in rats

	Control	Sildenafil	STZ	STZ + Sildenafil
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*	63.01 ± 0.87£※

Data are expressed as mean ± SE, *: P<0.05 as compared to the controls (Ia and Ib), £: P<0.05 as compared to the diabetic ratsIIa, ※: P<0.05 as compared to the controls (Ia and Ib).

DISCUSSION

This study examined the effect of oral administration of sildenafil citrate (20 mg/kg BW) for six weeks in relation to blood glucose level and biochemical and hematological parameters in STZ-induced diabetic rats.Injecting rats with STZ causes selective damage topancreatic β-cells in the islets of Langerhans, leading to hyperglycemia and many

complications [3]. We demonstrated that sildenafil significantly improved almost all measured parameters in the treated group compared to those of the diabeticrats that was not treated with sildenafil. Our findings are in agreement with previous studies [27, 34]. Sildenafil-treated normal rats exhibited a non-significant difference from normal control rats in all measured parameters, mirroring previous studies [35, 36].

The significant rise in fasting blood sugarconcentration in STZ-induced diabetic rats demonstrated in present study was the same as that shown in previous studies [37, 38]. It has been well documented that fasting hyperglycemia leads to a significant decrease in insulin levels due to the damage STZ causes to pancreatic β-cells [3]. After six weeks of treatment with sildenafil, the diabetic rats had significantly lower blood glucose levels. These findings agree with earlier studies demonstrating the part sildenafilplays in reducing blood glucose concentration at higher doses [39, 40]. Elsayed *et al*. (2014) used sildenafil (20 mg/kg BW)in diabetic rats and recorded a reduction in their blood glucoseconcentration. The mechanism of hypoglycemia brought on by sildenafil could occur through the inhibition of PDE5 and stimulation of the NO-cGMP pathway [41], NO being considered a possible second messenger for the stimulatory effect of insulin in carbohydrate metabolism. Furthermore, NO mimics insulin in stimulating the transport and oxidation of glucose [42]. On the other hand, Milani (2005) found that Sild (1 mg/kg BW) did not lower elevated serum glucose levels in male albino rats [43]. Young *et al*. (2003) also reported no significant change in serum glucose concentration in diabetic rats given sildenafil (5 mg/kg BW) [44]. Cheng *et al.* (2011) administered sildenafil to diabetic rats(12 mg/kg BW) and recorded non-significant changes in blood glucose values [45]. Determining whether the effect of sildenafil on blood sugar is dose dependent requires further investigation on the exact dose and mechanism of action. Analyzing HbA1c in blood can indicate an individual's average blood glucose concentration over the previous two months, which is the predicted half-life of red blood cells [46]. The HbA1c test is now recommended as a standard means of screening for and monitoring diabetes [47]. Our findings showed that the higher percentage of HbA1c found in group IIaand the significant decrease in group IIb are directly proportional to the increased and reduced concentration of blood glucose in diabetic and Sild-treated rats, respectively.

Liver damage is frequently found in patients with DM. This can include abnormal levels of liver enzymes and acute liver failure [38,48]. Fluctuating levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) occur primarily when these enzymes leak from the cytosol of hepatocytes into the bloodstream. In the present study, the increased activity in the marker of liver injury (AST) could reflect hepatocyte damage in STZ-induced diabetic rats. This finding is in line with those of previous studies [38, 49]. However, we found that treatment with sildenafil improved serum biomarker levels of liver injury. This study supports the findings of previous reports which have indicated that sildenafil has the effect of moving AST levels significantly closer to normal values [50].

Urea and creatinine have been reported as metabolic waste products stemming from kidney damage [51]. In our study, significant variation was found in the urea and creatinine levels of diabetic rats compared to the normal rats, which is in line with previous studies. Nogueria *et al*. (2005) observed a significant rise in blood urea and creatinine levels in diabetic rats given STZ (45 mg/kg) [52]. Khan and Ola (2011) reported significantly higher serum urea and creatinine in rats that had been induced with diabetes seven days earlier [53]. Elevated creatinine concentration occurs with end-stage renal disease, along with histological changes [9]. In the present study, it could be assumed that the renal function in the diabetic rats had deteriorated, based on the significant increase in serum urea and creatinine valuesversus the controls. These higher levels reflect the severity of the clinical renal damage and injury to functioning nephrons associated with diabetic kidney disease [36, 54]. The increase could also be due to the disruption in the metabolism of glucose and generation of reactive oxygen species (ROS), which tend to occur with persistent and chronic high blood glucose levels in DM. However, treatment with sildenafil significantly reversed the impaired renal function as shown by closer-to-normal levels of serum urea and creatinine. This indicates that Sild may act to protect the kidneys of STZ-induced diabetic rats, which is documented in previous studies [36].

Hyperglycemia's association with oxidative stress stems from its disruption of the oxidant/antioxidant balance, which is implicated in kidney damage [4]. Treatment with antioxidant agents may be useful to enhance renal protection in diabetes or other kidney conditions [55]. In our study, elevated MDA concentrations were seenin the kidneys of diabetic rats after six weeks, which is indirect evidence of intensified production of free radicals. Our observations are consistent with the results of other studies that found high MDA levels, pointing indirectly to the cytotoxicity of increased oxidative stress in the cultures of renal tissues in a high glucose environment [56, 57]. Our observation that Sild improved induced elevation in MDA concentration supports the findings of Morsy *et al*. (2014). They showed that six consecutive days of Sild treatment (5mg/kg) significantly lowered the elevated MDA values in nephrotoxicity-induced rats [36]. Sild treatment also decreased MDA levels in rats with renal ischemia reperfusion injury [58]. This inhibitory effect Sild has on lipid peroxidation may due to the role it plays in suppressing iNOS expression [59].

In addition to this, the decreased CAT activity observed in the kidneys of diabetic rats in our study indicates enzymatic antioxidant mechanisms, which is in line with past studies [57]. The decrease in the renal antioxidant enzyme CAT is related to higher levels of free radicals, causing oxidative membrane protein damage [60]. Administration of sildenafil to STZ-induced diabetic rats significantly increased their CAT levels, thus suggesting Sild's antioxidant potential.Similar findings regarding the ability of Sild to increase catalase activity have been reported [28, 36]. In the present study, a decrease in CAT activity indicated higher oxidative stress in diabetic rats, while a noticeable elevation of CAT activity levels was seen in the diabeticrats given Sild treatment, which may be attributed to Sild's role in scavenging free radicals.

The RBCs in subjects with DM have high glucose concentrations, causing the glycation of hemoglobin, prothrombin, fibrinogen and other proteins that play a role in the clotting mechanism [13]. Although the administration of STZ in our study did not affect PT, a significant difference in

APTT was seen in the diabetic rats compared to normal controls (Table 3). PT and APTT measure the extrinsic and intrinsic coagulation pathways, respectively and are effective tests of the risk of clotting or excessive bleeding. Zhao*et al*. (2011) reported statistically significant variances in APTT between diabetic and normal groups [61].

Elevated fibrinogen levels have been reported in diabetic patients [19]. Likewise, Lippi *et al*. (2010) reported elevated fibrinogen levels and shorter APTT in subjects with diabetes [62]. Elevated plasma fibrinogen levels have also been demonstrated in cases of STZ-induced diabetes[53]. There are many factors involved in the biological mechanisms of diabetic thrombosis which are not yet fully understood [15]. Korte *et al*. (2000) found that patients with short APTT values were susceptible to thrombosis and have a higher risk of thromboembolism [63]. 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. At times, shortened APTT has been thought to be due to problematic venipunctures in the lab [18]. However, when circulating activated clotting factors accumulate in plasma (due to heightened in vivo coagulation activation), shorter APTT values may be observed [15, 17].

In contrast, fibrinogen values were significantly closer to normal indiabetic rats after six weeks of oral sildenafil treatment (Table 3). This reduction was highly significant ($P<0.05$) and suggests Sild's fibrinolytic potency. Noticeable changes in fibrin structure, resulting in clots that are denser and more resistant to fibrinolysis are associated with hyperglycemia and tissue glycation [19].

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. Risk factors for thrombotic events include deficiencies of proteins C and S. It is thought that these proteins undergo structural changes caused by non-enzymatic glycation, which results in dysfunction[22]. However, in the present study, these deficiencies in plasma protein C and S were significantly reversed after the administration of sildenafil to diabetic rats.

CONCLUSION

Although no adverse effects were detected for sildenafil consumption on all parameters measured in the healthy rats, significant effects wereseen in the diabetic rats treated with sildenafil for six weeks. Sildenafil reduces renal damage in rats in part through lowering oxidative stress by preserving near-normal levels of catalase and MDA as well as improving the serum concentrations of urea, creatinine, and AST in rats with STZ-induced diabetes. Furthermore, the coagulopathy and vascular complications associated with diabetes may be improved or prevented by sildenafil, as it acts to normalize the values of APTT and fibrinogen and increase the values of the anticoagulant proteins C and S. Further studies are required to examine the clinical impact of these findings more thoroughly.

Conflict of Interest

None

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