

Neifissa H. Meki
Eman A. Abd El-Ghffar
Essam M. Ibrahim
Hoda G. Hegazy
Marim S. A-E. Elabassy

The potential effect of sildenafil citrate on some biochemical and haematological parameters in alloxan-induced Type I diabetes in male rats

ABSTRACT:

Sildenafil citrate is a phosphodiesterase type 5 (PDE5) inhibitor, which increases cyclic guanosine monophosphate (cGMP). It is well established that the pathogenesis of diabetic mellitus is associated with abnormalities of nitric oxide (NO) generation. Many of the biological actions of NO are mediated by cGMP, which is rapidly degraded by phosphodiesterase's. The Aim of this study is to evaluate the effect of sildenafil citrate on some biochemical and haematological parameters in alloxan-induced diabetes in male albino rat. Diabetes was induced after an intraperitoneal (i.p.) injection of a single dose of alloxan (150 mg/kg B.W.). Sildenafil was administered daily at a dose of 10 mg/kg B.W. via oral gavages during a period of 14 days to normal healthy rats, as well as diabetic rats. Blood samples were collected after 7 and 14 days of treatment in all experimental groups. The present investigation showed that alloxan injection induced significant elevations in serum glucose level, the percent of glycol haemoglobin (HbA1c) and serum malondialdehyde (MDA) level. Moreover, alloxan administration resulted in prolongation of prothrombin time (PT), activated partial thromboplastin time (APTT), as well as the increase in plasma fibrinogen level. Meanwhile, protein C and protein S concentrations were significantly decreased due to alloxan treatment. Oral administration of sildenafil to diabetic rats improved most diabetic complications including hyperglycaemia, oxidative stress, haemoglycation and hyperfibrinogenaemia induced by alloxan. The modulatory effects of sildenafil obtained herein were partial, but significant and more pronounced when administered for 14 days. In addition, sildenafil administration to normal healthy rats was found to have no effect on the studied parameters. The present study showed that sildenafil may have beneficial effects against some diabetic complications. The mechanism by which sildenafil citrate exerts its effect on the studied parameters might be attributed to increase cGMP, which is brought out by its phosphodiesterase inhibitory action, and this is discussed in detail.

KEY WORDS:

Alloxan, Hyperglycaemia, Hyperfibrinogenaemia, Protein C, Protein S, Sildenafil citrate.

CORRESPONDENCE:

Eman A. Abd El-Ghffar

Zoology Department, Faculty of Science, Ain Shams University, Abbasseya 11566, Cairo, Egypt.

E-mail: eman_a@sci.asu.edu.eg

¹Neifissa H. Meki

²Essam M. Ibrahim

¹Hoda G. Hegazy

¹Marim S. A-E. Elabassy

¹Zoology Department, Faculty of Science, Ain Shams University, Abbasseya 11566, Cairo, Egypt.

²Clinical Pathology Department, Animal Health Research Institute, Giza, Egypt.

ARTICLE CODE: 13.01.17

INTRODUCTION:

Diabetes mellitus is a heterogeneous disease characterized by a chronic hyperglycaemic condition, with subsequent disturbances of fat and protein metabolism, resulting from insulin deficiency and/or resistance. The prevalence of diabetes is increasing rapidly worldwide and the World Health Organization (WHO) has predicted that the number of adult patients with diabetes

would have almost increased to 422 million in 2014 (WHO, 2016). In Egypt, diabetes prevalence that estimated by WHO is 6-7 million patients by the year 2030. There are two main types of diabetes mellitus. Type 1 diabetes known as insulin dependent diabetes mellitus, is caused by lack of insulin secretion by β cells of the islets of Langerhans in the pancreas. Type 2 diabetes known as non-insulin dependent diabetes mellitus or insulin resistance, is caused by decreased sensitivity of target tissues to insulin. In both types of diabetes mellitus, metabolism of all main foodstuffs is altered (Abd El-Ghffar, 2016).

Diabetes mellitus associated with both metabolic and vascular abnormalities especially myocardial infarction, cerebrovascular and peripheral vascular diseases. Abnormalities in endothelial and vascular smooth muscle cell function, as well as a propensity to thrombosis, contribute to atherosclerosis and its complications. Endothelial cells regulate vascular function and structure. Among the important biological substances synthesized by the endothelial cell is NO, which is constitutively produced by endothelial NO synthase (eNOS). Diabetes management with conventional treatment is not possible without harmful effect. Thus, the present study is an attempt to assess the efficacy and safety of oral sildenafil citrate as a NO donor, after one and two weeks of treatment, on either diabetic rats induced by alloxan or non-diabetic control on some haematological and biochemical studies in rats.

Pathogenesis of Diabetes mellitus involves both genetic and environmental factors. The long-term persistence of metabolic disorders can cause susceptibility to specific complications including hypercoagulopathy, arteriosclerosis, cardiovascular disease, peripheral vascular disease, and cerebrovascular disease (Keen *et al.*, 1999). Moreover, there is a relation between diabetes and growing different types of cancer (Garg *et al.*, 2013). Diabetes mellitus is associated with a broad range of clinical presentations, from being asymptomatic to ketoacidosis or coma, depending on the degree of metabolic disorder. Several potential mechanisms have been proposed to explain abnormal endothelium dependent vasodilation in patients with diabetes. These include: decreased synthesis of nitric oxide (NO) by the endothelium, increased inactivation of NO and decreased responsiveness of the nitric oxide-guanylate cyclase pathway at the level of the vascular smooth muscle (Williams *et al.*, 1996). The loss of endothelium-derived NO_x permits increased activity of the pro-inflammatory transcription factor nuclear factor kappa β , resulting in expression of leukocyte adhesion molecules and production

of chemokines and cytokines. These actions promote monocyte and vascular smooth muscle cell migration into the intima and formation of macrophage foam cells, characterizing the initial morphological changes of atherosclerosis.

NO is a biological mediator plays an important role in a variety of biological processes and is a fundamental component in the fields of biochemistry, physiology, immunology and neuroscience. NO is synthesized from L-arginine by the enzyme nitric oxide synthase (NOS). NO is a unique biological messenger molecule. It mediates, in part, the immune functions of macrophages, it is produced by endothelial cells to mediate blood vessel relaxation; and it also serves as a neurotransmitter in the central and peripheral nervous system (Zhao *et al.*, 2015). cGMP is an enzyme has an important role in the physiology of the vascular system. Phosphodiesterase-5 (PDE5) is primarily distributed within the arterial wall, smooth muscles of the lungs and penis (Mahre *et al.*, 2017). PDE5 inhibitors are well known being effective via the NO and cyclic guanosine monophosphate (cGMP) pathway and are widely used in the treatment of diabetic erectile dysfunction (Angulo *et al.*, 2010). Sildenafil citrate is the first effective oral PDE5 inhibitors treatment for erectile dysfunction of various etiologies including diabetes mellitus. Its pharmacological action is due to its ability to prolong the signalling actions of neurotransmitter NO in penile smooth muscle through raising the available cGMP pool by stimulating soluble guanylyl cyclase and by preventing its hydrolysis via PDE5. This action lead to relaxation of smooth muscle in the corpus cavernosum and to engorgement of the penis with blood, resulting in an erection (Webb *et al.*, 2000). Therefore, diabetic males suffered from erectile dysfunction are now treated routinely with Sildenafil. Additionally, sildenafil has several anti-oxidative properties (Yousry *et al.*, 2016). Recent data reported a potential application for sildenafil in many experimental models of diseases rather than erectile dysfunction (Balarini *et al.*, 2013; Yousry *et al.*, 2016). Sildenafil dilates epicardial coronary arteries, improves endothelial dysfunction and inhibits platelet activation in patients with coronary artery disease and acutely enhances flow-mediated vasodilation in patient with heart failure (Mahre *et al.*, 2017). The concept of the present study to evaluate the effect of sildenafil citrate as a nitric oxide donor on some haematological and biochemical parameters of diabetic rats induced by alloxan. Alloxan is the most prominent diabetogenic chemical used in diabetes research. It is a cytotoxic glucose analogues causing selective destruction of beta cells through generation of reactive oxygen species (ROS) (Misra *et al.*, 2015).

MATERIAL AND METHODS:

Experimental animals:

Adult male Wistar albino rats (*Rattus norvegicus*), weighing between 180 – 200 gm, were used through this study. They were obtained from medical research centre, Ain Shams University hospitals and housed in suitable cages under good ventilation condition and natural light regimen with food and water supplied *ad-libitum*. At least one week before use, rats were left to acclimatize to the environment

Alloxan monohydrate was purchased from Al-Gomhoria pharmaceutical company, Cairo, Egypt. In addition, sildenafil citrate was purchased from Pfizer, USA. All chemicals used were analytical grade, obtained from Sigma-Aldrich (St Louis, MO, USA).

Induction of diabetes:

Rats were injected i.p. with a single dose of alloxan monohydrate (150 mg/Kg B.W.) according to Misra and Aiman (2012) that was dissolved in 0.9% sterile saline sodium chloride, pH 7 to induce diabetes. After 7 days of alloxan injection, blood glucose level was determined using a portable glucose analyser, a serum glucose level of 200 mg/dl was considered diabetic. These diabetic animals were selected for experiments. Treatments were then carried on through a daily oral administration for a period of 14 days as a single daily dose.

Experimental design:

The animals were divided into 4 groups of 8 rats each.

Group 1: Rats of this group received a corresponding placebo and served as control.

Group 2: Each rat received a daily oral dose of sildenafil citrate (10 mg/kg B.W.) 7 for 14 days *via* gavages according to Cadirci *et al.* (2011).

Group 3: Each rat was injected i.p. with a single dose of alloxan (150 mg/kg B.W.) to induce diabetes.

Group 4: This group comprised diabetic rats treated with a daily oral dose of sildenafil citrate (10 mg/kg B.W.) for 14 days.

Collection of samples for analysis:

Blood samples were withdrawn from each fasted rat from retro-orbital veins under light ether anaesthesia two times, the first was after seven days of sildenafil treatment; the second one was after fourteen days of sildenafil treatment. Blood samples were collected from fasted animals in three different test tubes. The first was collected in a clean dry test tube for measuring serum glucose level and serum MDA level, the

second was collected in a tube containing ethylene diamine tetra acetic acid (EDTA) for the determination of HbA1c, while, the last one was collected in a tube containing sodium citrate solution as an anticoagulant to prepare platelets rich plasma for the determination of PT, APTT, Fibrinogen, protein C, and protein S.

Measurements:

Serum glucose level was measured according to the method of Trinder (1969) using Spectrum diagnostic kits (Spectrum, Cairo, Egypt).

The percent of HbA1c was determined according to Trivelli *et al.* (1971) using commercial kits (Vitro Company, Cairo, Egypt). Serum malondialdehyde (MDA) level was carried out according to method of Satoh (1978) using bio diagnostics kits (bio diagnostics, Cairo, Egypt). PT and APTT were measured according to Loeliger *et al.* (1985), Hoffmann and Neulendijk (1978), respectively, using Spectrum diagnostic kits (Spectrum, Cairo, Egypt).

Fibrinogen, protein C and protein S and were estimated according to Poon *et al.* (2012), Stocker *et al.* (1986), and Dahlbäck (1991), respectively, using commercial kits (Biomed kit, Cairo, Egypt).

Statistics:

Statistical presentation and analysis of the present study was conducted, using the mean, standard deviation, Paired t-test, and Analysis of variance (ANOVA) tests by SPSS V17.

Unpaired Student T-test was used to compare between related samples. ANOVA test (according to the computer program SPSS for Windows) was used for comparison among different times in the same group in quantitative data. In the entire test, $P < 0.05$ was considered as statistically significant.

RESULTS:

Diabetic rats (alloxan-treated) group showed significant increase ($P < 0.05$) in serum glucose, HbA1c and serum MDA levels after 7 and 14 days as compared to normal control group (Table 1 & Fig. 1). These changes in sera of diabetic rats due to hyperglycaemia, oxidative stress and impairment of β cell of pancreas function. Oral administration of sildenafil to diabetic rats group significantly alleviated ($P < 0.005$) the increment in serum glucose, HbA1c and serum MDA levels induced by alloxan. This ameliorative effect was time-dependent and more obvious after 14 days of sildenafil treatment.

Table 1. Effect of sildenafil on serum glucose, HbA1c and serum MDA levels in healthy and diabetic male rats (*Rattus norvegicus*).

Parameters	Groups	Periods	control group	Sildenafil-treated group	Alloxan-treated group	Alloxan + Sildenafil-treated group
Serum glucose (mg/dl) level		After 7 days	72.50 ± 0.76 ^a	73.25 ± 1.04 ^a (1.03)	2.23 ^b 250.13 ± (245.01)	± 2.12 ^{cA} 175.75 (142.41)
		After 14 days	72.13 ± 0.83 ^a	73.00 ± 0.76 ^a (1.21)	257.50 ± 0.76 ^b (256.99)	150.50 ± 2.62 ^{cB} (108.65)
HbA1c%		After 7 days	3.79 ± 0.09 ^a	3.85 ± 0.13 ^a (1.58)	5.17 ± 0.11 ^b (36.41)	5.31 ± 0.05 ^{bA} (40.11)
		After 14 days	3.80 ± 0.09 ^a	3.86 ± 0.13 ^a (1.58)	5.91 ± 0.04 ^b (55.53)	4.80 ± 0.02 ^{cB} (26.32)
Serum MDA (µmol/dl) level		After 7 days	1.21 ± 0.06 ^a	1.23 ± 0.04 ^a (1.65)	2.26 ± 0.03 ^b (86.78)	2.20 ± 0.02 ^{cA} (81.82)
		After 14 days	1.21 ± 0.06 ^a	1.22 ± 0.04 ^a (0.83)	2.77 ± 0.02 ^b (128.93)	1.77 ± 0.02 ^{cB} (46.28)

Data presented as means ± standard deviation. HbA1c: glycohemoglobin; MDA: malondialdehyd. Means with different small letters within the same row are significantly different at P value < 0.05. Means with different capital letters within the same column are significantly different at P value < 0.05. Numbers between parentheses indicate the percentage of change in comparison with control values.

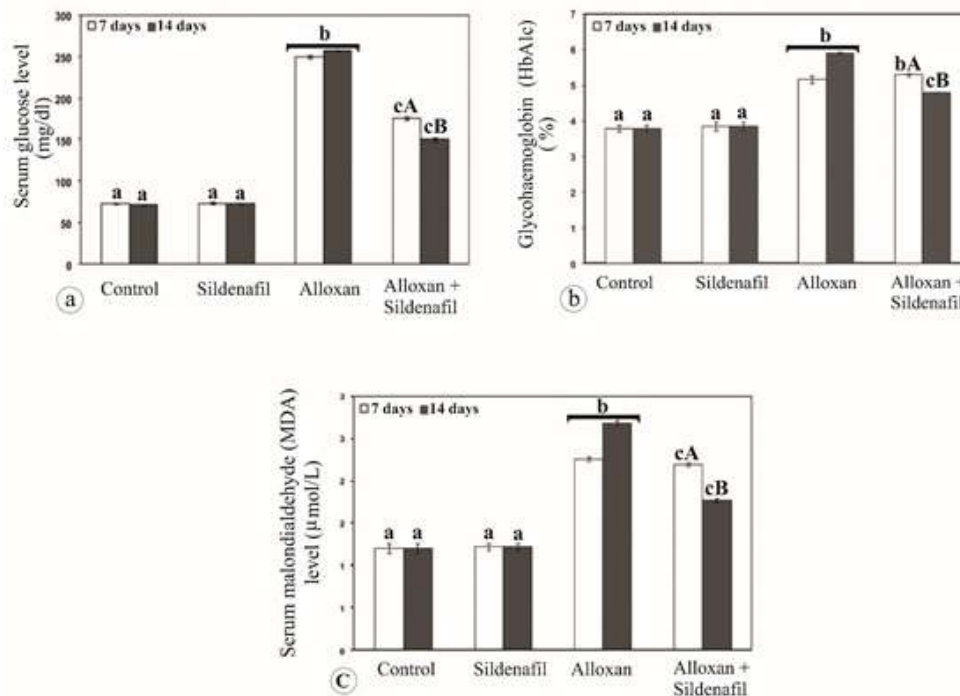


Fig. 1. Effect of sildenafil on serum glucose, HbA1c and serum MDA levels (a-c, respectively) in healthy and diabetic male rats (*Rattus norvegicus*). Data presented as means ± standard deviation represented by vertical bars. Within the different treatment groups, means with different small letters are significantly different at P value < 0.05. Within the different time groups (especially alloxan + sildenafil), means with different capital letters are significantly different at P value < 0.05.

As shown in table 2 and figure 2, diabetic rats group showed significant increase ($P < 0.05$) in PT, APTT and fibrinogen levels after 7 and 14 days as compared to normal control group. Oral administration of sildenafil to diabetic rats group significantly alleviated ($P < 0.005$) the increment in PT and APTT. This ameliorative effect was time-dependent and more obvious after 14 days of sildenafil treatment. In addition, the maximum effect that was obvious after 14 days of sildenafil treatment brought plasma PT level to normal value. Moreover, the significant increment ($P < 0.05$) of fibrinogen level in response to alloxan treatment was attenuated with sildenafil administration that brought fibrinogen level to

normal value after 7 and 14 days. While, diabetic rats group showed significant decrease ($P < 0.05$) in protein C and protein S after 7 and 14 days as compared to normal control group. Oral administration of sildenafil to diabetic rats induced a significant increase ($P < 0.05$) in protein C and protein S after 7 and 14 days of treatment. The ameliorative effect of sildenafil was time-dependent, with a maximum effect that was obvious after 14 days of treatment that brought plasma protein C level to normal value. Moreover, the significant decrement ($P < 0.05$) of plasma protein S in response to alloxan treatment was attenuated with sildenafil administration that brought plasma protein S level to normal value after 7 and 14 days.

Table 2. Effect of sildenafil on plasma PT, APTT, fibrinogen, protein C and protein S levels in healthy and diabetic male rats (*Rattus norvegicus*).

Parameters	Groups	Periods	control group	Sildenafil-treated group	Alloxan-treated group	Alloxan + Sildenafil-treated group
Plasma PT (sec.)		After 7 days	21.84 ± 0.19 ^a	21.81 ± 0.48 ^a (- 0.41)	28.58 ± 0.22 ^b (30.86)	26.42 ± 1.33 ^{cA} (20.97)
		After 14 days	21.72 ± 0.24 ^a	21.81 ± 0.48 ^a (0.41)	28.53 ± 0.24 ^b (31.35)	21.55 ± 0.49 ^{adB} (- 0.78)
Plasma APTT (sec.)		After 7 days	39.43 ± 0.38 ^a	39.76 ± 0.31 ^a (0.84)	52.00 ± 0.36 ^b (31.88)	47.34 ± 0.24 ^{cA} (20.06)
		After 14 days	39.43 ± 0.38 ^a	39.76 ± 0.31 ^a (0.84)	51.22 ± 0.22 ^b (29.90)	44.46 ± 0.10 ^{cB} (12.76)
Plasma fibrinogen (mg/dl)		After 7 days	229.19 ± 1.10 ^a	228.44 ± 1.58 ^a (- 0.33)	284.80 ± 1.43 ^b (24.26)	230.44 ± 0.37 ^{adA} (0.55)
		After 14 days	229.07 ± 0.95 ^a	228.44 ± 1.59 ^a (- 0.28)	287.53 ± 0.92 ^b (25.52)	230.03 ± 0.67 ^{adA} (0.42)
Plasma protein C (%)		After 7 days	58.92 ± 0.74 ^a	58.37 ± 1.32 ^a (- 0.93)	44.57 ± 0.73 ^b (- 24.36)	51.15 ± 1.09 ^{cA} (- 13.19)
		After 14 days	58.92 ± 0.74 ^a	58.59 ± 1.48 ^a (- 0.56)	38.84 ± 0.85 ^b (- 34.08)	57.50 ± 2.09 ^{adB} (- 2.41)
Plasma protein S (%)		After 7 days	63.23 ± 0.93 ^a	62.31 ± 1.36 ^a (- 1.46)	48.81 ± 0.57 ^b (- 22.81)	62.88 ± 0.80 ^{adA} (- 0.55)
		After 14 days	63.24 ± 0.94 ^a	62.32 ± 1.38 ^a (- 1.45)	40.03 ± 0.74 ^b (- 36.70)	63.00 ± 0.88 ^{adA} (- 0.38)

Data presented as means ± standard deviation. Means with different small letters within the same row are significantly different at P value < 0.05. Means with different capital letters within the same column are significantly different at P value < 0.05. Numbers between parentheses indicate the percentage of change in comparison with control values.

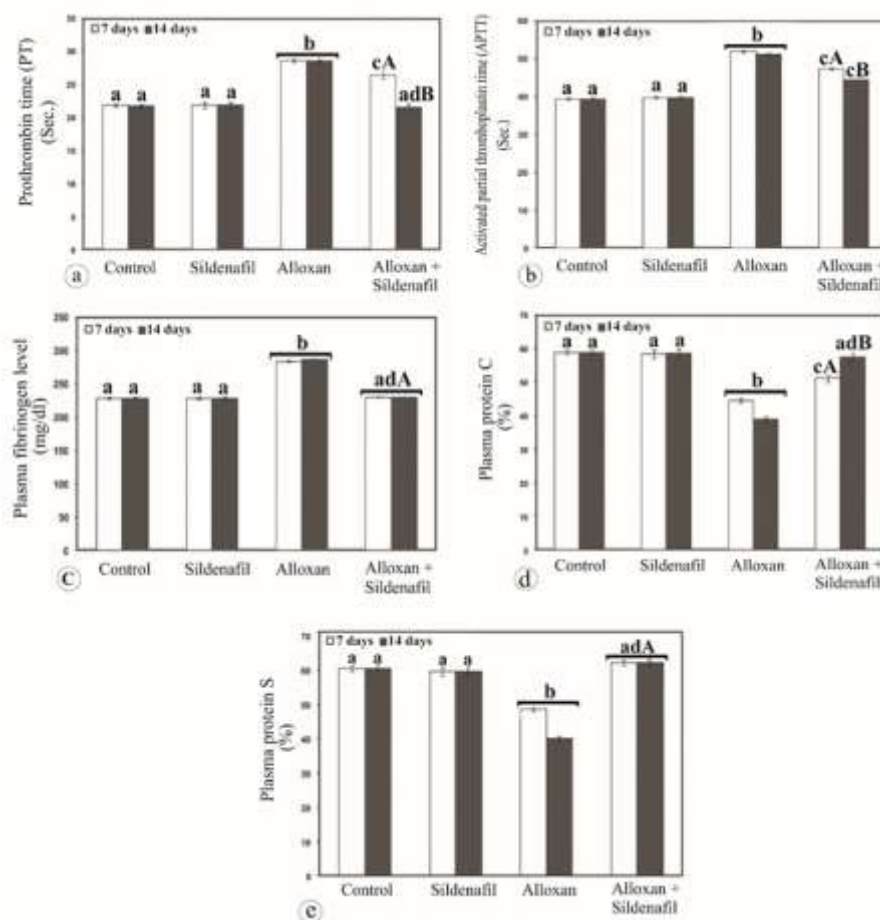


Fig. 2. Effect of sildenafil on plasma PT, APTT, fibrinogen, protein C and protein S (a-e, respectively) levels in healthy and diabetic male rats (*Rattus norvegicus*). Data presented as means ± standard deviation represented by vertical bars. Within the different treatment groups, means with different small letters are significantly different at P value < 0.05. Within the different time groups (especially alloxan + sildenafil), means with different capital letters are significantly different at P value < 0.05.

The percentages of changes of all parameters measured, compared with the control group, in alloxan-treated only group *versus* alloxan +sildenafil-treated after either 7 or 14 days groups were 51.00 ± 30.40 *versus* 36.52 ± 18.39 and 57.18 ± 33.97 *versus* 23.86 ± 13.53 , respectively, indicating that antidiabetic activity of sildenafil after 14 days exceeded that of 7 days treatment in alloxan-induced diabetes in rat model.

All the above parameters measured in this study did not significantly alter ($P > 0.05$) in healthy rats that received sildenafil either after 7 or 14 days compared with the control group. Therefore, no deleterious effects were detected for the dose of sildenafil used in the present study either after 7 or 14 days (Tables 1 & 2 and Figs 1 & 2).

DISCUSSION:

The present study indicated that, diabetic rats group (alloxan-treated) showed significant increase in serum glucose level, HbA1c, serum MDA level, PT, APTT, and fibrinogen, and decreased plasma protein C and plasma protein S after 7 and 14 days compared to normal control group. Present finding of this study are in agreement with other previous studies (Berliner *et al.*, 2002; Eteng *et al.*, 2008; Daisy *et al.*, 2009; Behnam-Rassouli *et al.*, 2010; Madan *et al.*, 2010; Abdulrahman and Dallatu, 2012; Kanthlal *et al.*, 2014; Mahre *et al.*, 2017). Alloxan is the most prominent diabetogenic chemical used in diabetes research. It is a cytotoxic glucose analogue causing selective destruction of β cells of islets of Langerhans in pancreas through generation of ROS which cause severe hypoinsulinaemia (Type 1 diabetes) that is responsible for the hyperglycaemia and many complications (Syiem *et al.*, 2002). The increased level of HbA1c (%) is directly proportional to the increased level of blood glucose in diabetic experimental rats (Mohan *et al.*, 2013; Kanthlal *et al.*, 2014).

Weak defence system of the body due to diabetic condition makes the body unable to counteract the enhanced ROS generation, imbalance between ROS and antioxidant pathways lead to domination of the condition of oxidative stress (Pandey *et al.*, 2010; Matough *et al.*, 2012; Abdel-Alaa *et al.*, 2017). Diabetes represents a state of increased oxidative stress, which is mainly based on the evidence of increased lipid peroxidation, or by indirect evidence of reduced antioxidant reserve. In animal models, decreased antioxidant enzyme levels and enhanced lipid peroxidation have been well-documented in alloxan-induced diabetes (Prince *et al.*, 1998). There is a strong positive correlation between MDA (index of polyunsaturated fatty acid oxidation) and

increased production of ROS and decreased activities of endogenous radical scavengers that lead to increased glycation of haemoglobin/proteins and oxidation of coagulation factors as shown in the present study.

The pathogenic mechanism of the clotting activation in diabetes is not completely clear. ROS are believed to be responsible for haematological complications develop in diabetes consist mainly of abnormalities in the function, morphology and metabolism of RBC, WBC, and platelets (Abd El-Ghffar, 2016). There is evidence suggests that certain haematological indices are altered in patients with diabetes mellitus (Dallatu *et al.*, 2009). In patient with diabetes mellitus, persistent hyperglycaemia exposes red blood cells to elevated glucose concentration, thus resulting in glycation of haemoglobin, prothrombin, fibrinogen, and other proteins involved in clotting mechanisms (Thanopoulou *et al.*, 2010). The glycation results in the incomplete activation and function of the clotting cascade (Qin *et al.*, 2004). Glycation of intrinsic and extrinsic clotting proteins will decrease the availability of these proteins which affect the clotting capacity. PT and APTT are haematological indices that give an insight into the coagulation status of patients (Hinchcliff *et al.*, 2004).

Prothrombin time (PT) is a laboratory screening test used to detect disorders involving the activity of the Fibrinogen (factors I), Prothrombin (factors II), Proaccelerin (factors V), Proconvertin (factors VII), and Stuart-Prower (factor X) of the extrinsic and common pathways (Hinchcliff *et al.*, 2004; Furlanello *et al.*, 2006). Also, APTT measures the activities of fibrinogen (factors I), prothrombin (factors II), proaccelerin (factors V), Fibrin stabilizing factor (factors VIII), Antihemophilic factor (factors VIII), Plasma thromboplastin antecedent (factors XI), and Hageman factor (factors XII) of the intrinsic and common pathways (Iazbik *et al.*, 2001). Fibrinogen (Factor I), acute phase protein, is increased in diabetic patients (Zachary and Bloomgarden, 2011). An increase in fibrinogen plasma levels is also considered an independent risk factor for cardiovascular disease. The glycation of fibrinogen results in the formation of a denser fibrin clot with finer fibres that is resistant to fibrinolysis (Soares *et al.*, 2010).

The circulatory disturbances in diabetes are characterized by alternation in platelet count and activity, fibrinolytic aberration, haemorrhagic factors and changes in endothelial metabolism (Kim *et al.*, 2013).

Proteins C and S, besides their known anticoagulant properties, have multiple actions such as anti-apoptotic and anti-

inflammatory activities, regulation of gene expression and stabilization of endothelial barrier protection (Wypasek and Undas, 2013). Hyperglycaemia may induce the glycation of the anticoagulants (protein C and protein S) and their function may be impaired. The function of protein S is to stop the action of two activated clotting proteins (factor V_a and VIII_a). In case of protein S deficiency, these clotting proteins remain activated and increased the tendency for blood to clot. Deficiencies of protein S and protein C are considered risk factors for thrombotic events. It is speculated that the non-enzymatic glycation of the protein causes structural changes that lead to dysfunction (Soares *et al.*, 2010).

The above findings supported the cytotoxic effect of alloxan shown in the present study. On the other hand, oral administration of sildenafil to diabetic rats induced significant decrease in serum glucose level, serum MDA, PT, APTT, and fibrinogen after 7 and 14 days, as well as HbA1c after 14 days. Moreover, oral administration of sildenafil to diabetic rats induced significant increase in plasma proteins C and S after 7 and 14 days of treatment. The results of this study found that, the ameliorative effect of sildenafil (at doses of 10 mg/kg body weight) was time-dependent and more obvious after 14 days of treatment in alleviating diabetic manifestations (hyperglycaemia, hyperfibrinogenaemia, oxidative stress, glycation of haemoglobin/ proteins and oxidation of coagulation factors) of diabetic rats. The results of this study agree with other previous studies (Aversa *et al.*, 2007; Khowailed *et al.*, 2012; El Sayed *et al.*, 2014; Mahre *et al.*, 2017).

Hypoglycaemia induced by sildenafil could be produced via the inhibition of PDE5 and stimulation of NO-cGMP pathway, NO was considered a second messenger for the stimulatory effect of insulin in carbohydrate metabolism (Mahre *et al.*, 2017). Furthermore, NO has insulin like effect in stimulating glucose transport and oxidation (Young *et al.*, 1997; Khan *et al.*, 2004). During blockage of cGMP breakdown by sildenafil, exogenous NO donors bypass autoregulation of vascular NO release, resulting in increased and potentially

uncontrolled vasodilation (Schalcher *et al.*, 2002). The ability of sildenafil to control hyperglycemia which promotes free radicals production or directly inactive free radical species (Mohamed and Faddah, 2007). There are supported by previous studies showed that, even sildenafil is poor hyperglycaemic control (Ojewole *et al.*, 2006) it has long-term protective effects on oxidative stress (Devan *et al.*, 2004), it inhibits the formation of ROS by direct inhibition of oxidases activities (Muzaffar *et al.*, 2005; Shukla *et al.*, 2005).

The anti-oxidative action of sildenafil may have an important role in modifying the vascular complication associated with diabetic state as it has transient vasodilatory properties *in vivo* (Loureiro-Silva *et al.*, 2006; Ojewole *et al.*, 2006). Sildenafil as a NO donor can help in preventing vascular complications associated with diabetic state which is clearly proven in this results through decreasing the values of PT, APTT, and fibrinogen and increasing the values of natural endogenous anticoagulants including protein C and protein S according to Webb *et al.* (2000) and Aversa *et al.* (2007). Oral administration of sildenafil citrate showed a good successful efficient capacity to improve the diabetic complications due to their action as a NO donor and PDE5 inhibitor. This results in an increase of cGMP, which promotes vasodilation.

No adverse/side effects were detected for sildenafil consumption on all parameters measured of healthy rats. These finding of the present study are in agreement with other previous authors (Rinaldi *et al.*, 2013; Morsy *et al.*, 2014). In general, the modulatory effects of sildenafil on some biochemical/haematological parameters measured in the present study were partial, but significant, and time-dependent. Therefore, it may be more beneficial if the time increased in future studies.

ACKNOWLEDGEMENTS:

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors. The authors have no potential financial conflict of interest.

REFERENCES:

- Abd El-Ghffar EA. 2016. Ameliorative effect of glabridin, a main component of *Glycyrrhiza glabra* L. roots in streptozotocin induced Type 1 diabetes in male albino rats. *Indian J. Tradit. Knowle.*, 15(4): 570-579.
- Abdel-Alaa A, Sirdah M, Abd El-Ghffar EA, Abughali AS. 2017. Evaluation of lipid peroxidation and antioxidant enzyme activities in type 2 diabetic Palestinian patients from the Gaza strip. *Int. J. Adv. Res.*, 5(2): 87-93.
- Abdulrahman Y, Dallatu MK. 2012. Evaluation of prothrombin time and activated partial thromboplastin in patients with diabetes mellitus. *Niger. J. Basic and Appl. Sci.*, 20(1): 60-63.
- Angulo J, González-Corrochano R, Cuevas P, Fernández A, La Fuente JM, Rolo F, Allona A, Sáenz de Tejada I. 2010. Diabetes exacerbates the functional deficiency of NO/cGMP pathway associated with erectile

- dysfunction in human corpus cavernosum and penile arteries. *J. Sex. Med.*, 7(2 Pt 1): 758-768.
- Aversa A, Bruzziches R, Vitale C, Marazzi G, Francomano D, Barbaro G, Spera G, Rosano GM. 2007. Chronic sildenafil in men with diabetes and ED. *Drug Metab. Toxicol.*, 3(3): 451-464.
- Balarini CM, Leal MA, Gomes IB, Pereira TM, Gava AL, Meyrelles SS, Vasquez EC. 2013. Sildenafil restores endothelial function in the apolipoprotein E knockout mouse. *J. Transl. Med.*, 11: 3.
- Behnam-Rassouli M, Ghayour MB, Ghayour N. 2010. Microvascular complications of diabetes. *J. Biol. Sci.*, 10(5): 411-423.
- Berliner JI, Rybicki AC, Kaplan RC, Monrad ES, Freeman R, Billett HH. 2002. Elevated levels of Factor XI are associated with cardiovascular disease in women. *Thromb. Res.*, 107(1-2): 55-60.
- Cadirci E, Halici Z, Odabasoglu F, Albayrak A, Karakus E, Unal D, Atalay F, Ferah I, Unal B. 2011. Sildenafil treatment attenuates lung and kidney injury due to overproduction of oxidant activity in a rat model of sepsis: a biochemical and histopathological study. *Clin. Exp. Immunol.*, 166(3): 374-384.
- Dahlbäck B. 1991. Protein S and C4b-binding protein: components involved in the regulation of the protein C anticoagulant system. *Thromb. Haemost.*, 66(1): 49-61.
- Daisy P, Santosh K, Rajathi M. 2009. Antihyperglycemic and antihyperlipidemic effects of *Clitoria ternatea* Linn. in alloxan-induced diabetic rats. *Afr. J. Microbiol. Res.*, 3(5): 287-291
- Dallatu MK, Anaja PO, Bilbis LS, Mojiminiyi FBO. 2009. Antioxidant micronutrient potentials in strengthening the antioxidant defense in alloxan-induced diabetic rats. *Niger. J. Pharm. Sci.*, 8(1): 89-94.
- Devan BD, Sierra-Mercado D Jr, Jimenez M, Bowker JL, Duffy KB, Spangler EL, Ingram DK. 2004. Phosphodiesterase inhibition by sildenafil citrate attenuate the learning impairment induced by blockade of cholinergic muscarinic receptors in rats. *Pharmacol. Biochem. Behav.*, 79(4): 691-699.
- El Sayed MES, Eid N, Kamel ASE. 2014. Beneficial effects of certain phosphodiesterase inhibitors on diabetes mellitus in rats. *B. Fac. Pharm. Cairo Univ.*, 52(2): 179-189.
- Eteng MU, Bassej BJ, Atangwho IJ, Egbung GE, Eyong EU. 2008. Biochemical Indices of macrovascular complication in diabetic rat model: Compared effects of *Vernonia amygdalina*, *Catharantus roseus* and chlorpropamide. *Asian J. Biochem.*, 3: 228-234.
- Furlanello T, Caldin M, Stocco A, Tudone E, Tranquillo V, Lubas G, Solano-Gallego L. 2006. Stability of stored canine plasma for hemostasis testing. *Vet. Clin. Pathol.*, 35(2): 204-207.
- Garg SK, Maurer H, Reed K, Selagamsetty R. 2013. Diabetes and cancer: two diseases with obesity as a common risk factor. *Diabetes Obes. Metab.*, 16(2): 97-110.
- Hinchcliff KW, Kaneps A, Hinchcliff K, Geor R. 2004. Equine sports medicine and surgery. In: Basic and clinical science of the equine athlete. WB Saunders, Philadelphia, PA, pp 1295-1302.
- Hoffmann JJML, Neulendijk PN. 1978. *Thrombos. Haemosta.* (Stuttgart) 39: 640.
- Iazbik C, Couto CG, Gray TL, Kociba G. 2001. Effect of storage conditions on hemostatic parameters of canine plasma obtained for transfusion. *Am. J. Vet. Res.*, 62(5): 734-735.
- Kanthlal SK, Kumar BA, Joseph J, Aravind R, Frank PR. 2014. Amelioration of oxidative stress by *Tabernamontana divaricata* on alloxan-induced diabetic rats. *Anc. Sci. Life*, 33(4): 222-228.
- Keen H, Clark C, Laakso M. 1999. Reducing the burden of diabetes: managing cardiovascular disease. *Diabetes Metab. Res. Rev.*, 15(3): 186-196.
- Khan RN, Hamoud H, Warren A, Wong LF, Arulkumaran S. 2004. Relaxant action of sildenafil citrate (Viagra) on human myometrium of pregnancy. *Am. J. Obstet. Gynecol.*, 191(1): 315-321.
- Khowailed A, Mohammad O, Elattar S, Gaber S. 2012. Effect of sildenafil on gonadotrophin and sex steroids in fructose induced diabetes in female rats. *Med. J. Cairo Univ.*, 80(2): 243-252.
- Kim JH, Bae HY, Kim SY. 2013. Clinical marker of platelet hyperreactivity in diabetes mellitus. *Diabetes Metab. J.*, 37(6): 423-428.
- Loeliger EA, Poller L, Samama M, Thomson JM, Van den Besselaar AM, Vermynen J, Verstraete M. 1985. Questions and answers on prothrombin time standardisation in oral anticoagulant control. *Thromb. Haemost.*, 54(2): 515-517.
- Loureiro-Silva MR, Iwakiri Y, Abalde JG, Haq O, Groszmann RJ. 2006. Increased phosphodiesterase-5 expression is involved in the decreased vasodilator response to nitric oxide in cirrhotic rat livers. *J. Hepatol.*, 44(5): 886-893.
- Madan R, Gupt B, Saluja S, Kansra UC, Tripathi BK, Guliani BP. 2010. Coagulation profile in diabetes and its association with diabetic microvascular complications. *J. Assoc. Physicians India*, 58: 481-484.
- Mahre MB, Umaru B, Ngulde SI, William A, Atela EP, Patrick AA, Middah YH, Zangoma Y. 2017. Haematological changes and wound healing effects of sildenafil citrate in diabetic albino rats. *J. Vet. Sci.*, 15(1): 20-26.
- Matough FA, Budin SB, Hamid ZA, Alwahaibi N, Mohamed J. 2012. The role of oxidative stress and antioxidants in diabetic complications. *Sultan Qaboos Univ. Med.*, 12(1): 5-18.
- Misra M, Aiman U. 2012. Alloxan: An unpredictable drug for diabetes induction? *Indian J. Pharmacol.*, 44(4): 538-539.
- Mohamed AM, Faddah LM. 2007. Therapeutic effects of sildenafil citrate (viagra) and/or

- vitamin E on some brain disorders of alloxan induced diabetes mellitus rats. *J. Med. Sci.*, 7(6): 932-941.
- Mohan Y, Jesuthankaraj GN, Thangavelu NR. 2013. Antidiabetic and antioxidant properties of *triticum aestivum* in streptozotocin-induced diabetic rats. *Adv. Pharmacol. Sci.*, Article ID 716073, pp. 9.
- Morsy MA, Ibrahim SA, Amin EF, Kame MY, Rifaai RA and Hassan MK. 2014. Sildenafil ameliorates gentamicin-induced nephrotoxicity in rats: role of iNOS and Enos. *J. Toxicol.*, Article ID 489382, pp. 7.
- Muzaffar S, Shukla N, Srivastava A, Angelini GD, Jeremy JY. 2005. Sildenafil citrate and sildenafil nitrate (NCX 911) are potent inhibitors of superoxide formation and gp91phox expression in porcine pulmonary artery endothelial cells. *Br. J. Pharmacol.*, 146(1): 109-117.
- Ojewole JA, Drewes SE, Khan F. 2006. Vasodilatory and hypoglycemic effects of two pyrano-isoflavone extractives from *eriosems kraussianum* N. E. Br. (Fabaceae) rootstock in experimental rat models. *Phytochemistry*, 67(6): 610-617.
- Pandey KB, Mishra N, Rizvi SI. 2010. Protein oxidation biomarkers in plasma of type 2 diabetic patients. *Clin. Biochem.*, 43(4-5): 508-511.
- Poon KW, Lyng FM, Knief P, Howe O, Meade AD, Curtin JF, Byrne HJ, Vaughan J. 2012. Quantitative reagent-free detection of fibrinogen levels in human blood plasma using Raman spectroscopy. *Analyst*, 137(8): 1807-1814.
- Prince PSM, Menon VP, Pari L. 1998. Hypoglycemic activity of *Syzygium cumini* seeds, effect on lipid peroxidation in alloxan diabetic rats. *J. Ethnopharmacol.*, 61(1): 1-7.
- Qin X, Goldfine A, Krumrei N, Grubissich L, Acosta J, Chorev M, Hays AP, Halperin JA. 2004. Glycation inactivation of the complement regulatory protein CD59-A possible role in the pathogenesis of the vascular complications of human diabetes. *Diabetes*, 53(10): 2653-2661.
- Rinaldi B, Donniacuo M, Sodano L, Gritti G, Signoriello S, Parretta E, Berrino L, Urbanek K, Capuano A, Rossi F. 2013. Effects of sildenafil on the gastrocnemius and cardiac muscles of rats in a model of prolonged moderate exercise training. *PLoS One*, 8(7): e69954.
- Satoh K. 1978. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Clin. Chim. Acta*, 90(1): 37-43.
- Schalcher C, Schad K, Brunner-La Rocca HP, Schindler R, Oechslein E, Scharf C, Suetsch G, Bertel O, Kiowski W. 2002. Interaction of sildenafil with cAMP-mediated vasodilation in vivo. *Hypertension*, 40(5): 763-767.
- Shukla N, Jones R, Persad R, Angelini GD, Jeremy JY. 2005. Effect of sildenafil citrate and a nitric oxide donating sildenafil citrate derivative, NCX 911, on cavernosal relaxation and superoxide formation in hypercholesterolaemic rabbits. *Eur. J. Pharmacol.*, 517(3): 224-231.
- Soares AL, Sousa MDO, Fernandes APSM, Carvalho MDG. 2010. Hemostatic changes in patients with type 2 diabetes mellitus. *Rev. Bras. Hematol. Hemoter.* 32 (6): 482-488.
- Stocker K, Fischer H, Meier J, Brogli M, Svendsen L. 1986. Protein C activators in snake venoms. *Behring Inst. Mitt.*, 79: 37-47.
- Syiem D, Syngai G, Khup PZ, Khongwir BS, Kharbuli B, Kayang H. 2002. Hypoglycemic effects of *Potentilla fulgens* L in normal and alloxan-induced diabetic mice. *J. Ethnopharmacol.*, 83(1-2): 55-61.
- Thanopoulou A, Karamanos B, Archimandritis A. 2010. Glycated hemoglobin, diabetes, and cardiovascular risk in nondiabetic adults. *N. Engl. J. Med.*, 362(21): 2030-2031.
- Trinder P. 1969. Determination of glucose in blood using glucose oxidase with an alternative oxygen receptor. *Ann. Clin. Biochem.*, 6: 24-27.
- Trivelli LA, Ranney HM, Lai HT. 1971. Hemoglobin components in patients with diabetes mellitus. *N. Engl. J. Med.*, 284(7): 353-357.
- Webb DJ, Muirhead GJ, Wulff M, Sutton JA, Levi R, Dinsmore WW. 2000. Sildenafil citrate potentiates the hypotensive effects of nitric oxide donor drugs in male patients with stable angina. *J. Am. Coll. Cardiol.*, 36(1): 25-31.
- WHO. 2016. Global report on diabetes. World Health Organization, Geneva, 2016. Available on: <http://www.who.int/mediacentre/factsheets/fs312/en/>
- Williams SB, Cusco JA, Roddy MA, Johnstone MT, Creager MA. 1996. Impaired nitric oxide-mediated vasodilation in patients with non-insulin-dependent diabetes mellitus. *J. Am. Coll. Cardiol.*, 27(3): 567-574.
- Wypasek E, Undas A. 2013. Protein C and protein S deficiency – practical diagnostic issues. *Adv. Clin. Exp. Med.*, 22(4): 459–467.
- Young ME, Radda GK, Leighton B. 1997. Nitric oxide stimulates glucose transport and metabolism in rat skeletal muscle in vitro. *Biochem. J.*, 322(Pt 1): 223–228.
- Yousry MM, Farag EA, Omar AI. 2016. Histological study on the potential effect of sildenafil on the kidney and testosterone level in experimentally induced diabetes in male rats. *J. Cytol. Histol.*, 7(4): 1-8.
- Zachary T, Bloomgarden MD. 2011. Diabetes and cardiovascular disease. *Diabetes Care*, 34: 24-30.
- Zhao Y, Vanhoutte P M, Leung SW. 2015. Vascular nitric oxide: Beyond eNOS. *J. Pharmacol. Sci.*, 129 (2): 83-94.

تأثير عقار سترات السيلدينافيل على بعض المعايير الفيزيولوجية والدموية في ذكور الجرذان البيضاء المصابة بمرض السكري

نفيسة حسين مكي*، إيمان علي عبد الغفار*، عصام محمد إبراهيم**، هدى جمال حجازي*،
مريم صابر عبد القادر العباسي*

* قسم علم الحيوان، كلية العلوم، جامعة عين شمس، مصر
** قسم الباثولوجيا الاكلينيكية، معهد بحوث صحة الحيوان، الجيزة، مصر

الثرثومبولاستين الجزئي النشط والفيبرينوجين في البلازما. وأيضاً إلى أدى إلى أحداث انخفاض معنوي في بروتين C وبرتوتين S. كما أظهر العلاج بالسليدينافيل عن طريق الفم إلى تحسين في معظم المضاعفات لمرض السكري مثل الزيادة المعنوية للجلوكوز والأكسدة وتجلط البروتينات وفرط الفيبرينوجين في الدم الناجمه عن طريق الألوكسان في ذكور الجرذان. وبوجه عام فإن التأثيرات المحسنة للسليدينافيل في هذه الدراسة كانت جزئية ولكنها معنوية وتزداد بزيادة الوقت. لم يتسبب تناول السليدينافيل إلى أحداث اي تأثيرات ضارة على جميع القياسات في الجرذان الصحاء. دعمت هذه الدراسة الحالية إفتراض أن السليدينافيل قد يكون له آثار مفيدة ضد مضاعفات مرض السكري. ونرجع الالية تأثير سترات السليدينافيل على قياستنا الى قدرته على زيادة الجوانزين الحلقى أحادي الفوسفات (cGMP) الذي قد يثبت بواسطة أنزيم الفسفودايستريز النوع الخامس.

سترات السليدينافيل من مثبطات أنزيم الفسفودايستريز النوع الخامس، مما يزيد الجوانزين الحلقى أحادي الفوسفات (cGMP). ومن الثابت أن الآلية المرضية لمرض السكري مرتبط بتشوهات لتوليد أكسيد النيتريك (NO). تهدف الدراسة الحالية إلى تقييم تأثير سترات السليدينافيل على بعض القياسات البيوكيميائية والدموية في مرض السكري المستحث بعد حقن جرعة واحدة من الالوكسان (150 مليجرام / كجم من وزن الجسم) في العشاء البريتوني لذكور الجرذان البيضاء. ثم تعامل الجرذان الاصحاء وكذلك الجرذان المصابة بمرض السكري بجرعة يومية 10 مغ/كغ من وزن الجسم من سترات السليدينافيل عن طريق الفم خلال فترة 14 يوماً للجرذان الاصحاء وكذلك الجرذان المصابة بمرض السكري. تم أخذ عينات الدم بعد 7 أيام وبعد 14 يوماً من المعاملة في كل مجموعات التجربة. أوضح البحث الحالي أن الألوكسان أحداث إرتفاع في قيم الجلوكوز في مصل الدم، والهيموجلوبين السكري وأنزيم المالون داي الدهيد (MDA) وزمن البروثرومين وزمن