Pharmacological study on the effect of sildenafil on the vascular reactivity and renal function in streptozotocin-induced diabetes in male albino rats

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Abstract: Diabetes is a serious metabolic disorder with micro and macrovascular complications that results in significant morbidity and mortality. The present study aimed to assess the possible protective role of sildenafil in the DM induced vascular and biochemical changes by investigating the effects of sildenafil on the isolated rat aorta reactivity and the biochemical changes induced by STZ experimentally induced diabetes mellitus in rats. After 6 weeks of treatment, blood samples were withdrawn for measurement of fasting blood glucose level, HbA1c, serum insulin level, blood urea, serum creatinine and the vascular reactivity of rat's aortae to the vasoconstrictive agent (norepinehrine) and the vasodilator agent (acetylcholine) was measured. **The results** showed that thr treatment with sildenafil significantly improved the acetylcholine – induced vasodilatation and attenuate the noradrenaline-induced contraction of the isolated rat aorta in the diabetic rats as compared to diabetic untreated rats. Urea and creatinine levels were decreased significantly in the sildenafil treated diabetic rats as compared with those of the diabetic untreated rats. However treatment of the diabetic rats with sildenafil did not produce significant effect on blood glucose or serum insulin as compared with the diabetic untreated rats. **In conclusion** sildenafil improves the disturbed renal functions and vascular reactivity in the diabetic rats.

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1. Introduction and aim of the work

Diabetes mellitus (DM), is a group of metabolic disorders in which there are high blood sugar levels over a prolonged period. **(WHO, 2013)** . Acute complications can include diabetic ketoacidosis, hyperosmolar hyperglycemic state, or death. **(Kitabchi et al., 2009)** . Serious long-term complications include cardiovascular disease, stroke, chronic kidney disease, foot ulcers, and damage to the eyes **(WHO, 2014).** The vascular complications of diabetes are the most serious manifestations of the disease. Atherosclerosis is the main reason for impaired life expectancy in patients with diabetes whereas diabetic nephropathy and retinopathy are the largest contributors to end-stage renal disease and blindness, respectively. **(Christian Rask-Madsen and George L. King, 2013).** The endothelium is important in maintaining vascular homeostasis and preventing the development of atherosclerosis. However, perturbation of its activity may lead to erectile dysfunction which, if left untreated, could progress to atherosclerotic lesion formation and subsequent cardiac events **(Aamer et al,2010).** During the development of diabetes a number of biochemical and mechanical factors converge on the endothelium, resulting in endothelial dysfunction and vascular inflammation. In the presence of insulin resistance, these processes are potentiated and they provide a basis for the macrovascular disease seen in diabetes **(Hartge et al, 2007).** Shi et al.,2012, reported that sildenafil can increase the levels of testosterone, F.S.H and L.H in the serum of D.M rats**. Ramirez et al.,2015**, reported that sildenafil can improve insulin sensitivity in human**.** So the present study aims to investigate and evaluate the ability of sildenafil in decreasing the hazards of diabetes mellitus and its complications in the experimentally streptozotocin-induced D.M. in rats, by testing the fasting blood sugar, HbA1c, serum insulin level, kidney functions as well as the vascular reactivity by recording the contractile response of rat aorta to norepinephrine and its relaxant response to ACh.

2. Materials and Methods

Experimental animals:

Adult male albino rats were chosen as an animal model for this study. Rats were brought from animal house, Faculty of Medicine, Assiut University, Assiut, Egypt, and were maintained on a balanced diet with water supply freely in clean containers. They were kept for two weeks to adapt to the laboratory conditions before the start of the experiment. Eighty age-matched male albino rats with initial body weights ranging from 150 to 200g were used.

Drugs and chemicals:

Acetylcholine (ACH) (Fluka, Switzerland), **sildenafil** (Pfizer Chemical Company, USA), **norepinephrine (NE)** (Sigma Chemical Company – Aldrich, USA), **streptozotocin (STZ)** (MP biomedicals, LLC- France).

Experimental protocol:

The rats were divided into 3 groups (10 rats each) as following**:**

Group I: Normal untreated non diabetic rats injected intraperitoneally by 0.1 M (pH 4.5) citrate buffer and received 1ml saline (vehicle) orally for 6 weeks.

Group II: Diabetic rats treated with saline (vehicle) orally for 6 weeks.

Group III: Diabetic rats treated with sildenafil (10 mg/kg/day) **(Aborayag et al., 2013)** orally for 6 weeks**.**

Procedures:

1-Experimental induction of diabetes mellitus:

Diabetes was induced in rats by single intraperitoneal injection of streptozotocin (STZ) at a dose of 50 mg/kg body weight dissolved in 0.1 M (pH 4.5) citrate buffer **(Chatzigeorgiou et al., 2009).** While the control group was given equivalent volume of citric acid buffer. STZ induces diabetes within 2 days by destroying β cells. Diabetes was confirmed through detecting blood glucose concentration by glucose oxidase method using glucometer with glucose test strip (One Touch Basic). Two days after STZ injection, rats with blood glucose levels of more than 250 mg/dl were considered diabetic and included in the study **(Chatzigeorgiou et al., 2009).**

2- Collection of blood samples:

A-The animal was anaesthetized with ether by placing the rat in an anesthetic box filled with ether vapor which was maintained by periodically applying liquid ether to a cotton wool on the base of the box. When surgical stage of anesthesia was reached (judged by loss of withdrawal reflexes), the animal was removed and placed on a table and blood was collected from the retro-orbital plexus using capillary tube (0.75-1.0 mm internal diameter) inserted in the medial canthus medial to the eye globe.

B-After six weeks, rats were fasted overnight and blood was collected from carotid artery after sacrificing of animals. The blood was collected into a dry clean graduated glass centrifuge tube. It was rapidly set to centrifuge at 5000 r.p.m for 10 minutes about half of the supernatant serum was sucked out into a clean dry glass serology tube using Pasteur pipette.

3-Preparation of the isolated aortic rings:

On the day of experiment, animals were killed by a blow on the head and cutting the throat. Abdominal and thoracic walls were opened. The thoracic aorta was dissected and cut, placed in dish containing Kreb's-Henseleit solution of the following composition (mM/L): (NaCl 118.4, KCl 4.69, kH2PO4:1.17, MgSO4 1.18, CaCl 2.52, glucose 11.10 and NaHCO3 25) aerated with carbogen (95% oxygen and 5% carbogen dioxide), cleaned from the surrounding attached tissues and cut into small rings (about 4mm length). The aortic rings were suspended in an isolated organ bath (30 ml capacity) containing Kreb's-Henseleit solution maintained at 37°C and aerated with carbogen. Aortic rings were subjected to an initial tension 1g, and were kept in the organ bath (for equilibration) for approximately 90 minutes, the physiological solution was renewed every 15 minutes. Response of the aortic rings to drugs were measured isometrically with a Grass FT O3 force-displacement transducer, and recorded on a polygraph. The viability and stability of the tissue were checked by two equal contractile responses to the same concentration of norepinephrine (10^{-7}). Norepinephrine contained 1% Hcl to prevent auto-oxidation. Tissues were then washed several times and allowed to relax to base line level. Cumulative dose–response curves to norepinephrine were performed on each ring, diluted solutions of norepinephrine (1×10^{-8} to 1×10^{-3} and 3×10^{-8} to 3×10^{-3}) were used. During performing the dose-response curves of norepinephrine each dose was added after reaching the plateau of the response of the previous dose. Each ring was serially washed after obtaining the maximum response to baseline and equilibrated. For relaxation study; aortic rings were precontracted by norepinephrine (10^{-6}) this concentration produced a submaximal response. When the response reached its plateau, cumulative concentration response curves of acetylcholine (1×10^{-8} to 1×10^{-3} and 3×10^{-8} to 3×10^{-3}) was done and each ring was serially washed after obtaining the maximum response to reach the baseline and equilibrated. During performing the dose-response curves of acetylcholine each dose was added after reaching the plateau of the response of the previous dose.

4- Biochemical measurements:

1- Blood glucose measurements:

Principle of the test*:* The blood glucose level was determined by enzymatic colorimetric method **(Trinder and Ann 1969).** Using diamond diagnostic kits.

2-Glycated Hemoglobin measurements:

Intended Use For the measurement of the glycated hemoglobin (HbA1c) in human whole blood HbA1c is a glycated product of hemoglobin A0 (HbA1c), the predominant form of hemoglobin in adults. Measurement of the percentage of HbA1c reflects the mean blood glucose concentration over the preceding one to two months, and is therefore considered to be an important diagnostic marker for

monitoring blood glucose levels. **Japan Diabetes Society,2010.**

5- Renal function tests:

A-Serum urea measurements:

Serum urea level was done by urease – Colorimetric method **(Batton and Crouch 1977).** Using Egyptian company for biotechnology kits.

B-Serum creatinine level:

Serum creatinine level was measured by kinetic method **(Young 1995).**

Statistical analysis:

Statistical analysis was done using the computer program (SPSS). The quantitative data were presented in the form of mean ± standard error (S.E). Statistical analysis of data was performed by using one–way analysis of variance (ANOVA) followed by Tukey-Kramer test for differences between means. A value of $P < 0.05$ was used as a criterion for statistical significance.

3. Results

Effects of sildenafil on the vascular reactivity

Cumulative concentration-response curves elicited by norepinephrine and acetylcholine on the isolated aortic ring preparations obtained from the normal untreated rats, the diabetic untreated rats, diabetic treated with sildinafil (10mg/kg/day p.o. for 6 weeks. The results show that the contractile response of the aorta was increased significantly ($P< 0.001$) in the diabetic untreated rats as compared with the normal rats, and decreased significantly ($P<0.01$) in the diabetic rats treated with sildinafil in comparison with the diabetic untreated rats, with no significant ($P> 0.05$) difference between the response of the aorta of the diabetic rats treated with sildinafil and the normal rats aortae (table-1 and figure-1).

Table (1): Effects of treatment with sildenafil on the contractile response of the diabetic rat's isolated aortae to norepinephrine (NE).

Groups	NormaL untreated	Diabetic untreated	Diabetic treated with sildinafil
- Log conc. of Norepinephrine	*Contraction (g)*	*Contraction (g)*	*Contraction (g)*
$1x10^{-8}$	0± 0.00**	0.9± 0.05	0.39± 0.09*
$3x10^{-8}$	0.02± 0.05**	1.95± 0.04	0.85± 0.08 *
$1x10^{-7}$	0.04± 0.03**	2.14± 0.05	0.94± 0.06 *
$3x10^{-7}$	0.1± 0.04**	2.29± 0.03	1.04± 0.05 *
$1x10^{-6}$	0.17± 0.02**	2.38± 0.07	1.12± 0.01 *
$3x10^{-6}$	0.35± 0.05**	2.51± 0.05	1.28± 0.01 *
$1x10^{-5}$	0.82± 0.03**	2.6± 0.02	1.58± 0.03 *
$3x10^{-5}$	1.25± 0.04**	2.66± 0.01	1.85± 0.02 *
$1x10^{-4}$	1.25± 0.02**	2.66± 0.04	1.85± 0.05 *

Each value represents mean ± SE (standard error) of 7 – 9 rats.
*Significant difference from the diabetic untreated rats
** Highly significant difference from the diabetic untreated rats

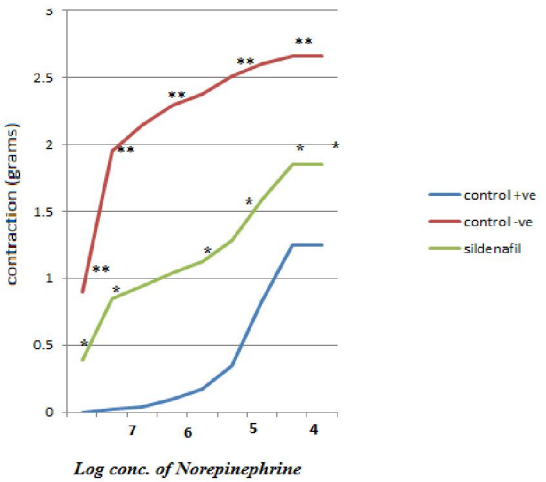

Figure-1: Effects of treatment with sildenafil on the contractile response of the diabetic rat's isolated aortae to norepinephrine (NE).

Each value represents mean ± SE (standard error) of 7 – 9 rats.

*Significant difference from the diabetic untreated rats.

** Highly significant difference from the diabetic untreated rats.

Effects of sildenafil, on the relaxant response of the noradrenaline - precontracted isolated rat aortae of the diabetic rats to acetylcholine:

Cumulative concentration-response curves elicited by acetylcholine on the norepinephrine precontracted aortic ring preparations obtained from normal untreated rats, the diabetic untreated rats, diabetic treated with sildinafil (10mg/kg/day p.o. for 6

weeks). The results show that the relaxant response of the aorta was decreased significantly (P< 0.001) in the diabetic untreated rats in comparison with the normal rats, and increased significantly (P< 0.01) in the diabetic rats treated with sildenafil in comparison with the diabetic untreated rats, with no significant (P> 0.05) difference between relaxant response of the aortae of the diabetic rats treated with metformin and normal rats aortae (table-2 & figure-2).

Table (2): Effect of treatment with sildenafil on the relaxant response of the rat's isolated aortae to acetylcholine.

Groups	Normal Untreated	Diabetic untreated	Diabetic treated with Sildinafil
-Log conc. of Ach	Relaxation (% of residual tone)	Relaxation (% of residual tone)	Relaxation (% of residual tone)
1x10-8	60.12± 0.05**	100± 0.03	67.01± 0.09*
3x10-8	45.25± 0.06**	98.38± 0.05	53.5± 0.03 *
1x10-7	41.63± 0.02**	89.91± 0.06	47.66± 0.04 *
3x10-7	38.01± 0.09**	84.92± 0.02	43.82± 0.05 *
1x10-6	36.2± 0.01**	80.88± 0.04	38.4± 0.07 *
3x10-6	30.48± 0.03**	76.86± 0.01	33.09± 0.02 *
1x10-5	24.48± 0.04**	70.87± 0.09	27.09± 0.08 *
3x10-5	20.32± 0.08**	57.81± 0.07	24.82± 0.01 *
1x10-4	20.33± 0.07**	57.84± 0.08	24.81± 0.06 *

*Significant difference from the diabetic untreated rats
** Highly significant difference from the diabetic untreated rats

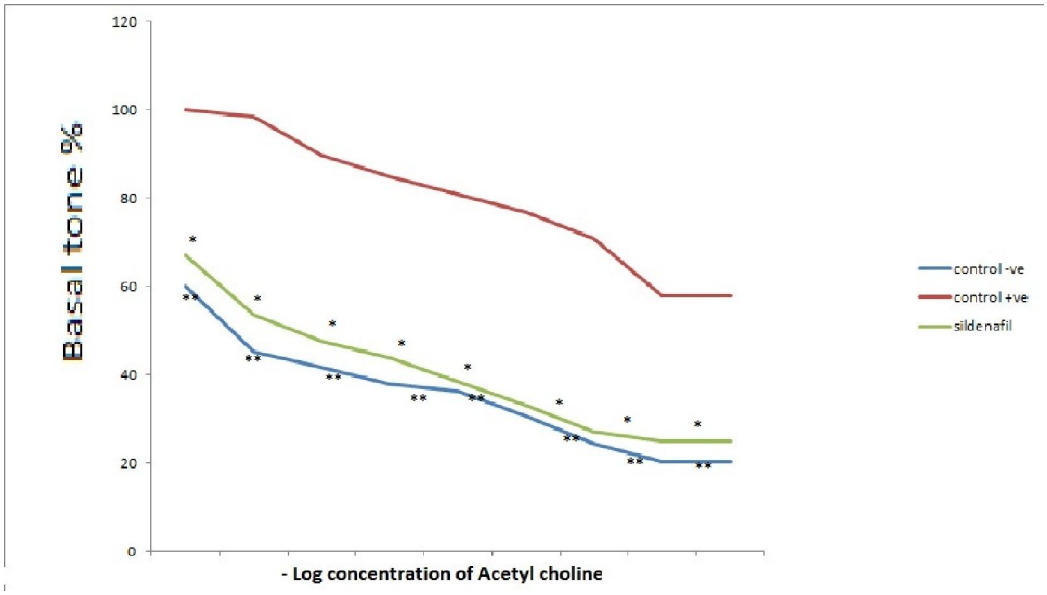

Figure-2: Effect of treatment with sildenafil on the relaxant response of the rat's isolated aortae to acetylcholine.
*Significant difference from the diabetic untreated rats
** Highly significant difference from the diabetic untreated rats

Effects of sildenafil on the biochemical changes induced by diabetes mellitus in rats:

1-Fasting blood glucose obtained from the normal untreated rats, the diabetic untreated rats, diabetic rats treated with sildenafil (10mg/kg/day p.o. for 6 weeks. The results showed that fasting blood glucose was increased significantly (P< 0.001) in the diabetic untreated rats in comparison with the normal untreated rats. Oral treatment of the diabetic rats with sildinafil decreased fasting blood glucose insignificantly (P> 0.05) as compared with diabetic untreated rats, and increased significantly (P< 0.05) as compared with the normal untreated rats. as shown in table (3 & figure-3).

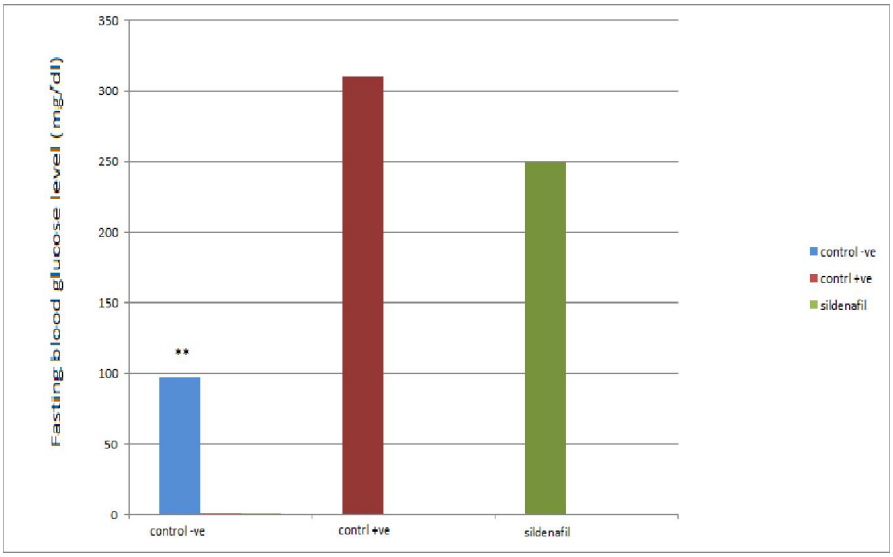

Figure-3: **Effects of treatment with sildenafil, on fasting blood glucose**
Ach value represents mean ± SE of 7 – 9 rats.
*Significant difference from the diabetic untreated rats
** Highly significant difference from the diabetic untreated rats

Table (3): Effects of treatment with sildenafil, on fasting blood glucose

Groups	Fasting blood glucose level (mg/dl)
Normal untreated	**97.59 ± 0.04****
Diabetic untreated	**310.58 ± 0.07**
Diabetic treated with Sildinafil	**249.57 ± 0.08**

Each value represents mean ± SE of 7 – 9 rats.
*Significant difference from the diabetic untreated rats
** Highly significant difference from the diabetic untreated rats

2-Effects of treatment with sildenafil on glycosylated hemoglobin of the rats

Table-4: Effects of treatment with sildenafil on glycosylated hemoglobi*n*

Groups	Glycosylated hemoglobin (HbA1c) %
Normal untreated	**5.66± 0.06****
Diabetic untreated	**12.47± 0.05**
Diabetic treated with Sildinafil	**10.54± 0.03**

Each value represents mean ± SE of 7 – 9 rats.
*Significant difference from the diabetic untreated rats
** Highly significant difference from the diabetic untreated rats

Glycosylated hemoglobin obtained from the normal untreated rats, the diabetic untreated rats, diabetic rats treated with sildinafil (10mg/kg/day p.o. for 6 weeks). The results showed that glycosylated hemoglobin was increased significantly (P< 0.001) in the diabetic untreated rats in comparison with the normal untreated rats. Oral treatment of the diabetic rats with sildinafil decreased glycosylated hemoglobin insignificantly (P > 0.05) as compared with diabetic untreated rats (table 4 & figure-4).

Effect of sildenafil on serum insulin level

Serum insulin obtained from the normal untreated rats, the diabetic untreated rats, diabetic rats treated with sildinafil (10mg/kg/day p.o. for 6 weeks. The results showed that that serum insulin level was decreased significantly (P< 0.001) in the diabetic untreated rats in comparison with the normal untreated rats. Oral treatment of the diabetic rats with sildinafil did not affect the insulin release (table-5 & figure-5).

Table (5): Effects of treatment with sildenafil on serum insulin level of the rats:

Groups	Serum insulin level (µIU/mL)
Normal untreated	**24.77± 0.09****
Diabetic untreated	**10.47± 0.04**
Diabetic treated with Sildinafil	**12.43± 0.07**

Each value represents mean ± SE of 7 – 9 rats.
*Significant difference from the diabetic untreated rats
** Highly significant difference from the diabetic untreated rats

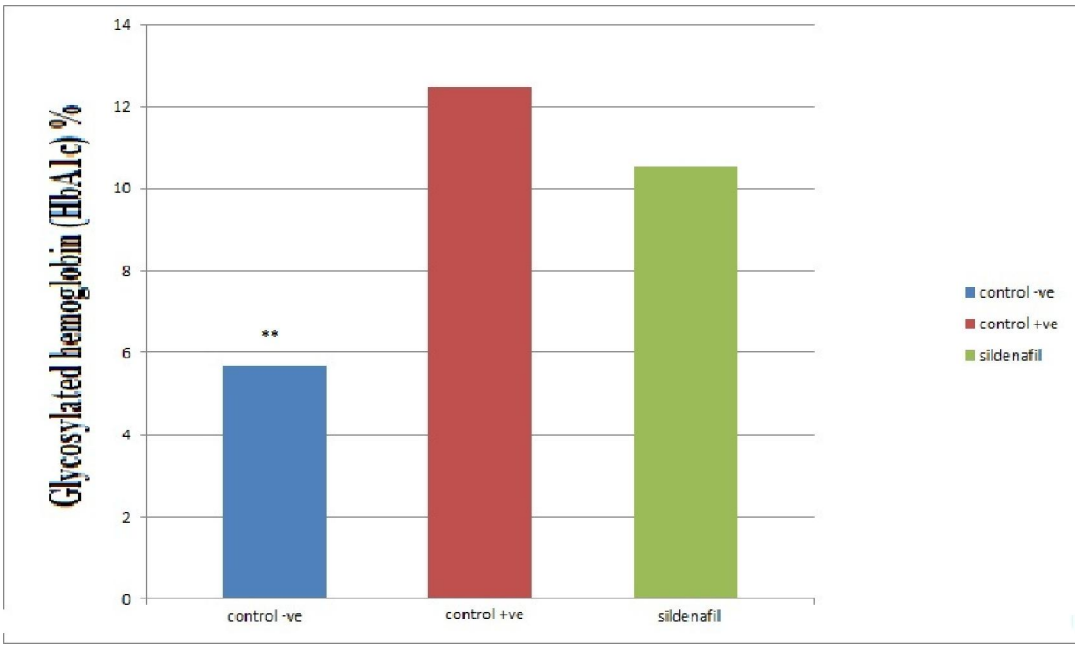

Figure-4: **Effects of treatment with sildenafil on glycosylated hemoglobi**
Each value represents mean ± SE of 7 – 9 rats.
*Significant difference from the diabetic untreated rats
** Highly significant difference from the diabetic untreated rats

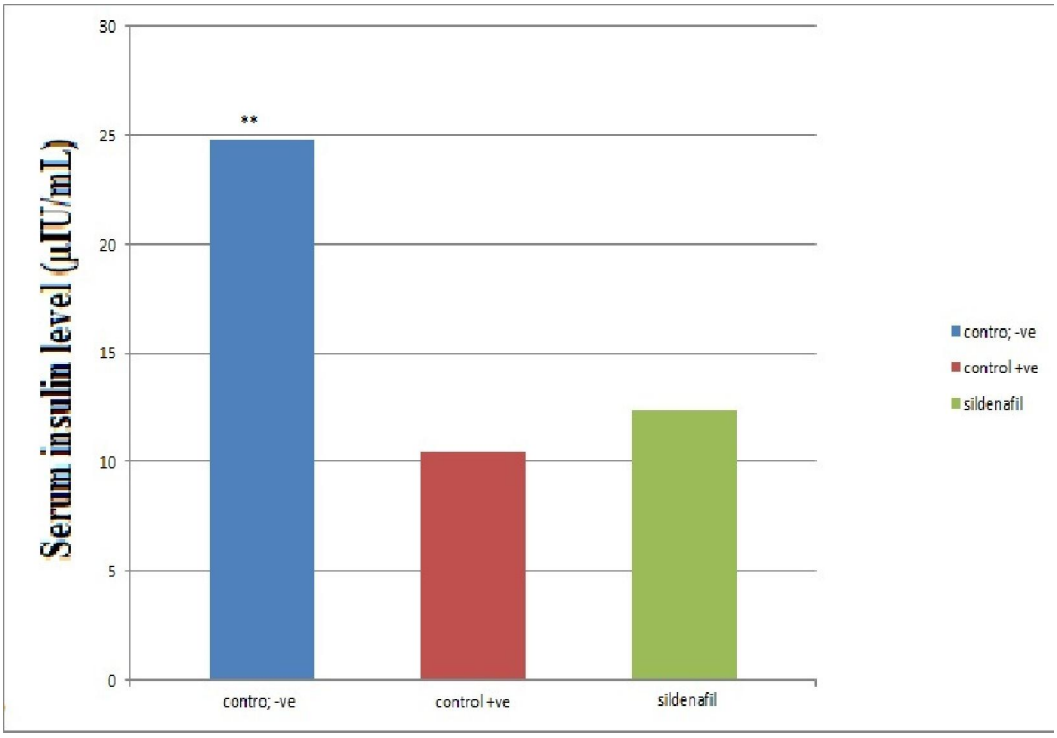

Figure-5: Effects of treatment with sildenafil on serum insulin level of the rats:
Each value represents mean ± SE of 7 – 9 rats.
*Significant difference from the diabetic untreated rats
** Highly significant difference from the diabetic untreated rats

Effects of sildenafil on serum creatinin*e*

Serum creatinine obtained from the normal untreated rats, the diabetic untreated rats, diabetic rats treated with sildinafil (10mg/kg/day p.o. for 6 weeks). The results showed that serum creatinine was increased significantly (P< 0.001) in the diabetic untreated rats in comparison with the normal untreated rats. Oral treatment of the diabetic rats with sildinafil decreased serum creatinine significantly (P< 0.05) as compared with diabetic untreated (Table—6 & Figure-6).

Table (6): Effects of treatment with sildenafil on serum creatinine of the rats:

Groups	Serum creatinine (mg/dl)
Normal untreated	**0.76± 0.09****
Diabetic untreated	**2.10± 0.08**
Diabetic treated with Sildinafil	**1.72± 0.1 ***

Each value represents mean ± SE (standard error) of 7 – 9 animals

*Significant difference from the diabetic untreated rats

** Highly significant difference from the diabetic untreated rats

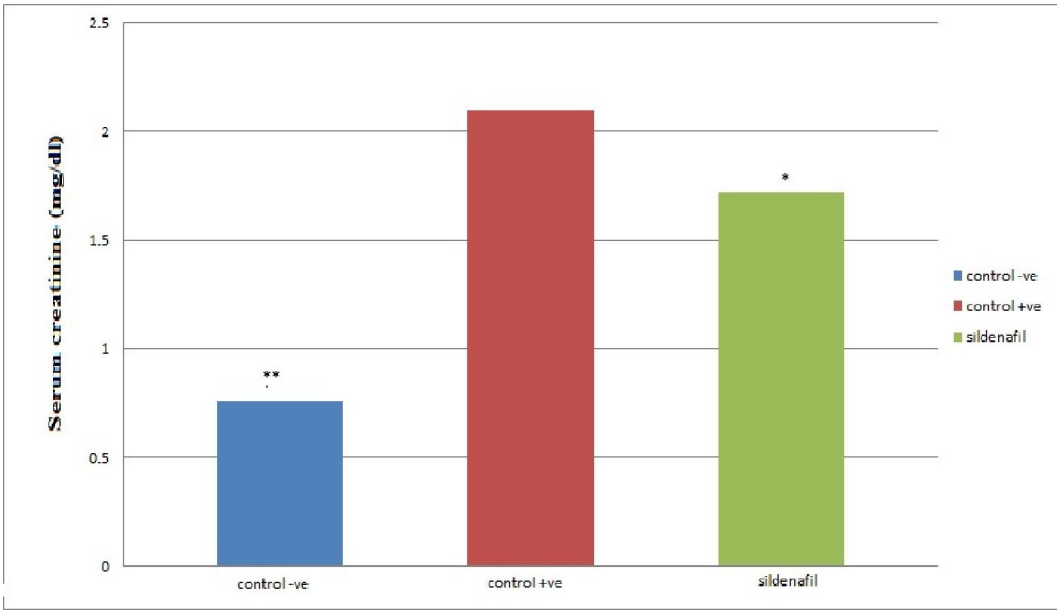

Figure-(6): **Effects of treatment with sildenafil on serum creatinine of the rats:**

Each value represents mean ± SE (standard error) of 7 – 9 animals

*Significant difference from the diabetic untreated rats

** Highly significant difference from the diabetic untreated rats

Effects of sildenafil, on serum urea*:*

Serum urea obtained from the normal untreated rats, the diabetic untreated rats, diabetic rats treated with sildinafil (10mg/kg/day p.o. for 6 weeks. The results showed that serum urea was increased significantly (P< 0.001) in the diabetic untreated rats in comparison with the normal untreated rats. Oral treatment of the diabetic rats with sildinafil decreased serum urea significantly (P< 0.05) as compared with diabetic untreated rats (table-7 & figure-7).

Table (7): Effects of treatment with sildenafil on serum urea of the rats:

Groups	Serum urea (mg/dl)
Normal untreated	38.34± 0.08**
Diabetic untreated	97.41± 0.07
Diabetic treated with Sildinafil	80.48± 0.1 *

Each value represents mean ± SE (standard error) of 7 – 9 animals

*Significant difference from the diabetic untreated rats

** Highly significant difference from the diabetic untreated rats

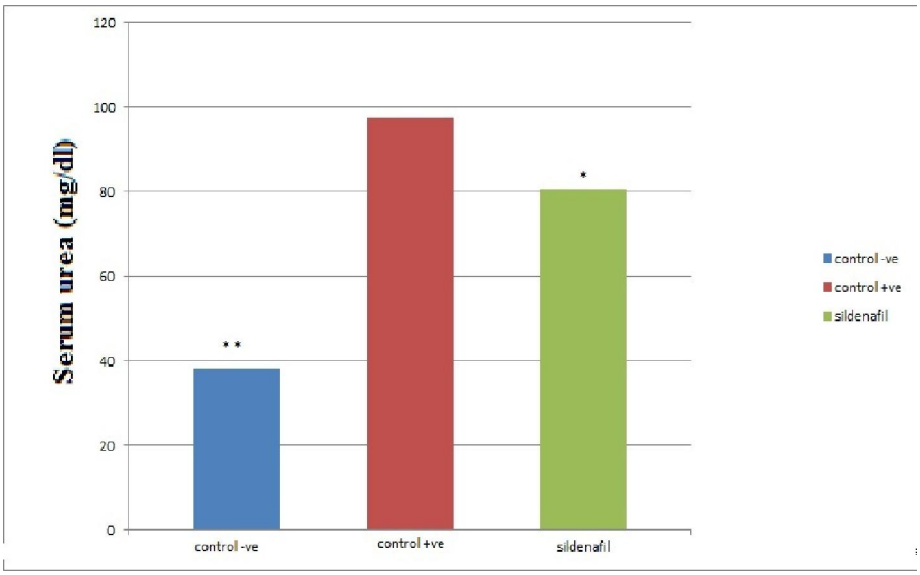

Figure-7: Effects of treatment with sildenafil on serum urea of the rats:
Each value represents mean ± SE (standard error) of 7 – 9 animals
*Significant difference from the diabetic untreated rats
** Highly significant difference from the diabetic untreated rats

4. Discussion

Acute complications of D.M. include diabetic ketoacidosis, hyperosmolar hyperglycemic state, or death. **(Kitabchi et al., 2009)**. Serious long-term complications include cardiovascular disease, stroke, chronic kidney disease, foot ulcers, and damage to the eyes **(WHO,2014).** The vascular complications of diabetes are the most serious manifestations of the disease. Atherosclerosis is the main reason for impaired life expectancy in patients with diabetes whereas diabetic nephropathy and retinopathy are the largest contributors to end-stage renal disease and blindness, respectively **(Christian Rask-Madsen and George L. King, 2013).** During the development of diabetes a number of biochemical and mechanical factors converge on the endothelium, resulting in endothelial dysfunction and vascular inflammation. In the presence of insulin resistance, these processes are potentiated and they provide a basis for the macrovascular disease seen in diabetes **(Hartge et al, 2007).** In the present work the effect of sildenafil treatment is studied in diabetic rats to show its possible protective effect on the vascular reactivity of diabetic rats aorta and its possible normalization effect on biochemical changes induced by diabetes in rats. The present results show that the contractile response of the rat's isolated aortae induced by norepinephrine (NE) is increased significantly in the diabetic untreated rats in comparison with the normal rats. This result is in agreement with **White and Carrier , 1990,** who reported that arteries from STZ-diabetic (10-12 weeks) rats developed greater contractile force in response to norepinephrine than in normal rats and also with **Jun Yoshino et al, 2010,** who found that the contractile response to norepinephrine was also enhanced by diabetic induction**.** Results of the present study are also in agreement with **Miranda et al, 2010,** who proved that actions of norepinephrine, phenylephrine or serotonin have been shown to be enhanced**.** The increased aortic contractile responses of diabetic rats may be due to impaired endothelial function **(Olukman et al., 2010)**, enhanced sensitivity of calcium channels **(Chang et al., 1993**), increased vasoconstrictor prostanoids due to increased superoxide anions, and increased sensitivity to adrenergic agonists **(Abebe,2008).** Studies have shown that in diabetes mellitus, glucose metabolism and protein glycosylation produce free oxygen radicals. Increased free radicals and reduced antioxidant defense have an important role in causing atherosclerosis and increased permeability and sclerosis of blood vessels. In addition, in diabetic patients, production of free radicals through autoxidation of glucose, activation of cyclooxygenase pathway, and production of active oxygen by carbohydrates and lipids are increased **(Yildirim and Buyukbingol 2003)**. On the other hand the results obtained by the present study are in disagreement with the results obtained by other workers; **Cameron and Cotter 1992**, found that diabetes reduced maximal tension production, particularly for responses to phenylephrine and serotonin which may suggest increased alpha 2-adrenoreceptor-mediated responses. The increased aortic contractile responses of diabetic

rats may be due to impaired endothelial function **(Potenza et al., 2009),** increased calcium influx through voltage-dependent L-type Ca2+ channels **(Pinho et al., 2010),** increased myofilament Ca2+ sensitivity **(Kizub et al., 2010),** increased vasoconstrictor prostanoids due to increased superoxide anions, and increased sensitivity to adrenergic agonists **(Ahmad and Beg, 2013),** and oxidative stress **(Tabit et al., 2010).** The voltage-dependent Ca2+ channels (VDCCs) are involved in KCl-induced contraction **(Niazmand et al., 2014).** The present study show that the acetylcholine induced relaxation of the noradrenaline precontracted isolated rat aortic ring preparations is decreased significantly in the diabetic untreated rats in comparison with the normal rats, in agreement with **Natsuko Ikubo et al,2011**, who found that the acetylcholine induced relaxation of the noradrenaline precontracted isolated rat aortic ring preparations is decreased significantly in the diabetic untreated rats in comparison with the normal rats. Also **Cinar et al., 2001**, found that induction of diabetes significantly impaired endothelium-dependent relaxations to acetylcholine in aortic rings. In diabetes, vascular responses to acetylcholine, norepinephrine, and volatile anesthetics are altered in mesenteric resistancearteries, **(Jun Yoshino et al, 2010).** The vascular functions are altered by DM. Even short-term hyperglycemia can lead to augmented contractile responses, which has been confirmed also in our experiment. Impaired endothelium dependent vasodilation has been demonstrated in various vascular beds of different animal models of diabetes and in humans with type 1 and type 2 diabetes. This may arise from several mechanisms: decreased production or enhanced inactivation of nitric oxide (NO), impairment of its diffusion to the underlying smooth muscle cells or enhanced production of EDCFs (endothelium-derived constricting factors) **(De Vriese et al. 2000; Taddei et al. 2002; Grassi et al. 2005; Beckman 2004).** These results suggest that the decrease in release of nitric oxide may be responsible for vascular complications in STZ-HFD diabetic rat. **(Chirag Prajapati and Falguni Majmudar,2015).** Diabetes significantly reduced the relaxation in response to acetylcholine in mesenteric arteries as compared with control **(Li-Mei Zhao et al, 2014).** Impaired endothelium-dependent relaxation in STZ-induced diabetic rat might be due to enhance blood glucose level and reduce blood insulin level. It has been shown that hyperglycemia leads to tissue damage by several mechanisms, including the advanced glycation end product (AGE) formation, increased polyol pathway flux, apoptosis, and reactive oxygen species (ROS) formation **(Hartge et al., 2007).** Some damaging effects of diabetes on vascular tissue of diabetic animals are also believed to be due to promoted oxidative stress **(Paneni et al., 2013).** The initial trigger, i.e., high glucose concentrations change vascular function, is the imbalance between NO bioavailability and accumulation of ROS, which leads to endothelial dysfunction. Indeed, hyperglycemia-induced production of superoxide anion (O2-) inactivates NO to form peroxynitrite (ONOO-), a potent oxidant which easily penetrates across phospholipid membranes and induces substrate nitration **(Luscher et al., 2003)**. The present results show that the contractile response of the aorta induced by noradrenaline is decreased significantly in the diabetic rats treated with sildenafil in comparison with the diabetic untreated rats. The present work shows that the relaxant response of acetylcholine on noradrenaline precontracted aortic ring preparations was increased significantly in the diabetic rats treated with sildenafil in comparison with the diabetic untreated rats. The results obtained by the present study are in agreement with the results obtained by other workers**; Lu Luo et al, 2011,** found that the cumulative vasoconstrictive curves showed an increase in vascular tension to alpha-1 agonist of the isolated thoracic aortic rings from the STZ-injected rats relative to control. An increase in contractile tone to phenylephrine and a decrease in relaxant tone to acetylcholine was found in the thoracic aorta. The increased vascular tone was reduced by CPU0213 and sildenafil, also **A Scha¨fer1 et al,2008**, demonstrated that sildenafil treatment rapidly and chronically improves vascular relaxation in diabetic rats. Acute application of sildenafil is sufficient to enhance the impaired vascular NO/cGMP signalling in diabetes **(A Scha¨fer et al,2008).** Chronic treatment of diabetic rats with sildenafil resulted in a significant improvement of endothelium-dependent as well as -independent vasorelaxation indicating improved signalling through the NO/cGMP-signalling cascade, even more than 24 h after the last dosing **(A Scha¨fer et al,2008).** A recent study in hypertensive patients demonstrated that sildenafil had the potential for chronic treatment in addition to its specific local indication as acute supportive treatment in erectile dysfunction **(Oliver et al., 2006).** Endothelial dysfunction is a common feature in cardiovascular diseases characterized by an imbalance between NO and ROS. Oxidant stress is a major cause of reduced endothelial NO bioavailability in diabetes and is involved in the pathogenesis and progression of diabetic tissue damage **(Guzik et al., 2002; Landmesser et al., 2006).** Endothelial nitric oxide (NO) modulates vascular tone at rest, facilitates vasodilation during stress and inhibits platelet aggregation by activating intracellular guanylate cyclase, which in turn generates cyclic guanosine 5′-monophosphate (cGMP) (**Quyyumi, 1998; Andrews**

et al., 2001). Vascular smooth muscle cGMP levels are regulated by the activity of phosphodiesterase type 5 (PDE5), enhances the effect of NO in experimental models (**Beavo, 1995**). Increased expression of NAD (P)H oxidase subunits, enhanced NAD (P)H oxidase and protein kinase C activity as well as increased levels of the endogenous eNOS inhibitor asymmetric dimethyl-arginine result in enhanced oxidative stress and reduced NO bioavailability in diabetes (**Hink et al., 2001).** Treatment with sildenafil reduced lipid peroxidation and increased total antioxidant capacity in plasma of diabetic rats (**Milani et al., 2005**). The present work shows that fasting blood glucose is increased significantly in the diabetic untreated rats in comparison with the normal rats. This finding is in agreement with the results obtained by other workers **Siti et al., 2001,** who shows that administration of STZ to rats caused destruction of the β cell of pancreas and lead to reduction of insulin secretion, with increasing in plasma glucose levels which is significantly high as compared to the control rats. The present work shows that in the diabetic rats treated with sildenafil, fasting blood glucose have no significant change in comparison with the diabetic untreated rats. The present work are in agreement with the results obtained by other workers **A Scha¨fer et al., 2008**, Diabetes-induced increases in blood glucose were unaffected by sildenafil treatment of diabetic animals, while the reduction in body weight was slightly further lowered following treatment with sildenafil. **Yoshihiro et al., 2011,** showed that there were no significant changes in plasma glucose concentrations and BW in the OLETF-CON (Otsuka Long-Evans Tokushima Fatty) and OLETF-SIL rats throughout the study period. The present work are in disagreement with the results obtained by other workers **Aborayag et al., 2013,** showed that treatment of diabetic rats with both 5 and 10 mg/kg sildenafil produced a pronounced amelioration of the elevated serum blood glucose levels. **Mostafa et al., 2014**, showed that Sild (10, 20 mg/ kg) significantly lowered SBG level starting from 2 h after the first dose and continued its hypoglycemic effect up to 2 weeks of daily drug administration. The present work shows that glycosylated hemoglobin is increased significantly in the diabetic untreated rats in comparison with the normal rats. The present work is in agreement with the results obtained by other workers **Siti Balkis Budin et al., 2007**, who claimed that administration of STZ (50 mg/kg) increased significantly FBG and HbA1c levels than in normal untreated rats. In STZ induced diabetes, hyperglycemia and oxidative stress have been implicated in the etiology and pathogenesis of disease complications **(Bynes and Thorpe., 1996).** The mechanism by which STZ destroys β-cells of the pancreas and induces hyperglycemia is still unclear. One of the actions which have been attributed to STZ is depletion of intracellular nicotineamide dinucleotide (NAD) in islet cells. In addition, STZ has been shown to induce DNA strand breaks and methylation in the pancreatic islet cells. STZ administration also associated with the generation of reactive oxygen species causing oxidative damage to the pancreatic islet cells **(Coskun et al., 2005)** The present work shows that in the diabetic rats treated with sildenafil, glycosylated hemoglobin have no significant change in comparison with the diabetic untreated rats. This finding in agreement with the results obtained by other workers **Wang et al., 2015,** who discovered that treatment of the diabetic mouse with sildenafil at a dose of 10 mg/kg (orally administered, o.p.), every day for 8 weeks did not significantly alter blood glucose levels, A1C, triglyceride and animal body weight**.** and **Chopp et al., 2016**, who discovered that treatment with -PDE- 5i- Tadalafil did not significantly alter animal body weight, blood glucose levels, A1C and triglyceride levels compared to the saline treatment. Our results are supported by a clinical study done by **Nalinee Poolsup et al., 2015,** who reported that chronic use of PDE-5i compared with placebo or no active treatment in T2DM patients (ii) reporting of HbA1c or glycated haemoglobin or fasting plasma glucose (FPG).the results showed HbA1c were analysed as only one study reported FPG. PDE- 5i had no beneficial effect on HbA1c. This finding in disagreement with the results obtained by other workers **Neifissa et al., 2017**, who reported that 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. The present work shows that serum insulin were decreased significantly in the STZ-induced diabetic rats in comparison with the normal untreated rats. The present results are in agreement with the results obtained by **Asri-Rezaei et al., 2015**, who claimed that serum insulin levels were significantly decreased by STZ. who intended to induce diabetes by intraperitoneal (i.p.) injection of 50 mg/kg of streptozotocin (STZ) and was confirmed by blood glucose levels higher than 250 mg/dL. Also **Essam et al., 2017,** who claimed that STZ selectively destroys pancreatic b-cells, inhibits synthesis and release of insulin and causes the onset of DM. **Nasry et al., 2013**, who proved that streptozotocin significantly increased serum glucose level and significantly decreased serum insulin level as compared to normal control value. The present work shows that in the diabetic rats treated with sildenafil, serum insulin did not affected significantly in comparison with the diabetic untreated rats. This finding in agreement with the results obtained by other workers **Aborayag et al., 2015**, who recorded that

values of fasted diabetic rats showed a highly significant decrease in serum insulin concentration as compared with the normal control rats. Treatment with both 5 and 10 mg/kg sildenafil produced an insignificant increase in serum insulin concentration. Also, **Shafiee-Nick and Pyne, Furman,1995**, who found that (PDE1/PDE5 inhibitor) did not modify glucose-induced insulin release from pancreatic islets. This finding in disagreement with the results obtained by other workers **Mostafa et al., 2014**, noted that The lowest dose of Sild (5 mk/kg) significantly increased serum insulin level to 824%, 203.78%, 383.73% of diabetic control after 2 h, 1 week, 2 weeks of daily drug administration respectively. However, Sild (10 mg/kg) significantly elevated serum insulin level to 961.6%, 228.1%, 468.73% of diabetic control after 2 h, 1 week, 2 weeks of daily drug administration respectively however a dose of 20 mg/kg significantly raised serum insulin level to 1408%, 400%, 573.46% of diabetic control after 2 h, 1 week, 2 weeks of daily drug administration respectively. The present study shows that serum urea and creatinine are increased significantly in the diabetic untreated rats in comparison with the normal untreated rats. Our results are in agreement with the results obtained by other workers; **Mohamed et al., 2010,** showed that diabetic animals had higher blood pressures, increased serum glucose, urea and creatinine when compared with control animals. Hyperglycemia is a known cause of renal pathophysiology (**Osterby 1992**). It enhances oxidative stress and directly affects mesangial cells to develop glomerulopathy (**Heidland et al., 2001**). The increase of serum creatinine in DM is due to hyperglycemia that causes osmotic diuresis and depletion of extracellular fluid volume (**Patel et al., 2009**). Oxidative stress has been suggested to play an important role in the pathogenesis of diabetic nephropathy. Diabetic nephropathy is a state in which oxidative stress increases and antioxidant status is reduced, as has been documented (**Horie et al., 1997).** Oxidative stress in diabetic kidney is usually associated with tissue damage that interferes with proper organ function, causing an increase in urinary protein excretion and blood urea nitrogen **(Montero et al., 2000**). Our result show that in the diabetic rats treated with sildenafil, serum urea and creatinine were decreased significantly in comparison with the diabetic untreated rats. Our results were in agreement with the results obtained by other workers; **Nageh et al., 2016**, who proved that treatment of the diabetic rats with sildenafil alone as 3 mg/kg/day; dissolved in filtered water administered orally for 8 weeks showed significant decrease in serum creatinine and BUN levels. And also **Yoshihiro et al., 2011**, who noted that OLETF (male Otsuka Long-Evans Tokushima Fatty-diabetic rats) were administered sildenafil as 2.5 mg·kg−1 in drinking water 28 weeks which significantly attenuated these changes in serum Cr ($P < 0.05$) and BUN ($P < 0.001$) levels in OLETF rats. Sildenafil significantly reverse oxidative stress changes associated with DN (significant increase in SOD activity) and, these results are in agreement with other reports **Garcia et al. 2014,** indicate that Sildenafil treatment normalize the oxidative stress imbalances indicating that NO/cGMP pathway can modulate the activation of NADpH oxidase indicating a direct involvement of NO/ cGMP pathway in the modulation of oxidative stress **(Nageh et al., 2016).** This might be based on the ability of Sildenafil to increase NO/cGMP activity that results into renal vasodilation. Thus, Sildenafil may restore glomerular filtration **(Lau et al., 2007).** Sildenafil significantly suppress inflammatory response manifested by a significant decrease in level of IL-1b and, these results are in agreement with other reports **Garcia et al. 2014; Raposo et al., 2013**. It may exert its anti inflammatory effect mainly through iNOS inhibition by CGMP-iNOS feedback **(Raposo et al., 2013).**

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