

# Effect of sildenafil citrate on prediabetic and diabetic albino rats treated with metformin

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Diabetes Miletus (DM) is a global epidemic disease. It is estimated that there are already 415 million adults aged 20–79 years diabetics worldwide. Sildenafil citrate is a phosphodiesterase type 5 (PDE5) inhibitor, which increases cyclic guanosine monophosphate (cGMP) and metformin (MET) is a biguanide used for the treatment of type 2 diabetes which increases peripheral insulin sensitivity.

**Aim:** This study aims to assess the effect of sildenafil citrate and metformin on lipid profile and glycemic control in diabetic and prediabetic albino rats.

**Materials and methods:** Adult male albino rats are used and divided into nine groups each group consists of 10 rats, diabetes is induced by feeding a high-fat diet (HFD) for an initial period of 2 weeks followed by a single intraperitoneal injection of (35 mg/kg) Streptozotocin. Prediabetes is induced by feeding (HFD) and glucose in water for a period of 2 weeks. Sildenafil was given in a dose of (5 & 10 mg/kg/day orally for 4 weeks), metformin was given in a dose of (50 & 100 mg/kg/day orally for 4 weeks) using oral gavages to normal healthy rats, diabetic and prediabetic rats. Blood samples were collected after 4 weeks of treatment in all experimental groups.

**Results:** Combined administration of sildenafil and metformin on diabetic rats improving hyperglycemia, oxidative stress, and hyperlipidemia induced by streptozotocin than the administration of metformin or sildenafil alone.

**Conclusion:** Sildenafil has beneficial effects against some diabetic complications. The present study showed that sildenafil with metformin has beneficial effects against diabetic complications.

## Introduction

Diabetes Meletus (DM) is worldwide epidemic disease. It is estimated that there are about 415 million adults aged 20–79 with diabetes, including 193 million who are undiagnosed. A further 318 million adults have impaired glucose tolerance, which puts them at high risk of developing the disease. By the end of 2015, If this high rate not stoped, by 2040 there will be 642 million people living with the disease It is estimated that 5%–10% of diagnosed cases are of Type I diabetes

mellitus (DM), while the remaining 90%–95% are of Type II DM .

Erectile dysfunction (ED) is defined as the persistent inability to attain and maintain an erection sufficient to permit satisfactory sexual activity and is a common complication of DM in men. The prevalence of ED of any degree in men aged 40–70 years was predestined to be 52%. ED occurs at an earlier age in men with DM than in men in the general population. ED in diabetes is often complex and caused by several mechanisms including vascular disease, autonomic neuropathy, hormonal imbalance, and psychogenic factors. The risk of ED increases with duration of diabetes and is directly related to poor glycemic control and the presence of other risk factors such as hypertension, smoking, and hyperlipidemia.

Treatment options for ED include psychological management, vacuum constriction devices, intracavernosal injections, transurethral drug delivery, penile prostheses, vascular surgery, and modification of medication contributing to the problem. However, many of these treatments have limited acceptability to users. The ideal goal in the treatment of ED is restoration of erectile capacity using a minimally invasive and safe treatment. Sildenafil is the first oral drug to be marketed specifically for the treatment of ED. Sildenafil has the potential to enhance erectile activity under conditions of sexual stimulation. It does not have a direct effect on libido or smooth muscle.

The use of Sildenafil in patients with DM is highly increased and its effect on glycated hemoglobin and fasting plasma glucose are still inconclusive.

This study is an attempt to assess the effect of daily oral sildenafil citrate on lipid profile and glycemic control in diabetic and prediabetic rats with metabolic syndrome, whether Sildenafil might represent new therapeutic approaches for preventing and treating metabolic syndrome.

## **MATERIAL AND METHODS:**

This work was done at faculty of medicine, Al-Azhar University (Assiut branch), pharmacology department.

**Experimental animals:** Ninety adult male albino rats were chosen as an animal model for this study with initial body weight (BW) ranging from 160–180 g. Rats were purchased from animal house, Faculty of Medicine, Assiut University, Assiut, Egypt, and were maintained on standard diet of known composition, and water supply freely in clean containers. They were kept for two weeks to adapt to the laboratory conditions before the start of the experiment. The animals were housed in standard polypropylene cages with stainless steel good, aerated covers and maintained under controlled room temperature with 12 hrs light-dark cycle.

**Induction of diabetes:** Type 2 DM was induced by feeding a high fat diet (HFD) for 2 weeks followed by an intraperitoneal injection of 35 mg/kg b.wt streptozotocin dissolved in citrate buffer pH 4.5 . After that, the rats were kept for next 48 hours on oral 10% glucose solution on top of their chaw to prevent hypoglycemia . After Seven days the blood glucose levels for injected rats were measured. Rats having blood glucose level  $\geq 200$  mg/dl, after 2 hours of glucose intake were considered diabetic .

**Induction of prediabetes:** Prediabetes in rats were induced by feeding a high fat diet (HFD) (45% energy from fat) and glucose in water for period of 2 weeks. Development of prediabetes was confirmed by development of obesity, hyperinsulinemia, and insulin resistance and not frank hyperglycemia or DM, in these cases the rats considered prediabetic.

**Experimental design:** The experimental animal was divided into nine groups, each group comprising ten rats designated as follows:

**G1:** Normal control rats.

**G2:** Diabetic control rats.

**G3:** Diabetic rats treated with metformin 100mg/kg b.wt orally for 4 weeks.

**G4:** Diabetic rats treated with Sildenafil citrate 10 mg/kg b.wt orally for 4 weeks.

**G5:** Diabetic rats treated with Sildenafil citrate 10 mg/kg b.wt and metformin 100mg/kg b.wt orally for 4 weeks.

**G6:** Prediabetic control rats.

**G7:** Prediabetic rats treated with metformin 50 mg/kg b.wt orally for 4 weeks.

**G8:** Prediabetic rats treated with Sildenafil citrate 5 mg/kg b.wt orally for 4 weeks.

**G9:** Prediabetic rats treated with Sildenafil citrate 5 mg/kg b.wt and metformin 50mg/kg b.wt orally for 4 weeks.

**Collection of samples for analysis:** At the end of the 6<sup>th</sup> week, food was withdrawn from the rats and they were fasted for 8 h, but had free access to water, and then anesthetized with thiopental (40 mg/kg ip.). Blood samples were collected from orbital venous plexus in non-heparinized tubes. Blood samples were centrifuged at 2500 rpm for 15 min, and the clear samples of blood serum were separated and stored at -80°C until used for the measurement of the levels of blood glucose (BG) level (mg/dl), insulin level (pmol/L), total cholesterol (TC) (mg/dl), LDL-cholesterol (mg/dl), HDL-cholesterol (mg/dl) and triglycerides (TG) (mg/dl).

**Biochemical studies: Serum glucose level** was measured by glucose oxidase method using Blood glucose (BG) kit (Egyptian Company for Biotechnology-Egypt).

**Insulin** estimated using enzyme linked immunosorbent assay ELISA methods using Insulin kit (Sigma-Aldrich Co. LLC-USA) .

**Serum cholesterol** level was done by enzymatic -colometric test with lipid clearing factor) using Serum cholesterol kit (Egyptian company for biotechnology-Egypt). **Serum triglycerides** were estimated by an enzymatic colorimetric test with GPO-PAP method using Serum triglycerides kit (Egyptian Company for Biotechnology-Egypt) . Serum high density lipoprotein (**HDL**) was estimated by enzymatic colorimetric method Using Serum high density lipoprotein (HDL) kit (Egyptian Company for Biotechnology-Egypt) .The serum LDL-cholesterol was estimated according to using the following equation; **LDL-cholesterol** in mg/dl= Total cholesterol - Triglyceride/5-HDL-cholesterol .

**Statistical analysis:** The obtained data were subjected to analysis of variance according to the procedures outlined by Snedecor and Cochran. The mean values were compared according to Duncan's multiple range test (DMRT) . The data were analysed using Co Stat software for windows (version 6.3), (Co Hort Software, Monterey, Calif).

## Results

### Changes of BW, BG and insulin level among different groups.

#### A-In diabetic groups:

The obtained data showed a significant ( $p < 0.01$ ) increase of body weight in G4 than diabetic

control group with no significant ( $P > 0.05$ ) change between G3, G5 and diabetic control group as shown in table (1) and figure (1). The BG level showed a significant ( $p < 0.01$ ) lower level in all diabetic treated groups than control diabetic group with significant ( $p < 0.01$ ) lower level of G5 than G4 and G3 groups as shown in table (1). There was significant ( $p < 0.01$ ) increase of serum insulin level in G4 and G5 than diabetic control group with no significant ( $P > 0.05$ ) change between G3 and diabetic control group as shown in table (1).

### B-In prediabetic groups:

The obtained data showed a significant ( $p < 0.01$ ) reduction of body weight in G7 than prediabetic control group with no significant ( $P > 0.05$ ) change between G8, G9 and prediabetic control group as shown in table (1). There was significant ( $p < 0.01$ ) lower level in Serum blood glucose level in all prediabetic treated groups than prediabetic control group with no significant ( $P > 0.05$ ) difference between all treated groups as shown in table (1). The serum insulin level showed a significant ( $p < 0.01$ ) increase in G8 and G9 than prediabetic control group with no significant ( $P > 0.05$ ) change between G7 and prediabetic control group as shown in table (1).

Insulin ( IU /dl)	BG (mg/dl)	B w (g)	Groups
12.67 c $\pm$ 2.16	89.33 d $\pm$ 12.50	170.17 c $\pm$ 12.74	<b>G1</b>
4.17 e $\pm$ 0.4	230.00 a $\pm$ 35.78	134.00 e $\pm$ 15.59	<b>G2</b>
4.87e $\pm$ 0.92	119.67 b $\pm$ 14.24	131.83 e $\pm$ 12.55	<b>G3</b>
10.1 d $\pm$ 1.12	124.17 b $\pm$ 16.27	155.17 d $\pm$ 8.71	<b>G4</b>
10.6 d $\pm$ 0.83	110.17 c $\pm$ 7.31	137.33 e $\pm$ 12.75	<b>G5</b>
15.17 b $\pm$ 2.87	110.67 c $\pm$ 8.64	197.33 a $\pm$ 10.41	<b>G6</b>
15.92 b $\pm$ 2.56	98.67 d $\pm$ 11.08	178.33 b $\pm$ 10.63	<b>G7</b>
25.08 a $\pm$ 2.85	98.50 d $\pm$ 7.67	201.83 a $\pm$ 10.69	<b>G8</b>
25.33 a $\pm$ 3.55	91.33 d $\pm$ 5.47	194.33a b $\pm$ 7.23	<b>G9</b>
282.43**	180.2 **	117.65**	<b>F-test</b>
0.00	0.00	0.00	<b>P .</b>

**Table 1.** Changes of body weight, blood glucose and insulin level among different groups.

Where **G1**: Control untreated; **G2**: Diabetic untreated rats; **G3**: Diabetic rats treated with metformin rats; **G4**: Diabetic rats treated with Sildenafil citrate; **G5**: Diabetic rats treated with Sildenafil citrate & metformin; **G6**: Prediabetic untreated rats; **G7**: Prediabetic rats treated with metformin; **G8**: Prediabetic rats treated with Sildenafil citrate; **G9**: Preiabetic rats treated with Sildenafil citrate & metformin.

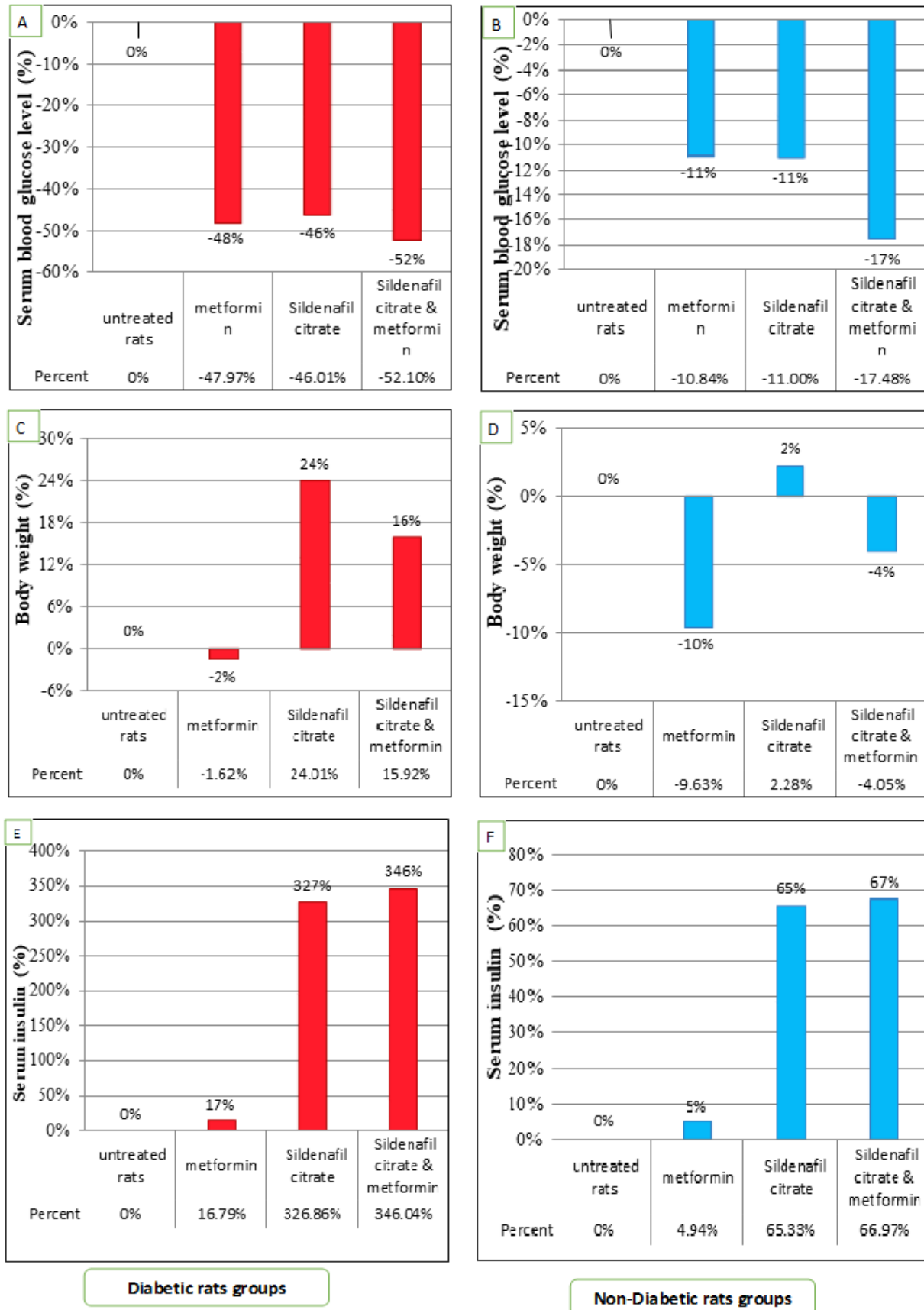
\*\* indicate  $P < 0.01$ . Means followed by a same letter are not significantly different at the 1% level by DMRT.

-letter (a) refer to highest mean and letter (b) referred to subsequent lower mean and so on.

### Comparison between body weight percentages in all groups

#### A-In diabetic groups:

The control diabetic group value was calculated a 100% to compare between it and all treated groups.



**Fig. (1):** Percentage of body weight, Blood glucose and insulin level for different diabetic and prediabetic rats treated groups compare to diabetic and prediabetic untreated groups.

### Figure 1.

The obtained data from fig (1-A) showed that in G3 body weight decreased by 2% but in G4 and G5 body weight increased by 16% and 3% respectively. The fig (1-D) showed that G3, G4 and G5 glucose level decreased by 48%, 46% and 52% respectively. Fig (1-E) showed that G3, G4 and G5 serum insulin level increased by 17%, 142% and 154% respectively.

### B-In prediabetic groups:

The control prediabetic group value was calculated a 100% to compare between it and all treated groups.

The obtained data from fig (1-B) showed that G7 and G9 body weight decreased by 10% and 2% but in G8 body weight increased by 2% respectively. Data from fig (1-E) showed that G7, G8 and G9 glucose level decreased by 11%, 11% and 17% respectively. Fig (1-F) showed that G7, G8 and G9 serum insulin level increased by 5%, 65% and 67% respectively.

### Changes of serum cholesterol , triglycerides, HDL and LDL level among different groups.

#### A-In diabetic groups:

The obtained data showed a significant ( $p < 0.01$ ) lower level in Serum cholesterol in all diabetic treated groups than control diabetic group with significant ( $p < 0.01$ ) lower level of G5 than G3 and G4 groups as shown in table (2). There was a significant ( $p < 0.01$ ) lower level in Serum triglycerides in all diabetic treated groups than control diabetic group with significant ( $p < 0.01$ ) lower level of G5 than G3 and G4 groups as shown in table (2). Serum HDL was significantly ( $p < 0.01$ ) increased in all diabetic treated groups than control diabetic group with significant ( $p < 0.01$ ) increase level of G5 than G3 and G4 groups as shown in table (2). The obtained data showed a significant ( $p < 0.01$ ) lower level in Serum LDL in all diabetic treated groups than control diabetic group with significant ( $p < 0.01$ ) lower level of G5 than G3 and G4 groups as shown in table (2).

LDL (mg/dl)	HDL (mg/dl)	Triglycerides (mg/dl)	Cholesterol (mg/dl)	Groups
20.00 $i \pm 2.83$	37.00 $a \pm 2.83$	50.50 $h \pm 3.74$	66.00 $h \pm 2.83$	G1
139.3 $a \pm 5.16$	22.00 $f \pm 2.83$	141.83 $a \pm 4.63$	187.67 $a \pm 6.02$	G2
76.17 $c \pm 4.27$	30.17 $d \pm$	70.83 $e \pm 2.94$	88.83 $d \pm 2.94$	G3
47.33 $e \pm 5.89$	29.33 $d \pm 3.50$	98.33 $c \pm 4.32$	93.50 $c \pm 3.74$	G4
40.33 $f \pm 4.13$	34.00 $b \pm 2.83$	65.17 $f \pm 2.94$	81.33 $e \pm 4.32$	G5
81.00 $b \pm 6.93$	25.83 $e \pm 2.94$	115.33 $b \pm 4.32$	117.83 $b \pm 5.72$	G6
53.33 $d \pm 4.32$	32.00 $c \pm 2.83$	64.50 $f \pm 3.74$	74.50 $g \pm 3.74$	G7
36.17 $g \pm 6.38$	30.6 $cd \pm 2.42$	74.17 $d \pm 5.28$	78.17 $f \pm 5.72$	G8
30.83 $h \pm 4.63$	34.83 $b \pm 2.94$	55.83 $g \pm 4.63$	68.17 $h \pm 5.72$	G9
1223.5**	60.59**	1303.7**	1585.02**	F-test
0.00	0.00	0.00	0.00	P.

**Table 2.** Changes of serum cholesterol, triglycerides, HDL and LDL level among different groups.

Where **G1**: Control untreated; **G2**: Diabetic untreated rats; **G3**: Diabetic rats treated with metformin rats; **G4**: Diabetic rats treated with Sildenafil citrate; **G5**: Diabetic rats treated with Sildenafil citrate & metformin; **G6**: Prediabetic untreated rats; **G7**: Prediabetic rats treated with metformin; **G8**: Prediabetic rats treated with Sildenafil citrate; **G9**: Prediabetics rats treated with



Sildenafil citrate & metformin.

\*\* indicate  $P < 0.01$ . Means followed by a same letter are not significantly different at the 1% level by DMRT.

-letter (a) refer to highest mean and letter (b) referred to subsequent lower mean and so on.

### **B-In prediabetic groups:**

The obtained data showed a significant ( $p < 0.01$ ) lower level in Serum cholesterol in all prediabetic treated groups than control prediabetic group with significant ( $p < 0.01$ ) lower level of G9 than G7 and G8 groups as shown in table (2). There was significant ( $p < 0.01$ ) lower level in Serum triglycerides in all prediabetic treated groups than control prediabetic group with significant ( $p < 0.01$ ) lower level of G9 than G7 and G8 groups as shown in table (2). The HDL level showed a significant ( $p < 0.01$ ) increase in all prediabetic treated groups than control prediabetic group with significant ( $p < 0.01$ ) increase level of G9 than G7 and G8 groups as shown in table (2). The obtained data showed a significant ( $p < 0.01$ ) lower level in Serum LDL in all prediabetic treated groups than control prediabetic group with significant ( $p < 0.01$ ) lower level of G9 than G7 and G8 groups as shown in table (2).

### **Comparison between serum cholesterol, triglycerides, HDL and LDL level among different groups.**

#### **A-In diabetic groups:**

The control diabetic group value was calculated a 100% to compare between it and all treated groups.

The obtained data from fig (2-B) showed that G3, G4 and G5 serum cholesterol level decreased by 53%, 50% and 57% respectively. Fig (2-D) showed that G3, G4 and G5 serum triglycerides level decreased by 50%, 31% and 54% respectively. Serum HDL showed that G3, G4 and G5 level increased by 37%, 33% and 55% respectively fig (2-F). There was decreased in serum LDL level in G3, G4 and G5 by 45%, 66% and 71% respectively as shown in fig (2-H).

#### **B-In prediabetic groups:**

The control prediabetic group value was calculated a 100% to compare between it and all treated groups. Fig (2-A) showed that G7, G8 and G9 serum cholesterol level decreased by 37%, 34% and 42% respectively. Serum triglycerides level in G7, G8 and G9 decreased by 44%, 36% and 52% respectively Fig (2-D).

The obtained data from fig (2-E) also showed that G7, G8 and G9 serum HDL level increased by 24%, 19% and 35% respectively and serum LDL level in G7, G8 and G9 decreased by 34%, 55% and 62% respectively as shown in fig (2-G).

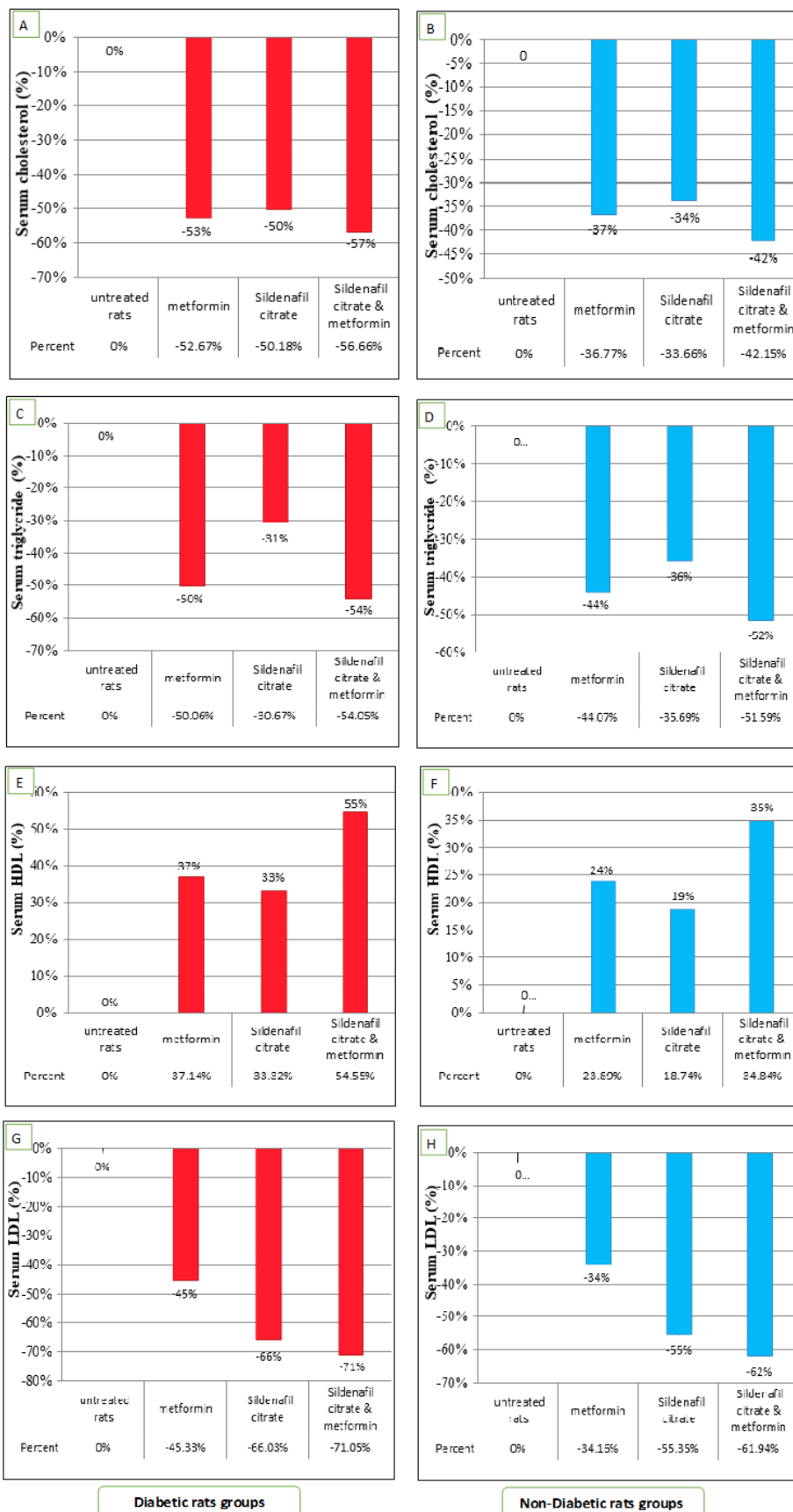


Fig (2): Comparison between serum cholesterol, triglycerides, HDL and LDL level for different diabetic and prediabetic rats treated groups compare to diabetic and prediabetic untreated groups



**Figure 2.**

## Discussion

The results present study showed that administration of sildenafil citrate to prediabetic and diabetic albino rats treated with metformin has no significant changes on BW. The BW of diabetic rats treated with metformin by decreased by 2% and in diabetic rats treated with Sildenafil citrate and (Sildenafil citrate & metformin) by 16% and 3% respectively in comparison with diabetic untreated rats as shown in fig (1). Body weight decreased by 10% and 2% in prediabetic rats treated with metformin and (Sildenafil citrate& metformin) but in prediabetic rats treated with Sildenafil citrate body weight increased by 2% respectively in comparison with prediabetic untreated rats as shown in fig (1). In the diabetic and prediabetic rats treated with metformin, the results of our work are in agreement with the results obtained by Mogul et al., 2003 and Mamikutty et al who reported that weight loss in obese women who were treated by metformin for 6 months was 5%. In the diabetic and prediabetic rats treated with Sildenafil citrate, the results of our work agree with the results obtained by Johann et al. & Dilworth et al. & Nisoli et al. who found that there is no effect of Sildenafil citrate on body weight [8, 20, 21].

In the diabetic and prediabetic rats treated with metformin and sildenafil, there is no significant change ( $P < 0.05$ ) on body weight in comparison with control diabetic and prediabetic rats. Our results are in agreement with Bruckbauer et al. who reported that combination of metformin and sildenafil did not change body weight. Tran and Howes found that Sildenafil citrate did not give any change in body weight of diabetic induced rats and Chronic phosphodiesterase (PDE)-5 inhibitors lead to increase energy expenditure, that improve energy balance and body weight reduction that may enhance insulin action.

To explain this, it should be noted that increasing cGMP is a fundamental mechanism of action of sildenafil citrate. A study on rat hepatocytes cGMP indicated that sildenafil citrate increases hepatocytes in vitro level but does not significantly alter glycogenolysis and gluconeogenesis. Nitric oxide has been suggested as a second messenger molecule for the stimulatory effect of insulin in carbohydrate metabolism.

**Mitschke et al.** found that, chronic treatment (12 weeks) with the PDE-5 inhibitor sildenafil improved insulin sensitivity. **Lizhi**, reported that PDE 5-inhibitors, lead to increase SIRT1 signaling in mice that lead to increase energy expenditure in skeletal muscle cells, improve energy balance and increase insulin action in both diet-induced obese, insulin resistant mice and diabetic patients.

**The effect on serum glucose level and serum insulin level:** The results present study showed that showed that the blood glucose level decreased in diabetic rats treated with Sildenafil citrate, metformin and (Sildenafil citrate & metformin) by 48%, 46% and 52% respectively in comparison with diabetic untreated rats and serum insulin level increased by 17%, 142% and 154% respectively in comparison with diabetic untreated rats. In prediabetic groups there were no significant difference in serum blood glucose level of prediabetic groups as glucose level decreased by 11%, 11% and 17% respectively in comparison with diabetic untreated rats and serum insulin level increased by 5%, 65% and 67% respectively in comparison with prediabetic untreated rats as shown in fig (1). The result of the current study is in agreement with results of Ayala et al. who found that chronic inhibition of PDE-5 enhance insulin action in obese rats, which may explained by PDE-5 inhibition may improve insulin action and prevent endothelial dysfunction. There is evidence supports the insulin resistance and type 2 diabetes may caused by endothelial dysfunction. Also endothelial dysfunction lead to impairing glucose uptake by muscles is by a decreasing the NO levels and cGMP production. Thus, preventing a decrease in cGMP levels, resulting in improved insulin action and glucose uptake by muscles. This effect may be related to central nervous system

as sildenafil has been found to cross the blood brain barrier, and PDE-5 expression has been detected in the brain. Thus, signaling through cGMP in the central nervous system may play a role in the regulation of insulin action and energy homeostasis as stated by Ayala et al.

**Hill et al.** observed a significant improvement in insulin sensitivity in the severely obese subjects and they suggest that this is a class effect of PDE5 inhibitors

Also, these findings are agreement with El Sayed et al who found that sildenafil citrate elevated serum insulin and decreased blood glucose level in dose dependent manner and they explained it by Inhibition of cGMP hydrolysis that activates cGMP-dependent protein kinase G, which phosphorylates and activates hormone-sensitive lipase then increases free fatty acid (FFA) availability and energy expenditure.

The results of this study disagree with the results obtained by other workers they showed that sildenafil citrate has in significant effect in reducing the blood glucose level of diabetic rats [35-37].

The results of this study showed that, combination of metformin and sildenafil has more significant reduction ( $P < 0.01$ ) on serum blood glucose of the diabetic rats more than metformin alone or sildenafil alone but there is no significant ( $P < 0.05$ ) difference on serum blood glucose in the prediabetic treated rats in comparison with control prediabetic rats. Also, combination of metformin and sildenafil has more significant increase ( $P < 0.01$ ) on serum insulin level of the diabetic and prediabetic rats more than metformin alone but there is no significant ( $P < 0.05$ ) difference on serum insulin level between combination and sildenafil alone.

In patients with DM2, an increase in free radicals caused by various risk factors (such as dyslipidemia, obesity and hyperglycemia) might enable the oxidative stress that reduces production and availability of NO with the subsequent appearance of ED .

Inhibitors of PDE-5, such as sildenafil, require adequate levels of NO to be effective. Because IR states have decreased levels of NO and insulin-sensitizing agents improve IR, we postulate that, in patients with ED and IR and a poor response to sildenafil, the addition of metformin can optimize the response to sildenafil, improving endothelial function.

We hypothesize that metformin and Sildenafil citrate improve endothelial function by reducing blood sugar levels, the present study also confirmed that metformin and Sildenafil citrate have a similar curative effect in reducing blood sugar levels, improving islet function and reducing the IR index. More ever, combination of metformin and seldinafil is more successful at improving endothelial function than Sildenafil citrate alone and metformin alone, the reason for which is currently unclear and requires further study.

**The effect on lipid profile: the results of the present** showed that, in diabetic rats treated with metformin, Sildenafil citrate and (Sildenafil citrate& metformin) serum cholesterol level decreased by 53%, 50% and 57% respectively in comparison with diabetic untreated rats, serum triglycerides level decreased by 50%, 31% and 54% respectively in comparison with diabetic untreated rats, serum HDL level increased by 37%, 33% and 55% respectively in comparison with diabetic untreated rats and serum LDL level decreased by 45%, 66% and 71% respectively in comparison with diabetic untreated rats as shown in fig (2). prediabetic rats treated with metformin, Sildenafil citrate and (Sildenafil citrate& metformin) serum cholesterol level decreased by 37%, 34% and 42% respectively in comparison with prediabetic untreated rats, serum triglycerides level decreased by 44%, 36% and 52% respectively in comparison with prediabetic untreated rats, serum HDL level increased by 24%, 19% and 35% respectively in comparison with prediabetic untreated rats and serum LDL level decreased by 34%, 55% and 62% respectively in comparison with diabetic untreated rats as shown in fig (2).

In the diabetic rats treated with Sildenafil citrate, our results agree with **Mitschke et al.** who

stated that diabetic rats treated with Sildenafil citrate decreased significant of serum triglycerides, serum total cholesterol level and serum LDL level, while serum HDL level increased significant in comparison with diabetic untreated rats. This positive effect of sildenafil could be due to increased appearance of brown-like adipocytes in white adipose tissue.

The significant decrease in the serum lipid levels in treated diabetic rat might have been due to the increase insulin sensitivity following sildenafil administration.

There is an evidence that use of PDE-5 inhibitors could overcome the oxidative stress-induced cellular dysfunctions and apoptosis associated with lipid peroxidation derivatives by increasing cyclic nucleotides. Polte and Schroder, reported an antioxidant property for NO donors in vascular endothelium through concerted action of cGMP and cAMP. In addition, the hypolipidemic and insulin sensitizing effects of sildenafil may be attributed to its anti-inflammatory effect. It was shown that sildenafil promotes browning of white adipocytes in vitro.

In the prediabetic rats treated with Sildenafil citrate, our results are in agreement with Ayala et al. who stated that prediabetic rats treated with Sildenafil citrate decreased significant of serum triglycerides, serum total cholesterol level and serum LDL level, while serum HDL level increased significant in comparison with prediabetic untreated rats and this improving effect of sildenafil may be explained by its direct effects of on metabolism other than eNOS activation, Improving insulin function and increase lipoprotein lipase activity.

Bennani-Kabchi et al explained the significant decrease in plasma TAG to the increased rate of lipolysis that is mediated by increase of plasma lipase activity.

The results of this study showed that, combination of metformin and sildenafil has more significant reduction ( $P < 0.01$ ) on serum cholesterol, triglycerides and LDL level of the diabetic and prediabetic rats more than metformin alone or sildenafil alone. Also, combination of metformin and sildenafil has more significant increase ( $P < 0.01$ ) on serum HDL level of the diabetic and prediabetic rats more than metformin alone and sildenafil alone.

Our results are in agreement with Lizhi, who investigate the Synergistic effect of Leucine-Metformin-Sildenafil on Nonalcoholic Steatohepatitis.

## Conclusion

Findings of the present study indicate that sildenafil citrate exerts hypoglycemic effect on diabetic albino rats. In addition to its hypoglycemic effect, it also has hypolipidemic effect on both diabetic and prediabetic albino rats and these effects may be attributed to its anti-inflammatory effect. Moreover, our study revealed that combination of Sildenafil citrate and Metformin has more effect in prevention and treatment of diabetes mellitus in albino rats than treatment by Sildenafil alone or Metformin alone.

Further studies are required for further evaluation and establishment of the beneficial effects of sildenafil citrate in treatment and prevention of vascular and other complications of diabetes mellitus and mechanisms of actions.

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