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Effect of sildenafil citrate (Viagra) on the lipid profile of the normal and experimentally Induced Diabetic Male Rats

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Abstract

The current study was designed to determine effectiveness of sildenafil citrate (viagra) on lipid profile of normal and experimentally induced diabetic male rats, the current study was carried out on (36) mature male mature rats, they were divided randomly into six equal groups, 6 of each group. The first group was administrated orally with (0.2 ml) of normal physiological saline (0.9% Nacl), the second group was injected (IP) by (0.5 ml/animal) with alloxane (125 $mg.kg^{-1}$), the third and fourth groups were administrated orally with viagra at doses (4 and 8 $mg.kg^{-1}$), respectively, while the fifth and sixth groups injected (0.5 ml/animal) with alloxane (125 $mg.kg^{-1}$), after one week, the (fifth and sixth) group were administrated orally with viagra at doses (4 and 8 $mg.kg^{-1}$), respectively. The experiment was continued for 21 days. At the end of the experimental period, the animals were sacrificed, the blood samples were collected and the serum concentration of total cholesterol (T.C.), triglycerides (T.G.), high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL) and atherogenic index were determined in the serum. The results showed a significant increase in the serum level of T.C., T.G., LDL, VLDL and atherogenic index to the normal and diabetic rats treated with viagra and decrease in HDL concentration of the normal and diabetic rats administered with viagra when compared this two groups with control and diabetic groups respectively.

Key Words: Sildenafil, Alloxane, lipid profile, Male rats .

Introduction

Diabetes mellitus is a clinical syndrome characterized by hyperglycemia due to absolute or relative deficiency of insulin. This can arise in many different ways **(**Nhanes, 1997**).** Lack of insulin affects the metabolism of carbohydrate, protein and fat and causes a significant disturbance of water and electrolyte homeostasis. Death may result from acute metabolic decomposition, while long- standing metabolic derangement is frequently associated with permanent and irreversible functional and structural changes in the cells of the body, with those of the vascular system being particularly susceptible. These changes lead to the development of well- defined clinical entities, the so- called "complications of diabetes" (Christopher *et al*, 2002), which characteristically affect the eye, the kidney and the nervous system (Susman & Helseth, 1997). Some patients may develop acute life threating hyperglycemia episodes, such as ketoacidosis or hyperosmolar coma (Burtis & Ashwood, 1994), furthermore, the diabetes mellitus causes erectyldysfunction (Wessells *et al*., 2006). Sildenafil (Viagra) is widely used to treatment of erectile dysfunction (ED) (Rosen & Kostis, 2003). It has also become increasingly apparent that sildenafil may also be effective in treating pulmonary hypertension (Zhao *et al*., 2001; Sebkhi *et al*., 2003; Ghofrani *et al*., 2004; Madden *et al*., 2004). The therapeutic action of sildenafil is mediated through the inhibition of type 5 phosphodiesterase (PDE 5), which increases cyclic GMP (cGMP) levels in response to nitric oxide (NO) (Jeremy *et al*., 1997), thereby augmenting the relaxation of vascular (and cavernosal) smooth muscle tissue (Rosen & Kostis, 2003). In the present study we aimed to evaluated the effect of sildenafil administration on cholesterol, triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL) and atherogenic index levels in normal and experimentally induced diabetic male rats.

Material and method

Laboratory animals:

Male Wister rats weighing 170-185g with (8-10 weeks) old (obtained from animal house of biology department / college of science / university of Thi-Qar / Iraq) were used in this study. The animals were housed in standard polycarbonate cages in a temperature-controlled room (20–22°C) with 12 h light/12 h dark cycle. Animals were acclimated at least 5 days before experiments with free access to food and water *add libitum*.

Induction of diabetes mellitus

The animals were fasted for 24 hr and diabetes was induced by a single intraperitoneal (IP) injection of alloxan monohydrated (BDH, England) dissolved in D.W at a dose of 125 $mg.kg^{-1}$ body weight in a volume of 0.5 ml. The diabetic state was confirmed 7 day after alloxan injection by the blood serum. Sugar value was greater than 200 mg/dl (hyperglycemia). Surviving rats with a fasting blood glucose level higher than 200 mg /dl were included in the study.

Sildenafil solution preparation

Sildenafil citrate (Broun&Burk, England) was isolated from Viagra tablets previously and dissolved in 0.9 % saline.

Experimental groups

The animals were divided into six groups (6 male rats per each group). The groups were treated as following:

1- First group control (G1): Normal rats administered orally 0.2 ml normal saline (0.9% NaCl)

2- Second group (G2): Diabetic rats induced by single dose of alloxane injected (IP) and then administered 0.2 ml of (0.9% NaCl) after one week of diabetic induction

3- Third group (G3): Normal rats administered ($4mg.kg^{-1}$) Viagra orally in 0.2 ml of normal saline.

4- Fourth group (G4): Normal rats administered ($8mg.kg^{-1}$) Viagra orally in 0.2 ml of normal saline.

5- Fifth group (G5): Diabetic rats administered with ($4 mg.kg^{-1}$) Viagra orally in 0.2 ml of normal saline after one week of diabetic induction.

6- Sixth group (G6): Diabetic rats administered with ($8 mg.kg^{-1}$) Viagra orally in 0.2 ml of normal saline after one week of diabetic induction.The experiment continues for 21 days.

Blood sample collection

After 21 days of experiment, the blood samples were collected from heart of animals of each group, and the serum was separated by centrifugation at 3000 rpm for 15 min. Serum was assayed either immediately or storedat-4°C.

Biochemicalparameters

Total cholesterol in the serum was measured by enzymatic method (Richmond, 1973; Fasce, 1982), with the (biolabo) kit, France).Total triglycerides in the serum were measured by enzymatic method (Young, 1975; Tietz, 1987) with the (biolabo kit, France). HDL-Cholesterol in the serum was measured by enzymatic method (Burstein, 1970; Tietz, 1999) using (biolabo kit, France).Very low density lipoprotein (VLDL) is estimated as triglyceride ÷5 (Tietz, 1987). After the measurement of total cholesterol, HDL–cholesterol and VLDL, LDL–cholesterol is calculated as total cholesterol minus Very Low Density Lipoprotein + High Density Lipoprotein (Koren, 1955). **LDL-C (mg/dl) = Total Cholesterol – (HDL + VLDL).** Atherogenic Index Level is calculated as LDL divided HDL (Wilson *et al*., 1998; Al-Zamely, 2001). **Atherogenic Index = LDL / HDL.**

Results

The results showed a significant increase ($p<0.01$) in the serum T.C. concentration of the normal administered group with Viagra (4 and 8 $mg.kg^{-1}$) when compared with the control group. while the results showed that there was a significant ($p<0.01$) increase in the serum T.C. concentration of the diabetic animals administered Viagra (4 and 8 $mg.kg^{-1}$ when compared with diabetic rats group (Table 1).The statistical analysis demonstrated that there was a significant increase ($p<0.01$) in serum T.G concentration of the animals group administered Viagra (8 $mg.kg^{-1}$) when compared with control group, as well as the normal rats which administered Viagra (4 $mg.kg^{-1}$) revealed a rising in serum T.G concentration did not reach the significance when compared with control group, While the results showed a significant increase ($p<0.01$) in serum T.G concentration of the diabetic group administered Viagra (4 and 8 $mg.kg^{-1}$) when compared with diabetic group (Table1).

Table 1: Effect of Viagra on the serum T.C. and normal and experimentally T.G. in the induced diabetic male rats

Treated groups	T.C. Concentration (mg/dL)	T.G. Concentration (mg/dL)
G1	56.30 ± 2.23 [a]	38.51 ± 1.49 [a]
G2	82.78 ± 1.66 [b]	46.53 ± 1.83 [c]
G3	70.00 ± 0.81 [c]	42.07 ± 1.79 [cd]
G4	78.83 ± 2.36 [b]	60.03 ± 0.64 [b]
G5	84.78 ± 2.63 [ab]	52.01 ± 1.63 [b]
G6	91.73 ± 2.40 [a]	64.05 ± 1.57 [a]
LSD	7.30	5.34

Values are means ± S.E.Different letters refer to significant differences ($p<0.01$)

The results of the serum HDL-C concentration in the animals group administered Viagra (4 and 8 $mg.kg^{-1}$) demonstrated that non-significant ($p>0.01$) decrease when compared with control group. While the obtained data showed non-significant ($p>0.01$) difference in serum HDL-C concentration in the diabetic group administered with Viagra (4 and 8 $mg.kg^{-1}$) when compared with diabetic group (table 2). The statistical analysis showed a significant ($p<0.01$) increase in serum LDL-C concentration in both animals group administered (4 and 8 $mg.kg^{-1}$ Viagra) compared with control while no significant difference were observed in the concentration of LDL-C in diabetic animals group treated with (4 $mg.kg^{-1}$ Viagra) compared with diabetic group. On the other hand the concentration of LDL-C increased significantly ($p<0.01$) in both (4 and 8 $mg.kg^{-1}$ Viagra) treated groups compared with control. The results demonstrated a significant ($p<0.01$) increase in serum VLDL-C concentration in the animals group administered Viagra (4 and 8 $mg.kg^{-1}$) compared with control but no significant difference was observed in VLDL-C concentration of diabetic group treated with (8 $mg.kg^{-1}$ Viagra) compared with animal group treated with (8 $mg.kg^{-1}$ Viagra) only. While the statistical analysis showed that there was a significant ($p<0.01$) increase in the serum VLDL-C concentration in the diabetic group administered with Viagra (4 and 8 $mg.kg^{-1}$) when compared with diabetic group (table 2).The obtained results demonstrated that there was a significant ($p<0.01$) increase in the atherogenic index concentration in blood serum of the animals group administered Viagra (4 and 8 $mg.kg^{-1}$) when compared with control group . While the results showed that a significant ($p<0.01$) increase in the atherogenic index concentration in blood serum of the diabetic group administered Viagra (8 $mg.kg^{-1}$) when compared with control group, also the results showed no significant increase in the atherogenic index concentration in blood serum of the diabetic group administered with viagra (4 $mg.kg^{-1}$) when compared the diabetic group only (table 2) .

Table2: Effect of Viagra on the serum HDL, LDL, VLDL and ATH in the normal and experimentally induced diabetic male rats

Animals group	HDL concentration (mg/dL)	LDL concentration (mg/dL)	VLDL concentration (mg/dL)	ATH index (mg/dL)
G1	44.80 ±1.71 [a]	3.79 ± 0.54 [e]	7.70 ± 0.29 [d]	0.09 ± 0.02 [e]
G2	39.12 ± 0.89 [b]	34.36 ± 1.21 [b]	9.31 ± 0.36 [c]	0.88 ± 0.04 [b]
G3	44.03 ± 0.84 [a]	17.55 ± 0.41 [d]	8.41 ± 0.35 [cd]	0.39 ± 0.01 [d]
G4	43.65 ±1.27 [a]	23.18 ± 1.15 [c]	12.01 ± 0.12 [a]	0.54 ± 0.04 [c]
G5	37.60 ± 0.87 [b]	36.78 ± 1.86 [b]	10.40 ± 0.33 [b]	0.98 ± 0.04 [b]
G6	36.18 ±1.45 [b]	42.74 ± 1.27 [a]	12.18 ± 0.32 [a]	1.19 ±0.06 [a]
LSD	4.20	4.08	1.06	0.14

Values are means ± S.E.Different letters refer to significant differences ($p<0.01$)Same letters refer to No significant differences ($p<0.01$)

Discussion

The increase of total cholesterol concentration in diabetic rats group when compared with control group agreement with data of Inawati and Winarno (2008) and Tenpe and Yeole, (2009) who observed an increase in T.C concentration in alloxane-induced diabetic mice, and this increase was observed in many studies investigate with diabetic patients especially type 2 diabetes (Menik *et al*., 2005; Idogun, 2007). The increase levels of cholesterol and other lipids occur when the cells of the body stop responding properly to the insulin, this is called insulin resistance or syndrome. Insulin resistance causes higher levels of insulin, blood sugar, and free fatty acids (Cahová *et al*., 2007). The abnormal high concentration of T.C in diabetic subject is due mainly to an increase in the mobilization of free fatty acids from peripheral fat depots, since insulin inhibits the hormone sensitive lipase (Kim *et al*., 2006). The statistical analysis demonstrated increase in T.C concentration level in both diabetic rats and normal rats

groups administered Viagra (4,8 mg.kg$^{-1)}$, this result is attributable to action of viagra which causes increase in the catabolism of lipids (Ayala *et al.*,2007) and release Acetyl Co-A which is the precursor to synthesis of cholesterol. The obtained results show increase in T.G. concentration level of alloxane-induced diabetic rats. These results are in agreement with Inawati and Winarno, (2008) who observed hypertriglyceridaemia which is associated with diabetes mellitus. The cause of the increase in T.G level may be due to the decrease in lipoprotein lipase (LPL) activity in adipose tissue, and the insulin necessary for the activity of the enzyme which acts on fragment and analysis of T.G at normal condition, therefore with hyperglucose and hypoinsulemia which cause the decrease in this enzyme lead to the accumulation of T.G which is considered as an alternative source for energy (Kovar *et al*., 2004; Daisy*etal*., 2009). Hypertriglyceridemia was observed with hyperglycemia and exceeded lipid absorption by small intestine. One of the causes of hypertriglyceridemia in diabetes mellitus is excessive production of very low density lipoprotein (VLDL) and chylomicrons; this may be due to increased influx of glucose and fatty acids into the liver, where they are converted into triglycerides. In addition, diabetes is often accompanied by catabolic disorders of VLDL triglycerides, which are correlated with severity of hyperglycemia. Excessive lipid absorption from small intestine was considered to be one of the causes of hyperlipidemia complicating diabetes mellitus (Omae *et al*., 2006). The increase of T.G concentration was observed when normal and diabetic rats were administered Viagra (4 and 8 mg.kg^{-1}), this is due to the Viagra which accelerate lipolysis in adipose tissue which lead to release excessive of T.G Cox & Nelson, 2000; Murray *et al*., 2003; Ayala *et al*., 2007). (The present study demonstrated the decrease of HDL-C concentration in alloxane-induced diabetic rats, these results are in agreement with El-Hazmi *et al.* (1999) who observed decrease in HDL-C concentration in diabetes mellitus patients, and this may be due to the hypoinsulemia and hyperglycemia in blood which may stimulate HDL-C metabolism and hepatic lipase (HL) which is affected by dysfunction of metabolism and then acts on fragment of HDL-C molecules (Tan *et al*., 2000). Also, it is known that HDL-C plays a key role in the protection against oxidative damage of membranes and lipid metabolism by transporting cholesterol from peripheral tissue to the liver through a process known as reverse cholesterol transport (El-Tantawy and Hassanin, 2007). The data showed that the administration of Viagra caused the decrease of HDL-C concentration in normal and diabetic rats, this due to engage of HDL in transport of T.C. from blood to liver there for the HDL while fall gradually along the period of experimental. The present data revealed the increase of LDL-C concentration in alloxane-induced diabetic rats when compared with control group that is similar to Shirdel *et al.* (2009). It is well known that LDL-C plays an important role in arteriosclerosis and that hypercholesterolemia is associated with a defect relating to lack of LDL receptors (Yadav *et al.*, 2008), also this increase may increase the synthesis of chylomicron and very low density lipoproteins VLDL (Aziz, 2009), due to excessive catabolism of protein and amino acids that are released and used for gluconeogensis, which also stimulates lipolysis in adipose tissue which gives rise to hyperlipedemia (Jothivel *et al*., 2007). Also the elevation of serum TC and LDL-C has been implicated as a primary risk factor for cardiovascular disease (Edijala *et al*., 2005). The oral administration of viagra produces the increase of LDL-C concentration level. These results may be due to an increase of acetyl Co-A which is the precursor to T.C. synthesis and the increase of T.C. stimulates the liver to synthesis of LDL which is necessary to transport of T.C. from the liver to the adipose tissue cross the blood there for elevate concentration of LDL intheblood. The results of present study showed the significant increase of VLDL-C concentration in alloxane-induced diabetic rats compared with control group. This result is similar with Niu and Evans (2008) who found that diabetes induced experimentally in rabbits affected lipoproteins concentration. The increase of VLDL-C concentration level may be due to inhibiting of lipoprotein lipase and reduction of VLDL-C metabolism, and then an increase in blood serum (Kovar *et al.*, 2004), or it may be that hypoinsulemia stimulate apolipoprotein formation Apo-C-III which is important to VLDL-C formation, also the reduction of lipolysis leads to an increase in VLDL-C (Chon *et al*., 1994). This result may be caused by decrease in VLDL receptors in diabetes state which prevent entering into the tissue and stay in blood stream (Iwasaki *et al*., 2005), while there was increase in VLDL-C concentration in normal and diabetic rats treated with viagra. Oral administration of this drug lead to increase VLDL-C concentration by increasing T.G., this lead to elevate of VLDL formation in liver and blood stream because the VLDL contain a large amount of T.G.

The increase of serum atherogenic index (ATH) in diabetic rats may be due to the decrease in the HDL and

increase in LDL LDL, this ratio is called risk factor (Grundy,1998; Stratton *et al*., 2000). The statistical analysis demonstrated the increase of (ATH) concentration level in both diabetic rats and normal rats group when administered with Viagra (4 and 8 $mg.kg^{-1)}$, this result is attributable to action of viagra which causes increase in the LDL and decrease in the HDL.

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