Effect of long-term administration of sildenafil on lipid profile and organ functions in hyperlipidemic rats

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Abstract. The aim of the present study was to elucidate the possible biochemical alterations in lipid metabolic profile and organ function profiles that may result from continuous treatment with the drug, sildenafil in normal and hyperlipidemic rats. Blood samples were taken for biochemical analysis on days 30, 45 and 60 of the experiment. Sildenafil (5.625 mg/kg) significantly decreased the serum lipid parameters including total lipid, triacylglycerols, cholesterol, HDL-C, LDL-C, VLDL-C concentrations of rats fed on fat-enriched diet. However, it increased their values in serum of negative control rats. In addition, administration of sildenafil to normal rats caused insignificant changes in serum liver enzymes ALT and AST concentrations all over the period of the experiment; as well as serum urea and creatinine; yet, it significantly decreased their serum concentrations in animals fed on fat-enriched diet compared to the +ve untreated ones, upon its administration starting from the 30th day of the experiment. However, concurrent administration of sildenafil with high-fat diet (group-iv) failed to guard against the rise in such liver and kidney function biomarkers. These data suggest that sildenafil may act as a mixed blessing drug; therefore it must be used carefully and under physician supervision to get its therapeutic benefits and guard against its adverse effects. (www.actabiomedica.it)

Key words: sildenafil, liver function, kidney function, lipid profile, erectile dysfunction, side effect

Introduction

Erectile dysfunction (ED) is the persistent inability to achieve and maintain an erection adequate for satisfactory sexual performance. The probability of erectile dysfunction increases with ageing and the presence of some disease conditions such as diabetes mellitus, hypertension, hypercholesterolemia, ischemic cardiac disease, depression and obesity (1). The pharmaceutical preparation sildenafil citrate (Viagra®) is being widely prescribed as a treatment for male ED (2). Sildenafil belongs to a class of compounds called phosphodiesterase (PDE) inhibitors. PDEs comprise a diverse family of enzymes that hydrolyze cyclic nucleotides, cyclic adenosine-5-monophosphate and cyclic guanosine-5-monophosphate (cAMP and cGMP) and therefore play a critical role in the modulation of second messenger signaling pathways (3).

Sildenafil is a potent and selective inhibitor of PDE-5, the predominant isozyme that metabolizes cGMP in the corpus cavernosum of the penis. cGMP is the second messenger of nitric oxide (NO) and a principal mediator of smooth muscle relaxation and vasodilatation in the penis. By inhibiting the hydrolytic breakdown of cGMP, sildenafil prolongs the action of cGMP. This results in augmented smooth muscle relaxation and hence, prolongation of the erection (4, 5).

However, the selectivity of sildenafil for PDE-5 has not been completely understood and may be partially lost upon sildenafil abuse. In addition, it is unclear that whether the enhancement of intracellular cGMP concentration does not generate major unwanted biological effects in tissues other than corpus

cavernosum or not, especially, those related to lipid metabolism and vital organ functions.

Therefore, the aim of the present study was to elucidate the possible biochemical alterations in lipid metabolic profile and organ function profiles that may result from continuous treatment with sildenafil in normal albino rats and those rendered hyperlipidemic by long-term supplementation of fat-enriched diet.

Material and methods

Sildenafil

Sildenafil was provided as sildenafil citrate which is a white to off-white crystalline powder of a molecular weight of 666.7. It is present in the drug market as the patent preparation Viagra® that is an oral therapy for ED, formulated as blue, film-coated rounded-diamond-shaped tablets equivalent to 25 mg, 50 mg and 100 mg of sildenafil for oral administration. However, in the present study, it was obtained as a kind gift from SIGMA Pharmaceutical Co., Quesna, Egypt, in its powder form. The dosage range for human is 25~100 mg/day, orally, according to the severity of the condition. The average dose prescribed for human (62.5 mg/day) was converted to equivalent dose for rat according to Paget & Barnes (6), where a rat dose is equivalent to human dose multiplied by 0.018 as a conversion factor considering body weight and surface area. So, the rat dose was calculated as 5.625 mg/kg body weight. Thus, a rat weighing 200 g received a daily dose of 1.125 mg suspended in 0.5 ml distilled water orally using a gastric tube of suitable size.

Atorvastatin

Atorvastatin (an inhibitor of cholesterol synthesis) used in the present study was produced by E.P.I.C.O (Egyptian Pharmaceutical International Company), 10th of Ramadan City, Egypt, under the commercial name ATOR®. It is presented as tablets containing 10, 20 and 40 mg of the drug. Atorvastatin was suspended in distilled water (0.66 mg/ml; 10 mg tablet in 15 ml) and each rat was administered 0.5 ml of the prepared suspension daily using a gastric tube. This amount is equivalent to the dosage rate of 1.8 mg/kg body weight daily (converted from human dose after Paget and Barnes) (6) to the corresponding groups as explained later.

Ezetimibe

EZE (an inhibitor of cholesterol absorption) used in the present study was a kind gift from SIGMA pharmaceuticals, Quesna, Egypt. It was obtained as a pure powder. EZE was dissolved in 20% ethanol; where 10 mg of EZE were dissolved firstly in 5 ml absolute alcohol, and then the alcoholic EZE solution was completed to 25 ml by distilled water. Each rat within the target group received 0.5 ml of the prepared solution which is equivalent to a dosage rate of 1 mg/kg. body weight, orally, once daily (7).

Diets

Composition of the basal and fat-enriched diets supplemented to rats is shown in the table 1.

Experimental animals

Seventy albino rats of both sexes aging 6-8 weeks of approximate weights 180-210 g were used in this study. Rats were kept in separate cages and allowed to a plenty of water and diets at room temperature. After one week of acclimatization, rats received different treatments as follows:

Group I: Rats were fed on normal diet and received no drugs; kept as negative control.

Table 1.

Feed ingredients	Level (%) in basal diet	Level (%) in fat-enriched diet
Oil	15.00	15.00
Yellow corn	44.15	44.15
Soya bean meal (44%)	20.51	20.51
Wheat bran	12.33	12.33
Cholesterol	0.00	1.00
Coconut oil	0.00	2.00
Molasses	3.00	3.00
Common salt	0.50	0.50
Lysine	0.18	0.18
DL-Methionine	0.74	0.74
Min.-Vit. Premix	1.50	1.50
Ground limestone	1.09	1.09
Total	100.00	100.00

Group II: Rats were fed on fat-enriched diet and received no drugs; kept as positive control for all experimental groups.

Group III: Rats were fed on normal diet and received sildenafil after 30 days from the start of the experiment; kept as negative treated group.

Group IV: Rats were fed on fat-enriched diet and received sildenafil after 30 days from the start of the experiment; kept as positive treated group.

Group V: Rats were fed on fat-enriched diet and received sildenafil from the start of the experiment; kept as concurrent treatment group.

Group VI: Rats were fed on fat-enriched diet and received atorvastatin after 30 days from the start of the experiment; kept as a standard treated group.

Group VII: Rats were fed on fat-enriched diet and received EZE after 30 days from the start of the experiment; kept as standard treated group.

Sampling

Blood for serum was collected on the 30th, 45th and 60th days from the start of the experiment. Samples were collected from the venous plexus located at the medial canthus of the eye by means of heparinized capillary tubes. The collected blood was allowed to clot at room temperature for an hour; and then refrigerated for further an hour for clot retraction. Clear sera were separated by centrifugation at 3000 r.p.m. for 10 minutes and then collected in Eppendrof's tubes using automatic pipettes. Serum samples were kept in deep freezer (-20°C) for analysis of the following biochemical parameters:

Total lipids (TL), total cholesterol (TC), triacyglycerols (TAG), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), very low density lipoprotein-cholesterol (VLDL-C), aspartate aminotransferase (AST; SGOT), alanine aminotransferase (ALT; SGPT), urea and creatinine.

Biochemical analysis

The serum total lipids were determined according to the method described by Chaboral & Castellano (8) using a kit supplied by SPINREACT, Sant Esteve De Bas, Spain; total cholesterol was determined enzymatically according to the method described by Meiattini

(9) using a kit supplied by SPINREACT; HDL-L were determined according to the precipitation method described by Burstein et al., (10) using a kit supplied by SPECTRUM, Obour city, Egypt; TAG were determined enzymatically according to the method described by Young and Pestaner (11) using a kit SPINREACT. LDL-C and VLDL-C values were calculated using the formulae described by Friedewald et al., (12) and Bauer (13), respectively. Serum ALT and AST were quantitatively determined according to the method described by Murray (14, 15) using kits supplied by DIAMOND, Cairo, Egypt. Urea was quantitatively determined according to the method described by Kaplan (16) using a kit supplied by DIAMOND, Cairo, Egypt; while creatinine was quantitatively determined according to the method described by Murray (17) using a kit supplied by the same company.

Statistical analysis

Data were expressed as mean ± S.E which are calculated using a SigmaPlot® software. The obtained data were statistically analyzed using Student's *t*-test to express the differences between individual groups and control ones according to Snedecor and Cochran (18).

Results

Effect of sildenafil on serum total lipids

As shown in table 2, there was a significant increase in serum total lipid concentration in rats fed on fat-enriched diet, compared to rats received basal diet. While administration of sildenafil suspension to normal rats caused significant rise in serum total lipid concentration on the days 45 and 60 of the experiment if compared with the -ve control rats; yet, it significantly decreased serum total lipid concentration in animals fed on fat-enriched diet compared to the +ve untreated ones, on the same days. Administration of sildenafil from the start of the experiment along with fat-enriched diet failed to impede induction of hyperlipidemia in the concerned rats. However, the level of serum total lipid was a midway between −ve and +ve groups with significance (P<0.05) against −ve group;

Table 2. Effect of oral administration of 0.5 ml sildenafil suspension (equivalent to 5.625 mg/kg b.wt) daily on serum total lipids concentration (X±S.E; mg/dL) in albino rats fed on basal and fat-enriched diets (n=10)

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Day 30	319.42±15.22	680.86*±20.13	339.17±16.33	634.67*±13.73	603.36*±20.14	630.22*±25.45	650.04*±15.61
Day 45	347.55±23.11	690.43*±23.77	463.3*±37.91	590.88*¤±26.68	546.25*¤±18.26	480.09*¤±29.38	575.45*¤±30.23
Day 60	369.11±25.25	704.5*±21.60	481.5*±35.73	533.75*¤±15.8	530.16*¤±17.23	450.65*¤±14.44	532.25*¤±25.33

Group 1: -ve control   Group 2: +ve control   Group 3: -ve treated with sild
Group 4: +ve treated with sild   Group 5: +ve plus sild   Group 6: +ve treated with ator
Group 7: +ve treated with EZE   *(P<0.05 against –ve)   ¤ (P<0.05 against +ve)

and without significance against +ve group on the 30th day that turned significant later on.

Effect of sildenafil on serum cholesterol

As shown in table 3, there was a significant increase in serum cholesterol concentration in rats fed on fat-enriched diet, compared to rats received basal diet. While administration of sildenafil suspension to normal rats caused significant rise in serum total cholesterol concentration on the days 45 and 60 of the experiment if compared with the -ve control rats; yet, it significantly decreased serum cholesterol concentration in animals fed on fat-enriched diet compared to the +ve untreated ones, on the same days. Administration of sildenafil from the start of the experiment along with fat-enriched diet failed to impede induction of hypercholesterolemia in the concerned rats. However, the level of serum cholesterol was a midway between –ve and +ve groups with significance (P<0.05) against –ve group and without significance against serum +ve group.

Effect of sildenafil on serum triacylglycerols

As shown in table 4 there was a significant in-

crease in TAG concentration in rats fed on fat-enriched diet, compared to rats received basal diet. Daily oral administration of sildenafil significantly decreased serum TAG concentration in animals fed on fat-enriched diet compared to the +ve untreated ones. However, it significantly increased TAG concentration in animals kept on basal diet when fed from the day 30 (group 3) or from the start of the experiment (group 5).

Effect of sildenafil on serum lipoproteins

Tables 5, 6, and 7 show significant increases in serum LDL-C and VLDL-C and a significant decrease in HDL-C in the group of rats that was fed on fat-enriched diet all over the period of the experiment, compared to the corresponding control group.

Analysis of samples taken on the days 30, 45 and 60 in the present study, have shown that sildenafil administration revealed significant changes in serum lipoproteins in rats fed on both basal and high-fat diets. It caused significant decreases in LDL-C and VLDL-C concentrations and a significant increase in HDL-C if compared to the rats which were fed fat-enriched diet. On the other hand, it produced re-

Table 3. Effect of oral administration of 0.5 ml sildenafil suspension (equivalent to 5.625 mg/kg b.wt) daily for on serum cholesterol concentration (X±S.E; mg/dL) in albino rats fed on basal and fat-enriched diets (n=10)

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Day 30	70.57±5.60	139.58*±8.06	73.33±5.28	137.77*±12.77	123.33*±7.79	145.00*±5.36	135.33*±8.40
Day 45	73.50±4.03	160.75*±8.06	101.09*±8.83	115.9*¤±14.72	126.00*±15.10	98.33*¤±7.00	125.33*¤±14.40
Day 60	77.60±9.34	168.55*±14.69	98.70*±11.19	104.35*¤±15.01	118.33*±14.01	92.33*¤±7.00	117.34*¤±10.35

Group 1: -ve control   Group 2: +ve control   Group 3: -ve treated with sild
Group 4: +ve treated with sild   Group 5: +ve plus sild   Group 6: +ve treated with ator
Group 7: +ve treated with EZE   *(P<0.05 against –ve)   ¤ (P<0.05 against +ve)

Table 4. Effect of oral administration of 0.5 ml sildenafil suspension (equivalent to 5.625 mg/kg b. wt) daily on serum TG concentration (X± S.E; mg/dL) in albino rats fed on basal and fat-enriched diets (n=10)

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Day 30	89.75±11.94	196.38*±17.99	144.16*±7.98	171.99*±13.12	158.33*±25.9	187.75*±4.09	176.66*±24.53
Day 45	91.5±5.67	188.62*±14.86	140.16*±4.16	139.74*¤±4.63	149.53*±30.44	87.66¤±5.92	166.49*±9.64
Day 60	84.99±7.13	185.30*±16.99	116.66*±15.21	125.02*¤±7.14	145.83*±14.23	74.99¤±4.81	154.16*¤±10.48

Group 1: -ve control   Group 2: +ve control   Group 3: -ve treated with sild
Group 4: +ve treated with sild   Group 5: +ve plus sild   Group 6: +ve treated with ator
Group 7: +ve treated with EZE   *(P<0.05 against –ve)   ¤ (P<0.05 against +ve)

Table 5. Effect of oral administration of 0.5 ml sildenafil suspension (equivalent to 5.625 mg/kg b. wt) daily on serum HDL-C concentration (X± S.E; mg/dL) in albino rats fed on basal and fat-enriched diets (n=10)

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Day 30	40.85±3.35	22.32*±2.66	39.06±2.01	18.62*±3.04	26.06*±2.01	16.13*±3.67	17.46*±1.49
Day 45	42.15±4.18	19.24*±2.58	30.46*±1.49	27.05*±2.62	27.34*±1.49	35.33¤±4.63	25.54*±1.04
Day 60	41.73±1.81	15.33*±2.62	32.27*±3.46	32.18*¤±2.35	32.03*±4.10	38.12¤±1.80	31.62*¤±1.80

Group 1: -ve control   Group 2: +ve control   Group 3: -ve treated with sild
Group 4: +ve treated with sild   Group 5: +ve plus sild   Group 6: +ve treated with ator
Group 7: +ve treated with EZE   *(P<0.05 against –ve)   ¤ (P<0.05 against +ve)

Table 6. Effect of oral administration of 0.5 ml sildenafil suspension (equivalent to 5.625 mg/kg b. wt) daily on serum LDL-C concentration (X± S.E; mg/dL) in albino rats fed on basal and fat-enriched diets (n=10)

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Day 30	10.03±1.6	61.38*±3.38	13.95±1.36	64.85*±5.85	60.11*±6.00	59.16*±7.81	61.66*±7.33
Day 45	11.86±1.31	77.05*±5.70	34.33*±1.45	43.04*¤±3.11	64.74*±7.36	40.33*¤±5.63	57.17*¤±7.13
Day 60	16.11±1.13	81.14*±9.02	38.35*±1.23	40.53*¤±4.24	61.14*±8.15	35.55*¤±7.14	52.91*¤±9.05

Group 1: -ve control   Group 2: +ve control   Group 3: -ve treated with sild
Group 4: +ve treated with sild   Group 5: +ve plus sild   Group 6: +ve treated with ator
Group 7: +ve treated with EZE   *(P<0.05 against –ve)   ¤ (P<0.05 against +ve)

Table 7. Effect of oral administration of 0.5 ml sildenafil suspension (equivalent to 5.625 mg/kg b. wt) daily on serum VLDL-C concentration (X± S.E; mg/dL) in albino rats fed on basal and fat-enriched diets (n=10)

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Day 30	24.4±3.46	34.52*±5.33	23.83±1.59	34.99*±3.39	25.82±5.33	35.75*±4.00	36.83*±5.74
Day 45	21.58±3.48	35.38*±3.58	29.16*±1.59	29.12¤±2.30	27.83±2.39	29.86*¤±1.2	35.21*±1.91
Day 60	22.66±2.00	37.94*±3.27	30.83*±2.86	27.91¤±3.67	27.06±3.58	24.99¤±0.96	31.1*¤±4.64

Group 1: -ve control   Group 2: +ve control   Group 3: -ve treated with sild
Group 4: +ve treated with sild   Group 5: +ve plus sild   Group 6: +ve treated with ator
Group 7: +ve treated with EZE   *(P<0.05 against –ve)   ¤ (P<0.05 against +ve)

verse actions when administered to rats fed on basal diet.

Effect of sildenafil on liver function profile

Data of the present study (tables 8 and 9) demonstrate a significant increase in serum ALT and AST concentrations of rats fed on fat-enriched diet all over the two-month period of the experiment, compared to the control rats that received basal diet. Although administration of sildenafil to normal rats caused insignificant changes in serum liver enzymes concentration all over the period of the experiment; yet, it sig-

nificantly decreased their serum concentrations in animals fed on fat-enriched diet compared to the +ve untreated ones, upon its administration starting from the day 30th of the experiment. However, concurrent administration of sildenafil with high fat diet failed to guard against the rise in liver enzymes.

Effect of sildenafil on kidney function profile

Data of the present study (tables 10 and 11) demonstrate a significant increase in serum urea and creatinine concentrations of rats fed on fat-enriched diet all over the two-month period of the experiment, compared to the control rats that received basal diet. Although administration of sildenafil to normal rats caused insignificant changes in serum urea and creatinine concentrations all over the period of the experiment; yet, it significantly decreased their serum concentration in animals fed on fat-enriched diet compared to the +ve untreated ones, upon its administration starting from the 30th day of the experiment. However, concurrent administration of sildenafil with high fat diet failed to guard against the rise of serum urea and creatinine.

Table 8. Effect of oral administration of 0.5 ml sildenafil suspension (equivalent to 5.625 mg/kg b. wt) daily on serum SGPT (ALT) concentration (X± S.E; U/L) in albino rats fed on basal and fat-enriched diets (n=10)

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Day 30	14.32±2.09	25.75*±1.25	16.24±1.15	26.75*±1.25	20.50±1.00	28.76*±1.33	26.53*±1.44
Day 45	16.34±1.60	27.75*±2.13	17.33±1.33	22.12*¤±1.15	22.25±1.84	23.36*¤±1.33	23.65±2.17
Day 60	15.2±1.82	26.75*±1.86	15.70±1.46	20.75¤±2.75	22.67*±1.66	21.61*¤±1.33	22.73±3.66

Group 1: -ve control   Group 2: +ve control   Group 3: -ve treated with sild
Group 4: +ve treated with sild   Group 5: +ve plus sild   Group 6: +ve treated with ator
Group 7: +ve treated with EZE   *(P<0.05 against –ve)   ¤ (P<0.05 against +ve)

Table 9. Effect of oral administration of 0.5 ml sildenafil suspension (equivalent to 5.625 mg/kg b. wt) daily on serum SGOT (AST) concentration (X± S.E; U/L) in albino rats fed on basal and fat-enriched diets (n=10)

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Day 30	23.11±2.30	38.25*±2.39	22.50±0.86	37.70*±5.22	33.50*±2.59	40.5*±2.98	39.25*±2.39
Day 45	21.25±5.32	39.00*±4.07	24.25±2.39	31.75±4.11	33.95*±3.06	32.5±2.98	35.80*±1.63
Day 60	22.70±2.53	37.50*±4.38	23.09±4.11	30.12¤±1.90	29.25*±2.39	28.5¤±2.98	32.66*±4.09

Group 1: -ve control   Group 2: +ve control   Group 3: -ve treated with sild
Group 4: +ve treated with sild   Group 5: +ve plus sild   Group 6: +ve treated with ator
Group 7: +ve treated with EZE   *(P<0.05 against –ve)   ¤ (P<0.05 against +ve)

Table 10. Effect of oral administration of 0.5 ml sildenafil suspension (equivalent to 5.625 mg/kg b. wt) daily on serum urea concentration (X± S.E; mg/dL) in albino rats fed on basal and fat-enriched diets (n=10)

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Day 30	41.50±2.80	55.97*±2.99	43.50±1.85	57.47*±3.32	51.62±3.80	61.75*±2.00	59.04*±1.40
Day 45	43.00±1.88	58.68*±1.30	45.00±1.04	52.56¤±1.40	47.49±2.41	49.33¤±1.40	54.25*±2.00
Day 60	42.50±2.34	57.04*±2.00	47.50*±2.26	49.56¤±1.42	46.19¤±1.04	46.04¤±1.40	49.90±1.98

Group 1: -ve control   Group 2: +ve control   Group 3: -ve treated with sild
Group 4: +ve treated with sild   Group 5: +ve plus sild   Group 6: +ve treated with ator
Group 7: +ve treated with EZE   *(P<0.05 against –ve)   ¤ (P<0.05 against +ve)

Table 11. Effect of oral administration of 0.5 ml sildenafil suspension (equivalent to 5.625 mg/kg b. wt) daily on serum creatinine concentration (X± S.E; mg/dL) in albino rats fed on basal and fat-enriched diets (n=10)

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Day 30	1.10±0.17	1.80*±0.18	1.11±0.12	1.85*±0.09	1.64±0.08	1.95*±0.11	2.05*±0.05
Day 45	1.23±0.08	1.98*±0.12	1.30±0.17	1.75*¤±0.09	1.62±0.09	1.40¤±0.10	1.86*±0.11
Day 60	1.20±0.18	2.10*±0.18	1.39±0.12	1.57*¤±0.14	1.55±0.05	1.35¤±0.17	1.66*¤±0.06

Group 1: -ve control   Group 2: +ve control   Group 3: -ve treated with sild
Group 4: +ve treated with sild   Group 5: +ve plus sild   Group 6: +ve treated with ator
Group 7: +ve treated with EZE   *(P<0.05 against –ve)   ¤ (P<0.05 against +ve)

Discussion

Historically, the story starts when coffee was discovered about 2000 years ago by goat herders in Ethiopia. Caffeine was found to be the main active ingredient in coffee in 1960. Caffeine produced various biological actions on different body systems. The first discovered mechanism of these actions was phosphodiesterase (PDE) inhibition. This fact does not exclude that caffeine has effects on other non-PDE. From this little far beginning, it could be stated that caffeine was the first known PDE inhibitor (19).

Later on, it was shown that at least 15 PDE isoenzymes exist, and that caffeine inhibits most of them and thus acts as non-selective PDE inhibitor. Major research efforts have led to the production and development of compounds that are more selective and more potent in inhibiting particular PDEs (20).

Sildenafil (Viagra)® is the first commercialized compound in this class, which selectively inhibits PDE-5 that is present mainly in the corpus cavernosum of the penis. This has been followed by introduction of more or less similar drugs, including vardenafil (Levitra)® and tadalafil (Cialis)® (21). Vardenafil has a similar structure as sildenafil, but tadalafil structure is different. Part of the ring structure of sildenafil or vardenafil is similar to that of caffeine. The ring structure in sildenafil and vardenafil is similar to a ring structure in cyclic guanosine monophosphate (cGMP) giving the scientific basis of these drugs to be competitive inhibitors of cGMP for PDE-5. Inspite of the different structure of tadalafil, yet, its molecular mechanism of action is believed to be similar.

The normal pathway for penile erection is illustrated in figure 1. If the cGMP level in corpus cavernosum smooth muscle cells is not elevated sufficiently or if relaxation of smooth muscle in this tissue is deficient due to cholesterol deposition or hyperlipidemia, a condition of erectile dysfunction (ED) revealed (22, 23).

PDE-5 inhibitors enhance erectile function during sexual stimulation by penetrating into smooth muscle cells and inhibiting PDE-5. This results in decreased degradation of cGMP, which maintains sufficient cellular levels of cGMP in both corpus cavernosum and the vessels supplying it. This increases relaxation of the smooth muscle, which dilates the corporeal sinusoids resulting in increased blood flow, allowing an erection to occur. cGMP is the second messenger for nitric oxide (NO) that is released, upon sexual stimulation, from non-adrenergic, non-cholinergic neurons (NANC) supplying the penile tissue. PDE-5 inhibitors do not increase the NO level, but they potentiate it.
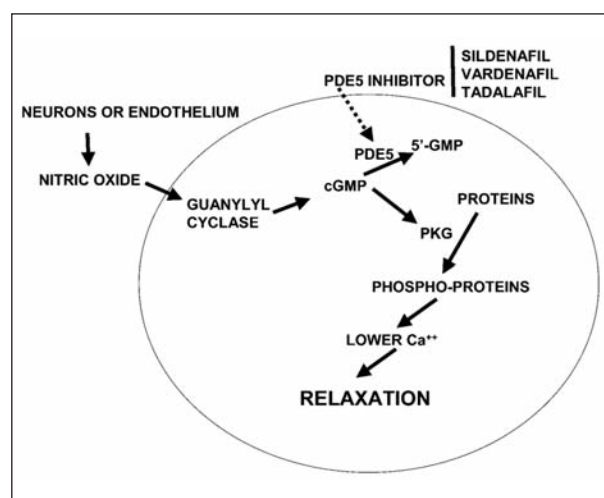

Figure 1. Normal pathway of penile erection

The vast use of PDE-5 inhibitors in ED which is usually associated with diabetic, cardiovascular and other metabolic abnormalities, led researchers to investigate their possible extra-sexual effects. Among them, is the present study. Other stimuli to do this research are recent animal studies that highlighted a possible interaction between chronic PDE-5 inhibition and glucose homeostasis which occurs through a marked improvement of high-fat diet-induced insulin resistance. In addition, it is unclear that whether the enhancement of intracellular cGMP concentration does not generate major unwanted biological effects in tissues other than corpus cavernosum or not, especially, those related to lipid metabolism and vital organ functions.

Data of the present study revealed that continuous administration of sildenafil affected lipid metablic profile and organ function of both normal and hyperlipidemic rats in a complex manner that needs further studies to be completely understood. Hyperlipidemic rat model was used to simulate more or less the health profile of most of ED patients, as the two conditions are closely related and interactive (24).

In the present study, hyperlipidemia, induced by continuous supplementation of high-fat diet, caused marked alterations (mainly increase except HDL-C which is decreased) in almost all lipid parameters of rat groups fed on such diet. Moreover, the obtained hyperlipidemia was associated with elevated markers for some organ dysfunction as liver, kidneys and aorta together with considerable histopathological changes in these organs (data not shown). These findings led us to use such rats as a model for hyperlipidemia to assess the possible modulating role of sildenafil that is used on a large scale either with physician prescription or, more dangerously, without prescription even in youth as a personal thinking of improving their sexual performance.

Data of the present study (table 2) demonstrate a significant increase in serum total lipid concentration of rats fed on fat-enriched diet all over the two-month period of the experiment, compared to the control rats that received basal diet. This result of hyperlipidemia is consistent to that have been reported previously in more than one species including, rats by Csont et al., (25), rabbits by Diaz et al., (26) and Hellal (27); and laying hens by Attia (28).

Data of the present study (table 3) demonstrate a significant increase in serum total cholesterol concentration of rats fed on fat-enriched diet all over the two-month period of the experiment, compared to the control rats that received basal diet. This result is consistent with those achieved by by Diaz et al., (26) who reported that, rabbits fed with the atherogenic diet showed marked increase in plasma total cholesterol. The result is also consistent with that of Abdel-Maksod et al., (29) who reported that mice and rats received cholesterol-enriched diet showed sever hypercholesterolemia, elevated plasma serum LDL-C and VLDL-C compared to those fed a normal diet. In addition, Attia (28) reported that administration of laying hens with diet rich in cholesterol diet led to marked elevation in plasma total cholesterol.

Rise in serum cholesterol might be attributed to the reduced catabolic rate of serum TC or reduced activity of hepatic cholesterol-7-alpha-hydroxylase, the rate limiting enzyme in bile acid synthesis from cholesterol (29). Moreover, the rise in serum TC observed in this study could be attributed to increased HMG-CoA reductase activity in the liver of animals fed on fat-enriched diet and the reduced rate of the clearance of LDL from circulation due to defective LDL receptors which associated with increase of plasma TC concentration (30). Similarly, Yoshie, et al., (31) stated that rabbits with high serum cholesterol concentrations developed intimal lesions similar to those of human atherosclerosis.

Although administration of sildenafil suspension to normal rats caused significant rise in serum total cholesterol concentration on the days 45 and 60 of the experiment if compared with the -ve control rats; yet, it significantly decreased serum cholesterol concentration in animals fed on fat-enriched diet compared to the +ve untreated ones, on the same days. Administration of sildenafil from the start of the experiment along with fat-enriched diet failed to stop induction of hypercholesterolemia in the concerned rats. However, the level of cholesterol was a midway between –ve and +ve groups with significance (P<0.05) against –ve group and without significance against +ve group. This data may be consistent with Sivasankaran et al., (32) who found that the long term combined administration of single daily doses of sildenafil plus ethanol increased

serum cholesterol compared to control rats. In our study, sildenafil behavior was atorvastatin-like rather than ezetimibe which are the standard antihyperlipidemic drugs used in this experiment. The antihypercholesterolemic effect of sildenafil in high-cholesterol group, therefore, may be explained on the basis of increasing the transfer of blood cholesterol to be used in bile synthesis and thus, biliary excretion of cholesterol or bile acids is increased resulting in reduced availability of cholesterol to be incorporated into lipoproteins (33); not on the basis of decreased cholesterol absorption inhibition. A speculation that cGMP might be related to enhanced cholesterol metabolism can not be excluded. Modulation of cholesterol concentration by sildenafil was assumed to be the reason of ameliorating the aortic degenerative atherosclerotic-like lesions in sildenafil-treated rats (data not shown).

Data of the present study demonstrated in table 4 showed a significant increase in TAG in animals kept on fat-enriched diet compared to their corresponding control. This result may be in accordance with those recorded by Brousseau, et al., (34), who found that a hyperlipidemic diet caused a significant increase of the plasma TAG and an increased content of cholesterol in the liver, despite the fact that the diet produced a cessation of endogenous cholesterol synthesis. Such significant rise in serum TAG level may be attributed to the decrease of activity of lipase which is an insulin-dependent enzyme involved in TAG clearance from plasma by mediating TAG lipolysis into glycerol and FFA (35). Another possibility is that such significant increase in triglycerides might be a consequence of over production of VLDL by the liver.

Daily oral administration of sildenafil significantly decreased serum TAG concentration in animals fed on fat-enriched diet compared to the +ve untreated ones. However, it significantly increased TAG concentration in animals kept on basal diet when fed from the day 30 (group 3) or from the start of the experiment (group 5). This data may be consistent with that of Sivasankaran et al., (32) who found that the long term combined administration of single daily doses of sildenafil plus ethanol increased serum TAG time-dependently along 45 days, compared to control rats.

The significant decrease in plasma TAG was explained previously by Bennani-Kabchi et al., (36) who related them to the increased rate of lipolysis that is mediated by increase of plasma lipase activity. However, Griffin et al., (37) stated that the low plasma TAG concentration might also reflect the low rate of hepatic lipogenesis or the use of plasma TAG by tissues other than adipose ones.

Data obtained in the present study in tables 5, 6 and 7 revealed significant increases in serum LDL-C and VLDL-C and a significant decrease in HDL-C in the group of rats that was fed on fat-enriched diet all over the period of the experiment, compared to the corresponding control group. These results are in accordance with those reported by Abdel-Maksod et al., (29) who reported that mice and rats receiving cholesterol-enriched diet showed sever elevated plasma LDL-C and VLDL-C compared to those kept on a normal diet. Abdel-Maksod and colleagues concluded that the elevated serum LDL-C and VLDL-C seemed to be related mainly to reduced catabolic rate that occurs when the production of LDL exceeds the capacity of LDL receptors present on hepatocytes; in other words when the efflux of cholesterol from the liver becomes more than its influx. Mahley & Habcombe (38) added that both dietary fat and cholesterol may change the lipoprotein content of serum and affect the different classes of lipoproteins, LDL and HDL and increases the content of cholesterol in VLDL. Analysis of samples taken on the days 30, 45 and 60 in the present study, have shown that sildenafil administration revealed significant changes in serum lipoproteins in rats fed on both basal and high-fat diets. It caused significant decreases in LDL-C and VLDL-C concentrations and a significant increase in HDL-C in compared to the rats which were fed fat-enriched diet. On the other hand, it produced reverse actions when administered to rats fed on basal diet. The improving effect of sildenafil may be explained by what was reported by Ayala et al., (39) that chronic sildenafil administration (12 weeks) counteracted the detrimental effects of a high-fat diet on endothelial function and insulin resistance by improving insulin function and energy balance. They added that effect persisted even in the presence of NO donors, suggesting direct effects of sildenafil on metabolism other than eNOS activation. Improving insulin function may increase lipoprotein lipase activity with the result of observed effects of silde-

nafil. The increasing effect of sildenafil in normal rats that were fed on basal diet remains to be understood. Additional mechanisms of the improving effect on lipoproteins that may be applied on sildenafil, may be, probably, decreasing the apoB which is the principal protein that comprises nearly 90% of total protein mass of LDL as stated by Ramadan et al., (40). In addition, it may be speculated that sildenafil may increase the preipheral and hepatic breakdown of cholesterol esters from VLDL and LDL. While the compositional change of HDL also suggests activation of Lecithin-cholesterol acyltransferase (LCAT) which must be stimulated firstly by exogenous cholesterol.

Data of the present study (tables 8 and 9) demonstrate a significant increase in serum ALT and AST concentrations of rats fed on fat-enriched diet all over the two-month period of the experiment, compared to the control rats that received basal diet. Although administration of sildenafil to normal rats caused insignificant changes in serum liver enzymes concentration all over the period of the experiment; yet, it significantly decreased their serum concentrations in animals fed on fat-enriched diet compared to the +ve untreated ones, upon its administration starting from the day 30th of the experiment. However, concurrent administration of sildenafil with high fat diet failed to guard against the rise in liver enzymes. These data may be explained on the basis of decreased ability of the diseased liver to metabolize sildenafil by hepatic microsomal cytochrome P450 which may result in increased efficacy of sildenafil with increasing its dose; while in normal rats the full metabolizing effect of their healthy liver may mask the effect of sildenafil. However, further studies are still needed for more understanding such discrepancy.

As for renal function tests, sildenafil treatment decreased significantly serum urea and creatinine concentrations which were elevated upon continuous high fat diet (tables 10 and 11; p<0.05). Such rise might be attributed to the nephritic changes occurred in the renal tissue upon cholesterol administration (histopathological finding not shown). These changes might be mildened upon sildenafil administration due its cholesterol lowering and renal artery dilating effects.

In conclusion, data of the present study suggest that sildenafil may act as a mixed blessing drug; therefore it must be used carefully and under physician supervision to get its therapeutic benefits and guard against its adverse effects.

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