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Effect of Sildenafil on Non-Alcoholic Fatty Liver

[1]Shereen Ezzelregal Alashry, [1]Mohammad A. Gaballah, [2]Hala Abdel Malek and [3]Adel I. Abd Elsalam
[1]Department of Dermatology, Andrology and STDs, Faculty of Medicine, Mansoura University, Egypt
[2]Department of Clinical Pharmacology, Faculty of Medicine, Mansoura University, Egypt
[3]Department of Internal Medicine, Faculty of Medicine, Mansoura University, Egypt

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Corresponding Author:
Mohammad A. Gaballah
Department of Dermatology,
Andrology and STDs,
Faculty of Medicine,
Mansoura University,
Mansoura, Egypt

ABSTRACT
Non Alcoholic Fatty Liver Disease (NAFLD) is now the commonest liver disease worldwide and can evolve into cirrhosis in a subgroup of patients. Sildenafil citrate is a specific phosphodiesterase-5 (PDE-5) inhibitor, which has been approved for treatment of erectile dysfunction in men. To examine the effect of sildenafil citrate on NAFLD in animal model and male patients with erectile dysfunction. Sixty male mice were divided into 4 groups: The 1-received a standard diet; 2-fed with a High-fat Diet (HFD); 3-HFD + sildenafil citrate; 4-standard diet + sildenafil citrate. The experiments were conducted for 16 weeks, after that, the mice in the treatment groups received 1.4 mg kg^{-1} sildenafil citrate base dissolved in distilled water daily for 8 weeks, through orogastric feeding tube. A case-control study enrolled 60 males with erectile dysfunction, who were divided into 30 patients with NAFLD and 30 patients without NAFLD (control group). They received sildenafil citrate 50 mg daily for 8 weeks. It was found that sildenafil administration improved liver enzymes, insulin resistance and lipids level compared to control group in animal model. There were insignificant changes as regard lipid profile, fasting serum insulin and liver enzymes (alkaline phosphatase, alanine 2-oxoglutarate aminotransferase) after sildenafil treatment for 8 weeks in NAFLD group in human study. There was significant decrease in aspartate 2-oxoglutarate aminotransferase (AST) level after sildenafil treatment in NAFLD. There were significant negative correlations between International Index of Erectile Function (IIEF) score and Body Mass Index (BMI), cholesterol and triglycerides levels. Sildenafil is well-tolerated and safe in NAFLD patients. However, it is less effective in NAFLD patients than in individuals without comorbidities. Further investigation is needed to test the effect of long-term sildenafil administration on insulin resistance and lipid profile.

Key words: Sildenafil, non-alcoholic fatty liver disease, erectile dysfunction

INTRODUCTION

Non Alcoholic Fatty Liver Disease (NAFLD) occurs in those, who don't consume alcohol in amounts considered harmful to the liver. The NAFLD is a clinicopathological condition characterized by the accumulation of triglycerides in hepatocytes and frequently associated with obesity, dyslipidemia, diabetes mellitus type 2 and insulin resistance (Berlanga *et al*., 2014). The NAFLD is a major public health issue due to its high prevalence worldwide. It ranges widely from 11-46% (Williams *et al*., 2011). The prevalence increases to 58% in overweight individuals and 98% in non-diabetic obese individuals (Machado *et al*., 2006).

The acronym is an umbrella term covering a spectrum of conditions ranging from harmless and non-inflammatory intracellular fat deposition (simple steatosis), to non-alcoholic

steatohepatitis (NASH). Patients with NASH are at-risk for advanced liver disease, since NASH is a potentially progressive and can evolve to cirrhosis in 12-25% of cases within 10 years and is associated with increased risk of cardiovascular and liver-related deaths (Harrison *et al*., 2003). The majority of patients with NAFLD are asymptomatic. The NAFLD may be detected via routine laboratory tests showing elevated liver enzymes or when an ultrasound is performed for various reasons and detects liver steatosis (Dowman *et al*., 2011). The NAFLD is considered a hepatic manifestation of metabolic syndrome. Metabolic syndrome (MetS) is diagnosed according to the revised Adult Treatment Panel-III (ATP-III), when at least three of five of the following are present: visceral obesity (waist circumference ≥102 cm in men or ≥88 cm in women), raised arterial blood pressure (>130/85 mm Hg), dysglycemia (fasting plasma glucose >100 mg dL^{-1}), increased triglyceride concentrations (>150 mg dL^{-1}), low High-Density Lipoprotein (HDL) (<40 mg dL^{-1} in men or <50 mg dL^{-1} in women) (Grundy *et al*., 2005).

These metabolic abnormalities constitute major risk factors for diabetes mellitus and coronary artery disease. The MetS may lead to Erectile Dysfunction (ED) through multiple mechanisms. The ED indicates the man's inability to attain or maintain erection sufficient for satisfactory sexual intercourse (Sanjay *et al*., 2015).

Sildenafil is a selective inhibitor of phosphodiesterase-5 (PDE-5), an enzyme, which catalyzes the breakdown of cyclic guanosine monophosphate (cGMP), an essential second messenger involved in smooth muscle relaxation. The PDE-5 inhibitors cause an accumulation of Nitric Oxide (NO) driven cGMP with subsequent vasodilatation in the corpus cavernosum and in pulmonary vasculature; therefore PDE-5 inhibitors are widely used treatment for erectile dysfunction (Leoni *et al*., 2013) and pulmonary hypertension. Moreover, intracellular cGMP-accumulation has been shown to reduce tissue injury in conditions associated with increased free radical release and oxidative stress (Dias-Junior *et al*., 2005). The aim of this study was to examine the effect of sildenafil citrate on non alcoholic fatty liver in mice and male patients with erectile dysfunction.

MATERIALS AND METHODS

Animal preparation: Sixty male mice, weighing 23-26 g at 8-10 weeks of age, were fed specific diet (Table 1) to induce fibrosing steatohepatitis. The mice were housed in plastic cages at 24±2°C at 45±5% humidity, 12/12 h light/dark cycle and were given free access to food and water throughout the experimental period.

Animal groups: After acclimation for one week by feeding of a basal diet, animals were divided into two groups of 30 mice each. A control group fed a purified powdered basal diet and an experimental group fed purified high-fat powdered diet containing suet powder at 37.5 g/100 g diet (high-fat diet) (Table 1). Both types of diet were purchased from (Segma-Aldrish, St. Louis). Experiments were performed according to the Guide for the Care and Use of Laboratory Animals (Institute for Laboratory Animal Research, National Research Council, Washington, DC: National Academy Press, No. 85-23, revised 1996). All protocols were approved by our local committee of Animal Care and Use According to Committee. Breeding mice (Medical experimental research center "MERC").

Table 1: Composition of the basal and experimental diet used

Elements	Basal diet w/w (%)	High-fat diet w/w (%)
Milk casein	20.00	24.48
DL-methionine	0.30	0.37
Corn starch	14.66	5.93
Granulated sugar	49.09	19.85
Cellulose	5.00	6.12
Suet powder	6.25	37.50
Vitamin mix (AIN-76)	1.00	1.22
Mineral mix (AIN-76)	3.50	4.28
Choline bitartrate	0.20	0.24
Calorie (kcal/100 g)	373.62	485.60
Ratio of fat/total calorie (%)	13.10	56.60

Specimens' collection: After treatment with the experimental diet for 16 weeks, the mice in the treatment groups received 1.4 mg kg^{-1} body weight of Sildenafil citrate base dissolved in distilled water daily for 8 weeks, through orogastric feeding tube. The mice were anesthetized with intraperitoneal injection of sodium pentobarbital, 50 mg kg^{-1} of body weight. Blood samples were collected for liver function test and insulin resistance measurement:

- Serum aspartate 2-oxoglutarate aminotransferase (AST), alanine 2-oxoglutarate aminotransferase (ALT), were measured with an automated analyzer
- Homeostasis model assessment of insulin resistance

The homeostasis model assessment of insulin resistance (HOMA-IR) was measured by obtaining glucose and insulin assay obtained after 7 h of food removal, the following formula were used:

- Fasting blood glucose (mg dL^{-1})×fasting insulin (μU mL^{-1})/405
- Serum lipid profile was performed using the commercially available kits

Study population: Cases and controls were recruited from patients sequentially seen at the outpatient clinic of dermatology, andrology and STDs, Mansoura University Hospital from January, 2013 to December, 2014. This case control study was performed according to the principles of Declaration of Helsinki and the procedures were according to

the instructions of the local ethics committee. The details of the study were explained to the patients and all subjects had provided written informed consent.

Cases included patients suffering from erectile dysfunction with BMI ≥30 kg m^{-2}, fatty liver was demonstrated by ultrasound and other liver diseases were ruled out (viral hepatitis B and C, auto-immune hepatitis, hepatitis caused by drugs) (n = 30). Controls were age- matched, patients suffering from erectile dysfunction without NAFLD and BMI ≤30 kg m^{-2} (n = 30).

Both groups received sildenafil citrate 50 mg daily for 8 weeks. Exclusion criteria were established before commencement of the study. Any patient with chronic renal disease, peripheral or autonomic neuropathy, cardiovascular disease, prostatic disease, history of pelvic trauma and surgery, mental illness and use of drugs or alcohol abuse, was excluded from the study. Endocrine causes of ED were also excluded. Similarly, those patients who had contraindications to PDE5 inhibitors (i.e. currently being treated with nitrites) were also excluded.

All patients were married with stable relationship. They provided detailed medical histories and had physical examinations before entry into the trial. Portions of an abridged 5-item version of the previously validated International Index of Erectile Function (IIEF-5) also known as the Sexual Health Inventory for Men (SHIM), was used to grade ED both before the trial and at its conclusion (8 weeks). International Index of Erectile Function (IIEF-5) is a multidimensional questionnaire consists of 5 questions for assessing ED. The erectile function score represents the sum of questions one through five of the IIEF-5 questionnaire, with a maximum score of 25; a score <21 indicates ED. The five questions assess erectile confidence, erection firmness, maintenance ability, maintenance frequency and satisfaction. The severity of ED is classified into five categories as severe (score 5-7), moderate (score 8-11), mild to moderate (score 12-16), mild (score 17-21) and no (score 22-25) ED (Rosen *et al*., 2002). We asked participants to estimate how long they remembered having ED and subsequently calculated the baseline IIEF-5 scores.

Examination included:

- Body Mass Index (BMI) is calculated for the participants in both groups using the following equation: weight (kg)/height (m^2). Subjects with BMI 19-25 kg m^{-2} are considered normal, 26-29 kg m^{-2} as overweight and ≥30 kg m^{-2} as obese
- Blood pressure will be taken with standard calibrated mercury manometers in the right arm of each individual in a sitting position after a rest of 5 min
- Waist circumference will be measured using tape measure: palpate hip bone to detect uppermost border of iliac crest then measuring tape will be placed around abdomen at this point

Laboratory investigations: Patient will be fasting for at least 12-14 h then 5 mL venous blood sample will be drawn from each patient into plain tube and left to clot. Serum will be separated by centrifugation into two aliquots; one will be used for routine analysis and the other aliquot will be stored at -21°C until insulin analysis.

The investigations include:

- Fasting Plasma Glucose (FPG) level
- Fasting serum insulin level measure using ELISA method by available kits
- Liver enzymes (alanine aminotransferase (ALT); aspartate aminotransferase (AST) and alkaline phosphatase (ALP)
- Lipid profile (Total cholesterol, Low-Density Lipoprotein (LDL), High-Density Lipoprotein (HDL) and Triglycerides)
- Insulin resistance will be estimated by HOMA-IR, calculated as follows; fasting serum insulin (μIU mL^{-1}) × fasting plasma glucose (mg dL^{-1})/405

Blood samples were collected for biochemical analysis before and after treatment with sildenafil for 8 weeks. Because of the high prevalence of hypogonadism in obese patients, baseline testosterone and prolactin levels were drawn and compared between both groups. Abnormal levels were excluded from the study.

Instructions regarding properties and correct administration of the study treatment were based on the Summaries of Product Characteristics (SPCs) and the European Association of Urology guidelines on male sexual dysfunction: One tablet (50 mg) per day at approximately the same time everyday on empty stomach, but no more than one tablet per day. During the 8 weeks treatment period, study visits were scheduled at 2 and 8 weeks; unscheduled visits occurred in case of efficacy or tolerability issues.

Statistical analysis: All the data were edited and processed using the SSPS version16. Data were expressed as Means±SD and compared by one-way analysis of variance (ANOVA) followed by Tukey test. The $p \leq 0.05$ was considered as the level of significance between non-treated and treated animal groups. In human population, comparison of continuous data was performed using independent-samples T test and the paired-samples T test. Correlations of the degree of improvement by sildenafil were performed using Pearson correlation. A p-value of <0.05 was considered statistically significant.

RESULTS

Results of animal study

Effects of Sildenafil on serum ALT and AST: Compared to the control group, serum ALT and AST were significantly higher in high fat-diet group. The increment of these parameters was significantly attenuated in high fat-diet +sildenafil group (Table 2).

Table 2: Comparison between animal groups

Groups	HOMA-IR	Serum ALT (U L^{-1})	Serum AST (U L^{-1})	Serum lipid profile		
				Triglyceride (mg dL^{-1})	LDL (mg dL^{-1})	HDL (mg dL^{-1})
Control	4.11±0.05	30.60±2.07	55±3.12	100. 23±6.90	82.090±3.42	60.27±3.06
Fatty liver model	12.24±0.07*	77.55±7.20*	154±6.80*	196.290±18.21*	0.160±7.34*	35.92±2.94*
Fatty liver +Sildenafil	7.30±0.04*	42.60±4.3*	89±3.50*	112.320±15.11*	0.110±5.17*	40.15±3.01*
Control +sildenafil	4.10±0.05	0.30±2.07	54±3.12	0.100±6.90	81.080±3.42	60.20±3.06

*p<0.05 is statistically significant versus its corresponding group

Table 3: Baseline demographics and clinical characteristics

Clinical characters	NAFLD group (n = 30)	Control group (n = 30)
Age (years)		
Mean±SD	54.8±8.7	53.63±7.5
Range	36-65	36-65
Age group		
20-40 years	3 (10%)	3 (10%)
40-60 ears	19 (63.3%)	16 (53.3%)
>60 years	8 (26.7%)	11 (36.7%)
Duration of marriage (years)		
Mean±SD	23.7±9.3	21.06±6.4
Range	4-40	4-30
ED duration (months)		
Mean±SD	54±17.18	52.13±16.65
Range	12-72	12-72
BMI, kg m^{-2}		
Mean±SD	34.89±2.87	26. 98±2.3
Range	(30.9-40.9)	(24-30)
Waist circumference (cm)		
Mean±SD	122.17±8.41	91.13±9.13
Fasting insulin (µU mL^{-1})		
Mean±SD	35.23±44.165	11.89±9.32
Fasting blood glucose (mg dL^{-1})		
Mean±SD	116.23±35.27	81.63±8.48
Comorbidities		
Smoking	10 (33.3%)	8 (26.6%)
Diabetes mellitus	7 (23.3%)	0
Hypertension	20 (66.7%)	0
IIEF-5 score before therapy		
No ED (22-25)	0	0
Mild ED(17-21)	2 (6.7%)	6 (20%)
Mild-moderate ED (12-16)	3 (10%)	10 (33.3%)
Moderate ED (8-11)	13 (43.3%)	10 (33.3%)
Severe ED (1-7)	12 (40%)	4 (13.3%)
IIEF-5 score after therapy		
No ED (22-25)	8 (26.7%)	18 (60%)
Mild ED (21-17)	22 (73.3%)	12 (40%)
Mild-moderate ED (12-16)	0	0
Moderate ED (11-8)	0	0
Severe ED (1-7)	0	0

NAFLD: Non alcoholic fatty liver disease, BMI: Body mass index, ED: Erectile dysfunction SD: Standard deviation

Effects of sildenafil on serum lipids: Table 2 shows the results compared to control group, serum triglycerides and LDL-cholesterol were significantly higher in the high fat group. This increase was significantly decreased in sildenafil-treated group.

Results of human study: The study included sixty patients, divided into two groups. All the patients completed the entire course of the study. Both groups were comparable to each other, as regard number, age of the patients, duration of marriage and duration of ED.

Table 4: Comparison between both groups

Clinical parameters	Control group n = 30 MV±SD	NAFLD group n = 30 MV±SD	t	p
Age	53.63±7.53	54.83±8.72	0.570	0.571
BMI	26.98±2.33	34.89±2.87	11.689	0.0001
Duration of marriage	21.06±6.41	23.70±9.32	1.274	0.208
Duration ED	52.13±16.65	54.00±17.18	0.427	0.671
ALP	76.06±6.79	80.60±11.60	1.847	0.070
SGOT	29.30±5.68	37.33±10.86	3.588	0.001
SGPT	31.10±7.92	39.23±9.48	3.603	0.001
Cholesterol	181.17±9.84	222.47±26.60	7.976	0.0001
Triglyceride	103.80±10.26	161.73±81.52	3.862	0.001
LDL	112.77±9.72	141.17±21.45	6.604	0.0001
HDL	48.30±3.20	45.53±2.88	3.512	0.001
IIEF score before therapy	11.33±3.63	9.06±3.03	2.621	0.011
IIEF score after therapy	21.16±2.11	19.76±2.29	2.452	0.017

NAFLD: Non alcoholic fatty liver disease, BMI: Body mass index, ED: Erectile dysfunction, p<0.05 is statistically significant SD: Standard deviation

The NAFLD group consisted of 30 participants. Their ages ranged from 36-65 years old with mean age 54.83±8.72 years. Sixteen patients (53.3%) aged between 40 and 60 years and 11patients (36.7%) above 60 years old. The duration of marriage ranged from 4-40 years. The duration of ED ranged from 12-72 months with mean 54±17.18 months. The mean value of BMI was 34.89±2.87 kg m^{-2}. Seven patients (23.3%) had frank DM. Twenty patients (66.7%) had HTN and 17 patients (56.6%) had collected the criteria of MetS. Twelve patients with NAFLD (40%) had severe ED. Thirteen patients (43.3%) had moderate ED and 3 patients (10%) had mild-moderate ED according to IIEF-5 score (Table 3).

There were significant differences between NAFLD group and control group as regard BMI (34.89±2.87 vs. 26. 98±2.3), waist circumference (122.17±8.41 vs. 91.13±9.13), serum fasting insulin (35.23±44.165 vs. 11.89±9.32) and fasting blood glucose (116.23±35.27 vs. 81.63±8.48) (Table 3).

The biochemical parameters including ALT, AST, cholesterol, triglycerides and LDL were significantly higher in NAFLD than control patients before and after sildenafil therapy. The HDL was significantly lower than control patients before and after sildenafil therapy (Table 4). There was significant decrease in AST level after sildenafil treatment for 8 weeks in NAFLD group.

International Index of Erectile Function (IIEF-5) score had significant positive correlation with IIEF-5 score after sildenafil therapy. The IIEF-5 score had significant negative correlation with BMI, cholesterol and triglycerides levels

Table 5: Correlations between IIEF score before therapy and BMI, lipid profile and IIEF score after therapy

	IIEF score before therapy	
Clinical parameters	r	p
BMI	-0.474	0.0001
Cholesterol	-0.270	0.037
Triglycerides	-0.292	0.023
LDL	-0.177	0.177
HDL	0.172	0.188
IIEF score after therapy	0.835	0.0001

BMI: Body mass index, $p<0.05$ is statistically significant

(Table 5). There were significant positive correlation between liver enzymes levels and cholesterol, triglycerides and LDL levels.

DISCUSSION

High fat diet, in animal model, induced pathology similar to human NASH, including; steatosis, hepatic inflammation and fibrous tissue formation. High fat diet for sixteen weeks also developed hyperglycemia and hyperinsulinemia, which mimics human metabolic syndrome process. Hepatic steatosis represents the hepatic part of the metabolic syndrome disorder and is accompanied by insulin resistance and elevated triglycerides (McCullough, 2006; Zhang *et al*., 2014).

In the present study, we demonstrated that sildenafil administration to high fat diet fed mice for 8 weeks improved liver enzymes, insulin resistance and lipids level via prevention of hepatic inflammation and improving insulin resistance. These data are in accordance with a work of Ayala *et al*. (2007) who found that in mice with high fat diet sildenafil improved insulin sensitivity measured with hyperinsulinemic-euglycemic clamp without increased levels of Akt-1phosphorylation.

Mammi *et al*. (2011) treated human umbilical vein endothelial cells with insulin in presence of glucose 30 mM and glucosamine 10 mM with or without sildenafil. They found that Insulin increased the expression of PDE5 and eNOS mRNA assayed by Real time-PCR. Akt-1 and eNOS activation was reduced in conditions mimicking insulin resistance and completely restored by sildenafil treatment. They concluded that sildenafil treatment can counteract this noxious effect by increasing NO production through eNOS activation and reducing oxidative stress induced by hyperglycemia and glucosamine.

Our study showed that compared to control group, serum triglycerides, LDL, cholesterol, in addition to, serum ALT and AST were significantly higher in the high fat diet fed group. Obesity and the related insulin resistance are frequently associated with an increased accumulation of lipids (triglycerides) in the liver. Increased numbers of lipid peroxidation markers have been observed in the liver, in animal models of diabetes and obesity (Furukawa *et al*., 2004; Svegliati-Baroni *et al*., 2006). A correlation between elevated LDL and low HDL and oxidative stress in animal models is well established. The LDL receptor-deficient mice fed a cholesterol-enriched diet developed elevated LDL levels and consequently oxidative stress (De Oliveira *et al*., 2011).

In the present study, we demonstrated that NAFLD patients aged from 36-65 years old with mean age 54.83±8.72 years. Sixteen patients (53.3%) aged between 40 and 60 years and 11patients (36.7%) above 60 years old. The mean value of BMI was 34.89±2.87 kg m^{-2}. Seven patients (23.3%) had frank DM. Twenty patients (66.7%) had HTN and 17 patients (56.6%) had collected the criteria of MetS. Conversely, Ahsan *et al*. (2015) conducted a cross-sectional study on 101 patients who met the inclusion criteria of MetS to estimate the prevalence of NAFLD and NASH. They found that there was a high prevalence of non-alcoholic fatty liver disease/non-alcoholic steatohepatitis in patients with metabolic syndrome and liver biopsy confirmed this in 90.3% patients who consented to this procedure.

Our study showed that 12 patients with NAFLD (40%) had severe ED. Thirteen patients (43.3%) had moderate ED and 3 patients (10%) had mild-moderate ED according to IIEF-5 score. Several recent reports have suggested a relationship between ED and MetS. Esposito *et al*. (2005) and Demir (2006) reported that men with MetS had an increased prevalence of ED. Moreover, they confirmed that IIEF scores significantly decrease as the number of components of MetS increases. Conversely, Bansal *et al*. (2005) showed that men with ED have a higher prevalence of MetS.

In the present study, we demonstrated that 8 patients with NAFLD (26.7%) had good response after sildenafil therapy i.e. IIEF score was 22-25, compared to 18 patients (60%) in the control group. These data are in accordance with a work of Suetomi *et al*. (2008), who demonstrated the negative impact of metabolic syndrome on the responsiveness to sildenafil. Although, the precise mechanisms remain unclear, visceral obesity and insulin resistance are characteristic and endothelial dysfunction seems to play a key role in ED. Insulin increased the expression of PDE5; this could explain the reduced response to sildenafil therapy in diabetic patients with elevated insulin resistance and hyperinsulinemia (Mammi *et al*., 2011). In the present study, we demonstrated that biochemical parameters including serum ALT, AST, cholesterol, triglycerides, LDL, in addition to, fasting insulin and fasting blood glucose were significantly higher in NAFLD patients while, HDL was significantly lower than control patients before and after sildenafil therapy. There was significant decrease in AST level after sildenafil treatment for 8 weeks in NAFLD group. Dadkhah *et al*. (2010) reported that PDE5 inhibitors are not curative, they are palliative. The treatment of erectile dysfunction should be directed not only toward improvement of erectile function (symptom), but also toward eradicating any medical conditions contributing to

erectile dysfunction. Adjunctive atorvastatin treatment in hypercholesterolemic men with erectile dysfunction improved the erectile function domain score and the response to oral Sildenafil.

CONCLUSION

Sildenafil is well-tolerated and safe in NAFLD patients. However, it is less effective in NAFLD patients than in individuals without comorbidities. Further investigation is needed to test the effect of long-term sildenafil administration on insulin resistance and lipid profile.

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