Research Article

Mechanisms Underlying the Protective Effect of Sildenafil in Metabolic Syndrome in Rats

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Abstract
Metabolic syndrome (MS) is described as the simultaneous occurrence of insulin resistance, abnormal serum lipid levels, hypertension and recently fatty liver. MS is also associated with erectile dysfunction that is treated with sildenafil. The current study investigated the effect of sildenafil on a rat model of MS induced by fructose overfeeding. Rats were divided into five groups: ١st group served as normal control, ٢nd group MS control group; ٣rd; ٤th and٥th groups treated by oral sildenafil in doses of ٥ mg/kg/day, ١٠ mg/kg/day, ٤٠ mg/kg/day; respectively for six weeks. Liver weight/body weight ratio (liver index), visceral fat index, insulin resistance (fasting blood glucose, fasting serum insulin and homeostasis model assessment of insulin resistance (HOMA-IR)), serum levels of lipids (triglyceride (TG), and high density lipoprotein (HDL), total cholesterol), oxidative stress (malondialdehyde (MDA), reduced glutathione (GSH) and catalase, and nitric oxide (NO)), tumor necrosis factor- α (TNF-α) and immunohistochemical assay of induced nitric oxide synthase (iNOS) and endothelial nitric oxide synthase (eNOS) in hepatic tissues were studied. Sildenafil in all doses reduced liver index and visceral fat index. Sildenafil (١٠ and ٤٠ mg/kg) improved fasting glucose level, fasting insulin level, (HOMA-IR).The protective effect of Sildenafil was associated with significant attenuation in oxidative stress as well as significant decrease in serum levels of TNF-α. Sildenafil increased eNOS and decreased iNOS expression in hepatic tissue. In conclusion, sildenafil was shown to be protective against MS as evidenced by improving lipid profile, improving insulin resistance, decreasing visceral fat index and liver index possibly via anti-oxidant, decrease serum levels of TNF-α as well as via modulation of nitric oxide.

Keywords: Sildenafil, Metabolic syndrome and Fructose overfeeding.

Introduction
Metabolic syndrome (MS) is characterized by a constellation of metabolic risk factors: obesity, dyslipidemia, elevated blood pressure, insulin resistance, and a prothrombotic and prion-flammatory state (Grundy et al., ٢٠٠٤).

Several lines of evidence have suggested that endothelial dysfunction, nitric oxide (NO) modulation, inflamation and increases in oxidative stress may be responsible for MS. However; the exact pathogenesis of MS is still not fully understood (Van Erk et al., ٢٠١٠). Erectile dysfunction is a highly prevalent disease which is also associated with components of MS such as hypertension, obesity, dyslipidemia and diabetes (Hatzimouratidis, ٢٠٠٦).

Sildenafil, a phosphodiesterase ٥ inhibitor (PDE-٥), which revolutionized erectile dysfunction treatment was also reported to reverse endothelial dysfunction and oxidative stress. Daily treatment with (PDE٥) inhibitors has beneficial effects on endothelial function in men with increased cardiovascular risk (Behr-Roussel et al., ٢٠٠٨).

The aim of the present work was to evaluate the possible mechanisms of the protective effect of sildenafil in MS induced by fructose in rats.

Materials & Methods
Animals: Adult male albino rats weighing ١٨٠-٢٠٠ g were used. They were allowed free access to standard laboratory food (El-Nasr Company, Abou-Zaabal, Cairo, Egypt) and water for one week before the experiment, as adaptation. All experimental protocols were approved by the board of the faculty of medicine, minia university.

Drugs & kits: Sildenafil (a generous gift from Pfizer, Egypt). Epitope **specific antibody to nitric oxide synthase** (iNOS), endothelial nitric oxide (eNOS) and caspase-٣ monoclonal mouse antibodies were purchased from Lab Vision Laboratories.

Experimental protocol: Fructose was added to drinking water (١٠٪) and also to rats chow diet (١٠٪) for ٦ weeks for induction of MS. Rats were divided into five groups: ١st group served as normal control, ٢nd group MS control group; ٣rd; ٤th and٥th groups treated by sildenafil (orally by gastric tube) in three ascending doses (٥ mg/kg/day, ١٠ mg/kglday,٤٠ mg/kg/day); respectively for six weeks.

The drug doses, routes of their administration as well as time of administration were selected on the basis of pilot study as well as according to the previously published studies (Csont et al., ١٩٩٨; Cunha et al., ٢٠١٠; Dussault et al., ٢٠٠٩; Habre et al., ٢٠١١; Khayyal et al., ٢٠٠٩; Schäfer et al., ٢٠٠٨; Rizzo et al., ٢٠١٠; Nalbant et al., ٢٠٠٦; Hermann et al., ٢٠٠٣; Padi et al., ٢٠٠٣; Kim et al., ٢٠٠٦).

After ٦ weeks, rats were weighed, anesthetized with ether. Blood samples were collected from neck viens by decapitations and centrifuged. Sera were separated and stored at -٨٠ °C for further assessments. Livers were rapidly dissected, weighed and prepared for immune-histochemical examinations or stored at -٨٠°C for further investigation.

Visceral fat index: Visceral fat (adipose tissue surrounding the abdominal and pelvic organs) were dissected and weighed. Visceral fat index was calculated according to the following equation: (visceral fat weight (g) / body weight (g)) x ١٠٠ (Hansen et al., ١٩٩٧).

Liver index: Liver index was calculated (liver weight/body weight)× ١٠٠ (Xu. et al., ٢٠٠٦).

Insulin resistance index: fasting glucose was determined after a ١٢ h fasting period. Blood glucose concentration from the tail vein was measured using the Active blood glucose meter (Roche, Mannheim, Germany). Enzyme-Linked Immunosorbent Assay (ELISA) was used for determination of fasting serum insulin (Grassi and Pradelles, ١٩٩١; Lu et al., ٢٠١٠). Insulin resistance was estimated according to the Homeostasis Model Assessment (HOMA-IR) which was calculated (the fasting concentrations of glucose (mg/dl) × insulin (μIU/ml) / ٤٠٥ (BleCastillo et al., ٢٠١٢).

Lipid profile: serum TG, total cholesterol and HDL (Spectrum, Egypt) were measured using an enzymatic colorimetric kits (Biodiagnostic, Giza, Egypt), Egypt).

Serum level of alanine amino transferase: serum levels of alanine amine transferase (ALT) were assayed spectrophotometrically using comercially available kits (Randox laboratories, UK).

Assessment of NO in the hepatic tissue: NO in form of nitrite was determined spectro-photo-metrically using Greiss reagent systems. NO_X was assayed by measuring nitrite ($NO_٢^-$) level, one of the stable end products of NO oxidation using Griess reagent, method described by Sogut et al., (٢٠٠٣).

Determination of serum level of tumor necrosis factor-alpha (TNF-α): TNF-α was measured using an ELISAKit (ID Labs Inc., Canada) according to manufacture instruction. It depends up on using wells coated with a polyclonal antibody specific for rat TNF-α. After incubation with the rat TNF-α antigen and a biotinylated polyclonal antibody and washing to remove the unbound enzyme, a substrate solution was added to induce a colored reaction product. The intensity of this colored product was directly proportional to the concentration of rat TNF- α present in the samples. The values were read at ٤٥٠ nm in an ELISA reader.

Oxidative stress parameters (lipid peroxides, reduced glutathione (GSH) and catalase): Lipid peroxides was determined using the thiobarbituric acid method described by Buege and Aust (١٩٧٨).The method depends on measuring the malondialdehyde (MDA) equi-valent substances which are breakdown products of lipid peroxides. The thiobarbituric-MDA adduct forms colored complexes when extracted with n-butanol/ pyridine; the absorbance of which is read at ٥٣٢ nm using Bausch & Lomb Spectronic ٢٠٠٠ spectro-photometer (Rochester, NY, USA). Reduced glutathione and catalase in serumwere measured using

colorimetric kits(Bio-diagnostic) acco-rding to the method described by Beutler et al. (١٩٦٣) and Aebi, (١٩٨٤) respectively.

Immunohistochemical assay of iNOS and eNOS in liver: immunehistochemistry was performed using induced nitric oxide synthase (iNOS), and endothelial nitric oxide (eNOS) monoclonal antibodies (Lab Vision Laboratories) according to the method described by Sun et al., (٢٠٠٩) and Ilker et al., (٢٠١٠).

Statistical analysis

Results were expressed as means ± standard error of mean (SEM). One-way analysis of variance (ANOVA) followed by the Tukey's test. P-values less than ٠.٠٥ were considered significant. Graph Pad Prism was used for statistical calculations (version ٥.٠٣ for Windows, Graphpad Software, San Diego CaliforniaUSA, www.graphpad.com).

Results

Effect of sildenafil on body weight, liver index, visceral fat index: There was no significant change in body weight between all groups. In MS group significance, there was a significant increase in liver index and visceral fat index compared to control group. Sildenafil (٥, ١٠ and ٤٠ mg/kg) significantly reduced liver index and visceral fat index compared to MS group (Table ١).

Effect of sildenafil on insulin resistance: There was a significant increase in fasting blood glucose, fasting serum insulin and HOMA-IR in MS group as compared to control group. Sildenafil (١٠ and ٤٠ mg/kg) significantly lowered the pervious parameters to near normal values (Table ٢)

Effect of sildenafil on triglycerides, high density lipoprotein, cholesterol and ALT: In MS group, there were significant increases in serum TG, cholesterol, and ALT and a significant decrease in serum HDL as compared to control group. Sildenafil (٤٠ mg/kg) significantly reduced serum TG, cholesterol and, ALT and significantly increased HDL (Table ٣).

Effects of sildenafil on serum levels of TNF-α, catalase, GSH and MDA: There was a significant increase in TNF-α in MS group as compared to control group. Sildenafil (١٠ and ٤٠ mg/kg) significantly reduced TNF-α level. There was a significant reduction in catalase and GSH as well as a significant increase in MDA in MS group compared to control group. Sildenafil, dose dependently improved the oxidative stress parameters (Table ٤).

Effect of sildenafil on NO and MDA in hepatic tissue: MS rats showed significant reduction of liver total nitrites (NO end product) and a significant increase in MDA compared to control group. Sildenafil (٥, ١٠ and
٤٠ mg/kg) significantly increased total to control group. Sildenafil (٥, ١٠ and ٤٠ mg/kg) significantly increased total nitrites and reduced MDA significantly compared to MS group (Table ٥).

Effect of sildenafil on Immunohistochemical assay of iNOS and eNOS in liver: eNOS immunereactivity in rat liver was strong in control group and weak in MS group. This was evident by the significant reduction in semi-quantitative scoring in MS group as compared to control group. Immunoreactivity of eNOS was weak in sildenafil (٥ mg/kg)-treated group, however, it was strong in both sildenafil (١٠ and ٤٠ mg/kg)-treated groups in which semi-quantitative scoring was significantly increased as compared to MS group (Table ٥, Fig. ١).

iNOS immunoreactivity in rat liver were weak in control group and strong in MS group. The semi-quantitative score showed that MS group was significantly higher as compared to control group. Strong iNOS immunoreactivity was noticed in sildenafil (٥ mg/kg)-treated group, however, it was weak in both sildenafil (١٠ and ٤٠ mg/kg)-treated groups in which semi-quantitative scoring was significantly reduced as compared to MS group (Table ٥, Fig. ٢)

Table (١): Effects of sildenafil on total body weight, liver index, visceral fat index

Group	Body weight (g)	Liver index	Visceral fat index
Control	٢٢٤ ± ٦.٧٢	١.٦٨ ± ٠.١٧	٠.١١ ± ٠.٠١
MS	٢٥٨ ± ١٤.١	٣.٦٣ ± ٠.١٨[a]	٢.٣٦ ± ٠.١٧[a]
Sil.٥	٢٢٦ ± ٥.٩٧	٢.٩١ ± ٠.١٢[a, b]	١.٠٤ ± ٠.١٩[a, b]
Sil.١٠	٢٢٤ ± ٣.٤٨	٢.٩٠ ± ٠.٠٨[a, b]	٠.٧٨ ± ٠.٠٥[b]
Sil.٤٠	٢٢٥ ± ٥.٢١	٢.٦٠ ± ٠.٠٩[a, b]	٠.٥٩ ± ٠.٠٥[b]

Values represent the mean ± SEM of observations from ٦ animals.
Control, control group; MS, metabolic syndrome non-treated group; Sil.٥, sildenafil (٥ mg/kg)-treated group; Sil.١٠, sildenafil (١٠ mg/kg)-treated group; Sil.٤٠, sildenafil (٤٠ mg/kg)-treated group.
[a]Significantly different from control group at $p < ٠.٠٥$,[b]significantly different from MS group at $p < ٠.٠٥$.

Table (٢): Effect of sildenafil on fasting blood glucose, fasting serum insulin, homeostasis assessment model of insulin resistance (HOMA-IR)

Group	Fasting blood glucose (mg%)	Fasting serum insulin (µIU/ml)	HOMA-IR
Control	١٠٣ ± ٤.٤٨	١٢.٤ ± ٠.٨٦	٣.١٦ ± ٠.٣٢
MS	١٣٩ ± ٤.٨٧[a]	٢٢.٨ ± ١.٨٤[a]	٧.٨٨ ± ٠.٨٣[a]
Sil.٥	١٢٩ ± ٣.١٢[a]	٢٢.٩ ± ٢.٠[a]	٧.٤٢ ± ٠.٧٢[a]
Sil.١٠	١١٧ ± ٣.٩٩[b]	١٢.٧ ± ١.٢٠[b, c]	٣.٧٤ ± ٠.٤٢[b, c]
Sil.٤٠	١٠٨ ± ٢.٨٠[b]	١٤.٠ ± ١.٠٧[b, c]	٣.٧٨ ± ٠.٤٤[b, c]

Values represent the mean ± SEM of observations from ٦ animals.
Control, control group; MS, metabolic syndrome non-treated group; Sil.٥, sildenafil (٥ mg/kg)-treated group; Sil.١٠, sildenafil (١٠ mg/kg)-treated group; Sil.٤٠, sildenafil (٤٠ mg/kg)-treated group. HOMA-IR: homeostasis assessment model of insulin resistance [a]Significantly different from control group at $p<٠.٠٥$, [b]significantly different from MS group at $p<٠.٠٥$,[c] significantly different from sil.٥ group at $p<٠.٠٥$.

Table (٣): Effect of sildenafil on triglycerides, high density lipoprotein, total cholesterol, Alanine aminotransferase enzyme (ALT).

Group	Triglycerides (mg/dl)	High density lipoprotein (mg/dl)	Total cholesterol (mg/dl)	(ALT) (U/l)
Control	٧٥.٣ ± ٣.٣٨	٣٧.٠ ± ٣.٢٦	١٣٠ ± ٤.٥٧	١٨.٣ ± ٢.٤٦
MS	١٨٦ ± ٧.٤٤[a]	١٥.٥ ± ١.٧٧[a]	٢٦٦ ± ٧.٧٩[a]	٥١.٣ ± ٤.٥٨[a]
Sil.٥	١٧٨ ± ٥.٢٧[a]	١٨.٣ ± ٢.٤٠[a]	٢٧٠ ± ١٤.٣[a]	٥١.٠ ± ٤.٤٣[a]
Sil.١٠	١٦٥ ± ٧.٨٤[a]	١٩.٨ ± ٣.٦٨[a]	٢٧٨ ± ١٣.٧[a]	٣٤.٢ ± ٣.٢٨[b,c]
Sil.٤٠	١٢٤ ± ٥.٣[abcd]	٣٨.٠ ± ٥.٨٢[b,c,d]	١٩٠ ± ١٢.٨[a,b,c,d]	٢٤.٦ ± ٣.٥٠[b,c]

Values represent the mean ± SEM of observations from ٦ animals.
Control, control non diseased group; MS, metabolic syndrome non-treated group; Sil.٥, sildenafil (٥ mg/kg)-treated group; Sil.١٠, sildenafil (١٠ mg/kg)-treated group; Sil.٤٠, sildenafil (٤٠ mg/kg)-treated group. [a]Significantly different from control group at $p < ٠.٠٥$,[b]significantly different from MS group at $p < ٠.٠٥$, [c]significantly different from sil.٥ group at $p < ٠.٠٥$, [d] significantly different from sil.١٠ group at $p < ٠.٠٥$.

Table (٤): Effects of sildenafil on serum levels of TNF-α, catalase, GSH, MDA

Group	TNF-α (pg/ml)	Catalase (U/dl)	GSH (mg/dl)	MDA(nmol /L)
Control	١١.٨ ± ١.٧١	٣٥.٢ ± ٤.٠٦	٥٣.٣ ± ٤.٨٠	٢٢٧ ± ١٩.٢
MS	٣١.٧ ± ١.١٦[a]	١٨.٤ ± ١.٠٢[a]	٢٨.٦ ± ٣.١١[a]	٣٣٤ ± ٢٤.٢[a]
Sil.٥	٢٦.٧ ± ٢.٣١[a]	٢٧.٩ ± ٣.١٩[b]	٤٧.٠ ± ٣.٦٥[b]	٢٣١ ± ٢٠.٩[b]
Sil.١٠	٢١.٠ ± ١.٨٦[a, b]	٢٨.٧ ± ٣.٧٦[b]	٥٢.٠ ± ٤.٤٩[b]	٢٠٧ ± ٩.٢٩[b]
Sil.٤٠	٢٠.٣ ± ١.٩٩[a, b]	٣٣.٦ ± ٢.٨٠[b]	٥٤.٧ ± ٣.٦٩[b]	٢٣٢ ± ٢٧.١[b]

Values represent the mean ± SEM of observations from ٦ animals.
Control, control non diseased group; MS, metabolic syndrome non-treated group; Sil.٥, sildenafil (٥ mg/kg)-treated group; Sil.١٠, sildenafil (١٠ mg/kg)-treated group; Sil.٤٠, sildenafil (٤٠ mg/kg)-treated group.[a]Significantly different from control group at $p < ٠.٠٥$,[b]significantly different from MS group at $p < ٠.٠٥$.

Table (٥): Effect of sildenafil on hepatic tissue content of NO, MDA, and immunohistochemical expression of iNOS, eNOS and caspase-٣

Group	*MDA (nmol /g tissue)*	*NO (nmol/g tissue)*	*iNOS expreesion*	*eNOS expression*
Control	٢٥.٤ ± ٢.٠٩	١٢٣ ± ٦.٣٦	١.٢٥ ± ٠.١٦	٣.٢٠ ± ٠.٣٧
MS	٤٨.٢ ± ٣.٥٥[a]	٨٧.٢ ± ٥.٠٥[a]	٣.٨٨ ±٠.١٣[a]	١.٢٥ ± ٠.٢٥[a]
Sil.٥	٣٢.٠ ± ٢.٩٥[b]	١٢٣ ± ٧.٣٤[b]	٣.٥٠ ± ٠.١٩[a]	١.٦٠ ± ٠.٢٤[a]
Sil.١٠	٣١.٦ ± ٢.٩٩[b]	١٢٨ ± ٤.٢٤[b]	٢.٢٥ ± ٠.٢٥[a,,b]	٢.٧٥ ± ٠.٢٨[b]
Sil.٤٠	٢٨.٧ ± ٤.٨٧[b]	١٢٤ ± ١٠.٩[b]	٢.٠٠ ± ٠.٢٧[b,c]	٢.٦٠ ± ٠.٤٠[b]

Values represent the mean ± SEM of observations from ٦ animals.
Control, control non diseased group; MS, metabolic syndrome non-treated group; Sil.٥, sildenafil (٥ mg/kg)-treated group; Sil.١٠, sildenafil (١٠ mg/kg)-treated group; Sil.٤٠, sildenafil (٤٠ mg/kg)-treated group. [a]Significantly different from control group at $p < ٠.٠٥$,[b]significantly different from MS group at $p < ٠.٠٥$, [c] significantly different from sil.٥ group at $p < ٠.٠٥$.
Figure captions

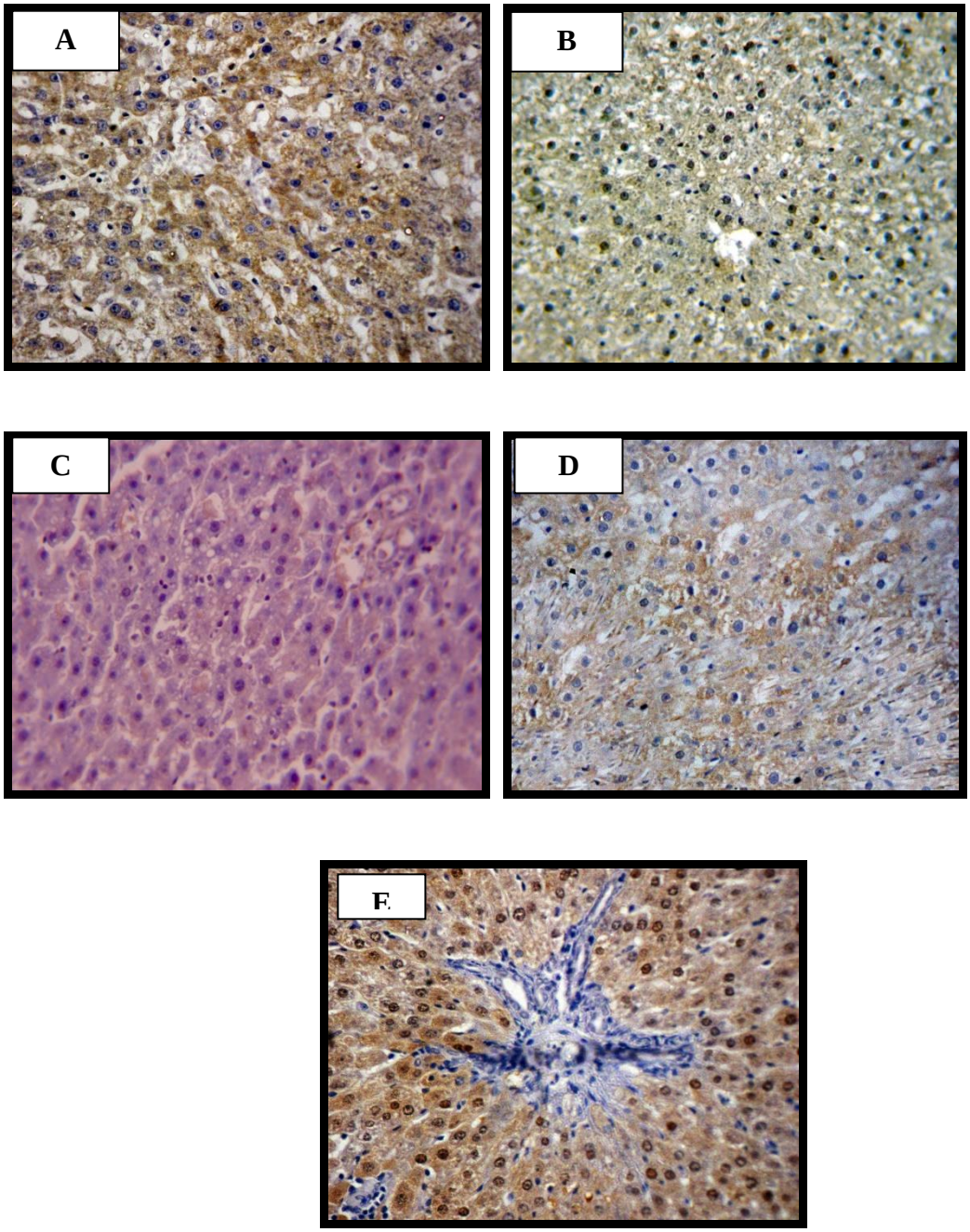

Figure (١): Effect of sildenafil on hepatic eNOS immunohistochemical staining in fructose fed rats

A, control group (the staining was strong); B, Metabolic syndrome non treated group (the staining was weak); C, sildenafil (٥ mg/kg/day)-treated group the staining was weak); D, sildenafil (١٠ mg/kg/day)-treated group (the staining was strong); E, sildenafil (٤٠ mg/kg/day)-treated group(the staining was strong.

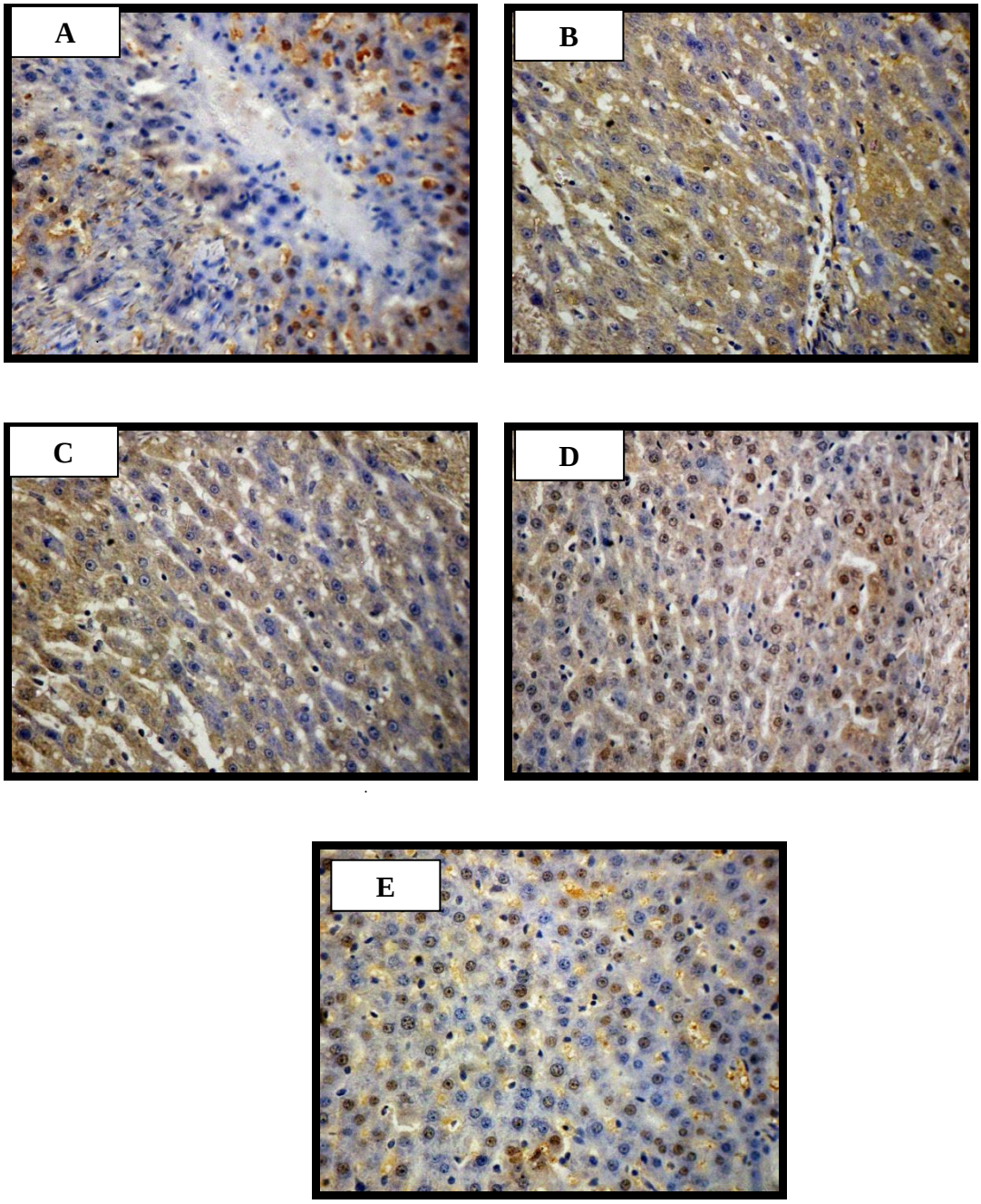

Figure (٢): Effect of sildenafil on hepatic iNOS immunohistochemical staining in fructose fed rats

A, control non diseased group (the stain was weak); B, Metabolic syndrome non-treated group (the stain was strong); C, sildenafil (٥ mg/kg/day)-treated group; D, sildenafil (١٠ mg/kg/day)-treated group(the stain was weak); E, sildenafil (٤٠ mg/kg/day)-treated group (the stain was weak).

Discussion

In the present study, fructose feeding caused development of MS as indicated by significant increase in fasting blood glucose, fasting serum insulin and HOMA-IR value which indicate development of insulin resis-tance (Borai et al., ٢٠١١).

Chronic exposure to fructose seems to indirectly cause hyperinsulinemia and obesity. Insulin receptor mRNA, and subsequent insulin receptor numbers in skeletal muscle and liver were reported to be significantly

lower in fructose fed rats compared to rats fed a standard chow diet (Catena et al., ٢٠٠٣).

Furthermore, disturbance in glucose transporters-٥ (GLUT٥) which is also a fructose transporter causing marked insulin resistance, implying a possible role of GLUT٥ receptors in the pathology of MS associated with fructose feeding and insulin resistance (Litherland et al., ٢٠٠٤).

Fructose-induced MS can be explained by the hexosamine hypothesis, where hexosamine flux is thought to regulate glucose and satiety-sensing pathways. With over-expression of glutamine: fructose-٦-phosphate amidotrans--ferase, the key regulatory enzyme in hexosamine synthesis, the liver produces excess fatty acids, skeletal muscle becomes insulin resistant, and hyperinsulinemia results. This pathway of excess hexosamine flux leads to long-term storage of energy, and eventually obesity and insulin resistance. (McClain, ٢٠٠٢).

The results of the current study showed that sildenafil reduced fasting insulin and fasting glucose levels and improved HOMA-IR which is a sensitive indicator of insulin resistance. This result is in agreement with Oudot et al., (٢٠١٠) who reported that sildenafil administration corrected hyperglycemia and hyper-insulinemia. In the same time, these results are in accordance with Rey Valzacchi et al. (٢٠١١) who reported that sildenafil co-administration significantly reduced HOMA-IR and fasting glucose level as compared to metformin alone.

In the MS group, there was a significant increase in serum TG, cholesterol and significant decrease in serum HDL as compared to control group. The exposure of the liver to large quantities of fructose leads to rapid stimulation of lipogenesis and TG accumulation, which in turn contributes to reduced insulin sensitivity and hepatic insulin resistance/ glucose intolerance. Because of its lipogenic properties, excess fructose in the diet can cause glucose and fructose malabsorption, and greater elevations in TG and cholesterol compared to other carbohydrates. It is believed that the ability of the liver to metabolize high doses of fructose is responsible for the disruption in energy stores (Daly et al., ١٩٩٧). Fructose-induced insulin resistance is consistent with the increased TG, very low density lipo-protein (VLDL) secretion, and atherosclerosis associated with chronic fructose feeding. Sildenafil in high dose (٤٠ mg/kg) significantly lowered triglycerides. This effect is mostly through antagonizing insulin resistance which is strongly linked to elevation of triglycerides induced by fructose administration (Czyzewska et al., ٢٠١٠)

In the presence of hyper-triglyceridemia, accelerated cholesterol ester transfer protein (CETP)-mediated lipid transfer generates TG-enriched HDL particles. This enhances HDL catabolism mediated by hepatic lipase and endothelial lipase which explained the reduction of HDL in FFR group. Similarly, reduction in triglycerides may explain the elevation of HDL noticed with sildenafil ٤٠mg/kg (Czyzewska et al., ٢٠١٠).

In the current work, fructose increased serum cholesterol which is in agreement with previous results of Mahmoud et al., (٢٠١٢). Sildenafil (٤٠ mg/kg) reduced serum cholesterol which can be attributed to improvement of insulin responsiveness affecting SREBP expression, which is responsible for regulating fatty acid and cholesterol biosynthesis by activating many enzymes involved in cholesterol biosynthetic pathways, such as HMG-CoA reductase (Brown and Goldstein, ١٩٩٧) and FAS (Bennet et al., ١٩٩٥**).** Expression of SREBP is enhanced by insulin in all three major insulin target tissues, liver, fat, and skeletal muscle (Kim et al., ١٩٩٨). Similarly, levels of SREBP are enhanced in the presence of hyper-insulinemia (Shimomura et al., ١٩٩٩). Fructose caused a gradual extended increase in SREBP activity (Matsuzaka et al., ٢٠٠٤).

In fructose fed rats, fructose markedly elevated liver and visceral fat indices. This can be explained by that lipid accumulation in liver and visceral adipose tissue is linked to insulin resistance and is linked also to hyper-triglyceridemia. Each of these risk factors can occur as a result of excessive fructose consumption. Visceral adiposity is known to be increased by fructose ingestion. It is associated with insulin resistance as a result of

the direct delivery of portal blood flow from visceral fat to the liver releasing free faty acids (FFAs). The greater lipolytic capacity of visceral than peripheral adipocytes releases more FFAs to the portal circulation. Furthermore, when visceral adipocytes enlarge, they become more insulin resistant than smaller adipocytes. Increased amounts of FFAs directly affect insulin signaling, diminish glucose uptake in muscle, and induce gluconeogenesis in the liver (El Mesallamy et al., ٢٠١٠).

Increased FFA release from adipose tissue or failure of FFA using tissues to remove them normally, lead to increased TG and FFA fluxes. Increased delivery of FFA to muscle reduces muscle glucose uptake and utilization by substrate competition or direct inhibition of glucose transport. Intracellular TG have been involved in beta cell failure which is called lipotoxicity phenomena. The rate of FFA to the liver is a major determinant of hepatic TG secretion. So the regulation of FFA distribution between FFA using tissues and the partition of FFA between storage and oxidation could be involved in the development of insulin resistance (Ziegler et al., ٢٠٠١).

Sildenafil (١٠ and ٤٠ mg/kg) lowered liver and visceral fat indices. This result is supported by Nseir et al., (٢٠١٠) who linked hyper-triglyceridemia, insulin resistance and inflammatory status with liver and abdominal fat accumulation and the former parameters were improved by sildenafil. High-fructose diets have induced fatty liver in rats and together with increases in hepatic lipid peroxidation and activation of inflammatory pathways in the liver of rats. The long-term administration of fructose to rats results in hepatic macro- and micro vesicular steatosis with a increase in hepatic triglycerides and an increase in hepatic cholesterol concentration.

In MS rats, there was a rise in ALT that reflects the liver damage. This finding is in agreement with previous work which reported elevation of ALT with fatty liver that recently considered a component of MS (Hamad et al., ٢٠١١).

TNF-α which is a marker of inflammation was increased in MS rat. This may play a role in liver injury and is correlated with the activation of hepatocyte apoptosis (Wang et al., ٢٠٠٣). Liver steatosis is a potential contributor to the low-grade inflammation associated with the MS (Gong et al., ٢٠١٢). Sildenafil, dose depentanlty, reduced ALT, and TNF-α serum levels. Cadrici et al. (٢٠١١) reported that sildenafil reduced serum TNF-α. Reduction in serum TNF-α has been linked to hepatic protection in several studies (Gong et al., ٢٠١٢; Kerner et al., ٢٠٠٥; Wang et al., ٢٠٠٣).

Excessive fructose intake was associated with an increase in oxidative stress. The increased production of MDA, the reduction of catalase activity and reduced glutathione in serum detected in the current work were supported by the findings of Ozdogan and co-workers (٢٠١٢). In the present results, fructose increased liver MDA and reduced liver nitrites denoting increased oxidative stress in this organ. This in agreement with Kostogrys and co-workers (٢٠١٠) and Pooranaperundevi and co-workers (٢٠١٠) who reported that MDA concentrations were significantly increased while nitrites were reduced in liver of fructose fed rat. Sildenafil increased serum GSH and reduced serum MDA. The antioxidant effects of sildenafil has been previously reported (Cadrici et., ٢٠١١; El-Far et al., ٢٠٠٩; Lee et al., ٢٠١٠; Luo et al. ٢٠١١).

In the present study, sildenafil increased hepatic tissue content of NO. NO is a key molecule with diverse functions including liver injury (Koeppel et al., ٢٠٠٧). Increased eNOS expression in hepatic tissue may explain the ability of sildenafil to increase NO content. At the same time reduction of iNOS is linked to antioxidant effects (Sahnon et al., ١٩٩٨).

In conclusion, sildenafil was shown to be protective against MS as evidenced by improving lipid profile, insulin resistance, visceral fat index and liver index. Such protection was associated with reduction of oxidative stress and TNF-α as well as modulation of eNOS and iNOS expression in hepatic tissue. These findings implying that, the most proposed mechanism of this

protective effect is through anti-oxidant, anti-inflammatory actions, and modulation of NO.

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