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Modulation activity of Vildagliptin on Hepatic Complications and Lipoprotein Abnormalities Associated with Insulin Resistance in Rats

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Abstract

Several predisposing factors have been incorporated into the worldwide increased prevalence of insulin resistance (IR), among them high caloric intake, sedentary lifestyle, aging, as well as genetic factors. Vildagliptin (VIL) is an oral hypoglycemic agent belonging to the dipeptidyl peptidase-4 inhibitor family. It has been reported to show multi-functional biological activities beyond its anti-hyperglycemic effect. The objective of the current study is to investigate the modulatory effect of VIL on hepatic complications, oxidative stress and lipoprotein abnormalities associated with IR in rats. In the current study, we have induced IR in rats through the combined administration of (10%) fructose in the drinking water with high-fat diet (HFFD) for 8 consecutive weeks. Animals were randomly allocated into three groups, the normal group, the HFFD, as well as the HFFD treated orally with VIL (10 mg/kg) for 8 consecutive weeks. Bodyweight indices, metabolic alterations, lipoprotein abnormalities, and oxidative stress biomarkers were markedly attenuated after VIL treatment. Furthermore, VIL significantly decreased the inflammatory response via inhibiting the nuclear factor-kappa B pathway. Moreover, VIL succeeded to ameliorate the hepatic DNA parameters. This study depicts that the oral daily treatment of the HFFD with VIL exerted a new modulatory activity on the hepatic complications and the lipoprotein abnormalities associated with IR through its complementary antioxidant, anti-inflammatory, and anti-hyperlipidemic effects.

Keywords: Insulin resistance; Vildagliptin, HFFD; hepatic complications; lipoprotein abnormalities; nuclear factor-kappa B; DNA Parameters.

Introduction:

The global prevalence of the metabolic syndrome is rapidly rising at an alarming rate, due to the wide incidence of insulin resistance (IR) that implicated in deficits in the biological response to endogenous or exogenous insulin **(Bautista et al., 2019)**.

IR is usually related to various physiological abnormalities including alterations in the levels of triglycerides (TG) and total cholesterol (TC); high and low-density lipoprotein cholesterol **(Amos, 2019)**. IR is found chiefly in patients with non-alcoholic fatty liver disease and nonalcoholic steatohepatitis (NASH) leading to hepatic cirrhosis and consequently hepatocellular carcinoma **(Lonardo et al., 2019)**. Hyperglycaemia attributed to peripheral IR, is responsible for elevated levels of free fatty acids within hepatocytes of NASH. In addition; Peripheral IR is implicated in hyperinsulinemia, preventing formation of very low-density lipoproteins. Moreover, IR is associated with inhibition β-oxidation of free fatty acids and promotion of lipogenesis **(Bessone et al., 2019)**.

Inflammatory mediators such as tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6), can trigger the nuclear factor-kappa B **(**NF-κB) pathway to establish a vicious cycle of chronic inflammation leading to induction of IR **(Baker et al., 2011)**. High fructose/ high fat diet (HFFD) is the model of choice that mimics the pathophysiological changes parallel to human IR. Where it disturbs lipid and glucose metabolism leading to marked metabolic deficits such as NASH and IR **(Tilg and Moschen, 2008)**. Moreover; it has

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been documented that HFFD is responsible for creating a redox imbalance due to significant generation and release of free radicals **(Lozano et al., 2016)**. Furthermore, HFFD model could be used to evaluate the efficacy of several drugs or compounds for the therapy of metabolic deficits **(Elmazar et al., 2013)**. Due to the several drawbacks of the current therapeutic treatment for metabolic syndrome especially metformin , such as high cost, daily high doses, GI disturbances, short term glycemic control and build –up of lactic acidosis . In addition, Metformin is contraindicated in patients with mild to moderate impairment in hepato-renal disorders. Thus, urgent necessities for exploring new therapeutic strategies are being mandatory. Vildagliptin (VIL), is the most promising drug in the treatment of metabolic syndrome due to its beneficial effects in preserving the pancreatic beta cell mass and function to enhance insulin secretion and differentiation of pancreatic beta-cells **(Buteau, 2008)**. Therefore, this study was conducted to evaluate the possible beneficial effect of VIL on IR induced by HFFD in drinking water for 8 weeks in rats. To achieve this aim; Percent change in body weight and hepatic weight/ body weight ratio were evaluated. Metabolic parameters and hepatic damage were assessed by serum hepatic biochemical parameters, elevated serum TG and TC levels. Similarly, oxidative stress biomarkers; which are due to an imbalance in redox homeostasis. Moreover, pancreatic damage which reflects the severity of hepatic injury is evidenced by inflammatory cytokines. Our study was also extended to examine the DNA parameters of hepatic and pancreatic tissues.

1. Materials and Methods

2.1 Experimental Animals

Juvenile male albino rats weighing 100–120 g were obtained from the Animal House Colony of the National Research Centre, (Dokki, and Giza, Egypt), housed under optimum laboratory conditions. Juvenile albino rats were provided with standard basal diet ad libitum with free access to water. The experiment was conducted in accordance with the ethical rules for standard experimental animal studies and the Medical Research Ethics Committee (MREC) of the National Research Centre under approval number (13110).

2.2 Drugs and Chemicals

Vildagliptin (VIL, purity 100%) and cholesterol powder were purchased from Sigma–Aldrich (St. Louis, Missouri, USA). Citric acid, fructose and diagnostic kits were obtained from El-Gomhouria Pharmaceutical Company and highly valuable commercial suppliers. (Cairo, Egypt).

Induction of insulin resistance

The initial weight of each rat was recorded then induction of IR via combining fructose (10%) in the drinking water with high fat diet (HFFD) for 8 weeks (composed of 14% lard , 1% cholesterol powder, 21% protein, 60% carbohydrates, 3% fibers in addition to 1% minerals and vitamins **(Axelsen et al., 2010).**

2.3 Experimental design

Thirty male Wistar albino rats, weighing 100-120 g, were divided into 3 groups (10 rat / group). Group I serves as a negative control group containing normal rats .Group II serves as positive group receiving HFFD for 8 consecutive weeks **(Axelsen et al., 2010)**. Group III receiving HFFD were treated orally with VIL (10 mg/kg per day) for 8 consecutive weeks **(Eom et al., 2016)**. Rats were weighed and blood samples were collected from the retro-orbital plexus of ether-anesthetized animals 24 hours after the last dose of the drugs. Sera was centrifuged at 3000 rpm at 4°C for 20 min for further metabolic and biochemical parameters.

- Serum biochemical assessment

The concentration of serum glucose was measured using glucose oxidase method, colorimetrically at 505 nm **(Trinder 1969)**. Serum insulin level was determined by Enzyme Immunoassay (EIA) kit **(Grassi and Pradelles 1991)**. Calculation of Homeostatic Model Assessment–Insulin Resistance (HOMA-IR) was evaluated via: HOMA-IR = fasting glucose value (mg/dl) × fasting insulin value (μU/ml)/405 **(Matthews et al. 1985).** Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) were measured colorimetrically at 510 nm according to the methods of Reitman and Frankel (1957). Similarly, serum levels of total cholesterol (TC) and triglycerides (TG) were estimated at 505 nm using a colormetric kit **(Richmond, 1973, Fossati and Prencipe, 1982) .**

Immediately after blood sampling, rats were sacrificed and portion of liver tissue was weighted and homogenized with ice-cooled PBS to prepare 20% w/v homogenate for assessment of TG **(Fossati and Prencipe, 1982)**, TC **(Richmond, 1973)** colorimetrically at 505 nm (492- 550 nm). Hepatic levels of lipid peroxides were estimated at 532 nm as thiobarbituric acid-reactive substances (TBARS) according to the method of Mihara and Uchiyama (1978) **(Bulaj et al. 1998; Ellman 1959).** Moreover, hepatic levels of reduced glutathione (GSH) were estimated colorimetrically at 412 nm using the methods of Ruiz-Larrea et al. (1994), depending on a reaction occurs between protein and non-protein thiol (–SH) groups (mainly GSH) react with ellman's reagent forming a stable

yellow color. In addition, hepatic levels of nitric oxide (NO) and super oxide dismutase were measured colormetrically (**Marklund and Marklund, 1974)**. Moreover, hepatic levels of TNF-alpha, IL-6 and NF-κB were determined with an enzyme-linked immunosorbent assay (ELISA) using a test reagent kit (Raybiotech) **(Akira et al., 1990, Bonavida, 1991)**. In addition, the DNA parameters of hepatic and pancreatic tissues were analyzed using the Leica Qwin 500 Image Analyzer LEICA Imaging Systems Ltd, Cambridge, England **(Li et al., 1995)**.

Statistical analysis

The results are expressed as mean ± SEM of six rats, and all measurable comparisons were made by using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. The data were analyzed with GraphPad Prism v. 8.0 (GraphPad Software, Inc., CA, USA). Difference was reported significant when p value is ≤ 0.05.

3. Results

3.1. Effect of VIL on body weight and hepatic weight/ body weight ratio of HFFD -induced IR in rats.

HFFD was associated with an increment in percent change in body weight and liver weight to body weight ratio reaching about 165% and 224% of the normal value, respectively. Oral treatment of HFFD with VIL (10 mg/kg) for 8 consecutive weeks showed a decrease in percent change in body weight and liver weight to body weight ratio reaching about 118% and 137% of the normal value, respectively (table 1).

3.2. Effect of VIL on metabolic parameters in HFFD -induced IR in rats.

Administration of highly saturated fats to the diet and high fructose (10%) to drinking water of rats was associated with elevated levels in the fasting serum glucose, insulin levels and HOMA-IR values reaching about 223%,7 fold and 20 fold of the normal value, respectively. Oral treatment of HFFD with VIL (10 mg/kg) for 8 consecutive weeks showed a decrease in fasting serum glucose, insulin levels and HOMA- IR values reaching about 135%, 191%, respectively and normalization in HOMA values (figure 1) .

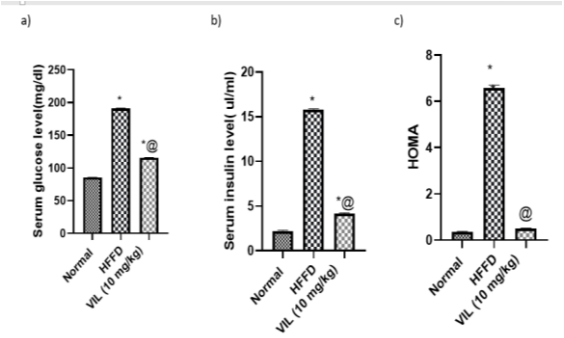

Figure (1): Effect of VIL on fasting serum glucose, insulin levels and HOMA values in HFFD -induced IR in rats

IR was rendered in rats by feeding high fructose (10%) with high fat diet (HFFD) for 8 consecutive weeks. oral treatment of HFFD with VIL (10 mg/kg; P.O) for 8 consecutive weeks. Twenty four hours after the last treatment, fasting serum glucose and insulin levels, in addition, HOMA values were evaluated. Results are expressed as mean ±SEM (n=6-8). *Significant difference from normal group *P<0.05*. @ Significant difference from group receiving HFFD *P<0.05*.

Table 1: Effect of VIL on percent change in body weight, hepatic weight / body weight ratio, hepatic lipid values and oxidative stress biomarkers in HFFD induced IR in rats

Groups	Percent change in body weight(g)	Hepatic weight/ body weight ratio	Hepatic TG (mg/g tissue)	Hepatic TC (mg/g tissue)	Hepatic GSH (nmol/g)	Hepatic MDA (nmol/g)	Hepatic NO (nmol/g)
Normal	127.3±2.71	2.76±0.05	79±0.26	24.88±0.03	32.88±0.03	90.66±0.17	6.86± 0.03
HFFD	210.8±1.65*	6.19±0.25*	121.5±0.51*	59.68±0.19*	10.81±0.09*	226.5±0.19*	45.15 ±0.25*
VIL (10mg/kg)	150.7±2.72*@	3.78±0.06*@	86.63±0.88*@	35.31±0.20*@	33.88±0.03@	91.7±0.02@	11.08 ±0.28*@

IR was rendered in rats by combining high fat diet with high fructose (10%) in drinking water for 8 consecutive weeks. Vildagliptin (VIL; 10 mg/kg; P.O) was administered orally for 8 consecutive weeks. Twenty-four hours after the last treatment, percent change in body weight, hepatic weight / body weight ratio; hepatic levels of TG, TC, GSH, MDA and NO were evaluated. Results are expressed as mean ±SEM (n=6-8). *Significant difference from normal group *P<0.05*. @ Significant difference from group receiving HFFD *P<0.05*.

3.3. Effect of VIL on serum AST and ALT values in HFFD -induced IR in rats.

Induction of IR with HFFD in rats was associated with elevated levels in serum hepatic enzyme AST and ALT values reaching about 4 fold and 3 fold of the normal value, respectively. Oral treatment of HFFD with VIL (10 mg/kg) for 8 consecutive weeks showed a decrease in serum hepatic enzyme AST and ALT values reaching about 109 % and 122 % of the normal value, respectively (figure 2) ..

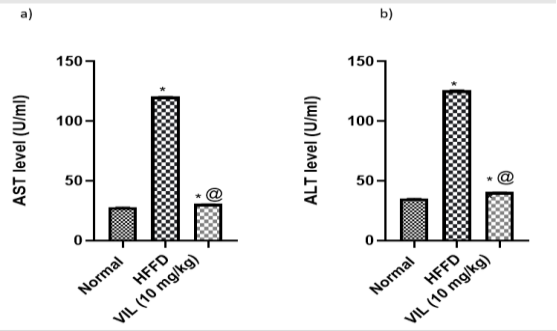

Figure (2): Effect of VIL on serum levels of AST and ALT in HFFD -induced IR in rats

IR was rendered in rats by feeding high fructose (10%) with high fat diet (HFFD) for 8 consecutive weeks. oral treatment of HFFD with VIL (10 mg/kg; P.O) for 8 consecutive weeks. Twenty four hours after the last treatment, serum levels of AST and ALT were evaluated. Results are expressed as mean ±SEM (n=6-8). *Significant difference from normal group *P<0.05*. @ Significant difference from group receiving HFFD *P<0.05*.

3.4. Effect of VIL on hepatic lipid values and oxidative stress biomarkers in HFFD -induced IR in rats

IR was rendered in rats by intake of high fat diet with high fructose 10% in drinking water for 8 consecutive weeks was associated with an increase in hepatic TG, TC, MDA and NO levels reaching about 153%, 239 %, 249% and 6 folds of the normal value, respectively. In addition, a decrease in hepatic GSH level reaching about 32% of the normal value. Oral treatment of HFFD with VIL (10mg/kg) for 8 consecutive weeks was associated with a decrease in hepatic TG, TC and NO levels reaching about 109%, 141 % and 161% of the normal value, respectively with normalization in hepatic GSH and MDA levels (table 1) .

3.5. Effect of VIL on hepatic inflammatory cytokines in HFFD -induced IR in rats

Rats received HFFD-induced IR was associated with an increase in hepatic TNF-α, IL-6 and NF-κB levels reaching about 4 folds, 5 folds and 4 folds of the normal value, respectively. Oral treatment of HFFD with VIL (10mg/kg) was associated with normalization of the fore mentioned parameters (table 2)

3.6. Effect of VIL on hepatic DNA parameters in HFFD -induced IR in rats

Liver isolated from rats receiving HFFD showed high proliferation index with high mean nuclear area. While rats receiving HFFD and treated orally with VIL (10 mg/kg) for 8 consecutive weeks showed normal mean nuclear area and moderate proliferation index close to that of the normal control group (figure 3).

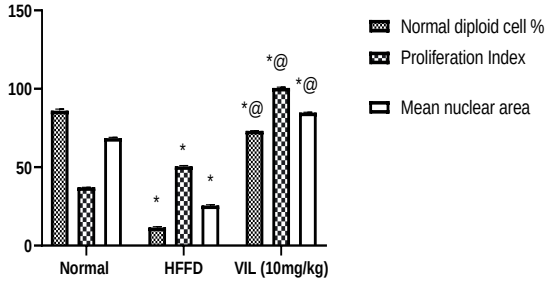

Figure (3): Effect of VIL on hepatic DNA parameters in HFFD -induced IR in rats

Table 2: Effect of VIL on hepatic inflammatory cytokines in HFFD -induced IR in rats

Groups	TNF-α (pg/g tissue)	IL-6 (pg/g tissue)	NF-κB (pg/g tissue)
Normal	9.32± 0.13	8.65 ±0.15	18.17 0±.26
HFFD	39.80± 0.22*	45.43± 0.13*	85.51± 0.43*
VIL (10mg/kg)	10.92± 0.14*@	9.03± 0.13*@	19.01± 0.23*@

IR was rendered in rats by combining high fat diet with high fructose (10%) in drinking water for 8 consecutive weeks. Vildagliptin (VIL; 10 mg/kg; P.O) was administered orally for 8 consecutive weeks. Twenty-four hours after the last treatment, TNF-α, IL-6 and NF-κB were evaluated. Results are expressed as mean ±SEM (n=6-8). *Significant difference from normal group *P<0.05*. @ Significant difference from group receiving HFFD *P<0.05*.

IR was rendered in rats by feeding high fructose (10%) with high fat diet (HFFD) for 8 consecutive weeks. oral treatment of HFFD with VIL (10 mg/kg; P.O) for 8 consecutive weeks. Twenty four hours after the last treatment, hepatic DNA parameters including normal diploid cell, proliferation index and mean nuclear area were evaluated. Results are expressed as mean ±SEM (n=6-8). *Significant difference from normal group *P<0.05*. @ Significant difference from group receiving HFFD *P<0.05*.

3.7. Effect of VIL on mean pancreatic islet area in HFFD -induced IR in rats

The mean pancreatic area in rats receiving HFHF is less than half of the normal control group. While it normalized in rats receiving HFFD and treated orally with VIL (10 mg/kg) for 8 consecutive weeks (figure 4).

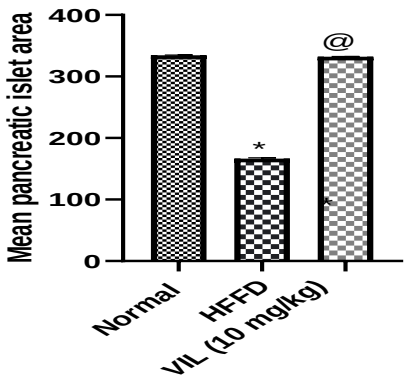

Figure (4): Effect of VIL on mean pancreatic islet area in HFFD -induced IR in rats

IR was rendered in rats by feeding high fructose (10%) with high fat diet (HFFD) for 8 consecutive weeks. oral treatment of HFFD with VIL (10 mg/kg; P.O) for 8 consecutive weeks. Twenty four hours after the last treatment, mean pancreatic islet area was evaluated. Results are expressed as mean ±SEM (n=6-8). *Significant difference from normal group *P<0.05*. @ Significant difference from group receiving HFFD *P<0.05*.

4. Discussion

Insufficient exercise and high caloric foods are the key predisposing factors for fat deposition, obesity and metabolic syndrome. IR is the main characteristic feature of metabolic syndrome that markedly increases the potential of cardiovascular diseases such as atherosclerosis **(Schmoyer and Siddiqui, 2017)**. The combined administration of high fructose intake with high fat diet (HFFD) is a reliable satisfactory model for experimentally induced-IR in rats. Our study showed that HFFD caused marked increase in percent change in body weight, liver weight to body weight ratio, as well as a marked elevation in the fasting serum glucose and insulin. Moreover, the induction of IR was evidenced by high HOMA-IR values. These effects could be attributed to the increased rate of gluconeogenesis and the reduced ability of insulin to suppress glucose production. Oral treatment of HFFD induced IR for 8 consecutive weeks with VIL (10 mg/kg per day) was associated with a marked decrease in percent change in body weight, liver weight to body weight ratio, and a marked decrease in serum glucose and insulin levels. **Hocher et al., (2012)** have attributed these effects to the ability of VIL to stimulate the pancreatic incretin hormone leading to the release of more insulin.

Excessive consumption of HFFD for 8 consecutive weeks was associated with several lipoprotein abnormalities such as hypertriglyceridemia and dyslipidemia **(Morsy et al., 2016)**. Our study revealed that HFFD was associated with excessive accumulation of TG and TC in the liver, thus showing the relationship between HFFD induced- IR and its effect on hepatic tissue **(Lozanoet al., 2016)**. Oral treatment with VIL succeeded to decrease TG and TC levels due to its ability to decrease the availability of fatty acids for oxidation. In addition, VIL is able to reduce absorption of dietary cholesterol from the small intestine with modulation of cholesterol metabolism **(Jia et al., 2019)**.

HFFD could be the main predisposing factor related to induction of hepatic biomarkers chiefly, AST and ALT that are considered intracellular indicative enzymes for evaluating not only hepatic damage but also, hepatic IR and metabolic syndrome **(Wahlang et al., 2019)**. Oral treatment of HFFD induced IR with VIL (10 mg/kg) for 8 consecutive weeks showed a prominent modulation in AST and ALT serum levels. Hepatocytes have a unique antioxidant cellular defenses strategy to overcome presence of reactive oxygen species (ROS) that are implicated in tissue injury, NASH and subsequently cell death **(Engel et al., 2019)**. HFFD model is one of the highly valuable predisposing factors that responsible for an increment in ROS and lipid peroxidation products leading to exhaustion of cellular defenses strategy **(Şahin et al., 2019)**. The findings of this study are in agreement with other investigations that reported that HFFD was associated with marked elevation in lipid peroxidation and significant reduction in antioxidant hepatic biomarkers **(Costa et al., 2019)**. Our treatment with VIL (10mg/kg) for 8 consecutive weeks succeeded to restore the antioxidant biomarkers, especially hepatic levels of GSH and MDA, thus decreasing oxidative stress and its complications. In addition, it has been postulated that excessive release of NO in HFFD may participate critically in β-cells lysis **(Haluzik and Nedvidkova, 2000)**. In the present study, VIL could significantly decrease hepatic NO levels and modulate the balance between oxidants and

antioxidants in hepatic tissue of HFFD induced IR in rats. The pronounced reduction in oxidative stress biomarkers and lipid peroxidation products as a result of supplementation with VIL as shown in the present study confirm the promising effect of VIL as antioxidant and scavenger of reactive intermediates, preserving cellular glutathione reservoirs**.** Besides, HFFD induced- IR is associated with an increment in the classical macrophages, that activated mainly within the adipose tissue leading to release of various pro-inflammatory cytokines (TNF-α, IL-6, and NF-κB) **(Korver et al., 2019)**. NF-κB is a key regulator of the inflammatory process and an intracellular target for hyperglycaemia and hyperlipidaemia, where abnormal triggering of NF-κB signaling pathway occurs in liver tissue leading to significant alterations in insulin signalling pathway, thus, IR occurs (Cai et al., 2005). Significant reduction of pro-inflammatory cytokines (TNF-α, IL-6, and NF-κB) as a result of supplementation with VIL (10mg/kg) for treatment of HFFD induced-IR, revealing the anti-inflammatory role of VIL in modulation of hepatic complications related with IR.

Finally, HFFD induced IR is associated with DNA damage, thus DNA cytometry is reliable marker of malignancy for abnormal DNA **(Paneni, 2014)**. Rats receiving HFFD and treated with VIL (10 mg/kg) for 8 consecutive weeks showed high proliferating index with normal mean nuclear area, Moreover, normal mean pancreatic area, indicating significant regeneration in the functions of hepatic and pancreatic tissues.

5. Conclusion

In the light of the obtained data, Using VIL as daily supplement could depict new intervention in modulation of hepatic complications and lipoprotein abnormalities associated with IR.

6. Conflict of Interest

The authors declare there were no conflicts of interest.

7. Funding

NA

8. Acknowledgments

NA

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