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A Comparative Study on the Effects of Incretin and Metformin on sugar Profile and Insulin Resistance in STZ-induced Diabetic Wistar Rats.

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ABSTRACT

Type 2 diabetes is a common and increasing global health problem. The purpose of this study was to comparatively evaluate the effects of metformin and Sitagliptinas anti-diabetic drugs on the sugar profile and insulin resistance in STZ-induced diabetic Wistar albino rats. In this study, 24 wistar albino rat neonates (9 ± 2 gr), were randomly divided in to 4 groups: The control (n=6), untreated diabetic (n=6), diabetic treated by metformin (n=6) and treated by sitagliptin (n=6). STZ was induced diabetic groups by intraperitoneal injection (i.p) at a dose of 90 mg/kg body weight. After induction of diabetes, rats were kept 8 weeks under the same conditions then enrolled. Group 3 were gavaged by 150 mg/kg/day dose of metformin and group 4 were gavaged by 100 mg/kg/day dose of sitagliptin for one month. At the end, blood was taken from each rat and percentage of glycated hemoglobin (HbA1C%) in the blood, serum levels of fasting blood sugar (FBS), fasting insulin and index of insulin resistance (HOMA-IR) were measured. The obtained quantitative data were finally analyzed through ANOVA and posthoc test between the groups under examination at significance level of P≤0.05. In diabetic rats, metformin therapy reduced blood concentration of HbA1C (-1.44 ± 0.28%), serum concentration of FBS (-139 ± 8 mg.dl), fasting insulin (-0.7 ± 0.1 µg.L) and HOMA-IR index (-0.099655 ± 0.116). Also in diabetic rats, sitagliptin therapy reduced blood concentration of HbA1C (-1.2 ± 0.1%), serum concentration of FBS (-130 ±10 mg.dl) and HOMA-IR index (-0.079598 ± 0.0086), but fasting serum insulin levels unchanged in the group that treated by sitagliptin compared with untreated diabetic group. As the results show, about the decreasing levels of FBS, HbA1C% and HOMA-IR index, there was no significant difference between 2 groups that treated with metformin and treated with sitagliptin in the end, but fasting insulin levels in diabetic rats treated with sitagliptin significantly (P ≤ 0.05) higher than the group treated with metformin. Fasting insulin levels indicate increased synthesis and secretion of insulin by sitagliptin through various mechanisms, including prevention of premature breakdown of GLP-1 by inhibition of enzymatic activity of DPP-4 and also increment of pancreatic β-cells activity. In other words, sitagliptin is effective on improving the sugar profile and insulin resistance like metformin. Based on the findings of this study and its relation to previous studies that have been done, it can be concluded that sitagliptin is suitable as a pharmaceutical composition and has beneficial antidiabetic effects. But since the effect of this drug is dependent on secretion of endogenous GLP-1, can be used only for patients with somewhat active β-cells.

Keywords: Diabetes Mellitus, Incretin, Metformin, Sitagliptin, sugar profile, insulin resistance, HOMA-IR

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INTRODUCTION

The prevalence of type 2 diabetes mellitus (T2DM) in the population is accompanied by uncontrolled obesity. Diabetes is crucial as a factor threatening public health in the world's population. The human and economic cost of the prevalence of diabetes can be associated with the chronic adverse complications of the disease. Some of these complications can be diabetic retinopathy, neuropathy and nephropathy, which can lead to blindness, kidney failure and high blood pressure in individuals with diabetes if left untreated (1).The risk of cardiovascular diseases in diabetics is 2 to 5-fold higher than that in non-diabetics, and may even lead to death (2). In most patients with type 2 diabetes, hyperglycemia is an outcome of β-cell inefficiency in the pancreas and reduced capacity of insulin secretion by these cells. In the body of individuals resistant against diabetes, the secretion of insulin from the β-cells rises due to the insulin resistance in the peripheral tissues. As for the genetically vulnerable individuals, however, insulin resistance is not compensated by increasing the functional capacity of β-cells, thus leading emergence of diabetic complications (3,4). There have so far been numerous methods proposed to measure the degree of insulin resistance and insulin sensitivity (5). For instance, the formula calculation of insulin resistance based on serum levels of glucose and insulin (homeostatic model assessment) (HOMA-IR) can provide a better indicator (6, 7).

During the past 2 decades, the prevalence of diabetes has led to development of eight categories of blood-glucose-reducing drugs with more effective control on blood glucose levels in patients with T2DM, ultimately, reducing the complications of diabetes (8). Given the fact that type 2 diabetes mellitus is a progressive disease with aggravating metabolic physical control over time, the long-run effects of therapeutic agents on glucose concentration is highly crucial. Safety, side effect profile, patient acceptance, treatment capacity and cost of treatment are the key factors contributing to long-term treatment of the chronic malignant disease (9).

Typically, it is difficult in most patients with type 2 diabetes mellitus to achieve an adequate level of blood glucose control and balance. In fact, the blood glucose control in the diabetic population has not remarkably progressed during the last. In other words, the average level of Hb1AC in many patients is more than 8% (10, 11). In addition, many therapeutic agents currently result in complications, including weight gain, hypoglycemia, gastrointestinal intolerance and peripheral tissue edema. Each of these complications can reduce the use of glucose-lowering drugs. They also challenge the fulfillment of treatment goals concerning blood glucose concentration (12, 13).

The neonatal albino Wistar rats with streptozotocin-induced diabetes are particularly useful for testing the insignificance of β-cell function in the development of type 2 diabetes (NIDDM). In this model, diabetes is induced by injection of STZ at different doses (80-100 mg/kg) at various age groups (0, 2 or 5 days of birth) leading to different degrees of β-cell destruction (14).

By comparing the performance of metformin (golden drug that is already widely used to treat type 2 diabetes) and incretine (a new generation of blood glucose lowering drugs) and investigating beneficial effects on sugar profile and insulin resistance, we can more accurately understand their mechanism and then finally apply new detection and treatment methods for type 2 diabetes patients using this drug family.

As an anti-hyperglycemic drug, metformin is widely prescribed in America (15) and Europe (16) for the treatment of diabetes. Moreover, it is the first option among oral glucose-lowering medications (17, 18). The role of metformin in reducing blood glucose levels is associated with diminished cardiovascular complications (19, 20). Metformin reduces blood glucose levels through decreasing hepatic glucose production as well as improving insulin resistance (21). In addition, previous studies indicated that the concentration of endogenous glucagon like peptide-1 (GLP-1) is increased in the range of 1.5 to 2-fold due to oral administration of metformin in non-diabetic obese patients (22). The effect of metformin on GLP-1 concentration is not based on inhibition of dipeptidyl peptidase-4 (DPP-4) (23, 24).

In 2007, metformin was widely used as a hypoglycemic drug in 54% of diabetic patients referred to US Medical Centers, both as monotherapy and in combination with other drugs, including insulin, sulfonylureas, Thiazolidinediones (particularly pioglitazone), and DPP-4 inhibitors (25).

In response to food intake, the digestive tract secretes a great deal of gastrointestinal hormones. For instance, GLP-1 and glucose-dependent insulinotropic peptide (GIP) are essential in regulation of blood glucose (26). Both GLP-1 and GIP lead to stimulation of insulin secretion from β-cells in response to glucose concentration. Moreover, GPL-1 can inhibit the glucagon secretion after meals. Later, it was found that the function of incretin hormones in patients with T2DM is somewhat impaired. Studies suggest that diminished effect of incretin indicate lower stimulation of pancreatic β-cells by these hormones. Moreover, the complication cannot be compensated through higher secretion of GIP (27, 28). This phenomenon rejects the GIP as alternative to blood glucose lowering drugs. Nevertheless, it has been shown that if GLP-1 is injected more than physiological amount, it can reduce the blood glucose level in individuals with type 2diabetes (29). The GLP-1 is dissolved by rapid digestive system and deactivated through DPP-4 activity. The DPP-4 enzyme gene can be expressed in many tissues. The presence of this enzyme, GLP-1 is inactivated in less than two minutes (30). This means that GLP-1 could not be applied as a clinical factor to treat diabetes, because it requires persistent administration for maintaining the function concentration. Therefore, any therapeutic benefit from the effects of GLP-1 on pancreatic β-cells requires the analogues of GLP-1, which are resistant to DPP-4, or alternatively the drugs that can maximize the effects of endogenous GLP-1 by inhibiting the DPP-4 (31).

There are numerous medications recognized so far as DPP-4 inhibitors, such as Sitagliptin, vildagliptin and Sazaglyptin (32). Sitagliptin is an oral medication and highly selective DPP-4 inhibitor demonstrating a new approach for the treatment of patients with type 2 diabetes. In contrast to the GLP-1 agonists, the DPP-4 inhibitors are prescribed orally at once a day dose after a meal. After 1 to 2 hours, the concentration of endogenous GLP-1 in these patients reached to a maximum amount within 2 to 3-fold higher than its concentration before food intake, thus curtailing the blood glucose levels after a meal (33).

This study was designed in line with several recent research on diabetes covering the effect of aqueous extract of saffron (Crocus Sativus) on serum biochemical parameters in rats with STZ-induced diabetes (34), association between beverage consumption pattern and lipid profile in diabetes (35), effect of aqueous extract on insulin secretion (36), Heat Shock Proteins in Diabetes(37) and effect of different plant extract on serum parameters in diabetes(38).

In fact, this study focused on changes in laboratory parameters including sugar profile and insulin resistance in Wistar rats in groups healthy, diabetic, and diabetic under treatments with metformin and Sitagliptin separately.

MATERIALS AND METHODS

Providing laboratory animals and diabetic injection: The baby albino Wistar male rats (purchased from an animal house of Jundishapur University, Ahvaz) with an average age 2 to 5 days divided randomly into two healthy and patient groups. The latter was intraperitoneally (i.p) injected STZ with a dose of 90 mg/kg body weight. Healthy group as a control only received physiological serum injection (14).

Classification of animals: After 48 hours, the rats with blood glucose levels greater than 250 mg/dl were considered diabetic and used in the experiment (26). After confirmation of diabetes and spent 8 weeks, the rates were divided into 4 groups of six : 1) Normal rats considered as a control after serum injection without STZ , 2) Diabetic rats without treatment , 3) Diabetic rats treated with metformin (150 mg/kg body weight) (38), and 4) Diabetic rats treated with sitagliptin (100 mg/kg body weight) (40, 41). The study continued up to the end of the one month.

Providing blood and serum samples: After one month, the rats were sacrificed and blood samples were taken from the Aortic input. The blood samples were kept at room temperature for 60 minutes and serum was isolated away through centrifuge at 3000 rpm for 15 at 25˚c. The serum falcons were kept at -20˚c until the analysis of biochemical parameters.

The experiment protocol was confirmed by the Council of animal ethics committee with instructions regarding the use and treatment of laboratory animals prepared by Ahvaz Jundishapur University of medical sciences.

Biochemical analysis: The serum glucose levels (FBS) were measured by commercial kit (*Pars Azmone, Tehran, Iran)*, an automatic analyzer (Abbott, model Alcyon 300, USA) and spectrophotometer device (at 505 nm) through enzymatic colorimetric method according to the following formula.

Fasting Blood Sugar (mg/dl) = [A (sample) / A (standard)] x 100 (mg/dl)

The fasting insulin level was determined by ELISA kit (Mercodia Corporation, Uppsala, Sweden) for the measurement of serum insulin in rats and based on direct sandwich immunoassay. In this test, after purification of insulin serum samples with the enzyme conjugate solution (proxidase conjugated rat monoclonal anti- insulin) and combination with substrate TMB (3, 3′, 5, 5′- tetra methyl benzidine), absorbance of the samples at 450nm was compared with the standard curve. Thus, the insulin concentration in serum samples was reported by µg/l unit.

The glycated hemoglobin (HbA1C) level in blood samples was measured using the ion exchange chromatography method with commercial kit (Bio Systems, S.A., Barcelona, Spain). According to this method, after purification of HbA1C and using spectrophotometer at 415nm, finally, HbA1C was reported as a percentage of total hemoglobin according to the following formula.

$$[(A\ HbA1C) \times (V\ HbA1C) /\ (A\ Hb\ Total) \times (V\ Hb\ Total)] \times 100 = HbA1C\%$$

As noted above, HOMA-IR was the measuring indicator for insulin resistance and β-cell activity calculated based on glucose concentration and serum level of fasting insulin. Insulin resistance index was first proposed in 1985 by Matthews and was calculated using insulin and glucose concentration of serum samples according to the following formula (6).

HOMA-IR: [insulin](in mu/l) x [glucose](in mg/dl) / 405

Statistical analysis: All the results were presented as mean ± SD. The differences between the mean values for each parameter in different groups were specified through one-way ANOVA at significance level of P≤0.05 using SPSS 16.0.

RESULTS

Figures 1- 4 show the changes in the serum concentration of sugar profile after one month of experiment. According to Figures 1 and 2, the glucose and HbA1C levels in the diabetic group (D) at significance level of P≤0.05 increased as compared to control group (H). At the end of experimental period, both parameters in the diabetic rats treated with metformin (D+M) and Sitagliptin (D+I) decreased at significance level of P≤ 0.05.But, there was no statistically significant association between the 2 diabetic groups treated with metformin (D+M) and sitagliptin (D+I) at end of the experiment.

According to Figure 3, the serum fasting insulin levels in diabetics (D) at significance level of P≤0.05 were higher than those compared to the normal group (H). At the end of one-month experimental period, the fasting serum insulin in diabetic rats treated with metformin reduced significantly at P≤0.05, But didn't change in the rats treated with sitagliptin (D+I). By comparing the two diabetic groups treated with metformin (D+M) and Sitagliptin (D+I), it was concluded that serum fasting insulin was higher in the treatment group under Sitagliptin (D+I) at P≤0.05.

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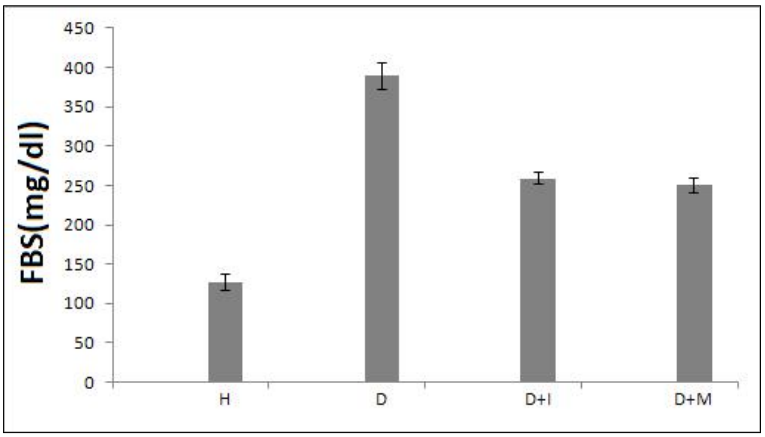
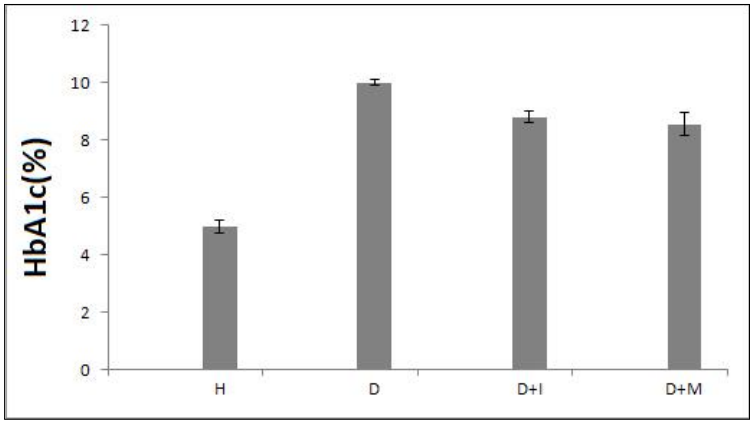


(1)



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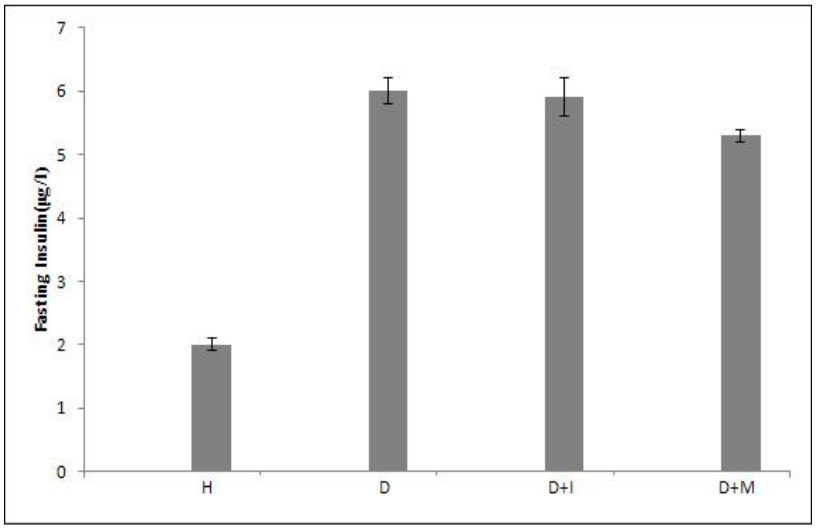

(3)

Figures 1 to 3. Illustrate the variations of serum levels of sugar profile in rats at the end of the one-month experiment.(1) FBS, (2) HbA1C%, (3)Fasting insulin. In 4 groups: healthy control (H), diabetic without treatment (D), diabetic treated by metformin (D+M) and diabetic treated by sitagliptin (D+I).

According to Figure 4, the insulin resistance index (HOMA-IR) increased in diabetics (D) as compared to the control group (H) at significance level of P≤0.05. At the end of the period, the HOMA-IR index decreased in diabetic rats treated with metformin (D+M) and Sitagliptin (D+I) at significance level of P≤ 0.05. According to data obtained, metformin and sitagliptin were effective in reducing insulin resistance and there were no significant differences between them.

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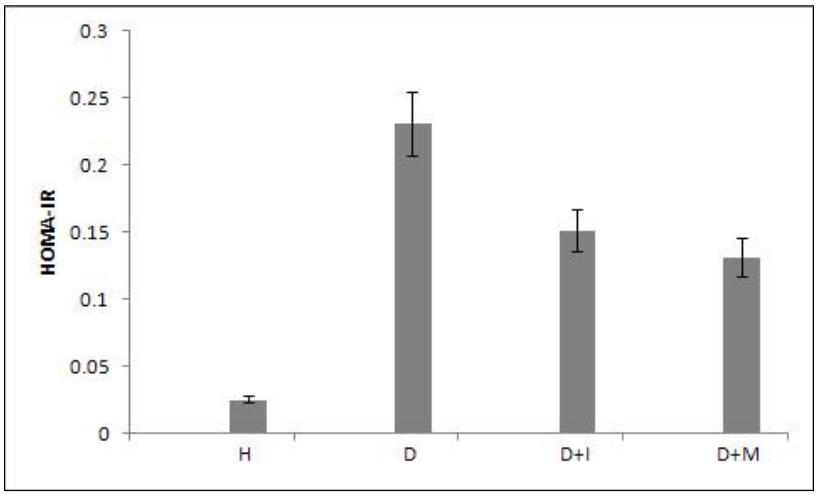

(4)

Fig 4: Alterations of HOMA-IR index in the rats at the end of the 30 days trial. In 4 groups: healthy control (H), diabetic without treatment (D), diabetic treated by metformin (D+M) and diabetic treated by sitagliptin (D+I).

DISCUSSION

In diabetic rats, metformin therapy reduced blood concentration of HbA1C (-1.44 ± 0.28%), serum concentration of FBS (-139 ± 8 mg.dl), fasting insulin (-0.7 ± 0.1 µg.L) and HOMA-IR index (-0.099655 ± 0.116). Also in diabetic rats, sitagliptin therapy reduced blood concentration of HbA1C (-1.2 ± 0.1%), serum concentration of FBS (-130 ±10 mg.dl) and HOMA-IR index (-0.079598 ± 0.0086), but fasting serum insulin levels unchanged in the group that treated by sitagliptin compared with untreated diabetic group.

Oral administration of metformin (150 mg/kg body weight) for one month improved the sugar profile and insulin resistance in STZ-induced diabetic rats. This indicates that metformin has anti-hyperglycemic effects.

Metformin mainly applies its anti-hyperglycemic effects due to decrease the hepatic glucose out put through inhibition of gluconeogenesis and increase peripheral glucose consumption (42). Increase the sensitivity of insulin receptors in target tissues is one of the possible mechanisms (43, 44). Such that metformin causes increment of tyrosine kinase receptor activity (45) and also increases the transport of glucose from the cell membrane by GLUT-4 (46, 47). In adipose tissue, metformin increases glucose uptake (48, 49).

The mechanism of metformin effect on β-cells function has not been defined, but several studies indicate that this drug caused the survival and preservation of β-cells (50).

Sitagliptin with 100 mg/kg body weight dosage for one month improved the sugar profile and insulin resistance in STZ- induced diabetic rats.

According to studies, sitagliptin inhibits DPP-4 enzyme and then prevents premature degradation of endogenous GLP-1.

GLP-1 is the insulin- releasing peptide that increases glucose dependent insulin secretion. In addition, GLP-1 inhibits glucagon secretion, stimulates biosynthesis of insulin, and develops β- cell mass and increases β-cells activity through gene expression of pdx-1 (51).

GLP-1 effects on increment of insulin secretion and reduction of glucose level through direct and indirect ways. In direct way, GLP-1 activates protein kinase- A enzyme (PKA), increases level of c AMP, and finally increases biosynthesis of insulin through binding to specific receptors on β- cells surface (52, 53). As well as, in the indirect method, GLP-1 increases insulin secretion through the portal and hepatic vagus nerve (53, 54). GLP-1 enters the portal circulation then activates glucose sensors and in this way sends signals to central

nervous system through afferents vagus nerve and brain send signals to pancreas through efferent vagus nerve that causes increment of insulin secretion from β- cells (53-55).

GLP-1 has a direct effect on α-cells and thus decreases glucagon secretion from pancreas, but doesn't at less than 65 mg/dl. GLP-1 induces satiety signal and reduces food intake through stimulation of pyloric sphincter receptors and reduction of gastric emptying rate (9, 56).

As the results show, about the decreasing levels of FBS, HbA1C% and HOMA-IR index, there was no significant difference between 2 groups that treated with metformin and treated with sitagliptin in the end, but fasting insulin levels in diabetic rats treated with sitagliptin significantly ($P \leq 0.05$) higher than the group treated with metformin. Fasting insulin levels indicate increased synthesis and secretion of insulin by sitagliptin through various mechanisms, including prevention of premature degradation of GLP-1 by inhibition of DPP-4 enzyme and also increment of pancreatic β-cells activity. In other words, sitagliptin is effective on improving the sugar profile and insulin resistance like metformin.

Based on the findings of this study and its relation to previous studies that have been done, it can be concluded that sitagliptin is suitable as a pharmaceutical composition and has beneficial antidiabetic effects. But since the effect of this drug is dependent on secretion of endogenous GLP-1, can be used only for patients with somewhat active β-cells.

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