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THE EFFECT OF SITAGLIPTIN AND MIRABEGRON ON SERUM LEPTIN, ADIPONECTIN, TUMOR NECROSIS FACTOR-α AND OXIDATIVE STRESS IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

Introduction: Mirabegron is a novel beta-3 receptor agonist. It has potential antiobesity and antidiabetic effects. The aim of the study was the evaluation of the possible antidiabetic, anti-inflammatory and antioxidant effects of mirabegron as well as its effect on serum leptin and adiponectin in diabetic rats alone and in combination with sitagliptin. **Materials and Methods***:* Five groups of Wistar rats were used. Diabetes was induced in group 2, 3, 4 and 5 using streptozotocin in dose of 100 mg/kg. The third, fourth and fifth group were treated for 20 days with sitagliptin (10 mg/kg), mirabegron (10 mg/kg) and combination of the 2 drugs, respectively. Blood samples were used for determination of blood glucose, serum level of leptin, adiponectin, TNF-α, malondialdehyde (MDA) and glutathione (GSH). **Results:** Streptozotocin caused induction of type-2 diabetes, increased TNF-α and MDA with reduction in the leptin, adiponectin and GSH. Sitagliptin caused a significant reduction of blood glucose, TNF-α and MDA with significant increase in leptin, adiponectin and GSH. Mirabegron caused a non-significant reduction of blood glucose, TNF-α and MDA with significant increase in the leptin and GSH. There was non-significant difference between the effect of the combination of sitagliptin and mirabegron and the effect of sitagliptin alone. **Conclusion:** Sitagliptin has a moderate anti-inflammatory and antioxidant effects. Mirabegron has a modest antidiabetic, anti-inflammatory and antioxidant effects and failed to normalize the low serum levels of leptin and adiponectin of streptozotocin induced diabetic rats. The addition of mirabegron to sitagliptin shows no additional or synergistic effects.

KEYWORDS: Adiponectin; Leptin; Mirabegron; Sitagliptin; Streptozotocin.

INTRODUCTION

Mirabegron is a highly selective β3-adrenoceptor agonist that is approved for the management of overactive urinary bladder symptoms. The drug is not only able to control the urinary symptoms of overactive urinary bladder disease in women, but also causes a significant improvement in the female sexual life.[1] It has a potential antiobesity and antidiabetic effects.[2] β_3-adrenergic receptors are widely expressed in both the brown and white adipose tissues.[3] Activation of the central β3-receptors by BRL37344, the β3-adreneroceptor agonist caused hypophagia in non-obese rat.[4] Treatment of obese mice with BRL-35135 which is a selective beta-3 adrenergic agonist for twenty days caused normalization of the glucose level and reduced plasma levels of insulin and free fatty acid. It also resulted in a 2 fold increase in beta-3 adrenergic receptors mRNA.[5]

Sitagliptin is one of the dipeptidyl peptidase-4 (DPP-4) enzyme inhibitors. It is active orally and approved for the treatment of diabetes mellitus type-2. By inhibiting the DPP-4 enzyme, sitagliptin elevates the level of incretin hormones as glucose-dependent insulinotropic polypeptide (GIP) and glucagon like peptide-1 (GLP-1).[6]

Incretins have an important role in the controlling of glucose homeostasis. When there is an elevation in the blood glucose levels, GIP and GLP-1 increase the synthesis and release of insulin. In addition, GLP-1 decreases the secretion of glucagon causing a reduction in glucose production by the liver.[7]

In 1995, Scherer and his co-workers indicated to the presence of a new protein and named it as adiponectin.[8] In cases of insulin resistance, type 2 diabetes, dyslipidemia, hypertension and obesity, there is a reduction in the serum levels of adiponectin.[9] Adiponectin increases insulin sensitivity leading to an increase in glucose metabolism. It reduces the plasma

level of triglycerides due to stimulation of the oxidation of fatty acid in the muscle and the liver.[10] Adiponectin has an important role in regulating the body weight and balance of energy. Insulin and insulin like growth factor-1 (IGF-1) can regulate the synthesis and the secretion of adiponectin.[11] There are 2 types of adiponectin receptors, adipoR1 which is expressed mainly in skeletal muscle, while adipoR2 is expressed mainly in the liver. In the pancreatic cells there is expression of the both types of the receptors.[12]

Leptin is a protein hormone that is secreted by the fat cells. Plasma leptin is responsible for maintaining the storage of body fat through the regulation of the intake of food and the expenditure of energy. Its plasma level is elevated with the increase in the total body fat. In the hypothalamus, leptin inhibits neuropeptide Y neurons causing anorexia.[13] In the peripheral tissues, leptin regulates the immune cell function, pancreatic beta cell, adipocytes and muscle cell functions.[14] Hyperleptinemia plays an important role in the pathogenesis of the complications of obesity as it is evident from the presence of high plasma leptin level in such cases.[15] Since mirabegron is a novel β3-adrenoceptor agonist that has limited studies about its pharmacological properties, so the aim of the present study was the evaluation of the possible antidiabetic, anti-inflammatory, antioxidant effects of mirabegron as well as its effect on serum leptin and adiponectin in streptozotocin induced diabetic rats alone and in combination with sitagliptin.

MATERIALS AND METHODS

1. Drugs and chemicals

Mirabegron was obtained in the form of a commercial tablet from Astellas Pharma Europe (Betmiga® 50 mg) and dissolved in normal saline. Sitagliptin was obtained in the form of a commercial tablet from Merck Sharp & Dohme, Italy (Januvia® 100 mg) and dissolved in normal saline. Rat Leptin ELISA kit (Abcam R– ab100773, Cambridge, UK), Rat Adiponectin ELISA kit (Abcam R– ab108784, Cambridge, UK) and Rat TNF-α ELISA Kit (cat number ab100784, lot number GR307317-1, Abcam Biochemicals, Cambridge, UK)), were used for determination of the serum levels of leptin, adiponectin and TNF-α, respectively. Glucose oxidase commercial kit (Sigma, St. Louis, Mo, USA) was used for measuring of the serum glucose. Ellman's reagent, reduced glutathione and malondialdehyde were obtained from Sigma Aldrich, USA. The source of 2-thiobarbituric acid and phosphate buffered saline was MP Biomedical, France. The rest of the chemicals were obtained from the local commercial sources and had an analytical grade.

2. Animals

Forty male Wistar Albino rats with a weight 150-200 g were used in the research. The animals were purchased from the house animal of the University of Assiut. The rats consumed the ordinary laboratory food and water *ad libitum*. Rats were kept in the laboratory for seven days before the start of experiment for adaptation. According to the guide for the care and use of laboratory animals of the National Institutes of Health (NIH 1985), the experiment was conducted. The research was approved by the ethics committee of the College of Medicine, University of Assiut (approval no 17300268).

3. Induction of diabetes

Diabetes was induced in rats of group 2, 3, 4 and 5 by single intraperitoneal (i.p.) injection of streptozotocin (STZ) in a dose of 100 mg/kg b.w.[16] STZ was dissolved in 10 mM of ice-cold sodium citrate buffer with pH 4.5. The solution was kept in ice and used within 5 minutes of its preparation.[17] 1gm of STZ was dissolved in 50 ml of citrate buffer, so 1 ml of the solution contained 20 mg of STZ. Animals were fasted over night before giving the STZ. After 72 hours of STZ injection, blood samples were collected from rat tail vein and measuring of blood glucose was done. Rats with blood glucose 200-300 mg/dl were considered as diabetic and were taken in the experiment.[17]

4. Animal grouping

5 groups of animals, 8 rats in each group were used in the experiment. The first group was injected i.p. with 1 ml of 10 mM of ice-cold sodium citrate buffer with pH 4.5. and used as a control non-diabetic group. The second group was injected by single i.p. injection of streptozotocin (STZ) in a dose of 100 mg/kg b.w. and used as a control diabetic group. The third group was diabetic rats and treated with sitagliptin 10 mg/kg/day orally using oral gavage needle for 20 days. The fourth group was diabetic rats and treated with mirabegron 10 mg/kg/day orally using oral gavage needle for 20 days. The fifth group was diabetic rats and treated with a combination of mirabegron (10 mg/kg orally) with sitagliptin (10 mg/kg orally) for 20 days.

The selection of the mirabegron and sitagliptin doses depended on the range of doses of previous investigations.[18, 19] After the end of 20 days of treatment, the animals were anesthetized using pentobarbital 50 mg/kg b.w. then decapitation of rats by cervical dislocation was done. Blood samples were collected then centrifuged for 10 minutes at 3000 revolutions/minute and stored at -20 °C till use in the assessment of blood glucose, serum levels of leptin, adiponectin, tumor necrosis factor-α and oxidative stress.

5. Determination of the blood glucose level

Determination of the blood glucose level was done spectrophotometrically by using of glucose oxidase/peroxidase method.[20]

6. Determination of the serum level of leptin

The level of leptin in serum was measured using the rat leptin ELSIA kit according to the instructions of the manufacturer. The tested samples, the control and the standards were placed into the wells and incubation for two hours at 37 ^{o}C was done. After five times of

washing, we added the enzyme-linked polyclonal antibody which was specific for the rat leptin of interest to the wells. Then we added the substrate solution after washing of the wells. At 450 nm, the enzymatic reaction was read. The standard curve was done and exclusion of the values which were below the standard curve was done.[21]

7. Determination of the level of serum adiponectin

The serum level of adiponectin was measured using the ELSIA kit of rat adiponectin according to the instructions of the manufacturer. The tested samples, the control and the standards were placed into the wells, and then incubation at 37 ^{o}C for two hours was done. The addition of the enzyme-linked polyclonal antibody to the wells was done after five times of washing. Then after washing of the wells, we added the substrate solution. The enzymatic reaction was read at 450 nm. The standard curve was done and exclusion of the values which were below the standard curve was done.[21]

8. Determination of the level of plasma tumor necrosis factor-α (TNF-α)

The level of TNF-α in plasma was measured using the rat TNF-α ELSIA kit according to the instructions of the manufacturer. The samples were added to the well that coated to TNF-α. Incubation at 37 °C for 2 ½ hours was done after gentle shaking. After plate washing, adding of a specific antibody for each well was done followed by re-incubation for 60 minutes. Washing of the plate was performed, and then we added streptavidin solution with re-incubation for 45 minutes. After washing of the plate, one-step substrate reagent was added. Finally, stop solution was added and the reaction was measured by an automated ELISA reader at 450 nm.[22]

9. Evaluation of the oxidative stress

Determination of the serum malondialdehyde (MDA)

The level of MDA was measured in serum of rats by the method indicated by Ohkawa et al. (1972). MDA level was measured spectrophotometrically after colorimetric reaction with thiobarbituric acid. MDA is a good marker for lipid peroxidation and oxidative stress.[23]

Determination of the serum reduced glutathione (GSH)

According to the method described by Boyne and Ellman (1972), the serum level of GSH was measured. Trichloroacetic acid 10 % was mixed with the rat serum then centrifuged at 5000 revolutions per minute at -4 ^{o}C for ten minutes. Then disodium hydrogen phosphate buffer (pH 8.4) and Ellman's reagent (0.25 ml) were added to supernatant. After incubation of the samples for 10 min, the absorbance of the color was measured spectrophotometrically at 412 nm.[24]

10. Statistical analysis

Data were represented as the mean ± S.E. of 8 observations. For detection if there was a statistically significant difference between the different groups, one way analysis of variance (ANOVA) was done. Tukey's post hoc test was used for multiple comparisons between the groups. The results were considered as statistically significant differences if $P < 0.05$. The analysis was done by the use of Prism software (Graph-Pad Software, version 7).

RESULTS

1. Effect of single i.p. injection of streptozotocin (STZ) 100 mg/kg, sitagliptin (SGT) 10 mg/kg/day orally for 20 days, mirabegron (MIRA) 10 mg/kg/day orally for 20 days and their combination on the blood glucose level

The results of this study showed that there was a significant ($p< 0.05$) increase in blood glucose in the rats of group 2 that was received single i.p. injection of streptozotocin as showed in figure 1. Treatment with sitagliptin 10 mg/kg/day orally for 20 days caused a significant ($p< 0.05$) reduction of blood glucose as compared with the streptozotocin treated group (figure 1). Treatment with mirabegron 10 mg/kg/day orally for 20 days caused a reduction in the blood glucose but this reduction was non-significant as compared with the streptozotocin treated group (figure 1). Treatment of the rats with the combination of sitagliptin and mirabegron for 20 days caused a significant ($p< 0.05$) reduction of blood glucose and this reduction was more than the reduction that was caused by the use of sitagliptin alone as showed in figure 1.

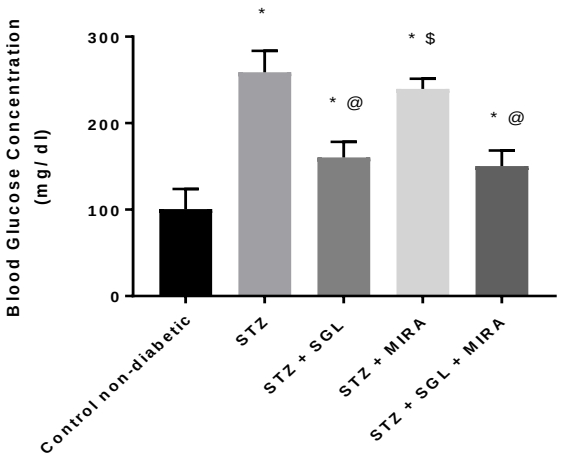

Figure 1: Effect of single i.p. Streptozotocin (STZ) 100 mg/kg, Sitagliptin (SGL) 10 mg/kg orally, Mirabegron (MB) 10 mg/kg orally and their combination for 20 days on the blood glucose level in rats

Results were represented as mean ± SE (every group consisted of 8 rats)

*$p < 0.05$ in comparison with control non-diabetic rats.

$^{@}p < 0.05$ in comparison with STZ treated rats.

$^{\$}p < 0.05$ in comparison SGL treated rats.

2. Effect of single i.p. injection of streptozotocin (STZ) 100 mg/kg, sitagliptin (SGT) 10 mg/kg/day orally for 20 days, mirabegron (MIRA) 10 mg/kg/day orally for 20 days and their combination on the serum leptin level in rats

Single i.p. injection of streptozotocin caused a significant ($p< 0.05$) decrease in level of serum leptin in rats as compared with the control rat group as showed in figure 2. Treatment with sitagliptin 10 mg/kg/day orally for 20 days caused a significant ($p< 0.05$) increase in the level of serum leptin as compared with the streptozotocin treated group and there was non-significant difference between the sitagliptin treated group and the control non-diabetic group (figure 2). Treatment with mirabegron 10 mg/kg/day orally for 20 days caused a significant increase in the level of serum leptin compared with the streptozotocin treated group but this increase was statistically less than caused by sitagliptin (figure 2). The use of the combination of sitagliptin and mirabegron for 20 days caused a significant ($p< 0.05$) elevation of the serum leptin as showed in figure 2.

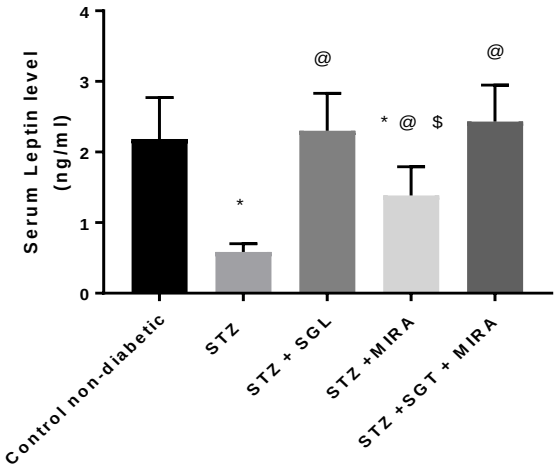

Figure 2: Effect of single i.p. Streptozotocin (STZ) 100 mg/kg, Sitagliptin (SGL) 10 mg/kg orally for 20 days, Mirabegron (MB) 10 mg/kg orally for 20 days and their combination on the serum leptin level in rats.

Results were represented as mean ± SE (every group consisted of 8 rats)

*$p < 0.05$ in comparison with control non-diabetic rats.

$^{@}p < 0.05$ in comparison with STZ treated rats.

$^{\$}p < 0.05$ in comparison SGL treated rats.

3. Effect of single i.p. injection of streptozotocin (STZ) 100 mg/kg, sitagliptin (SGT) 10 mg/kg/day orally for 20 days, mirabegron (MIRA) 10 mg/kg orally for 20 days and their combination on the serum adiponectin level in rats

The results demonstrated that single i.p. injection of streptozotocin caused a significant ($p< 0.05$) reduction in the level of rat serum adiponectin as compared with the control rat group (figure 3). Treatment with sitagliptin 10 mg/kg/day orally for 20 days caused a significant ($p< 0.05$) increase in the level of serum adiponectin as compared with the streptozotocin treated group (figure 3). Treatment with mirabegron 10 mg/kg/day orally for 20 days caused a non-significant increase in the level of serum adiponectin. The use of the combination of sitagliptin and mirabegron for 20 days caused a significant ($p< 0.05$) elevation of the serum adiponectin as showed in figure 3.

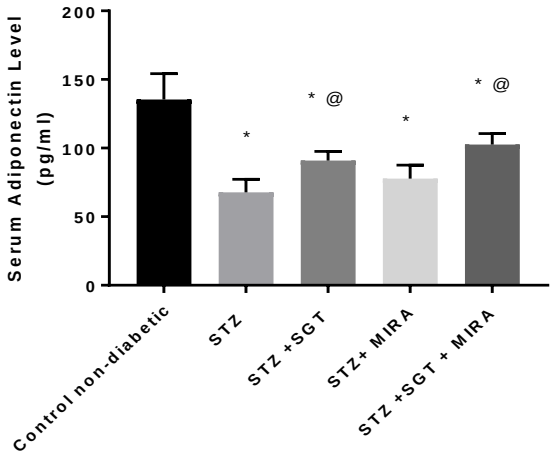

Figure 3: Effect of single i.p. Streptozotocin (STZ) 100 mg/kg, Sitagliptin (SGL) 10 mg/kg orally for 20 days, Mirabegron (MB) 10 mg/kg orally for 20 days and their combination on the serum adiponectin level in rats.

Results were represented as mean ± SE (every group consisted of 8 rats)

*$p < 0.05$ in comparison with control non-diabetic rats.

$^{@}p < 0.05$ in comparison with STZ treated rats.

$^{\$}p < 0.05$ in comparison SGL treated rats.

4. Effect of single i.p. injection of streptozotocin (STZ) 100 mg/kg, sitagliptin (SGT) 10 mg/kg/day orally for 20 days, mirabegron (MIRA) 10 mg/kg orally for 20 days and their combination on the serum level of tumor necrosis factor-α (TNF-α)

The injection of streptozotocin i.p. caused a significant ($p< 0.05$) increase in the level of rat serum TNF-α as compared with the control rat group (figure 4). Treatment with sitagliptin 10 mg/kg/day orally for 20 days caused a significant ($p< 0.05$) reduction in the level of serum TNF-α as compared with the streptozotocin treated group (figure 4). Treatment with mirabegron 10 mg/kg/day orally for 20 days caused a reduction in the level of serum TNF-α but this reduction was statistically non-significant in comparison with the streptozotocin treated group (figure 4). The use of the combination of sitagliptin and mirabegron for 20 days caused a significant ($p< 0.05$) decrease in the serum TNF-α (figure 4).

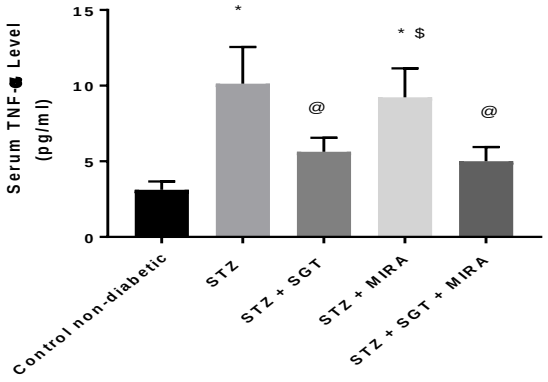

Figure 4: Effect of single i.p. Streptozotocin (STZ) 100 mg / kg, Sitagliptin (SGL) 10 mg/kg orally for 20 days, Mirabegron (MB) 10 mg/kg orally for 20 days and their combination on the level of serum TNF-α in rats.

Results were represented as mean ± SE (every group consisted of 8 rats)

*p < 0.05 in comparison with control non-diabetic rats.

@p < 0.05 in comparison with STZ treated rats.

$p < 0.05 in comparison SGL treated rats.

5. Effect of single i.p. injection of streptozotocin (STZ) 100 mg/kg, sitagliptin (SGT) 10 mg/kg/day orally for 20 days, mirabegron (MIRA) 10 mg/kg orally for 20 days and their combination on the serum level of malondialdehyde (MDA) in rats.

The results showed that streptozotocin i.p. caused a significant (p< 0.05) increase in the level of MDA of the rat serum in comparison with the control rat group (figure 5). Treatment with sitagliptin 10 mg/kg/day orally for 20 days caused a reduction in the level of the serum MDA but this reduction was statistically non-significant (figure 5). Treatment with mirabegron 10 mg/kg/day orally for 20 days caused a very weak reduction in the level of serum MDA and this reduction was also statistically non-significant in comparison with the streptozotocin treated group (figure 5). The use of the combination of sitagliptin and mirabegron for 20 days caused a significant (p< 0.05) reduction in the serum MDA compared to the streptozotocin treated group (figure 5).

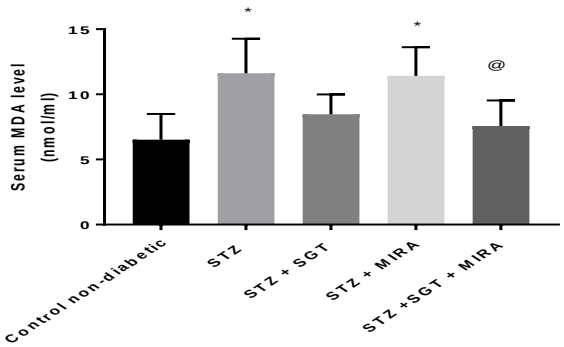

Figure 5 Effect of single i.p. Streptozotocin (STZ) 100 mg / kg, Sitagliptin (SGL) 10 mg/kg orally for 20 days, Mirabegron (MB) 10 mg/kg orally for 20 days and their combination on the level of serum MDA in rats.

Results were represented as mean ± SE (every group consisted of 8 rats)

*p < 0.05 in comparison with control non-diabetic rats.

@p < 0.05 in comparison with STZ treated rats.

$p < 0.05 in comparison SGL treated rats.

6. Effect of single i.p. injection of streptozotocin (STZ) 100 mg/kg, sitagliptin (SGT) 10 mg/kg/day orally for 20 days, mirabegron (MIRA) 10 mg /kg orally for 20 days and their combination on the serum level of reduced glutathione (GSH) in rats

Single i.p. injection of streptozotocin caused a significant (p< 0.05) reduction of the level of GSH of rat serum in comparison with the control rat group (figure 6). Treatment with sitagliptin 10 mg/kg/day orally for 20 days caused a significant (p< 0.05) elevation in the level of serum GSH compared to the streptozotocin treated group (figure 6). Treatment with mirabegron 10 mg/kg/day orally for 20 days caused also similar results as it caused a significant (p< 0.05) elevation in the level of serum GSH in comparison with the streptozotocin treated group (figure 6). The use of the combination of sitagliptin and mirabegron for 20 days caused a significant (p< 0.05) elevation in the level of serum GSH in comparison with the streptozotocin treated group (figure 6).

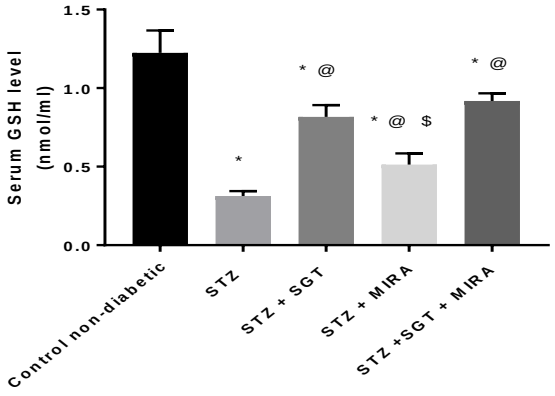

Figure 6 Effect of single i.p. Streptozotocin (STZ) 100 mg/kg, Sitagliptin (SGL) 10 mg/kg orally for 20 days, Mirabegron (MB) 10 mg/kg orally for 20 days and their combination on the level of serum GSH in rats.

Results were represented as mean ± SE (every group consisted of 8 rats)

*p < 0.05 in comparison with control non-diabetic rats.

@p < 0.05 in comparison with STZ treated rats.

$p < 0.05 in comparison SGL treated rats

4-DISCUSSION

The results of the study indicated that treatment of the rats with streptozotocin caused a significant increase in blood glucose compared with the control group. The results were in agreement with other reports that demonstrated that single injection of 100 mg /kg of

streptozotocin i.p. can cause hyperglycemia due to induction of type -2 diabetes mellitus in rats.[25] Streptozotocin and alloxan are well known agents that are widely used for experimental induction of diabetes. The type of diabetes induced by streptozotocin is dependent on the dose of streptozotocin and the used animal species.[26] The methods of induction of experimental diabetes using streptozotocin can be divided into three types: multiple small doses for several days, a single moderate dose and a single high dose. Multiple small doses (40-60 mg/kg for 5 days) are used for induction of mild type-1 diabetes mellitus as these doses cause a condition similar to autoimmune insulitis. Hyperglycemia that is caused by this method may be T-lymphocyte dependent or due to apoptosis of pancreatic β-cell.[27] A single moderate dose (100 mg/kg) is commonly used for induction of type-2 diabetes mellitus with preservation of pancreatic β-cell. Hyperglycemia induced by this type may be caused by increasing of the resistance to insulin.[25] A single high dose (150-200 mg/kg) of streptozotocin can cause destruction of most of the pancreatic β-cell with near complete absence of insulin secretion. This method is used for induction of type-1 diabetes mellitus.[28] Streptozotocin may act as a toxin for the pancreatic β-cell causing rapid destruction of the cells. Diabetes mellitus induced by the use of streptozotocin can cause chronic endogenous oxidative stress caused by the hyperglycemia.[29]

The results indicated that the treatment of the diabetic rats with sitagliptin 10 mg/kg/day orally for 20 days caused a significant reduction of blood glucose. Sitagliptin is a the DPP-4 enzyme inhibitor which elevates the level of incretin hormones that causes increase in the synthesis and release of insulin and decreases the secretion of glucagon causing a reduction in glucose production by the liver.[7] Inhibitors of DPP-4 now become the most commonly used oral antidiabetic medications for type-2 diabetic patients in Japan.[30] The results of the effect of sitagliptin on blood glucose were in accordance of other reports that indicated that sitagliptin caused an improvement in the oral glucose tolerance test of diabetic rats.[31] Sitagliptin is a well-tolerated antidiabetic drug and causes no hypoglycaemia or increase in body weight in type-2 diabetic patients.[32] Treatment with mirabegron 10 mg/kg/day orally for 20 days caused a modest reduction in the blood glucose of diabetic rats but this reduction was non-significant in comparison with the streptozotocin treated diabetic group. There were previous reports that indicated that β3-adrenoceptor agonists have a potential antidiabetic effect as Liu et al. (1998) reported that chronic treatment with CL-316243 which is a β3-adrenergic agonist normalized hyperglycemia and increased responsiveness to insulin with increasing the uptake of glucose in fat and muscle cells of obese rat.[33] β3-adrenergic agonist increased the defective oxidative capacity of mitochondrial in brown and white adipose tissues of diabetic animals, so increased expenditure of energy and oxidation of fat, so reduced level of plasma free fatty acids. This can cause enhancement of the utilization of glucose by the skeletal muscle.[34] The combination of sitagliptin and mirabegron caused a reduction of the blood glucose of the diabetic rats but there was no significant difference between the effect of this combination and the effect sitagliptin alone indicating that there was no synergistic effect of this combination regarding the effect on blood glucose.

Regarding the effect of the tested drugs on serum leptin, the use of streptozotocin caused a significant decrease in the level of serum leptin in rats as compared to the control non-diabetic rat group. The results were in agreement with the previous reports that indicated that streptozotocin-induced diabetes was associated with a reduction in the secretion of leptin due to decrease in the metabolism of glucose in the fat tissues.[35] The reduction of the leptin level in type-1 diabetes is associated with a decrease in insulin level.[36] Leptin can reduce the intake of food, body weight and fat with suppression of glucagon secretion which may be responsible for its antidiabetic effect.[37] In contrast, Wu et al. demonstrated that there was an elevation of plasma leptin level of diabetic rats compared with the control non-diabetic animals.[19] Treatment with sitagliptin 10 mg/kg/day orally for 20 days caused a significant increase in the level of serum leptin. There were limited studies about the effect of sitagliptin on leptin level in rats. However other studies demonstrated that insulin administration in diabetic rats increased the secretion of leptin through increasing of glucose uptake by the adipocytes.[38] Havel et al. (1998) reported that there was a marked reduction of plasma leptin level after induction of diabetes in rats using streptozotocin with restoration of plasma leptin level following insulin use.[39] In the present study, treatment with mirabegron 10 mg/kg for 20 days caused also a significant increase in the level of serum leptin compared with streptozotocin treated group. It may act by a mechanism similar to insulin through increasing of glucose uptake by the adipocytes. There were insufficient data about the effect of mirabegron on serum leptin.

The results demonstrated that streptozotocin caused a significant reduction in the level of rat serum adiponectin which was in agreement with other studies that demonstrated that the level of adiponectin in the serum is decreased in type-2 diabetes and negatively associated with the body mass index of the normal persons.[40] By contrast, there was an increase in the level of adiponectin in type-1 diabetes mellitus.[41] There was a reduction in the serum levels of adiponectin in cases of insulin resistance, type 2 diabetes, dyslipidemia, hypertension and obesity. Adiponectin is responsible for increasing the insulin sensitivity leading to an increase in glucose metabolism.[9] Treatment with sitagliptin for 20 days caused a significant increase in the level of serum adiponectin which was in accordance with the report of Hibuse et al. (2014) who demonstrated that there was an elevation in the serum level of adiponectin in Japanese

patients with type-2 diabetes mellitus after 3 months of treatment with sitagliptin.[42] Treatment with sitagliptin and vildagliptin caused a significant elevation in the level of adiponectin in 10 randomized controlled studies.[43] Treatment with mirabegron for 20 days caused a non-significant increase in the level of serum adiponectin but the combination between sitagliptin and mirabegron caused a more elevation of serum adiponectin than the effect of sitagliptin alone.

Regarding the effect on the inflammatory mediator; tumor necrosis factor-*α* (TNF-*α*), the injection of streptozotocin caused a significant increase in the level of rat serum TNF-α which may contribute in the pathogenesis of diabetes mellitus induced by streptozotocin. TNF-α is a cytokine that has an important role in the inflammatory process and secreted mainly by macrophage and adipocyte.[44] It causes inhibition of the transduction of insulin, and affects the metabolism of glucose.[45] Disturbance in the metabolism of TNF-α may have a role in the pathogenesis of insulin resistance and the onset and progression of type-2 diabetes mellitus.[46] Esposito et al. reported that there was an increase in the inflammatory markers as TNF-α, IL-1, IL-6 and IL-18 in the blood of patients with type 1 and type 2 diabetes mellitus.[47] Similar results were reported by Margoni and his group (2011) who demonstrated that there was an increase in the serum level of TNF-α in diabetic rats.[48] Treatment of diabetic rats with sitagliptin for 20 days in this study caused a significant reduction in the level of serum TNF-α which indicated that sitagliptin had anti-inflammatory effect in cases of diabetics cases. This is in accordance with the report of Marques et al. (2014) who reported that sitagliptin decreased the inflammatory process and reduce serum TNF-α in type 2 diabetic rats.[49] Treatment with mirabegron 10 mg/kg/day orally for 20 days caused a non-significant reduction in the level of serum TNF-α indicating that the drug had a very weak anti-inflammatory effect and the drug was ineffective in restoration of this inflammatory marker.

Regarding the effect of the tested drugs on the oxidative stress markers, the results demonstrated that streptozotocin caused a significant increase in the level of MDA of rat serum with a significant decrease in the level of GSH. Similar results were reported by Samarghandia et al. (2017) who demonstrated that streptozotocin injection caused an elevation in the MDA level, TNF-α and IL-6 with decrease in the GSH level.[50] Oxidative stress and free radicals have an important role in the diabetes pathogenesis and complications. The generation of oxygen free radical is enhanced by hyperglycemia.[51] Many reports indicated that streptozotocin causes imbalance between antioxidant and oxidant system. MDA is a product of peroxidation of lipid and binds to molecules causing disruption of pancreatic beta cells leading to disturbance in metabolism of glucose.[52] Treatment with sitagliptin 10 mg/kg/day for 20 days caused a reduction in the level of serum MDA and an elevation of serum GSH indicating that the drug had antioxidant effect. The results were in agreement with many other studies that indicated that sitagliptin had an antioxidant effects in experimental diabetic animals and diabetic human. In a study in diabetic rabbits, sitagliptin caused a significant reduction of aortic MDA level indicating a decrease in lipid peroxidation and reactive oxygen species generation. There was also a restoration of the depletion in GSH, so it maintaining the antioxidant reserves.[53]

Civantos et al. (2017) indicated that sitagliptin treatment caused a modulation of antioxidant response in the experimental diabetic kidney.[54] Alam and his co-workers (2015) indicated that there was an increase in the markers of oxidative stress in plasma such as nitric oxide and MDA in two kidneys and one clip (2K1C) and there was normalization of these markers after treatment of the rats with sitagliptin.[55] Treatment with mirabegron 10 mg/kg/day orally for 20 days caused a very weak and non-significant reduction in the level of serum MDA with a significant elevation in the level of serum GSH indicating that the drug has a modest antioxidant activity. There were limited studies regarding the antioxidant activity of mirabegron. However, there was a study that indicated that β3-adrenoceptor agonist that contained aryloxypropanolamine moiety had an antioxidant activity in vitro.[56] The combination of sitagliptin and mirabegron caused a significant reduction of the of the level of serum MDA with a significant elevation in the level of serum GSH of the diabetic rats but there was no significant difference between the effect of this combination and the effect of the sitagliptin alone indicating that there was no synergistic effect of this combination regarding the effect on oxidative stress.

5. CONCLUSION

The study had shown that sitagliptin has a moderate anti-inflammatory and antioxidant effects and able to normalize the serum levels of leptin and adiponectin of streptozotocin induced diabetic rats. Mirabegron has a modest or a weak antidiabetic, anti-inflammatory and antioxidant effects and failed to normalize the serum levels of leptin and adiponectin in cases of streptozotocin induced diabetic rats. The addition of mirabegron to sitagliptin shows no additional or synergistic effects.

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Conflict of Interests

The author declares no conflict of interests in preparing this research.

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