ADDITION OF SITAGLIPTIN TO REPAGLINIDE IMPROVES PANCREATIC ISLET PROLIFERATION AND INSULIN PRODUCTION IN EXPERIMENTALLY-INDUCED TYPE 2 DIABETES IN RATS

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ABSTRACT

Type 2 diabetes mellitus is a complex heterogeneous group of metabolic conditions characterized by increased levels of blood glucose due to impairment in insulin action and/or insulin secretion. Exploring new drug therapies will benefit in preventing morbidity and mortality associated with diabetes as well as the growing health care costs. Sitagliptin is a highly selective DPP-4 inhibitor that has been shown to improve glycemic control and beta cell function. Repaglinide is a short acting insulin sectretagogue stimulating insulin release. The purpose of this study was to evaluate the effect of combining sitagliptin (5 mg/kg) and repaglinide (0.15 mg/kg & 0.3 mg/kg) on improving hyperglycemia and enhancing the pancreatic function in high fat diet/streptozotocin-induced type 2 diabetes mellitus rat model. The current results highlight a significant improvement in glycemic control and pancreatic insulin production upon addition of sitagliptin to repaglinide therapy. Repaglinide either alone or in combination was effective in preserving islet cell integrity. Further, immunohistochemical staining revealed significant higher insulin content in the combination groups compared to corresponding monotherapies. The combination therapy had a positive effect in lowering serum lipids. In conclusion, the present study reinforces the view of using gliptins in combination with repaglinide to enhance the glycemic control and lipid profile in patients with type 2 diabetes.

Key words: insulin, repaglinide, sitagliptin, type 2 diabetes, rat model

INTRODUCTION

Hyperglycemia and diabetes are important causes of mortality and morbidity worldwide **(Danaei *et al.*, 2006)**. Type 2 diabetes mellitus accounts for 95% of diabetic people around the world **(Lysy et al., 2012)**. It is a chronic metabolic disorder characterized by a progressive decline in insulin action (insulin resistance), followed by the inability of β-cells to compensate for insulin resistance (pancreatic β-cell dysfunction) **(Li *et al.*, 2008; Gunasekaran and Gannon, 2011; Ashcroft and Rorsman, 2012)**. Decreased β cell mass resulting from increased apoptosis is also an important property of type 2 diabetes **(Butler *et al.*, 2003; Marchetti *et al.*, 2010)**. A direct correlation between reductions in β-cell mass and the occurrence of type 2 diabetes has been observed in pathological investigations of pancreases retrieved from patients with type 2 diabetes **(Butler *et al.*, 2003; Marchetti *et al.*, 2010)**. Therefore, when considering therapeutic intervention in type 2 diabetes, the agents of choice will be those that stimulate insulin secretion in a glucose dependent manner and maintain or enhance β-cell mass through either increased proliferation or decreased apoptosis.

A new class of antidiabetic drugs, incretins, has been developed. Incretins are gastrointestinal hormones, released in response to nutrient ingestion (mainly glucose and fat). They exert a wide range of effects, including stimulation of pancreatic insulin secretion in a glucose-dependent manner **(Cernea and Raz, 2011)**. The incretin effect is attributed largely to two hormones, glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide-1 (GLP-1) **(Mortensen *et al.*, 2003)**. Both hormones augment glucose-dependent insulin secretion from β-cells of the pancreas **(Gautier *et al.*, 2005)**. In addition, it has also been shown that GLP-1 inhibits glucagon secretion in a glucose dependent fashion **(Holst., 2000)**, promotes β-cell proliferation and differentiation and probably neogenesis, while enhancing resistance to apoptosis **(Li *et al.*, 2003)**. In type 2 diabetes, GLP-1 concentrations are reduced in response to a meal, making GLP-1 a favored potential therapeutic target. GLP-1 is rapidly degraded by dipeptidyl peptidase-4 enzyme (DPP-4) resulting in circulating half-life of the hormone in the range of minutes which precludes its use in diabetes therapy **(Field *et al.*, 2009)**. Accordingly, new drugs based on GLP-1 receptor (GLP1R) agonism and DPP4 inhibition have been approved for the treatment of type 2 diabetes **(Amori *et al.*, 2007)**.

Sitagliptin is a highly selective DPP-4 inhibitor that provides 24-h DPP-4 inhibition when dosed once daily **(Kim *et al.*, 2005; Herman *et al.*, 2007)**. In patients with type 2 diabetes, sitagliptin, both as monotherapy and when given in combination with other antihyperglycemic agents has been shown to improve glycemic control and measures of β-cell function and to be well-tolerated in large multinational, placebo-controlled and active-controlled trials **(Aschner *et al.*, 2006; Raz *et al.*, 2006)**. Repaglinide is a short acting insulin sectretagogue that functions through the inhibition of potassium ATP channels in the beta cells **(Hu, 2002)**. This drug stimulates insulin release only in the presence of glucose which means that it should be taken just before or with meals, and because of this mechanism of action it have been shown to improve postprandial hyperglycemia **(Rudovich et al., 2004)**.

Rats fed a high-fat diet (HFD) and treated with low-dose streptozotocin can serve as an experimental animal model for type 2 diabetes because of their impaired insulin secretion, glucose intolerance, insulin resistance and obesity **(Bonner-Weir and Smith, 1994; Luo *et al.*, 1998; Badole and Bodhankar, 2009)**. In the present study, we aimed to evaluate the effect of adding of sitagliptin to the insulinotropic agent, repaglinide, on improving the glycemic control and enhancing the pancreatic function in this rat model of type 2 diabetes mellitus.

MATERIALS & METHODS

Experimental animals

All experiments were carried out using male albino Wister rats weighing 180–210 g. Rats were housed in stainless steel cages in a normal light–dark cycle at around 25 ± 4 °C. Food was replaced daily and any uneaten portions were discarded. Body weights of rats were recorded every week. All experimental protocols were approved by the institutional Animal Care and Use Committee at the Faculty of Pharmacy, Suez Canal University, Ismailia, Egypt.

Drugs and chemicals

Streptozotocin (STZ) was provided by Sigma-Aldrich® (MO, USA) and dissolved in citrate buffer (0.1 M, pH = 4.5). Repaglinide powder was kindly provided by Al-Hekma Pharmaceutical Company (6th of October City, Egypt). Sitagliptin (Januvia® tablet) was

obtained from Merck Sharp & Dohme Ltd (Pavia, Italy). The two drugs were prepared as suspension in 1% Na-CMC. The feeding ingredients such as lard and sucrose were procured from the commercial sources. All the other chemicals and solvents used were of the highest analytical grade.

Induction of diabetes in rats

Rats were fed with a HFD for four weeks, prepared by adding 20 % sucrose (w/w) and 10 % lard (w/w) into basal diet and water *ad libitum*. Normal control rats were fed with a basal diet. After 4 weeks, animals were fasted overnight and injected with a freshly prepared STZ (35 mg/kg, i.p.) in a volume of 1 ml/kg **(*Srinivasan et*** al., **2005; Mansor *et al.,* 2013)**. Three days after STZ administration, blood glucose level of each rat was determined, hyperglycemia was confirmed and steady state of hyperglycemia was acquired after 10 days. Rats with fasting blood glucose above 130 mg/dl were included in the study. Serum glucose was determined from blood samples obtained by tail pricking and glucose was measured by Accu-check blood glucose meter (Roche Diagnostic, Germany).

Experimental design

Seventy male rats were randomly allocated into 7 groups, 10 rats each. Group I: Normal control rats, received (1 ml/kg, i.p.) citrate buffer (pH = 4.5). Group II: Diabetic control rats: fed with HFD followed by STZ (30 mg/kg) and received (2 ml/kg of 1% Na-CMC solution, p.o. control to drugs). Group III: Diabetic rats treated with sitagliptin (5 mg/kg/day, p.o.) **(Tahara *et al.,* 2009; Shang *et al.,* 2012; Joo *et al.,* 2013)**. Group IV: Diabetic rats treated with repaglinide (0.15 mg/kg/day, p.o.). Group V: Diabetic rats treated with repaglinide (0.3 mg/kg/day, p.o.) **(Gumieniczek *et al.,* 2011)**. Group VI: Diabetic rats treated daily with a combination of sitagliptin (5 mg/kg) + repaglinide (0.15 mg/kg). Group VII: Diabetic rats treated daily with sitagliptin (5 mg/kg) + repaglinide (0.3 mg/kg). All the pharmacological treatments were given for 28 days started 10 days after STZ injection at which the steady state of hyperglycemia was acquired. The body weights of the rats were monitored weekly.

Oral glucose tolerance test

Oral glucose tolerance test (OGTT) was carried out at the end of the 28th day. D-glucose (2.5 g/kg, p.o.) was administered to the above mentioned groups at the 4th h of pre-treatment with drugs. Serum glucose was determined before D-glucose administration (zero time), then 15, 30, 45 and 60 min after glucose administration **(Badole and Bodhankar, 2009)**.

Hematological and biochemical parameters

Blood samples were collected in three groups of tubes by cardiac puncture of anesthetized rats into (0.11 M) trisodium citrate in the ratio (9:1 V/V). Blood samples in the first group were processed immediately by centrifugation at 2000 × *g* for 10 min followed by plasma separation into clean test tubes. (1) Prothrombin Time (PT): was determined, as indicator for extrinsic coagulation pathway, by mixing plasma with a combined calcium/thromboplastin reagent DiaPlastin® (DiaMed, Switzerland) after incubating them separately at 37 °C for 5 min, then timing for formation of the initial clot. Analysis was performed on automated coagulation analyzer (Sysmex CA-1500, Semins, Dade Behring, IL, USA). (2) Activated Partial Thromboplastin Time (APTT): was estimated, as marker for intrinsic coagulation pathway, by mixing sample plasma with the reagent DiaCelin® (cephaloplastin, rabbit brain, with complexed kaolin) (DiaMed, Switzerland) followed by incubation for 3 min at 37 °C then 0.02 M calcium chloride and the timing for initial clot formation was recorded. Analysis was performed on automated coagulation analyzer

(Sysmex CA-1500, Semins, Dade Behring, IL, USA). The second group of blood samples was used to prepare platelet-rich plasma (PRP), samples were centrifuged immediately at 160 × *g* for 15 min at room temperature, then PRP was transferred into plastic tubes and the remaining blood was centrifuged at 3000 × *g* for 10 min to obtain platelet-poor-plasma (PPP). Platelet count in PRP was adjusted to (5×10^8/ml) with PPP. Platelet aggregation was measured by addition of 5 µg/ml collagen (Chrono-Log corp.) using a dual channel aggregometer (Clot 2, SEAC- Radium Company, Italy). Results were expressed as a percentage of aggregation, extent of aggregation was estimated by change in light transmission.

The third group of blood samples was left at room temperature to allow clotting and sera were separated after centrifugation at 3000 × *g* and kept at -80 °C until used for estimation of various biochemical parameters. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities as well as serum total cholesterol (sTC), triglycerides (sTGs), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) were determined using commercial enzymatic colorimetric assay kits (Bio-diagnostic®, Cairo, Egypt) using a UV-visible spectrophotometer (UV-1601-PC, Shimadzu, Japan).

Histopathological examination of the pancreas & morphometric analysis

The whole pancreas from each animal was dissected and removed and its wet weight was recorded. Then, it was washed immediately with saline and fixed in 10% buffered neutral formalin solution. After fixation, the tissues were processed by embedding in paraffin and sectioned at 5-µm thickness for histological analysis. Sections were stained with hematoxylin and eosin and examined under high power microscope (20×) and photomicrographs were taken. Tissue sections were examined by two pathologists who were blinded of the study groups.

Pancreatic immunohistochemistry

Immunostaining was performed using a streptavidin–biotin-immunoperoxidase complex method with 5-µm thick sections. Tissues were deparaffinized and heated in citrate buffer solution (0.01 m, pH = 6.0) for 15 min using microwave oven to retrieve antigens. Rabbit polyclonal antibodies against insulin and Ki-67 (Thermo Fischer Scientific, Fremont, CA 94538, USA) were employed. Sections were incubated with the corresponding primary antibody at 4 °C overnight. After conjugation with streptavidin–biotin–peroxidase complex (Broad spectrum LAB-SA detection system, Invitrogen®), DAB was applied as the final chromogen, thereafter, the sections were counterstained with hematoxylin and examined under a light microscope (Olympus CX21, Japan). The morphometric analysis were done manually by a stereological method with mathematical support using Image pro software (Nikon) to measure the insulin- positive stained area and total pancreatic area at 100 × magnification. All analyses were performed in a blinded fashion where the investigator was not aware of the treatment groups throughout the analysis. Insulin-positive staining was found to be consistently located as a composite unit within the islet.

9. Statistical analysis

Results were expressed as mean ± SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. The Statistical Package of Social Science (SPSS) program version 17, (Chicago, IL, USA) was used for the statistical analysis. The differences were considered significant at $P < 0.05$.

RESULTS

Percentage survival and percent body weight gain

The current results demonstrated that there were no significant differences in the percentage survival calculated in the experimental groups (Fig. 1A). On the other hand, percentage change in body weight was lower in diabetic rats compared to normal control group. Monotherapy with sitagliptin further decreased the percentage body weight gain compared to the diabetic group. Differently, monotherapy with repaglinide (0.3 mg/kg) produced greater percentage body weight gain compared to the diabetic group. Addition of sitagliptin to repaglinide promoted a lesser degree of weight gain compared to repaglinide (Fig. 1B).

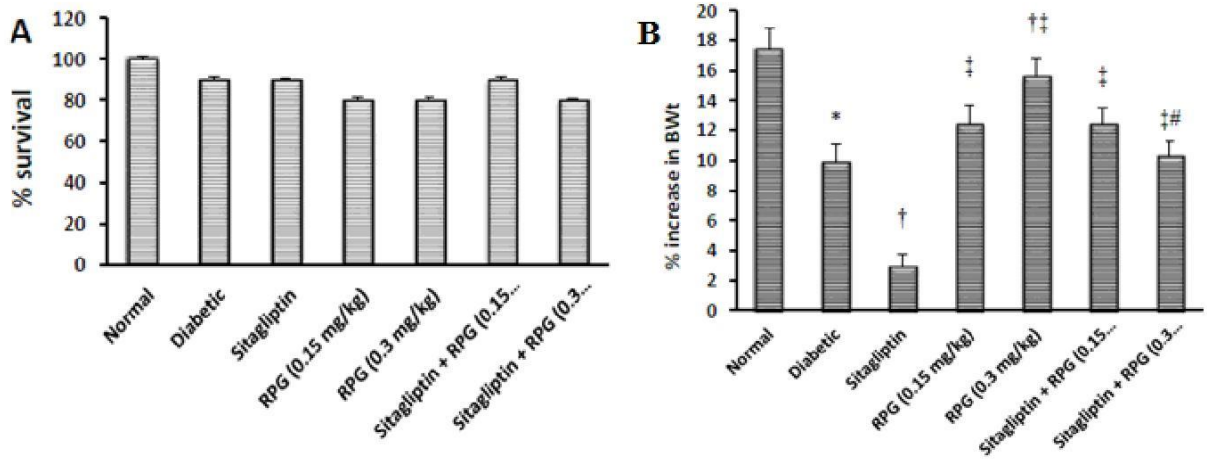

Fig. 1. Percent survival (A) and percent body weight gain (B) in the experimental groups. Data are presented as mean ± SEM and analyzed using one-way ANOVA followed by Tukey's multiple-comparisons test at $P < 0.05$. Compared to normal group, *Compared to diabetic group, †Compared to sitagliptin group, ‡Compared to repaglinide (0.15 mg/kg) group, $^{\$}$Compared to repaglinide (0.3 mg/kg) group.

Oral glucose tolerance test

To generate a non genetic model for diabetes, rats were fed with a HFD for four weeks followed by a low dose STZ injection. Diabetic rats showed greater AUC compared to the normal control group. Monotherapy with sitagliptin or repaglinide (0.15 or 0.3 mg/kg) or their combination decreased the measured AUC compared to diabetic rats. The combination of sitagliptin with repaglinide (0.15 or 0.3 mg/kg) showed smaller AUC compared to the corresponding monotherapies (Fig. 2).

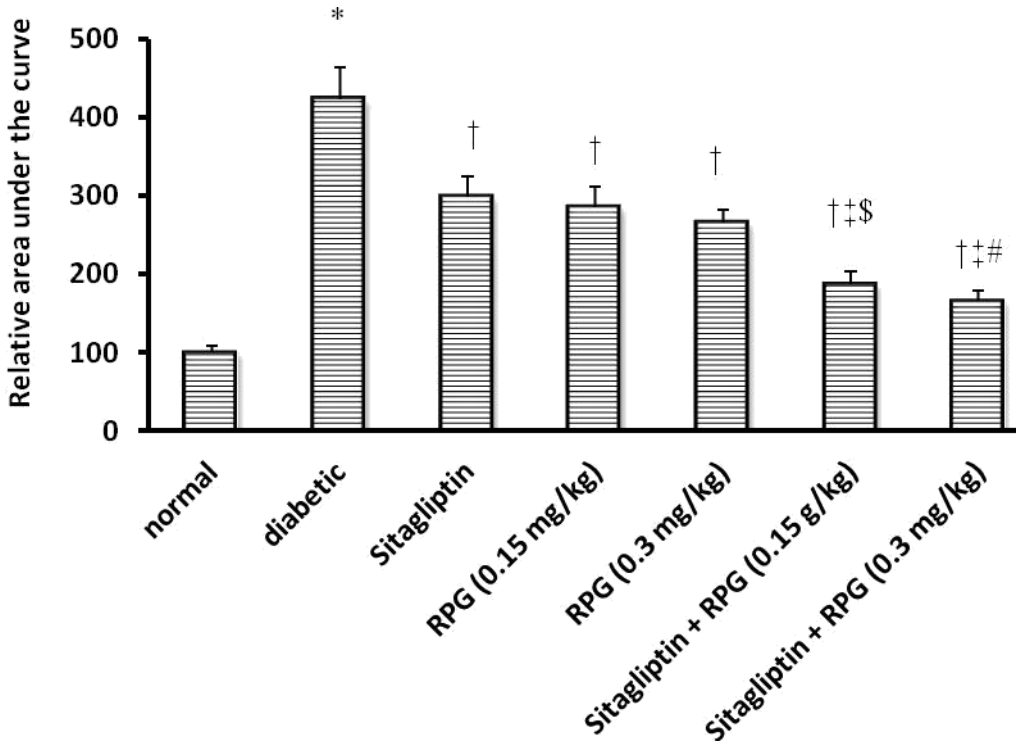

Fig. 2. Effect of sitagliptin + repaglinide (0.15 or 0.3 mg/kg) on the calculated area under the curve in the oral glucose tolerance test. RPG: repaglinide. Data are presented as mean ± SEM and analyzed using one-way ANOVA followed by Tukey's multiple comparisons test at $P < 0.05$. *Compared to normal group, †Compared to diabetic group, ‡Compared to sitagliptin group, $Compared to repaglinide (0.15 mg/kg) group, #Compared to repaglinide (0.3 mg/kg) group.

Hematological parameters, liver enzymes and lipid profile

These data highlighted that the PT value recorded in diabetic rats did not differ significantly from normal control group (Fig. 3A). Differently, the APTT was significantly higher in diabetic rats compared to normal control group. All the treatment regimens successfully ameliorated the APTT compared to the diabetic group (Fig. 3B). Regarding platelet aggregation, the measured aggregation percentage was greater in diabetic rats compared to normal control group. Monotherapy with sitagliptin, repaglinide (0.15 or 0.3 mg/kg) or their combination reduced the aggregation percentage compared to the diabetic group (Fig. 3C).

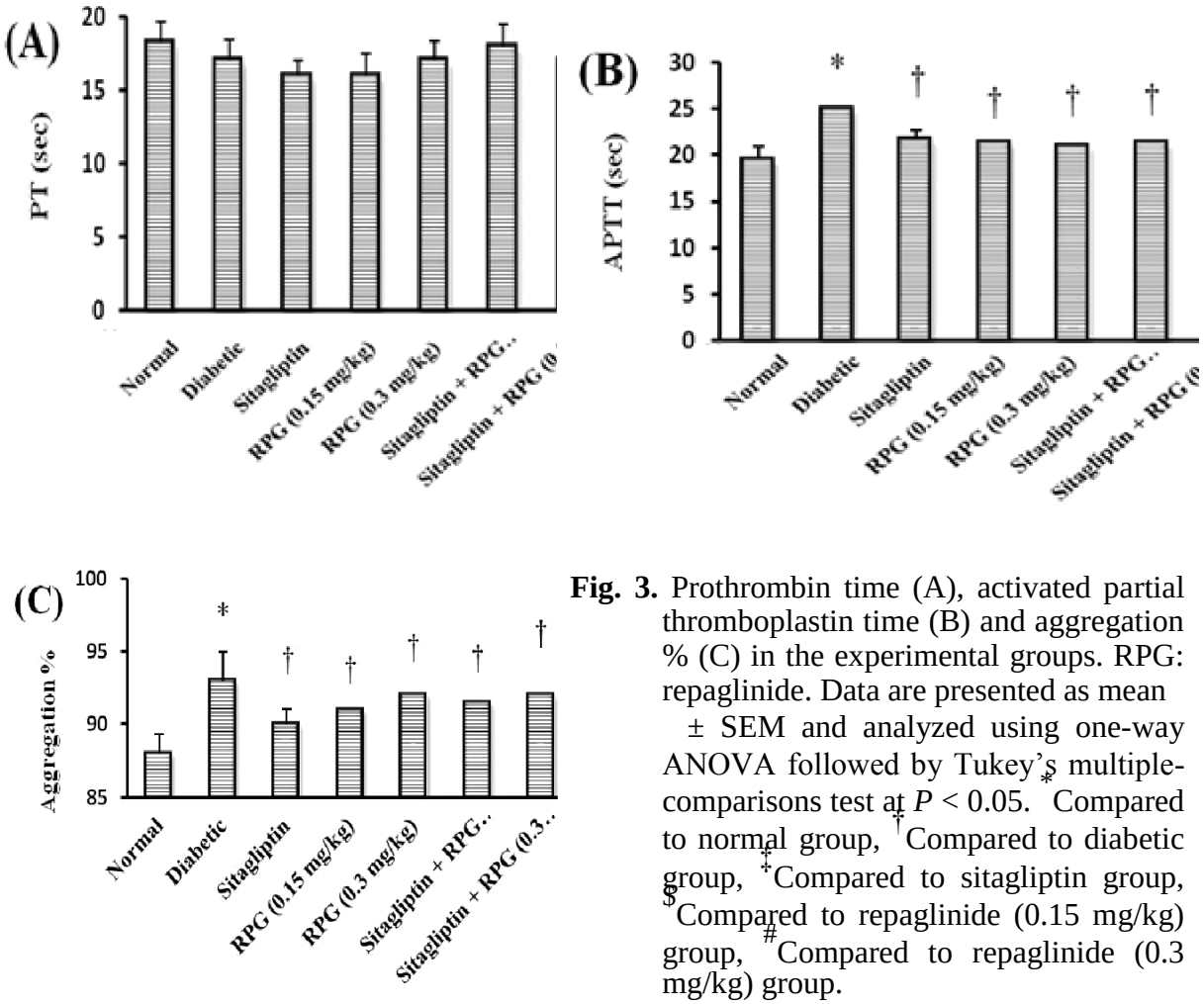

Fig. 3. Prothrombin time (A), activated partial thromboplastin time (B) and aggregation % (C) in the experimental groups. RPG: repaglinide. Data are presented as mean ± SEM and analyzed using one-way ANOVA followed by Tukey's multiple-comparisons test at $P < 0.05$. * Compared to normal group, † Compared to diabetic group, ‡ Compared to sitagliptin group, $ Compared to repaglinide (0.15 mg/kg) group, # Compared to repaglinide (0.3 mg/kg) group.

Determination of liver enzymes indicated greater levels of AST and ALP in diabetic rats compared to normal control group while no significant change occurred in the levels of ALT. Monotherapy with repaglinide (0.15 or 0.3 mg/kg) significantly reduced AST activity compared to diabetic rats. The combination of sitagliptin plus repaglinide (0.15 or 0.3 mg/kg) showed greater AST activity compared to monotherapies with repaglinide (0.15 or 0.3 mg/kg), respectively. None of the implemented agents induced a change in the activity of ALT or ALP (Table 1).

Estimation of serum lipid profile showed greater TC, TG and LDL but lower HDL in diabetic rats compared to normal control group. All the treatment regimens produced significant reductions in TC and LDL level compared to diabetic rats. Further, analysis of TG level demonstrated that all the treatment regimens-except for monotherapy with sitagliptin-successfully reduced its level in comparison to diabetic rats. Regarding HDL, the combination therapies ameliorated its level in comparison to diabetic rats (Table 1).

Table 1: Effect of drugs on serum liver enzyme activities and lipid profile in diabetic rats

Group	**AST (U/l)**	**ALT (U/l)**	**ALP (U/l)**	**sTC (mg/dl)**	**sTG (mg/dl)**	**LDL (mg/dl)**	**HDL (mg/dl)**
Normal	78 ± 4.4	29.2 ± 2.1	65.3 ± 6	69.3 ± 5	152.6 ± 10.3	34.5 ± 2.1	53.1 ± 4.3
Diabetic	92 ± 6.1*	36.3 ± 2.5	82.3 ± 9*	115.2 ± 8*	217.2 ± 18.2*	78.2 ± 5.4*	35.2 ± 2.8*
Sitagliptin	85 ± 6.3	38.2 ± 2.8	76.2 ± 8.1	89.2 ± 7.3†	199.2 ± 15.4	62.4 ± 4.8†	38.2 ± 3.1
RPG (0.15 mg/kg)	78 ± 5.2†	41.3 ± 3.2	73.1 ± 6.2	86.3 ± 5.7†	184.3 ± 13.2†	67.3 ± 5.7†	39.1 ± 2.8
RPG (0.3 mg/kg)	74 ±6.2†	46.4 ± 4.9	74.2 ± 6.3	88.2 ± 6.9†	175.2 ± 15.4†	60.8 ± 4.8†	37.8 ± 3.6
Sitagliptin + RPG (0.15 mg/kg)	94 ± 8.7$	43.2 ± 2.3	81.1 ± 7.2	81.3 ± 6.5†	173.2 ± 14.5†	52.2 ± 5.1†	41.8 ± 3.7†
Sitagliptin + RPG (0.3 mg/kg)	90 ± 5.2 #	47.2 ±5.3	85.3 ± 5.4	79.1± 5.5 †	165.4 ± 13.7†	47.3 ± 4.2 †‡#	46.9 ± 4.4 †#

ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, sTC: serum total cholesterol, sTG: serum triglycerides, LDL: low-density lipoprotein, HDL: high-density lipoprotein, RPG: repaglinide. Data are presented as mean ± SEM and analyzed using one-way ANOVA followed by Tukey's multiple comparisons test. *Compared to normal group at $P < 0.05$. †Compared to diabetic group at $P < 0.05$. ‡Compared to sitagliptin group at $P < 0.05$. $Compared to repaglinide (0.15 mg/kg) group at $P < 0.05$, #Compared to repaglinide (0.3 mg/kg) group at $P < 0.05$, *n = 6-10*.

Morphometric analysis

Morphometric analysis for pancreatic islets indicated that diabetic rats showed lower islet cell diameter compared to normal control group (Fig. 4A). Monotherapy with sitagliptin or repaglinide (0.3 mg/kg) produced significant increases (two-fold and three-fold, respectively) in the islet cell diameter (Fig. 4B). The combination of sitagliptin plus the high dose of repaglinide (0.3 mg/kg) produced a significant increase in islet cell diameter compared to diabetic rats as well as sitagliptin-treated rats (Fig. 4A&B).

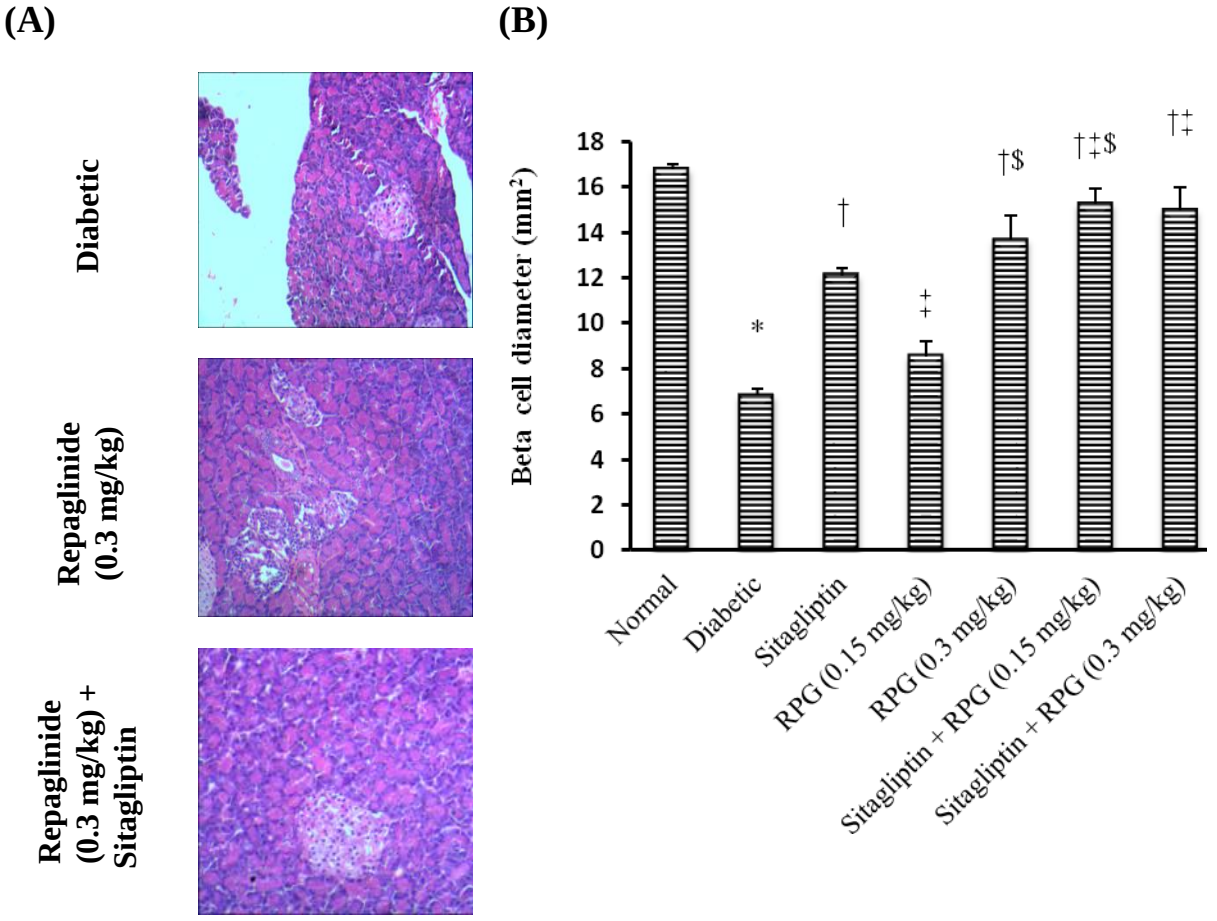

Fig. 4. A) Histopathological picture of pancreatic beta cell of the experimental groups [hematoxylin & eosin, at x100 magnifications]. **B)** A graphic presentation for the diameter of the pancreatic islets. RPG: repaglinide. Data are presented as mean ± SEM and analyzed using one-way ANOVA followed by Tukey's multiple comparisons test at $P < 0.05$. *Compared to normal group, †Compared to diabetic group, ‡Compared to sitagliptin group at $P < 0.05$, $^{\$}$Compared to repaglinide (0.15 mg/kg) group, $^{\#}$Compared to repaglinide (0.3 mg/kg) group.

Immunohistochemical analysis

Immunohistochemistry for insulin positive cells showed higher optical density in rats treated with repaglinide or combination therapies compared to diabetic rats (Fig 5A). The optical density for insulin positive cells was greater in all the study groups –except sitagliptin group- compared to diabetic rats. The effect of the combination therapies [sitagliptin + repaglinide (0.15 mg/kg)] or [sitagliptin + repaglinide (0.3 mg/kg)] on immunostaining for insulin was significantly greater than its corresponding monotherapies (Fig. 5B).

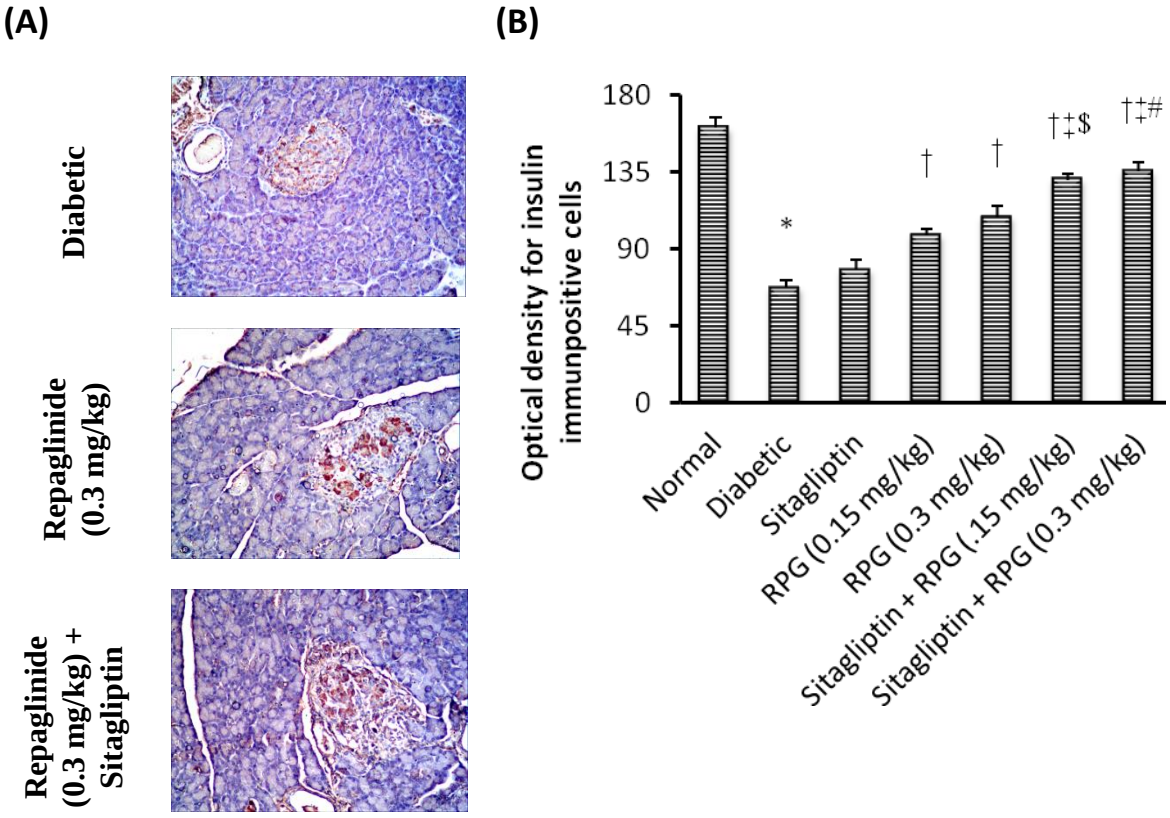

Fig. 5. A) Immunohistochemical staining for insulin in the pancreatic islet cells of the experimental groups (x 40). **B)** A graphic presentation for the optical density of the insulin immunpositive cells in the pancreatic islets in the experimental groups. RPG: repaglinide. Data are presented as mean ± SEM and analyzed using one-way ANOVA followed by Tukey's multiple comparisons test at $P < 0.05$. *Compared to normal group. †Compared to diabetic group, ‡Compared to sitagliptin group, $Compared to repaglinide (0.15 mg/kg) group, #Compared to repaglinide (0.3 mg/kg) group.

Furthermore, immunohistochemical staining for Ki-67 demonstrated that all the treatment regimens increased the number of immunpositive nuclei in pancreatic islet cells compared to diabetic group. The effect of the combination therapies was greater than that produced by monotherapy with sitagliptin or repaglinide (0.15 or 0.3 mg/kg) (Fig. 6A&B).

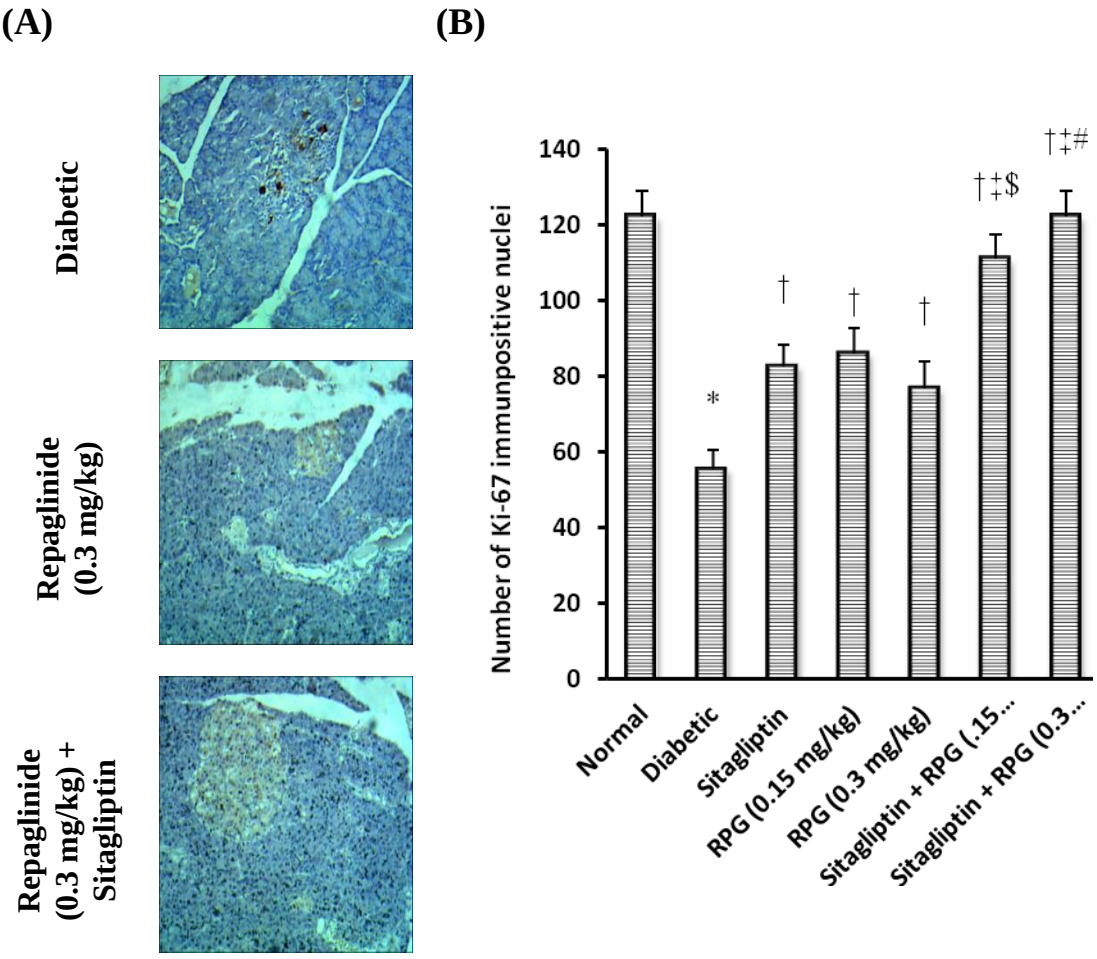

Fig. 6. A) Immunohistochemical staining for Ki-67 in the pancreatic islet cells of the experimental groups (x 100). **B)** A graphic presentation for the number of immunpositive nuclei in the pancreatic islets. RPG: repaglinide. Data are presented as mean ± SEM and analyzed using one-way ANOVA followed by Tukey's multiple-comparisons test at $P < 0.05$. *Compared to normal group, †Compared to diabetic group, ‡Compared to sitagliptin group, $^{\$}$Compared to repaglinide (0.15 mg/kg) group, $^{\#}$Compared to repaglinide (0.3 mg/kg) group.

DISCUSSION

Diabetes is a rising global hazard; the World Health Organization has predicted that 366 million people will have diabetes by 2030. Type 2 diabetes is a heterogeneous and polygenic metabolic disease that is usually associated with β-cell failure, so a key challenge is to find therapies that improve β-cell function **(Barroso *et al.*, 2003; Cuevas-Alvarez *et al.*, 2006; Smyth and Heron, 2006)**. In patients with type 2 diabetes, the long term treatment with a single antidiabetic drug often results in a poor maintenance of the glycemic control due to the deteriorating β-cell function in addition to the side effects of the existing antidiabetic drugs **(Hou *et al.*, 2012)**. Therefore, combining antidiabetic drugs with different or complementary mechanism of action can improve our control over hyperglycemia. The current study aimed to investigate the added benefits of combining two antihyperglycemic agents, sitagliptin and repaglinide, in a rat model for type 2 diabetes.

In this study, we used a HFD rat model with low dose of STZ (35 mg kg^{-1}) which can mimic the pathophysiological state of type 2 diabetes. HFD feeding induced insulin resistance and STZ injection induced β-cell dysfunction **(Srinivasan *et al.*, 2005)**, we observed the significant improvement in glycemic control and pancreatic insulin production

upon the addition of sitagliptin to repaglinide therapy. Treatment with repaglinide alone or in combination with sitagliptin counteracted the catabolic loss of body weight observed in the diabetic group. Sitagliptin therapy significantly reduced the body weight gain, which can be attributed to the DPP-4 inhibition and subsequently improved glucose tolerance **(Kim *et al.*, 2012)**. Alternatively, it can be attributed to its anorectic effect as GLP suppresses the hypothalamic appetite centers after meals and/or increased energy expenditure. Double incretin receptor knockout mice fed a HFD displayed the resistance to diet-induced obesity and increased energy expenditure associated with increased locomotor activity **(Hansotia *et al.*, 2007)**.

The combination therapy was also more effective in restoring glucose homeostasis and showed a lower AUC compared to the other treatment groups. All treatment regimens improved the islet architecture. Interestingly, repaglinide (0.3 mg/kg) as well as sitagliptin plus repaglinide (0.3 mg/kg) treated groups had the highest islet cell diameter. These results suggest that the repaglinide either alone or in combination is effective in preserving islet cell integrity. Alternatively, this could be attributed to the low dose of sitagliptin used in the study (5 mg/kg) and its short half-life in rats **(Kim *et al.*, 2005; Beconi *et al.*, 2007)**. Further evaluation of the β- cell mass through immunolabeling pancreatic sections with anti-insulin, revealed higher insulin content in all treated groups compared to the diabetic group. The β-cell mass in the combination groups were significantly higher than their corresponding monotherapies. This restoration of islet cell was likely due to the enhancement of the cell proliferation as the immunostaining for the cell proliferation marker anti-Ki67 showed a significant increase in all treated groups and a more potent effect in the combination therapy. Finally, the combination therapy showed a better lipid profile compared to the diabetic and the corresponding monotherapy groups.

Repaglinide is a carbamoyl methilbenzoil derivated acid that has been introduced to control the post-prandial hyperglycemia. Through its benzoic acid moiety, repaglinide initiates the insulin secretion by closing the ATP-sensitive K^{+} channels, thereby activating the Ca^{++} channels with increase in intracellular Ca^{++} influx. The increase in the intercellular calcium leads to a rapid insulin release **(Grell *et al.*, 1998; Owens *et al.*, 2000; Ambavane *et al.*, 2002)**. Repaglinide has a short half-life (4 h); therefore it is usually given as multiple daily doses before meals **(Owens *et al.*, 2000; Ambavane *et al.*, 2002; Rizzo *et al.*, 2004)**. On the other hand, Sitagliptin is a member of a new class of anti-hyperglycemic agents that can maintain the glycemic control in a glucose-dependent manner. It is an oral DPP-4 inhibitor that prevents the enzymatic degradation of incretins GLP-1 and GIP **(Drucker and Nauck, 2006; Han *et al.*, 2011)**. In previous studies done on animal models, GLP-1 and GIP have been reported to have positive effects on β-cell mass through promoting β-cell survival or stimulating islet neogenesis **(Farilla *et al.*, 2002; Tourrel *et al.*, 2002; Trumper *et al.*,2002)**. Thus, the beneficial effects of sitagliptin on β-cell mass and function in the current study could be partially mediated directly via increased incretin signaling. Additionally, sitagliptin was reported to increase the islet insulin content and improve the islet responsiveness to glucose stimulation **(Deacon and Holst, 2002; Deacon, 2007; Mu *et al.*, 2009)**. Further, in a 2-year study carried on human patients, sitagliptin was found beneficial in reducing the body weight and decreasing TC, TG and LDL **(Derosa *et al.*, 2012)**.

In conclusion, this combination therapy was found to be more efficient in improving the glycemic control in STZ-treated rats fed with HFD. Additionally, this combination therapy had also a positive effect in lowering TC, TG and LDL. Therefore, the complementary mechanisms of repaglinide and sitagliptin can be beneficial to lower the blood glucose in patients with uncontrolled type 2 diabetes and especially in patients with abnormalities in their lipid profile.

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اضافة عقار سيتاجليبتين الى عقار ريباجلينيد يحسن من تكاثر خلايا البنكرياس و انتاج الانسولين فى الداء السكرى من النوع الثانى و المستحدث تجريبيا فى الجرذان

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االاهداف: اختبار ما اذا كان اضافه جرعه صغيرة من عقار سيتاجلبتين الى عقار ريباجلينيد له قدره افضل على ضبط ارتفاع نسبه السكر في الدم وتحسين وظائف البنكرياس وذلك في الجرذان المصابه بداء السكري من النوع الثاني والمحدث عن طريق عقار الستربتوزتوسن مع نظام غذائى مرتفع فى نسبة الدهون.

الطريقة: أجريت هذه الدراسة في قسم علم الادويه والسموم، كلية الصيدلة، جامعتي قناة السويس- االاسماعيلية والازهر- القاهرة، وقد اختيرت ذكور الجرذان البيضاء لهذه الدراسة وقسمت الحيوانات الى سبع مجموعات تحتوي كل مجموعه على عشرة جرذان. و لقد قمنا بقياس و قت التجلط. كما قمنا بالتقدير البيوكيميائي لمحتوى مصل الدم لكل من: الانزيمات الناقله لمجموعة الامين وانزيم الفوسفاتيز القاعدي و الكولسترول و الدهون الثلاثية و البروتينات الدهنية مرتفعة الكثافة و منخفضة الكثافة. وقد أجري الفحص المجهري و فحص عن طريق تقنيات الكيمياء المناعية لشرائح من أنسجة البنكرياس لتقدير تأثير العقارات المستخدمة على خلايا البنكرياس.

النتائج: أشارت نتائج الدراسة الى تحسن ملحوظ في ضبط مستوى سكر الدم وافراز البنكرياس لهرمون الانسولين وذلك عند اضافه جرعة صغيرة من عقار سيتاجلبتين الى عقار ريباجلينيد. وقد وجد ايضا ان تعاطي كلا العقارين معا قد تسبب في انخفاض ملحوظ في مستوى الدهون في مصل الدم.

خاتمه: تساهم النتائج الحاليه في اظهار اهميه استخدام كلا العقارين (السيتاجلبتين والريباجلينيد) معا لتحسين القدرة على ضبط مستوى السكر في الدم وانخفاض مستوى الدهون في مصل الدم وذلك في المرضى المصابين بداء السكري من النوع الثاني.