Medicine Updates
Faculty of medicine
April 2020,volume 1, issue 1
https://muj.journals.ekb.eg
dean@med.psu.edu.eg
vice_dean_postgraduate@med.psu.edu.eg
DOI:10.21608/muj.2020.25106.1007
ISSN : 2682-2741

Submitted: 3/3/2020
Accepted : 4/3/2020
Pages:9-28

The Possible Antidiabetic Effects of Ranolazine Versus Glimepiride In STZ-Induced Type 2 Diabetes In Male Wistar Rats

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Abstract

Background: Type 2 diabetes is a major illness that distresses millions of people. The crucial causes of type 2 diabetes are insulin resistance and decreased insulin secretion

Objectives: To evaluate the possible effects of ranolazine versus glimepiride on blood glucose levels, HbA1c, nitric oxide and oxidative stress markers in STZ-induced type 2 diabetes in male Wistar rats and their effect on the histopathological picture of the pancreas.

Materials and Methods: Forty male Wistar rats were divided into four groups. The normal control group which received saline (1 mg/kg/day) for 5 weeks. The diabetic control group that received saline (1 mg/kg/day) for 5 weeks. The glimepiride-treated group that received glimepiride ((0.1 mg/kg/day)) once daily for 5 weeks and the ranolazine-treated group that received ranolazine (20 mg/kg) twice daily for 5 weeks. Body weight and fasting blood glucose levels were measured weekly for the 5 weeks, then blood samples were attained for several biochemical analysis: lipid profile, HbA1c, and AGEs. Then rats were sacrificed and the pancreatic tissues were attained for oxidative stress markers assessment, and for histopathological examination using hematoxylin and eosin stain.

Results: Ranolazine improved diabetes by reducing fasting blood glucose level, HbA1c, and AGEs. Furthermore, it improved the oxidative stress markers, and the histopathological picture of the pancreas.

Conclusion: Ranolazine has the potential to become an innovative agent for

treating type 2 diabetes patients.

Keywords: Diabetes mellitus, STZ, Oxidative stress markers, HbA1c. Keywords: Diabetes mellitus, STZ, Oxidative stress markers, Caspase-3, HbA1c.

Introduction

Type 2 diabetes is a major illness that affects millions of people. The principal causes of type 2 diabetes are insulin resistance and decreased insulin secretion **[1]**. About 2,623,000 people in Egypt are affected, with the anticipation of 6,726,000 people in 2030 **[2]**. Moreover, Type 2 diabetes is considered a risk factor for cardiovascular events and is a substantial predictor of cardiovascular morbidity and mortality **[3]**.

Ranolazine is an innovative drug for angina that decreases frequency of angina attacks and recovers exercise tolerance in the affected patients **[4]**. Ranolazine is a selective inhibitor of cardiac late sodium channels that result in decreasing the intracellular Na^{+} and Ca^{+2}, leading to its anti-anginal properties in myocardial ischemia. **[5]**. Ranolazine also has been revealed to lower HbA_{1c} in cardiac patients with comorbid diabetes **[6]**. In the Combination Assessment of Ranolazine In Stable Angina trial**,** ranolazine significantly lowered HbA1c levels by 0.7 ± 0.18% when it is given for 12 weeks irrespective of concomitant oral antidiabetic therapy. Furthermore, long-term ranolazine treatment shows preservation of β-cell and improvement of insulin secretion **[7]**.

Glimepiride is insulin secretagogue that used frequently for type 2 diabetes mellitus (DM) treatment. Glimepiride is a second-generation sulfonylurea acts directly by binding to the ATP-dependent potassium channels (K+ATP) on the β-cells. The closure of these channels by sulfonylureas results in depolarization of the β-cells and a successive calcium influx which leads to glucose-independent insulin release resulting in reduction of blood glucose level **[8]**.

Linking the fact that calcium channel antagonists and β-receptor blockers produce hyperglycmia and that ranolazine behaves as an add on treatment for beta-antagonists and calcium channel antagonists in anginal patients with the fact that ranolazine has been revealed to lower HbA_{1c} in patients with angina, ranolazine was logical applicant for study. Therefore, the current study was conducted to observe the possible antidiabetic effect of ranolazine and its mechanisms in STZ- induced type 2 diabetes in rats.

Materials and Methods

Experimental animals: Forty adult male wistar rats, weighing 180-200 g. They were accommodated in polyethylene cages with free access to standard animal diet and tap water *ad libitum*. The rats were kept under standard conditions of normal light-dark cycle and temperature adjusted between 25-30°C.

Induction of type-2 diabetes: Rats received a single injection of STZ (45 mg/kg, i.p.). Five days after STZ injection, rats with fasting blood glucose levels >200 mg/dl were designated as diabetic **[9]**. Then, rats divided into four groups of ten animals each. The normal control group that received saline (1 mg/kg/day) for 5 weeks. The diabetic control group that received saline (1 mg/kg/day) for 5 weeks. The glimepiride-treated group that received oral glimepiride (0.1 mg/kg/day) once daily for 5 weeks **[10]**. The ranolazine-treated group that received oral ranolazine (20 mg/kg) twice daily for 5 weeks **[11]**.

Body weights and blood glucose levels were measured weekly for the 5 weeks. At the end of 5 weeks treatment, blood samples were obtained for various biochemical analysis: HbA1c, Lipid profile (Total cholesterol, HDL, TG, LDL and VLDL), and advanced glycated endproducts (AGEs). Then rats were sacrificed and The pancreatic tissues were gained for oxidative stress markers (MDA, GSH) estimation, for histopathological examination using hematoxylin and eosin (H and E) stain.

Statistical analysis: All the grouped data were statistically estimated using statistical

package for social sciences (SPSS) program (windows version number 10) and were expressed as mean ± SEM. The gained data were analyzed by one-way ANOVA followed by Banferroni's multiple comparisons test. Data with a P value < 0.05 were considered statistically significant.

Results

Fig (1) Revealed that STZ-challenged rats showed significant surge in FBS levels in comparison to the normal control group ($p<0.05$) beginning from the start of the study and continued till the end of experiment. Treatment with either glimepiride or ranolazine significantly ($p<0.05$) lowered FBS levels as compared to diabetic control group; with significant ($p<0.05$) differences between the two treated groups.

Fig (2) Revealed that STZ-challenged rats showed significant reduction in BW in comparison with normal control group ($p<0.05$) starting from the fourth week. Treatment with glimepiride significantly ($p<0.05$) elevate BW as compared to diabetic control group starting from the second week. Treatment with ranolazine significantly ($p<0.05$) rise BW as compared to diabetic control group starting from the second week; with significant ($p<0.05$) differences between the two treated groups.

Table (2) Revealed that STZ-challenged rats showed a significant augmentation in cholesterol, LDL, TG, and VLDL levels accompanying by significant reduction in HDL levels compared to normal-control group ($p<0.05$). Treatment with either glimepiride or ranolazine significantly mitigated these parameters as compared to diabetic-control group ($p<0.05$) with significant ($p<0.05$) differences between the two treated groups in Cholesterol, TG, and LDL.

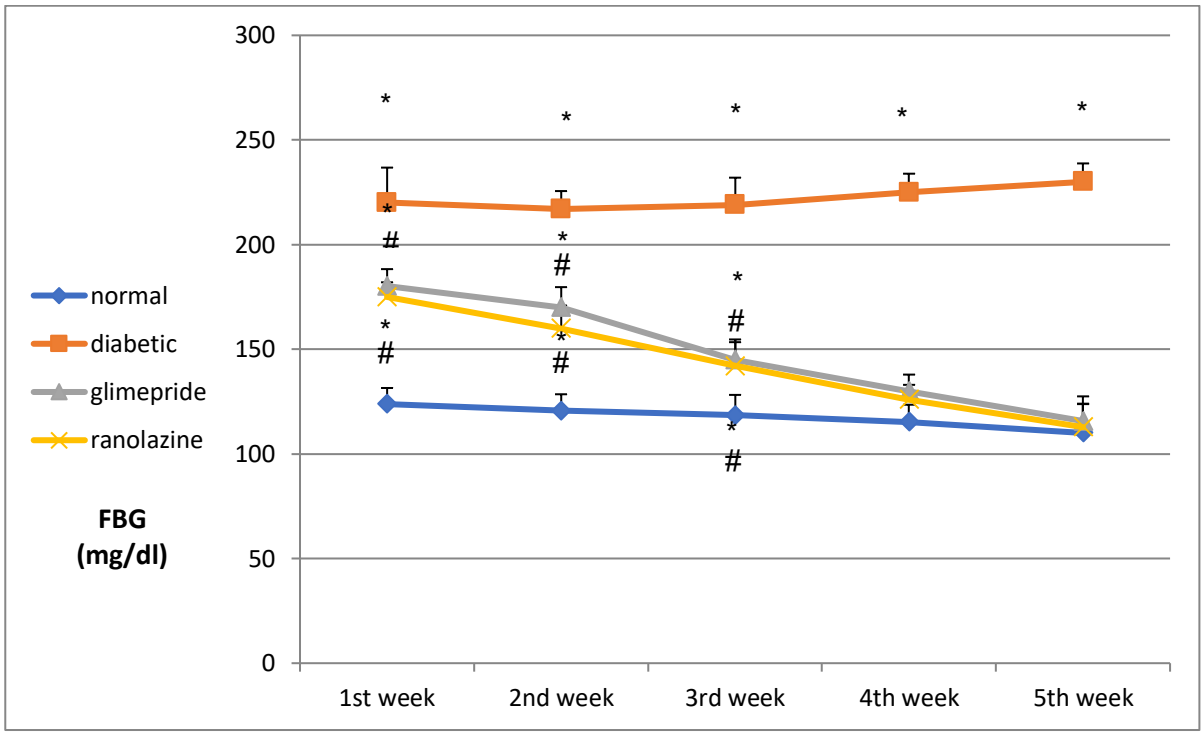
Fig (3) Showed that STZ-challenged rats was associated with significant ($p<0.05$) surge in HbA_{1c}, and AGEs levels in comparison with the normal control group. Treatment with either glimepiride or ranolazine significantly ($p<0.05$) ameliorated these augmented levels compared to diabetic control group with insignificant ($p>0.05$) differences between the two treated groups

Fig (4) Showed that STZ-challenged rats increased oxidative stress in the form of

significant ($p<0.05$) augmentation of MDA level and significant ($p<0.05$) decrease in GSH levels compared to normal control group**.** These lethal effects were significantly ($p<0.05$) improved with either glimepiride or ranolazine as compared to diabetic control group with significant *(p*<0.05) differences between the two treated groups on MDA, and GSH levels. Clearly, treatment with ranolazine revealed significant difference ($p<0.05$) in MDA, and GSH levels compared to glimepiride treated group.

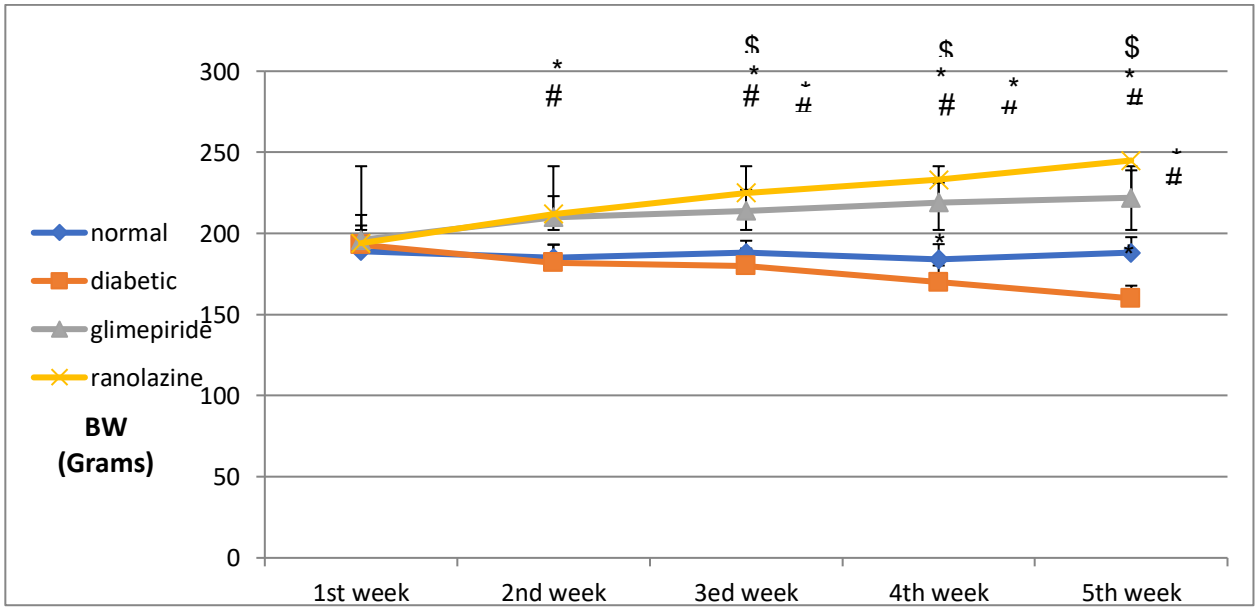
Fig (5A-D) Examination of H and E-stained islets of pancreas exhibited that: In normal-control group, there was preserved rounded contour of islets (C), the cells have eosinophilic cytoplasm **(Fig-5A)**. On the other hand, pancreatic islets cells from diabetic-control group showed lost rounded contour of islets with islets shrinkage due to reduction in the number of cells within each islet. The cells have eosinophilic cytoplasm, rounded to angulated nuclei with shrunken cells size. Most of cells showed vacuolar (hydropic) degeneration (V), with scattered deeply stained eosinophilic bodies (apoptotic bodies) (A) **(Fig-5B).** Treatment with glimepiride showed focally restored contour of some islets, others still have irregular contours and less cellular due to an increase in the number of cells within each islet. The cells have eosinophilic cytoplasm, rounded to angulated nuclei, and residual vacuolar (hydropic) degeneration, with apoptotic bodies **(Fig-5C)**. Treatment with ranolazine showed restored rounded contour of islets; having cellular islets due to an increase in the number of cells within each islet. The cells have eosinophilic cytoplasm, rounded regular nuclei, no vacuolar (hydropic) degeneration, with very few apoptotic bodies **(Fig-5D)**.

Figure 1: Effect of Glimepiride and Ranolazine on FBG (mg/dl) levels in STZ-induced diabetic rats:



Statistically significant difference versus normal control group (P-value < 0.05)
 # Statistically significant difference versus diabetic control group (P-value < 0.05)
$Statistically significant difference versus glimepiride-treated group (P-value < 0.05)

Figure 2: Effect of Glimepiride and Ranolazine on body weight (BW) (grams) in STZ-induced diabetic rats:



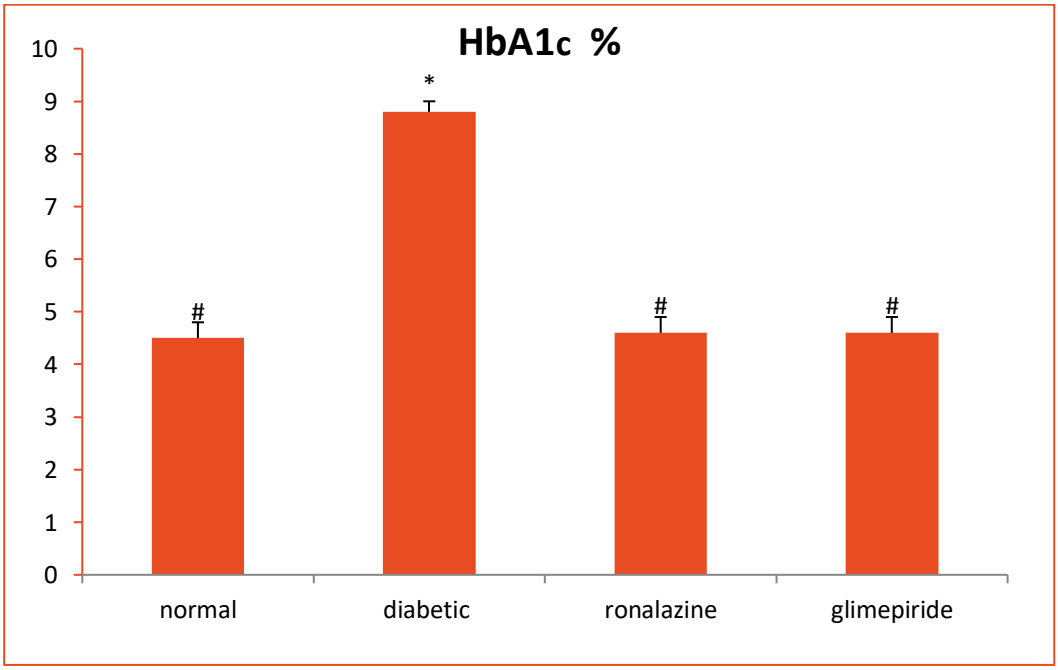
Statistically significant difference versus normal control group (P-value < 0.05)
 # Statistically significant difference versus diabetic control group (P-value < 0.05)
$ ***Statistically significant difference versus glimepiride-treated group (P-value < 0.05)***

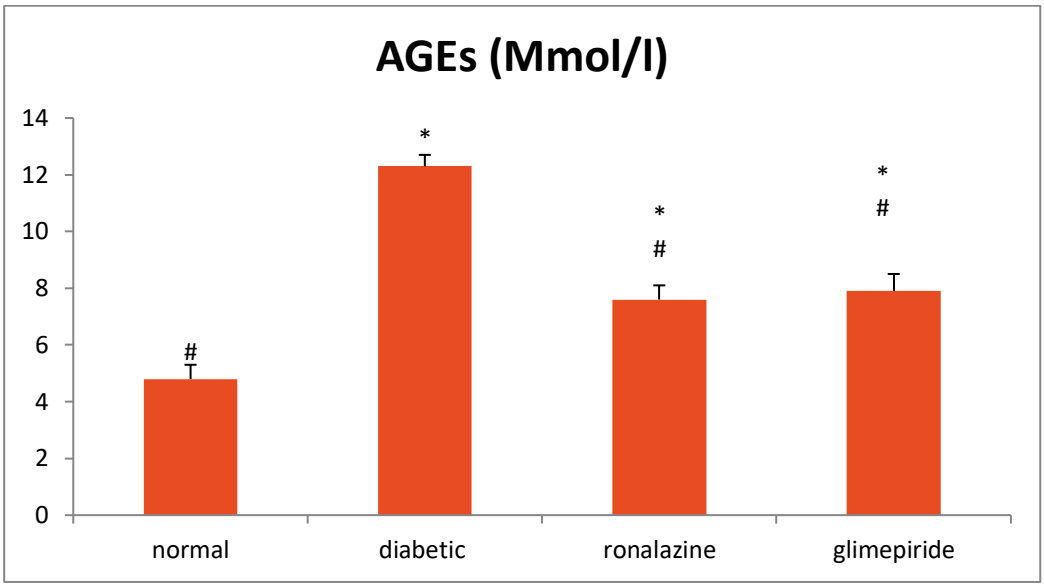
Table 1: Comparison of lipid profile (cholesterol (mg/dl), TG (mg/dl), HDL (mg/dl), LDL (mg/dl) and VLDL(mg/dl)) among the experimental groups.

	Cholesterol (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Normal	99±8	63±2	50±3.2	36±3.5	30±1.3
Diabetic control	220±6*	160±0.6*	30±1.6*	150±5.8*	69±4.1*
Glimepiride	115±9#	82±4.3*#	45±1.4#	66±10.9*#	38±2.2#
Ranolazine	100±5#	65±2.6#	49±1.3#	40±3.5#$	35±1.3#

Statistically significant difference versus normal control group (P-value < 0.05)
 # Statistically significant difference versus diabetic control group (P-value < 0.05)
$ ***Statistically significant difference versus glimepiride -treated group (P-value < 0.05)***

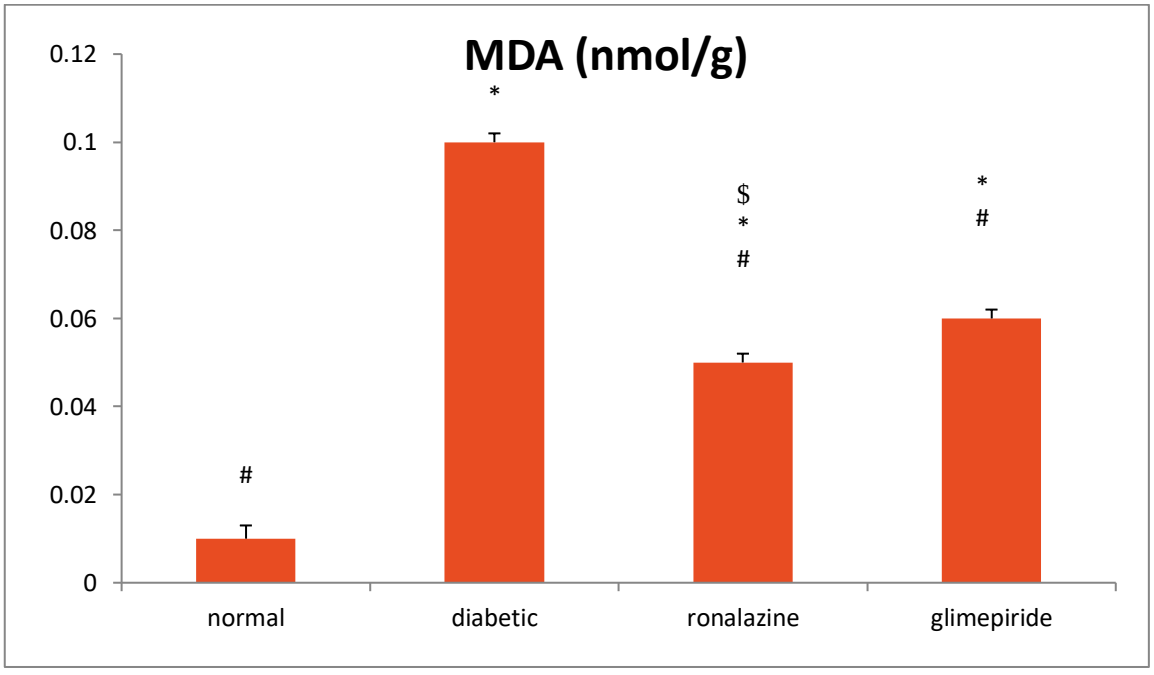
Figure 3: Effect of Glimepiride and Ranolazine on HbA_{1c}(serum %), and AGEs (Mmol/l) in STZ-induced diabetic rats:

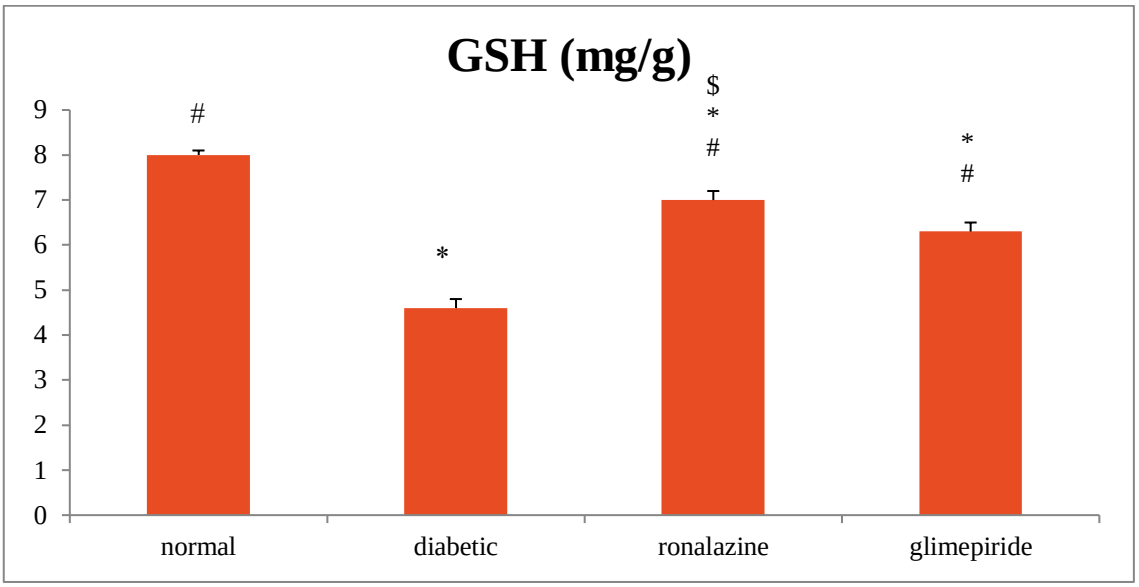




Statistically significant difference versus normal control group (P-value < 0.05)
 # Statistically significant difference versus diabetic control group (P-value < 0.05)
$Statistically significant difference versus glimepiride -treated group (P-value < 0.05)

Figure 4: Effect of Glimepiride and Ranolazine on oxidative stress markers (MDA (nmol/g), and GSH (mg/g) in STZ-induced diabetic rats:

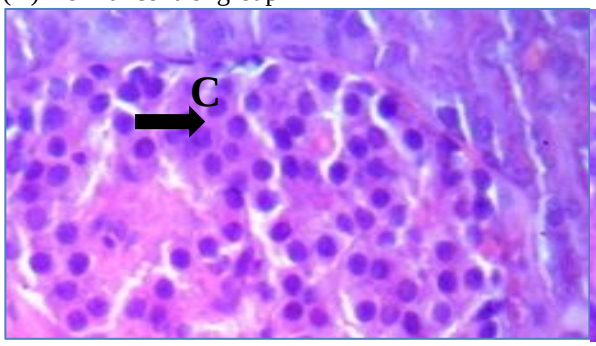




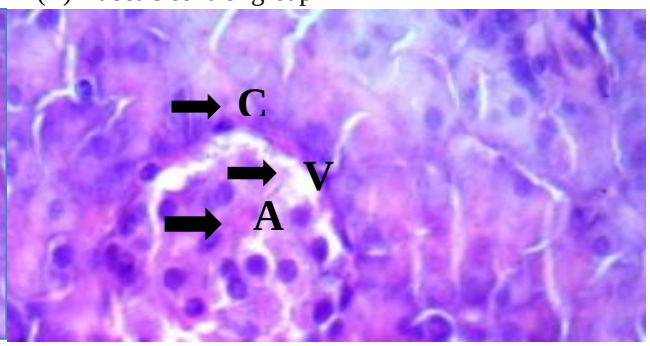
Statistically significant difference versus normal control group (P-value < 0.05)
 # Statistically significant difference versus diabetic control group (P-value < 0.05)
$ ***Statistically significant difference versus glimepiride -treated group (P-value < 0.05)***

Figure 5: Histopathological evaluation of pancreatic islets stained with H/E.

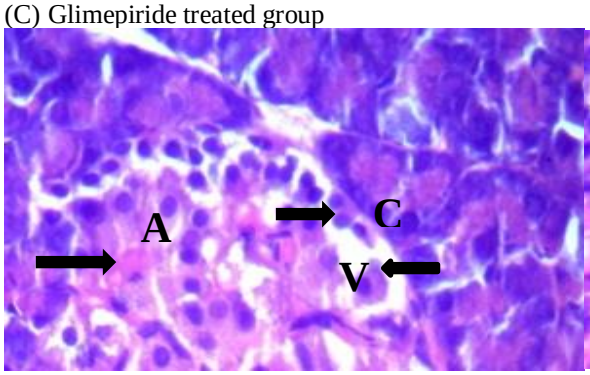
(A) Normal control group



(B)Diabetic control group



(C) Glimepiride treated group



(D)Ranolazine treated group

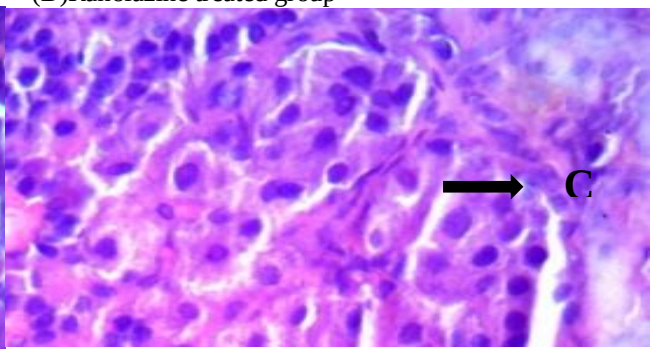


Fig. (5A-D) A photomicrograph of a section in pancreatic islets of a rat from all experimental groups **(H&EX400).**

Discussion

Ranolazine is an innovative drug for angina which surges exercise duration and reduces frequency of anginal attacks in chronic angina patients. Ranolazine likewise has advantageous effects in diabetics as noticed by significant declines in HbA_{1c} in the clinical trials **[12,13]**. This study was directed to assess the anti-diabetic effect of ranolazine in STZ-challenged rats, which causes moderate hyperglycemia due to damage of pancreatic β-cells.

Streptozotocin (STZ) is a commonly powerful alkylating agent that yields a selective lethal effect on β-cells of pancreas and brings DM in most experimental animals **[14].**

Glimepiride, which is used as a standard antidiabetic agent in the current study improved STZ-induced hyperglycemia via increasing insulin release from β-cells of pancreas and accelerating tissue uptake and consumption of glucose **[15]**. Ranolazine competently improved the harmful effects related to the STZ as proven by reducing FBG level and HbA_{1c} **[16].**

In accordance with **[17]** studies, the current study revealed that STZ-induced hyperglycemia produced noticeable increased level of serum TG, TC and LDL-cholesterol (LDL-C) and decreased level of serum HDL cholesterol (HDL-C). One conceivable explanation could be afforded by **[18]** who highlighted that, this hyperlipidemia may be due to elevated level of cortisol and insulin insufficiency, which have an imperative role in fat accumulation process.

In agreement with **[19]**, treatment with glimepiride proficiently lowered TC, TG and LDL-C concentrations and augmented HDL-C levels due to its stimulatory effect on the release of insulin. In accordance with **[20,21]**, the current study shown that the STZ produced hyperglycemia and hyperinsulinemia; that were competently ameliorated by treatment with either glimepiride or ranolazine.

Advanced glycated end products (AGEs) are involved in endothelial dysfunction

[22]. The augmented formation of AGEs establishes a possible mechanism of hyperglycaemia-induced diabetic complications **[23]**. In accordance with **[24,25]**, our results revealed that glimepiride treatment competently lowered the augmented AGEs levels. Moreover, the current results revealed the antiglycation outcome of ranolazine on glucose-induced AGE formation.

High levels of free radicals can produce Products of Lipid peroxidation such as MDA which are significant in the pathogenesis of DM complication **[26,27].** In the current study, a significant reduction in GSH and an elevation in MDA were observed in STZ-challenged rats. Treatment with either glimepiride or ranolazine produced a significant reduction in MDA and significant surge in GSH activity; reflecting the antioxidant characteristics of glimepiride and ranolazine. revealed that glimepiride may lessen the oxidative stress via acting as a free radical scavenger **[28]**.

The histopathological analysis of the pancreas using H/E assured the biochemical markers and revealed the islets' collapse. In harmony with **[29,30],** the histopathological examinations of islets of pancreas in STZ-challenged rats showed hypocellularity, severe size shrinkage in association with deteriorating changes in pancreatic duct lining cells in comparison to normal controls.

The worsening of glycemic control in diabetes is supposed to be related to β-cell mass loss and progressive deterioration of β-cell function. The major finding of this study is that treatment with ranolazine decelerates the diabetes progression by preserving β-cell mass in STZ-challenged rats. Thus, the role of ranolazine in preservation of β-cell could be due to preservation of normal blood glucose level after STZ treatment. Another explanation could be afforded by **[31]** who stressed that ranolazine may apply advantageous effects on damage of beta cell, which is reliant on the cytosolic Ca^{2+}. The cytosolic Ca^{2+} elevation surge antioxidatant enzymes and provide protection of beta-cells from the oxidative stress. Additionally, the augmented insulin sensitivity results in lowered blood glucose level and improvement of beta-cells environment.

In summary, the data from the current study reveals that ranolazine may apply possible ameliorating anti-diabetic effect by protecting β-cell mass and augmenting insulin sensitivity. In this setting, ranolazine has the potential to become a innovative agent for treating patients with both diabetes and angina. Nevertheless, more pre-clinical studies are required to further describe the probable antidiabetic effect of ranolazine and its underlying molecular mechanisms.

Conclusion

The results of this study showed that ranolazine improved diabetes by decreasing fasting blood glucose level, HBA_{1c}, and AGEs. Moreover, it improved the oxidative stress markers, the histopathological picture of the pancreas.

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