

Modulation of the Antidiabetic Effect of Glimepiride by Diazepam in Diabetic Rats

¹W.R. Mohamed, ¹G.A. El Sherbiny, ²H.F. Zaki and ²M.E. El Sayed

¹Dept. of Pharmacology and Toxicology, Faculty of Pharmacy, Beni-Suef University, Beni-Suef, Egypt. 62511

²Dept. of Pharmacology and Toxicology, Faculty of Pharmacy, Cairo University, Egypt 11562

Abstract: Anxiolytic drugs may influence glycemic control in diabetic subjects, so the present study was conducted to investigate the effect of glimepiride, a commonly used oral hypoglycemic, diazepam, a commonly used anxiolytic, and their combination in STZ-induced diabetic rats. Diabetes was induced by streptozotocin (50 mg/kg i.p.). Rats were divided into 5 groups namely: normal control, diabetic control, glimepiride (10 mg/kg p.o.), diazepam (5 mg/kg i.p.) or combination of both glimepiride and diazepam, respectively. All groups received daily treatments for 2 weeks including the normal group which received 1% Tween 80. Diazepam significantly improved the effect of glimepiride on the levels of serum glucose, insulin, C-peptide and liver glycogen content. In addition, combination of diazepam with glimepiride significantly improved the effect of the latter on oxidative stress biomarkers including serum lipid peroxides, blood glutathione levels and blood superoxide dismutase activity of diabetic rats. In conclusion, the present study revealed that diazepam increased the antidiabetic and the antioxidant actions of glimepiride which may be of considerable value in the treatment of diabetes mellitus where both reduction of associated anxiety and tight glycemic control are needed.

Keywords: Anxiety, diabetes, diazepam, glimepiride, insulin, oxidative stress

INTRODUCTION

Diabetes Mellitus (DM) is a group of metabolic disorders characterized by chronic hyperglycemia resulting from relative or absolute insulin deficiency with or without insulin resistance (Ryden *et al.*, 2007). Patients with DM experience different types of stresses caused by illness and its treatment. Stresses can arise from dietary adjustment, frequent blood checks, constricted social life, insulin injections and other complications of uncontrolled diabetes (Tak-Ying *et al.*, 2003; Ludman *et al.*, 2004). These stresses affect patient's work, social relationship, family, quality of life and mental health status (Lee *et al.*, 2006).

Although human studies on the role of stress in the onset and course of type-2 DM(T₂DM) are few (Esposito-Del Puente *et al.*, 1994), a large body of animal studies proved that stress produces hyperglycemia and can cause insulin resistance (Surwit *et al.*, 1992; Strommer *et al.*, 1998; Soop *et al.*, 2001). Moreover, drugs used for treatment of anxiety such as alprazolam and diazepam may affect glycemic control in diabetic subjects(Surwit *et al.*, 1986; Gomez *et al.*, 1999).

Anxiety stimulates damaging pathways, causing increased production of Reactive Oxygen Species (ROS) leading to lipid peroxidation, protein oxidation, DNA damage and cell death(Liu *et al.*, 1996; Liu and Mori, 1999; Heise *et al.*, 2003).

Glimepiride, a sulfonylurea drug used in T₂DM, acts by both pancreatic and extrapancreatic mechanisms (See *et al.*, 2003) . It possesses many advantages over other sulfonylureas including lower dosage, rapid onset, longer duration of action and lower insulin C-peptide levels (Muller *et al.*, 1994).

Diazepam is one of the benzodiazepines widely used in the treatment of anxiety disorders, depression and insomnia (Kim *et al.*, 2010). Diazepam binds to the α -subunit of γ -amino butyric acid type A receptors (GABA_A) and facilitates the inhibitory effect of GABA(DeLorey and Olsen, 1992).

Since the use of anxiolytics may influence glycemic control in diabetics, the present study was conducted to investigate the possible effect of diazepam and its combination with glimepiride on different parameters in streptozotocin (STZ)-induced diabetic rats. Parameters chosen to fulfill this aim included effects on serum levels of glucose, insulin and C-peptide as well as liver glycogen content.

As oxidative stress appears to be a common link between DM and anxiety, the study was extended to include assessment of certain oxidative stress biomarkers namely, serum malondialdehyde (MDA) and blood glutathione (GSH) levels as well as blood Superoxide Dismutase (SOD) activity in diabetic rats.

MATERIALS AND METHODS

Animals: Adult male Wistar rats (120-160 g) obtained from the National Research Centre (Giza, Egypt) were used in the current study. They were housed 10 per cage and maintained at $25\pm2^{\circ}\text{C}$ with free access to water and food, under a 12/12 h light-dark cycle. All procedures in this study were carried out according to guidelines of Ethics Committee of Faculty of Pharmacy, Cairo University.

Drugs and chemicals: Glimepiride and diazepam were provided as a gift from SEDICO Company (Egypt) and NILE Company (Egypt), respectively. They were both suspended in 1% Tween 80 and glimepiride was orally administered in a dose of 10 mg/kg (Ladriere *et al.*, 1997) whereas diazepam was intraperitoneally administered in a dose of 5 mg/kg (Cuparencu and Horak, 1993). STZ was purchased from Sigma-Aldrich (USA). Ortho-phosphoric acid from Merck (Germany). Thiobarbituric acid from Sigma-Aldrich (USA). Ellman's reagent from Sigma-Aldrich (USA).

Induction of experimental diabetes: Diabetes was induced in 12 h fasted rats with single i.p. injection of STZ (50 mg/kg) (Hounsom *et al.*, 1998) dissolved in citrate buffer (0.01 M, pH 4.5). Normal control group were injected with citrate buffer alone. Animals were considered diabetic when their blood glucose level exceeded 250 mg/dL (Cam *et al.*, 2003) and were included in the study after 72 h of STZ injection.

Experimental design: In addition to the normal group, diabetic rats were divided into 4 groups each consisting of 8 rats. The first group received 1% Tween 80 (control diabetic). The remaining 3 groups received glimepiride (10 mg/kg p.o.), diazepam (5 mg/kg i.p.) or combination of both glimepiride and diazepam, respectively. All groups received daily treatments for 2 weeks daily including the normal group which received 1% Tween 80.

At the end of the experiment rats were fasted overnight, blood and serum were collected and used for various biochemical estimations. Animals were sacrificed and liver isolated for liver glycogen content determination.

Biochemical estimations: Serum glucose level was estimated using glucose kit (Spinreact, Spain) and expressed as mg/dl whereas serum insulin and C-peptide

levels were assayed using radioimmunoassay kit (Coat-a-Count kit -DPC, Los Angeles, CA, USA) and expressed as $\mu\text{IU/mL}$ and ng/mL, respectively.

Liver glycogen was estimated according to the method described by (Kemp and Van Heijningen, 1954) and expressed as mg/g wet tissue.

Serum lipid peroxides level was estimated by determination of the level of Thiobarbituric Acid Reactive Substances (TBARS) that were measured as MDA, according to the method described by ((Mihara and Uchiyama, 1978) and expressed as nmol/mL. Blood SOD was estimated according to pyrogallol method described by (Marklund and Marklund, 1974) and expressed as ug/mL.

Blood GSH was estimated according to the method described by (Beutler *et al.*, 1963) and expressed as mg%.

Statistical analysis: The values of the measured parameters were presented as mean \pm S.E.M. Comparisons between different treatments were carried out using one way Analysis of Variance (ANOVA) followed by Tukey-Kramer multiple comparisons test. Differences were considered statistically significant when $p<0.05$.

RESULTS

Effect of two weeks of daily administration of glimepiride, diazepam and their combination on serum glucose, insulin and C-peptide levels in diabetic rats: STZ significantly increased serum glucose level while significantly decreased serum insulin and serum C-peptide levels of rats. Glimepiride and diazepam significantly decreased serum glucose level to 41.18 and 41.59% of the diabetic control value, respectively. The combination of diazepam with glimepiride significantly reduced serum glucose level as compared to glimepiride monotherapy.

Glimepiride and diazepam significantly increased serum insulin level to 196.25 and 191.13% of the diabetic control value respectively. The combination of diazepam with glimepiride significantly elevated serum insulin level as compared to glimepiride alone.

Glimepiride and diazepam significantly increased serum C-peptide level to 269.15 and 244.44% of the diabetic control value respectively. The combination of diazepam with glimepiride significantly elevated serum C-peptide level as compared to glimepiride alone. (Table 1)

Effect of two weeks of daily administration of glimepiride, diazepam and their combination on liver glycogen content in diabetic rats: STZ significantly decreased liver glycogen content of rats. Glimepiride and diazepam significantly elevated liver glycogen content to 264.59 and 231.12% of the diabetic

Table 1: Effect of two weeks of daily administration of glimepiride, diazepam and their combination on serum glucose, insulin and C-peptide levels in diabetic rats

Parameters Drugs & doses	Serum glucose (mg/dL)	Serum insulin (μ IU/mL)	Serum C-peptide (ng/mL)
Normal control (1% tween 80)	74.50 \pm 2.92	6.62 \pm 0.27	1.05 \pm 0.05
Diabetic control (STZ 50 mg/kg)	392.20 \pm 12.41*	2.93 \pm 0.05*	0.27 \pm 0.03 *
Glimepiride (10 mg/kg)	161.5 \pm 17.74 ^a	5.75 \pm 0.20 ^a	0.72 \pm 0.05 ^a
Diazepam (5 mg/kg)	163.10 \pm 11.12 ^a	5.60 \pm 0.06 ^a	0.66 \pm 0.01 ^a
Glimepiride (10 mg/kg)	88.98 ^a	8.60 ^{ab}	1.10 \pm 0.07 ^{ab}
+ Diazepam (5 mg/kg)			

Each value represents the mean \pm SE of the mean; Each group consists of 6-8 rats; *: Significantly different from normal control at p<0.05; ^a: Significantly different from diabetic control at p<0.05; ^b: Significantly different from glimepiride group at p<0.05

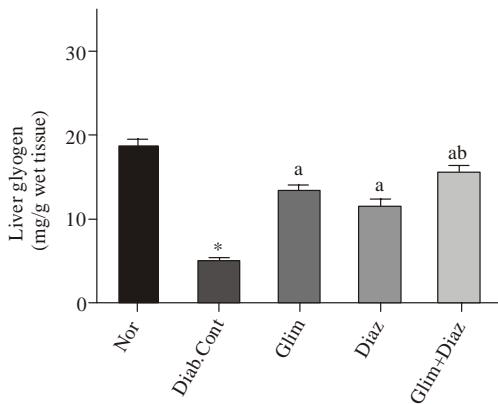


Fig. 1: Effect of two weeks of daily administration of glimepiride (Glim), diazepam (Diaz) and their combination (Glim+Diaz) on liver glycogen in diabetic rats (Diab.cont). Normal group is abbreviated by (Nor); Each value represents the mean \pm SE of the mean; Each group consists of 6-8 rats; *: Significantly different from normal control at p<0.05; ^a: Significantly different from diabetic control at p<0.05; ^b: Significantly different from glimepiride group at p<0.05

control value respectively. The combination of diazepam with glimepiride significantly increased liver glycogen content as compared to glimepiride alone. (Fig. 1)

Effect of two weeks of daily administration of glimepiride, diazepam and their combination on oxidative stress biomarkers in diabetic rats: STZ significantly increased serum lipid peroxides of rats. Glimepiride and diazepam significantly decreased serum lipid peroxides to 46.64 and 54.61% of the diabetic control value respectively. Combination of diazepam with glimepiride significantly lowered serum lipid peroxides compared to glimepiride alone. (Fig. 2)

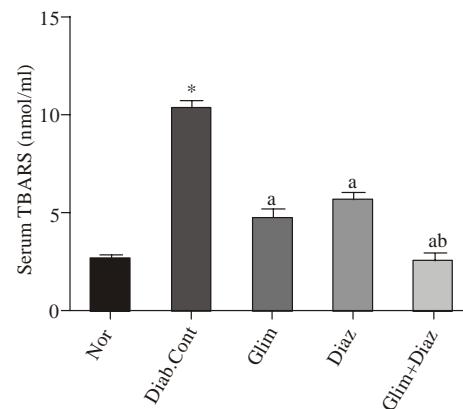


Fig. 2: Effect of two weeks of daily administration of glimepiride (Glim), diazepam (Diaz) and their combination (Glim+Diaz) on serum lipid peroxides in diabetic rats (Diab.cont). Normal group is abbreviated by (Nor); Each value represents the mean \pm SE of the mean; Each group consists of 6-8 rats; *: Significantly different from normal control at p<0.05; ^a: Significantly different from diabetic control at p<0.05; ^b: Significantly different from glimepiride group at p<0.05

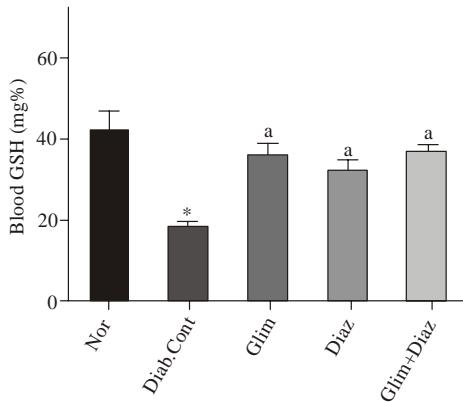


Fig. 3: Effect of two weeks of daily administration of glimepiride (Glim), diazepam (Diaz) and their combination (Glim+Diaz) on blood reduced glutathione (GSH) in diabetic rats (Diab.cont). Normal group is abbreviated by (Nor); Each value represents the mean \pm SE of the mean; Each group consists of 6-8 rats; *: Significantly different from normal control at p<0.05; ^a: Significantly different from diabetic control at p<0.05; ^b: Significantly different from glimepiride group at p<0.05

STZ significantly decreased blood GSH of rats. Glimepiride and diazepam significantly elevated blood GSH to 196.85 and 178.52% of the diabetic control value respectively. The combination of diazepam with glimepiride did not significantly affect the effect of glimepiride alone. (Fig. 3)

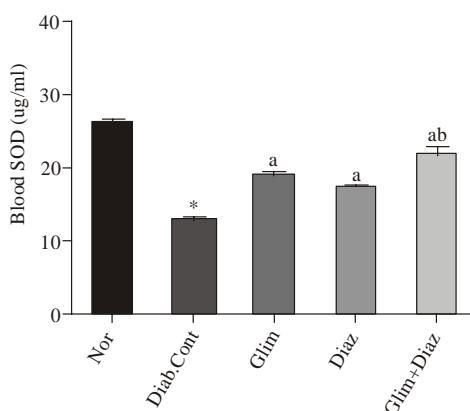


Fig. 4: Effect of two weeks of daily administration of glimepiride (Glim), diazepam (Diaz) and their combination (Glim+Diaz) on blood superoxide dismutase (SOD) in diabetic rats (Diab.cont). Normal group is abbreviated by (Nor); Each value represents the mean \pm SE of the mean; Each group consists of 6-8 rats; *: Significantly different from normal control at $p<0.05$; ^a: Significantly different from diabetic control at $p<0.05$; ^b: Significantly different from glimepiride group at $p<0.05$

STZ significantly decreased blood SOD of rats. Glimepiride and diazepam significantly elevated blood SOD to 150 and 136.99% of the diabetic control value respectively. The combination of diazepam with glimepiride significantly increased blood SOD as compared to glimepiride alone. (Fig. 4)

DISCUSSION

The present study revealed that STZ produced significant rise in blood glucose level while it significantly lowered serum insulin and serum C-peptide levels in rats. STZ is a diabetogenic agent (Rakieten *et al.*, 1963) that causes toxicity to β -cells of the pancreas (LeDoux *et al.*, 1986; Murata *et al.*, 1999). The severity and persistence of diabetes in experimental animals depends on STZ dose used (Tancrède *et al.*, 1983; Thulesen *et al.*, 1997). STZ dose of 50 mg/kg was used to induce type 2 diabetes mellitus in rats (Hounsom *et al.*, 1998; Kamalakkannan and Prince, 2006). Glimepiride exerted significant hypoglycemic effects as evidenced from its amelioration of STZ-induced changes in serum glucose, insulin and C-peptide levels. Similar results were previously reported both in clinical subjects and experimental animals (Mir *et al.*, 2008; Mowla *et al.*, 2009). The observed hypoglycemic effect of glimepiride could be attributed to its stimulant effect on insulin secretion. Glimepiride binds to specific receptors on pancreatic beta cells which lead to closure of potassium ATP channels and subsequent opening of calcium

channels leading to an increase in cytoplasmic calcium and stimulation of insulin release (Philipson and Steiner, 1995; Muller, 2005). It was shown that glimepiride caused restoration of morphology of beta cell of diabetic rabbits and it increased comparative percentage of beta cells as compared to diabetic rabbits (Mir *et al.*, 2008) this protective effect could be attributed to the antioxidant properties of glimepiride which was proven in the present study also it was proven that chemicals possessing antioxidant properties protect islets from toxic effects of STZ (Fernandez-Alvarez *et al.*, 2004; Coskun *et al.*, 2005). In addition, glimepiride directly stimulates glucose transport (Muller *et al.*, 1995), inhibits hepatic gluconeogenesis and activates insulin mediated glycogen synthesis as well as lipogenesis (Scholz *et al.*, 2001). It also improves insulin sensitivity (Kabadi and Kabadi, 2004).

Treatment of diabetic rats with diazepam, in the current study, significantly reduced serum glucose level meanwhile it increased serum insulin and serum C-peptide levels. A study reported that diazepam significantly decreased glucose level and increased plasma insulin level in STZ-induced diabetic rats (Gomez *et al.*, 1999). Several Studies revealed that GABA_A receptor complex found in human, porcine as well as rodent endocrine pancreas cells are functionally active (Atkinson and Maclare, 1993; Yang *et al.*, 1994; Marchetti *et al.*, 1996). GABA receptors can induce insulin release from isolated islets under the effect of positive modulators as benzodiazepines (Marchetti *et al.*, 1996). Benzodiazepines caused increased insulin secretion through binding membranes prepared from pig pancreatic islets (Marchetti *et al.*, 1996).

Present study revealed that combination of diazepam with glimepiride significantly improved the effect of the latter on serum glucose, insulin and C-peptide levels in diabetic rats. This additive effect has several advantages including reduction of the doses of glimepiride, decreased toxicity profiles and reduced economic cost.

Present results showed that glimepiride significantly elevated liver glycogen content of diabetic rats. Glimepiride stimulated glycogenesis in rat diaphragm (Muller *et al.*, 1995) and increased fasting liver glycogen content of obese rats (Mori *et al.*, 2008). The effect of glimepiride might be mediated via insulin-stimulated glycogen synthesis as glimepiride was shown to cause a dose-dependent increase of insulin-stimulated glycogen synthesis in cultured human skeletal muscle cells (Haupt *et al.*, 2002).

Similarly, treatment with diazepam significantly increased liver glycogen content of diabetic rats and its combination with glimepiride improved the effects of the latter on liver glycogen content. The mechanism of the elevated liver glycogen content by diazepam could be attributed to stimulated insulin secretion from pancreas as shown by results of the present study.

Injection of STZ in the present study resulted in significant increase in serum MDA level parallel to decrease in blood GSH level and SOD activity. The present results are in accordance with those obtained by other investigators (Krauss *et al.*, 2003; Elcioglu *et al.*, 2011). Several studies demonstrated that free radicals are excessively generated in hyperglycemia through many pathways including glucose auto-oxidation (Hamilton *et al.*, 2003), protein glycation ((Sakurai and Tsuchiya, 1988) and excessive activation of polyol pathway (Barnett *et al.*, 1986).

In the current study, treatment of STZ-diabetic rats with glimepiride significantly reduced serum MDA level while it significantly increased blood GSH level and SOD activity of diabetic rats. In a study performed by(Krauss *et al.*, 2003), glimepiride significantly decreased MDA level while it significantly increased GPX and SOD activities in erythrocytes of STZ-induced diabetic rats. The antioxidant activity of glimepiride could be explained through normalization of elevated hyperglycemia as glucose autooxidation is the major source of free radicals and peroxides production in diabetes (Krauss *et al.*, 2003).

In a similar fashion, treatment of diabetic rats with diazepam significantly reduced serum MDA level meanwhile it improved blood GSH level and SOD activity. Diazepam was shown to reduce oxidative stress biomarkers in brain of normal rats (Musavi and Kakkar, 2000). Diazepam significantly lowered MDA level and increased SOD activity in rats exposed to chronic mild stress (Nirmal *et al.*, 2008). On the other hand, the present results are not in harmony with the results of (Abdelmajeed, 2009) who reported an increase in kidney, liver and heart MDA contents by diazepam treatment. This discrepancy might be attributed to the difference in the dose used by the investigator which was twice that used in the present investigation.

The observed antioxidant activity of diazepam could be mediated through Peripheral Benzodiazepine Receptor (PBR) expressed on mitochondria (Zavala, 1997). PBR are involved in two aspects of mitochondrial oxidative processes first, protection of mitochondria from exogenous ROS; second, regulation of mitochondrial generation and/or release of ROS (Kunduzova *et al.*, 2004). Earlier investigations showed the role of PBR in oxidative processes through the involvement of this receptor in hematopoietic cells protection against apoptosis following H₂O₂ treatment (Carayon *et al.*, 1996). According to the findings of the present investigation combination of diazepam with glimepiride significantly improved the effect of the latter on oxidative stress biomarkers of diabetic rats. These results further support the additive effect between the two agents on glucose and insulin levels.

It was proved from previous studies that chemicals which possess antioxidant properties and free radical

scavengers properties can help in the regeneration of beta cells and protection of pancreatic islets against the cytotoxic effects of STZ (Fernandez-Alvarez *et al.*, 2004; Coskun *et al.*, 2005; Kamalakkannan and Prince, 2006). So the antioxidant properties of glimepiride and diazepam have a role in protection of beta cells from toxic effects of STZ and participate in their antidiabetic effects.

In conclusion, the present study revealed that diazepam increased the antidiabetic action of glimepiride which may be of considerable value in the treatment of DM where both reduction of associated anxiety and tight glycemic control are needed.

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