

Research Article



Angiotensin Inhibitors Potentiate the Hypoglycemic and Antioxidant Effects of Gliclazide in Rats

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ABSTRACT

Diabetes mellitus and hypertension are major public health problems which resulting in significant morbidity and mortality. In the present study, we evaluated the effect of concurrent administration of one of gliclazide with captopril or losartan on its antidiabetic activity and the oxidative stress parameters in streptozotocin (STZ)-induced diabetic rats. Hyperglycemia was induced in rates by the injection of STZ (50 mg/kg i.p.). Diabetic animals received gliclazide (10 mg/kg p.o.), captopril (10 mg/kg p.o.) or losartan (5 mg/kg, p.o.) as single treatments or in combinations. In addition, the combination effect was confirmed by the use of *in vitro* pancreatic islets. The combination effects were assessed by the measurement of serum glucose level, serum insulin level, liver glycogen content and oxidative stress parameters. *In vivo* and *in vitro* results showed that the combination of captopril or losartan with gliclazide results in significant decrease in serum glucose, increase in serum insulin, increase liver glycogen content and improvement of oxidative stress parameters. Combination of captopril or losartan with gliclazide potentiated its effect on lowering the serum glucose level of diabetic rats. This effect highlights the importance of dose lowering consideration upon combining these medications.

Keywords: Diabetes; Gliclazide; Captopril; Losartan; Serum glucose; Oxidative stress.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. DM affects approximately 170 million individuals worldwide and is expected to alter the lives of at least 366 million individuals within a future span of 25 years¹. Symptoms of marked hyperglycemia include polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision. Acute, life-threatening consequences of uncontrolled diabetes are hyperglycemia with ketoacidosis or the non-ketotic hyperosmolar syndrome². DM is a devastating illness with microvascular and macrovascular complications and may result in significant morbidity and mortality. Gliclazide is a sulphonylurea drug and used in the treatment of type II diabetes³. It is generally well tolerated, is associated with a relatively low incidence of hypoglycaemia and may have beneficial effects beyond the reduction of serum glucose³. Gliclazide effectively maintains glycemic control possibly by pancreatic and extrapancreatic effects⁴. Captopril is superior to a diuretic/ β -blocker antihypertensive treatment regimen in preventing cardiovascular events in hypertensive diabetic patients, especially in those with metabolic decompensation⁵. Captopril is frequently used in the treatment of patients with type II diabetes. These drugs also exert beneficial metabolic effects, causing an improved glucose tolerance⁶. Losartan was associated with a lower incidence of new-onset diabetes and lower total mortality. It is currently not known whether losartan

is superior to diuretics or calcium channel blockers as a first-line treatment of isolated systolic hypertension⁷. The aim of the present study is to measure the hypoglycemic and the antioxidant effect of captopril, an ACE inhibitor, and losartan, an angiotensin receptor blocker, in STZ-induced diabetes mellitus and their possible interactions with certain antidiabetic drugs namely, gliclazide.

MATERIALS AND METHODS

Animals

Adult albino rats weighing 200-250 g were obtained the animal house of Beni-Suef University, Beni-Suef, Egypt. The animals were housed in plastic cages with good aerated covers. Animals were allowed water *ad libitum*. Animals were fed pellet chow from El-Nasr chemical Co., Cairo, Egypt. All animal use and handling were done in accordance with protocols approved by the Faculty of Pharmacy, Beni-Suef University Animal Care and Use Committee.

Drugs and Chemicals

STZ was purchased from Sigma-Aldrich (St. Louis, MO, USA). Gliclazide, Captopril and Losartan were provided as a gift from Egyptian International Pharmaceutical Industries Company (EIPICO, Cairo, Egypt). For *in vivo* experiments, gliclazide was suspended in 2% tween 80 and administered orally in a dose of 10 mg/kg. For *in vitro* experiments, it was used in a dose of 40 μ mo/l. Captopril was dissolved in normal saline and orally administered in a dose of 10 mg/kg or used in a concentration of 100



$\mu\text{mo/l}$ for *in vitro* experiments. Losartan was dissolved in normal saline and orally administered in a dose of 5 mg/kg or in a concentration of 100 $\mu\text{mo/l}$ for *in vitro* experiments.

Induction of Experimental Diabetes

Rats were fasted for 18 h, the diabetes was induced by intra peritoneal injection of 50 mg/kg STZ. Streptozotocin must be freshly prepared by dissolving it in cold 0.1 M citrate buffer just before injection.

After injection of STZ, rats were fed on 5% glucose solution for 24 h to prevent hypoglycemia during the hyper-insulinemic phase caused by β -cells lyses.

Diabetes was confirmed by the presence of glucosuria using gluco-test strips at 24, 48, and 96 hours after STZ injection in order to ensure persistent hyperglycemia.

Islets Isolation

Islets of Langerhans were isolated by collagenase digestion technique according as mentioned before⁸.

Islets were isolated from non-fasting rats since fasting would diminish the responsiveness of the islets to stimulation of insulin secretion *in vitro*.

Rats were pretreated with intraperitoneal injection of pilocarpine nitrate (20mg/kg) 2-3 hours prior to islets isolation.

Pilocarpine allows the depletion of zymogens from the exocrine pancreatic tissues and thus minimizes the destruction of islets membrane structure which could occur during collagenase digestion of the tissue.

In vivo Experiments

Rats were divided into 7 groups; one normal control group and 6 diabetic groups as following: diabetic control group, gliclazide (10 mg/kg p.o.), captopril (10 mg/kg p.o.), losartan (5 mg/kg, p.o.), combination of gliclazide and captopril and combination of gliclazide and losartan.

In vitro Experiments

Islets were divided into 6 groups; normal control, gliclazide (40 $\mu\text{mol/L}$), captopril (/100 $\mu\text{mol/L}$), losartan (100 $\mu\text{mol/L}$), combination of gliclazide and captopril and combination of gliclazide and losartan.

Biochemical Analysis

Glucose assay kit was obtained from BIOLABO SA (Maizy, France), for the measurement of serum glucose level.

For the measurement of serum insulin level and for *in vitro* insulin measurement, insulin assay kit was used (Coat-A-Count[®], Diagnostic Products, Los Angeles, CA, USA).

Liver glycogen content was measured as mentioned before⁹. Blood GSH, SOD, MDA and nitric oxide levels were determined according to the methods described before¹⁰⁻¹⁴.

Statistical Analysis

The significance of difference between group means and the statistical analysis were determined using one-way ANOVA test followed by Tukey-Kramer multiple comparisons test, using *GraphPad Instat* (ISI software) and BC INSTAT computer programs (1993).

RESULTS

Effect on Serum Glucose Level

The normal control value of serum glucose level was 86.47 ± 4.79 mg%. Streptozotocin (50 mg/kg) significantly increased serum glucose level to 389 ± 13.83 mg% as compared to normal control value.

Gliclazide, captopril, losartan and combination of gliclazide and captopril significantly reduced serum glucose level to 54.45, 65.78, 63.37 and 35.19 % of the diabetic control value respectively.

In addition, the decrease in serum blood glucose level induced by the combination of gliclazide and captopril was significantly high compared to gliclazide alone. Combination of gliclazide and losartan significantly reduced serum glucose level to 40.03 % of the diabetic control value.

In addition, the decrease in serum blood glucose level induced by this combination was significantly high compared to gliclazide alone (Fig. 1).

Table 1: Effect of two weeks daily dose administration of gliclazide, captopril or losartan alone or in combination on oxidative stress parameters of streptozotocin-induced diabetic rats.

Treatments	Oxidative stress biomarkers			
	Plasma GSH (mg %)	Serum MDA (nmol/mL)	Plasma SOD ($\mu\text{g/ml}$)	Serum NO (μM)
Normal control	36.52 ± 2.95	1.54 ± 0.16	37.12 ± 2.82	108.9 ± 3.98
Diabetic control	$14.52 \pm 1.36^*$	$5.03 \pm 0.45^*$	$11.69 \pm 1.68^*$	$44.03 \pm 4.38^*$
Gliclazide	31.43 ± 1.75^a	$2.62 \pm 0.15^{a*}$	$23.38 \pm 1.84^{a*}$	$81.32 \pm 3.28^{a*}$
Captopril	$28.93 \pm 1.45^{a*}$	$3.41 \pm 0.17^{a*}$	23.38 ± 2.7^a	$67.27 \pm 3.83^{a*}$
Losartan	$28.3 \pm 0.92^{a*}$	$3.7 \pm 0.12^{a*}$	$21.59 \pm 2.39^*$	$68.44 \pm 2.27^{a*}$
Gliclazide + captopril	33.97 ± 1.8^a	1.95 ± 0.1^a	35.06 ± 2.37^{ab}	102.9 ± 3.81^{ab}
Gliclazide + losartan	32.32 ± 1.2^a	2.28 ± 0.12^a	32.47 ± 1.84^a	101.9 ± 3.05^{ab}

N= 6-8 rats per group.

Data were expressed as mean \pm SEM

Statistical analysis is carried out using one way ANOVA followed by Tukey-kramer multiple comparisons test.

*: Significantly different from normal control at $p < 0.05$

a: Significantly different from diabetic control at $p < 0.05$

b: Significantly different from gliclazide group at $p < 0.05$



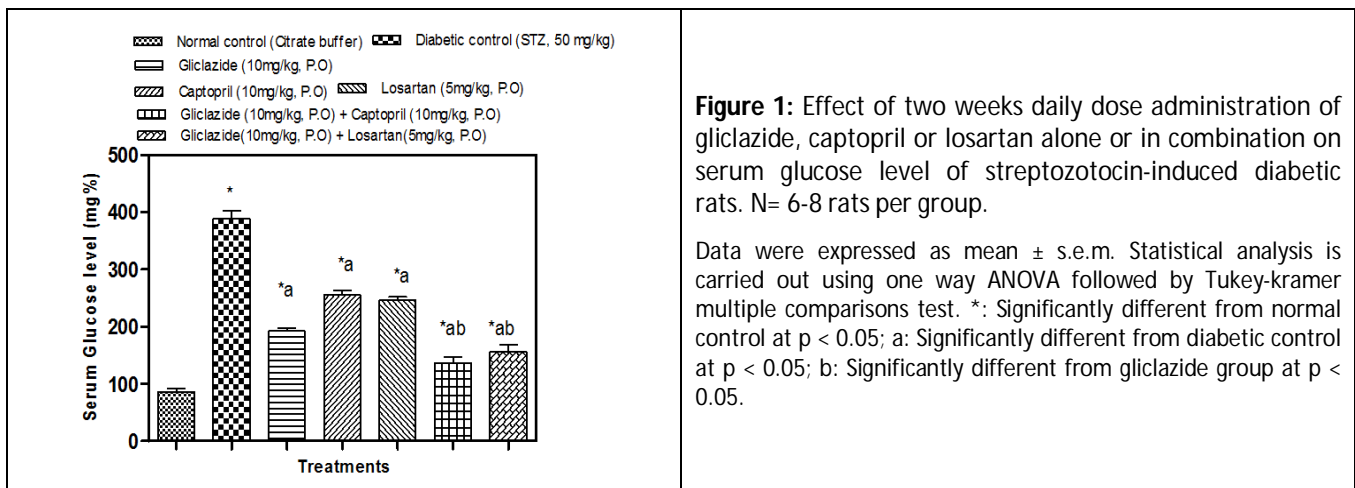


Figure 1: Effect of two weeks daily dose administration of gliclazide, captopril or losartan alone or in combination on serum glucose level of streptozotocin-induced diabetic rats. N= 6-8 rats per group.

Data were expressed as mean ± s.e.m. Statistical analysis is carried out using one way ANOVA followed by Tukey-kramer multiple comparisons test. *: Significantly different from normal control at p < 0.05; a: Significantly different from diabetic control at p < 0.05; b: Significantly different from gliclazide group at p < 0.05.

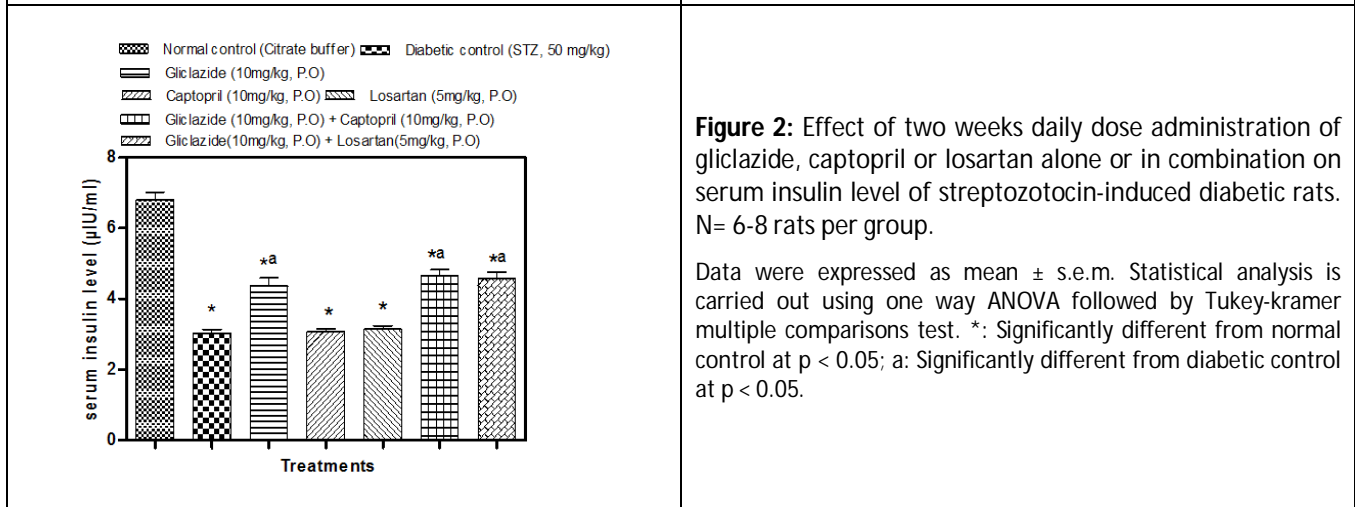


Figure 2: Effect of two weeks daily dose administration of gliclazide, captopril or losartan alone or in combination on serum insulin level of streptozotocin-induced diabetic rats. N= 6-8 rats per group.

Data were expressed as mean ± s.e.m. Statistical analysis is carried out using one way ANOVA followed by Tukey-kramer multiple comparisons test. *: Significantly different from normal control at p < 0.05; a: Significantly different from diabetic control at p < 0.05.

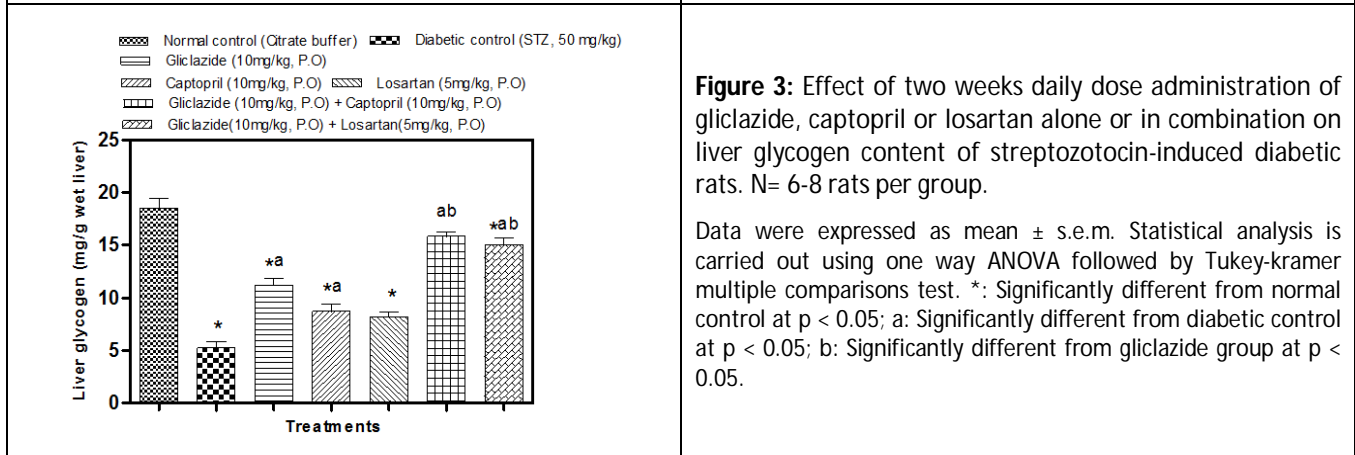


Figure 3: Effect of two weeks daily dose administration of gliclazide, captopril or losartan alone or in combination on liver glycogen content of streptozotocin-induced diabetic rats. N= 6-8 rats per group.

Data were expressed as mean ± s.e.m. Statistical analysis is carried out using one way ANOVA followed by Tukey-kramer multiple comparisons test. *: Significantly different from normal control at p < 0.05; a: Significantly different from diabetic control at p < 0.05; b: Significantly different from gliclazide group at p < 0.05.

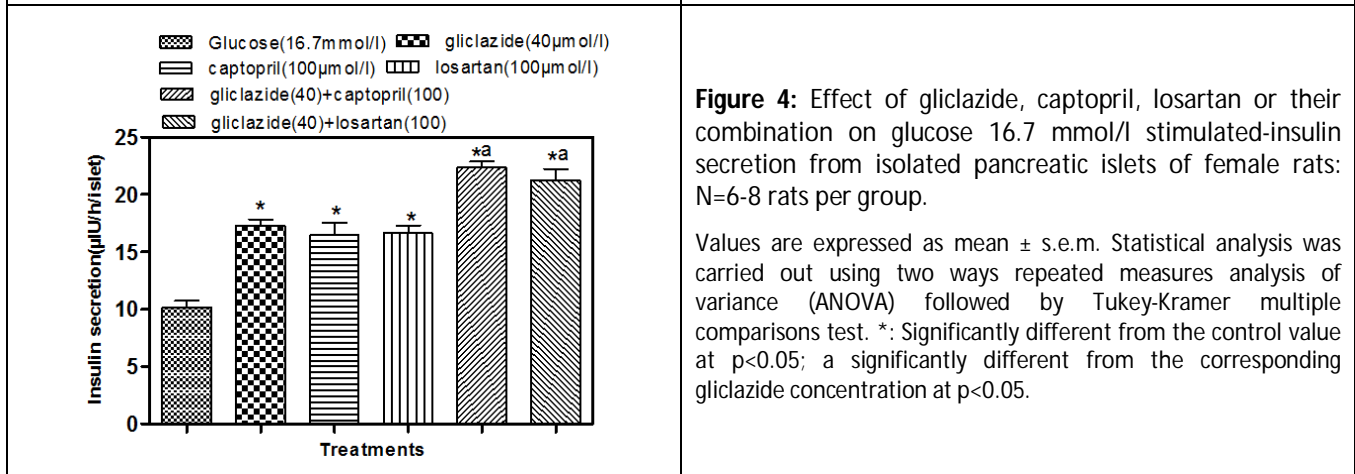


Figure 4: Effect of gliclazide, captopril, losartan or their combination on glucose 16.7 mmol/l stimulated-insulin secretion from isolated pancreatic islets of female rats: N=6-8 rats per group.

Values are expressed as mean ± s.e.m. Statistical analysis was carried out using two ways repeated measures analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test. *: Significantly different from the control value at p<0.05; a significantly different from the corresponding gliclazide concentration at p<0.05.

Effect on Serum Insulin Level

The normal control value of serum insulin level was $6.8 \pm 0.22 \mu\text{U/ml}$. Streptozotocin (50 mg/kg) significantly decreased serum insulin level to $3.03 \pm 0.11 \mu\text{U/ml}$ as compared to normal control value. Gliclazide significantly increased serum insulin level to 147.19 % of the diabetic control value. Captopril and losartan did not show any significant effect on serum insulin level as compared to the diabetic control value. Combination of gliclazide and captopril significantly increased serum insulin level to 153.8 % of the diabetic control value. In addition this effect was not significantly different from gliclazide alone. Combination of gliclazide and losartan significantly increased serum insulin level to 151.49 % of the diabetic control value. In addition this effect was not significantly different from gliclazide alone (Fig. 2).

Effect on Liver Glycogen Content

The normal control value of liver glycogen content was $18.49 \pm 1 \text{ mg/g}$ wet tissue. Streptozotocin (50 mg/kg) significantly decreased liver glycogen content to $5.26 \pm 0.58 \text{ mg/g}$ wet tissue as compared to normal control value. Gliclazide and captopril significantly increased liver glycogen content to 212.17, 166.1 % of the diabetic control value respectively. However, losartan did not show any significant effect on liver glycogen content as compared to the diabetic control value. Combination of gliclazide and captopril significantly increased liver glycogen content to 281 % of the diabetic control value. In addition, the increase in liver glycogen content induced by this combination was significantly high compared to gliclazide alone. Combination of gliclazide and losartan significantly increased liver glycogen content to 286.31 % of the diabetic control value. In addition, the increase in liver glycogen content induced by this combination was significantly high compared to gliclazide alone (Fig. 3).

Effect on Blood Glutathione (GSH)

The normal control value of blood GSH was $36.52 \pm 2.95 \text{ mg\%}$. Streptozotocin (50 mg/kg) significantly decreased blood GSH to $14.52 \pm 1.36 \text{ mg\%}$ compared to normal control. Gliclazide, Captopril and losartan significantly increased blood GSH to 216.46, 199.25 and 194.9 % of the diabetic control value respectively. Combination of gliclazide and captopril significantly increased blood GSH to 233.95 % of the diabetic control value. In addition this effect was not significantly different from gliclazide alone. Combination of gliclazide and losartan significantly increased blood GSH to 222.59 % of the diabetic control value. In addition this effect was not significantly different from gliclazide alone (Table 1).

Effect on Serum Malondialdehyde (MDA)

The normal control value of serum MDA level was $1.54 \pm 0.16 \text{ nmol/ml}$. Streptozotocin (50 mg/kg) significantly increased serum MDA level to $5.03 \pm 0.45 \text{ nmol/ml}$ as compared to normal control value. Gliclazide, captopril and losartan significantly reduced serum MDA level to

52.09, 67.79 and 73.56 % of the diabetic control value respectively. Combination of gliclazide and captopril significantly reduced serum MDA level to 38.77 % of the diabetic control value. In addition this effect was not significantly different from normal control or gliclazide alone. Combination of gliclazide and losartan significantly reduced serum MDA level to 45.33 % of the diabetic control value. In addition this effect was not significantly different from normal control or gliclazide alone (Table 1).

Effect on Blood Superoxide Dismutase (SOD)

The normal control value of blood SOD level was $37.12 \pm 2.82 \mu\text{g/ml}$. Streptozotocin (50 mg/kg) significantly decreased blood SOD level to $11.69 \pm 1.68 \mu\text{g/ml}$ as compared to normal control value. Gliclazide and captopril significantly increased blood SOD to 200 and 200 % of the diabetic control value respectively. Losartan did not show any significant effect on blood SOD as compared to the diabetic control value. Combination of gliclazide and captopril significantly increased blood SOD to 299.91 % of the diabetic control value. In addition, the increase in blood SOD induced by this combination was significantly high compared to gliclazide alone. Combination of gliclazide and losartan significantly increased blood SOD to 277.76 % of the diabetic control value. In addition this effect was not significantly different from gliclazide alone (Table 1).

Effect on Serum Nitric Oxide Level

The normal control value of serum nitric oxide level was $108.9 \pm 3.98 \mu\text{M}$. Streptozotocin (50 mg/kg) significantly decreased serum nitric oxide level to $44.03 \pm 4.38 \mu\text{M}$ as compared to normal control value. Gliclazide, captopril and losartan significantly increased serum nitric oxide level to 184.69, 152.78 and 155.44 % of the diabetic control value respectively. Combination of gliclazide and captopril significantly increased serum nitric oxide level to 233.7 % of the diabetic control value. In addition, the increase in serum nitric oxide level induced by this combination was significantly high compared to gliclazide alone.

Combination of gliclazide and losartan significantly increased serum nitric oxide level to 231.43 % of the diabetic control value. In addition, the increase in serum nitric oxide level induced by this combination was significantly high compared to gliclazide alone (Table 1).

Effect on Glucose 16.7 mmol/l Stimulated-Insulin Secretion from Isolated Pancreatic Islets of Female Rats

Incubation of islets in Krebs Ringer HEPES medium containing 16.7 mmol/l glucose significantly increased insulin secretion to $10.18 \pm 0.57 \mu\text{U/h/islet}$. Gliclazide in a concentration of 40 $\mu\text{mol/l}$ significantly increased stimulated-insulin secretion to 169.94% of the respective control value. Captopril in a concentration 100 $\mu\text{mol/l}$ significantly increased stimulated-insulin secretion to 161.79 % of the control value. Losartan in a concentration 100 $\mu\text{mol/l}$ significantly increased stimulated-insulin



secretion to 163.36 % of the control value. Combination of gliclazide (40 $\mu\text{mol/l}$) and captopril (100 $\mu\text{mol/l}$) significantly increased stimulated-insulin secretion to 219.74 % of the control value. In addition, the increase in serum insulin level induced by this combination was significantly high compared to gliclazide alone. Combination of losartan (100 $\mu\text{mol/l}$) and gliclazide (40 $\mu\text{mol/l}$) significantly stimulated-insulin secretion to 208.94% of control value. In addition, the increase in serum insulin level induced by this combination was significantly high compared to gliclazide alone. Combination of gliclazide and losartan increased stimulated-insulin secretion in a dose dependant manner. It could be concluded that captopril or losartan synergizes gliclazide when they are administered together in vitro (Fig. 4).

DISCUSSION

STZ administration resulted in a significant increase in serum glucose level and decrease in serum insulin level due to the destruction of pancreatic islets in addition to reduced liver glycogen content in rats¹⁵. Results of our study revealed that STZ significantly increased serum MDA level while significantly decreased blood GSH level, blood SOD activity and serum nitric oxide level. The administration of gliclazide significantly decreased serum glucose level and increased serum insulin level. This effect was confirmed by the *in vitro* studies which revealed that gliclazide enhanced glucose-stimulated insulin secretion from pancreatic islets.

Gliclazide exerts the hypoglycemic effect through pancreatic action by stimulating or strengthening of endogenous insulin secretion in response to physiologic secretagogues¹⁶ or extrapancreatic action through increasing the glucose utilization in muscles and adipose tissues and increasing glucose uptake to skeletal muscle cells¹⁷. These effects are possibly related to its ability to restore insulin-stimulated glucose transporter 4 (GLUT4) translocation in peripheral tissues¹⁸.

Findings of the present work revealed that gliclazide significantly elevated liver glycogen content of diabetic rats. This effect could be through enhancement of insulin-stimulated glycogen synthesis¹⁹. In addition, gliclazide significantly reduced serum MDA level and increased blood GSH level, blood SOD activity and serum nitric oxide level of diabetic rats. Gliclazide possibly exerts such antioxidant effects due to the characteristics of its molecular structure²⁰. The data of the present study showed that captopril and losartan significantly decreased serum glucose level but did not significantly alter serum insulin level in STZ-induced diabetic rats after repeated doses administration for two weeks. Angiotensin II has effects on GLUT4 and insulin receptors in insulin-sensitive tissues such as muscle, liver and adipose tissue so it may cause insulin resistance²¹. Captopril increased the insulin-mediated disposal of glucose and increased insulin sensitivity²². ACEI increased insulin responsiveness, thus being beneficial in insulin-

resistant states. This metabolic effect is due to elevated systemic kinin levels²³. The regulation of local blood flow and glucose uptake to the tissues was mediated by kinins²⁴. Captopril modulates the early steps of insulin signaling by increasing receptor autophosphorylation in the liver and muscle of rats²⁵.

ARB treatment improves insulin resistance by modification of adipose tissue thereby blunting the development of diabetes²⁶. The *in vitro* studies of the present investigation revealed that captopril and losartan enhanced basal and glucose-stimulated insulin secretion from isolated rat pancreatic islets in a dose dependent manner. Captopril increased islet blood flow and insulin secretion in addition it increased glucose tolerance²⁷. Losartan selectively increased insulin synthesis and insulin release in rat islets²⁸. Findings of the present work revealed that captopril but not losartan significantly elevated liver glycogen content of STZ-induced diabetic rats after repeated doses administration for two weeks. The present data are in accordance with those obtained by other investigator. Captopril enhanced insulin-stimulated glycogen synthesis in skeletal muscle and liver²⁹. Angiotensin II exerts its actions on insulin-sensitive tissues such as liver, muscle and adipose tissue²¹. Captopril increased the insulin-mediated disposal of glucose in these tissues²². Results of the present investigation revealed that captopril and losartan significantly reduced serum MDA level of STZ-induced diabetic rats after repeated doses administration for two weeks while significantly increased blood GSH level, blood SOD activity and serum nitric oxide level of STZ-induced diabetic rats after repeated doses administration for two weeks. Similar results obtained by previous investigators. ACE inhibitors augmented the activities of antioxidant enzymes in diabetic rats³⁰. Ang II-mediated generation of reactive oxygen species (ROS) has been suggested to be involved in several diabetic complications³¹. The inhibition of Ang II production reduces oxidative stress in STZ-induced diabetes³¹. Captopril-treated diabetic rats had lower lipid peroxidation and increased NO release³². Inhibition of lipid peroxidation could be one of the protective mechanisms of renin-angiotensin axis inhibition in diabetic kidney tissues³². Captopril increased MDA content, SOD and catalase and decreased MDA concentration in streptozotocin-induced diabetic rats³³. Together, these findings highlight the interaction between captopril or losartan with gliclazide on lowering the serum glucose level of diabetic rats. This effect underlies the importance of dose lowering consideration upon combining these medications.

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