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Research Article

Possible Study of Drug-Drug Interactions between Lisinopril and Gliclazide in Experimental Animals

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ABSTRACT

The most often problem in type II diabetic patients coexists with hypertension, the incidence of hypertension among diabetic patients is high. In this case, use a combination of medicine unavoidable (Polypharmacy). In such situations, this may be leading to drug-drug interactions. Gliclazide (sulphonylurea) and lisinopril (ACE inhibitors) widely used in Type II diabetes associated with hypertension. The present study was aimed to evaluate the presence of any drug-drug interaction features between single and multiple dose treatment of lisinopril (1mg/kg) on antidiabetic activity of gliclazide (3.7 mg/kg) in both normal and streptozotocin induced diabetic rats and in normal rabbits. Selected doses of gliclazide, lisinopril and their combination given to animals via oral route with one week washout periods in between treatments. The blood samples were collected from rats and rabbits at different time intervals 0, 4, 8, 12, 16, 20, and 24 hours and were investigated for serum glucose and insulin level by GOD/POD and insulin levels kits. Gliclazide was produced a significant effect on blood glucose reduction in multiple doses treatment of lisinopril and gliclazide in normal and diabetic rats, whereas, single dose treatment was produced a significant effect on blood glucose in diabetic rats only, in combination, chronic use of lisinopril increases the effect of gliclazide in normal and diabetic rats. Thus, it can be concluded that lisinopril and gliclazide maybe ought to be avoidable or may need dose adjustment when used concomitantly. However, further studies are necessary.

Keywords: Drug interaction, Gliclazide, Lisinopril, Diabetes mellitus, Streptozotocin

INTRODUCTION:

Use of multiple medications in the same patients (Polypharmacy) nowadays is a common practice all over the world to treat chronic disorders like diabetes mellitus and hypertension. These practices are acceptable to treat single and multiple disorders, which occur simultaneously.

Polypharmacy sometimes unavoidable. Recently, the standard of healthcare in most chronic conditions achieved by administrate that multiple drug therapy[1] A drug-drug interaction defined as an interaction between one or more medications that administrate concomitantly, which lead to the change of the pharmacokinetic/pharmacodynamic properties of any medications. Interactions may occur with over-the-counter and prescription drug, foods, fruits, vitamins, diseases, and genetics. [2]

A high number of medications becomes a suitable therapy when patients diagnosed with diabetes mellitus. These consist of medications for dyslipidemia, hypertension, and glucose control. Therefore, many medications may be overwhelming, and it is authoritative that patients are carefully educated about their drug regimen. Patients in such case have several worries when various drugs are started, these concern are possible adverse reactions, prescribing error sand the cost of medications. [1]

Diabetes Mellitus (DM) is a most common disease in the world, especially in third world countries such as Yemen. Diabetes mellitus is a metabolic disease characterized by hyperglycemia due to a defect in the secretion of the insulin from the pancreas, or defect in the metabolism of carbohydrates and lipids in the body. The most common types of diabetes are type I diabetes mellitus and type II diabetes mellitus, Type II diabetes is caused by either insulin resistance or a defect in insulin secretion from beta cells in the pancreas or both. [3]Diabetes and hypertension are both lead to cardiovascular disease, especially when the nephropathy is present. ACE inhibitors –such as lisinopril- consider first choice drugs for hypertension associated with diabetes. Diabetic patient who used anti-diabetic such as sulphonylurea (gliclazide) needs to continuous monitoring of blood glucose levels to prevent the occurrence of complications of diabetes, such as cardiovascular disease and many other complications such as neuropathy, retinopathy, and

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neuropathy. Hypoglycemia should be avoided due to its dangerous effect that can cause convulsions, coma and even death, therefore drug interactions of various drugs with oral anti-diabetic medications should be studied carefully. Such knowledge enables physicians and pharmacists to prevent such risks or even reduce the occurrence of these interactions through doses adjustment or by using alternative medications [4]

MATERIALS AND METHODS

Drugs and Chemicals:

Lisinopril drug was purchased from (Hetero Drugs, India) Gliclazide and streptozotocin powders were purchased from Sigma Aldrich, Glucose and insulin estimation kits were purchased from Roche Diagnostics. All other chemicals used were an analytical grade.

Experimental Animals:

The study was conducted on healthy adult male, white rabbits with a weight range from 1.2-1.8 kg, and healthy albino waster rats weighing 250±20g. Rabbits were obtained from the animal house of Faculty of Pharmacy at University of Science and Technology; Rats were obtained from the animal house (Sana'a University-Department of Biology). Animals were acclimatized to the laboratory conditions for seven days before commencement of treatment. They were kept in a clean cage with free feed and water under observation. The study protocol and experimental procedures were reviewed and approved by the University Ethics Committee (Reg.No:42/2015) before any experiment could be conducted. Concerning the use of lisinopril and gliclazide in clinical practice, dose was calculated according to the pilot study and the method of (Paget's and Barnes, 1964) [5]

Study Design:

Normal Rabbits' Study:

A group of six adult male, white rabbits with a weight range from 1.2-1.8 kg was used in the study. They were given 3 ml of normal saline orally (through an oral gavage tube). Blood samples were collected at 0, 4, 8,12,16,20, and 24 h from the marginal ear vein. Blood glucose and insulin levels were measured by using glucose oxidase/peroxidase (GOD/POD).[6] and insulin kits (Cobas 6000 e501, Roche Company). The animals were left for a one-week rest period. Then, they were given 3.7 mg/kg body weight gliclazide orally (through an oral gavage tube). Blood samples were collected at 0, 4, 8,12,16,20, and 24 h. Blood glucose and insulin levels were measured. The animals were left for another one-week washout period. The same group of animals was given 1mg/kg of lisinopril and 3.7 mg/kg of gliclazide (30 minute interval) orally (single dose treatment). Blood samples were collected at the same time intervals as mentioned previously, Blood glucose and insulin levels were measured. This step was followed by another washout period for another one week to give multiple doses of 1 mg/kg Lisinopril and 3.7 mg/kg gliclazide (30 minute interval) for one week. Blood samples were collected at the end of the week at 0, 4, 8,12,16,20, and 24 h time intervals. The same pharmacodynamic parameters (blood glucose and insulin levels) were measured to detect the presence of any drug-drug interaction features between the two tested medications.

Normal Rats' Study:

A group of six adult male healthy albino waster rats weighing 250±20 g was used in the study. They were given 3 ml of normal saline orally (through an oral gavage tube). Blood samples were withdrawn at 0, 4, 8,12,16,20, and 24 h from the retro orbital.[7]

The same procedure that conducted in normal rabbits was repeated in normal rats.

Diabetics Rats' Study:

Induction of Diabetes in Rats:

For induction of type II diabetes mellitus streptozotocin (STZ) was dissolved in citrate buffer; that it should be freshly prepared directly before use by mixing 21.75 ml of sodium citrate solution (1.5 g sodium citrate in 50 ml distilled water) and 26.75 ml of citric acid solution (1.05 g citric acid in 50 ml distilled water).and completing the volume to 100 ml distilled water. Then, pH was adjusted to 4.5.[8].Streptozotocin 35mg/kg of body weight was administrated intraperitoneally.[9] After 48 hours, a group of six rats with blood glucose levels more than 250 mg/dl was selected for the study.

The same procedure that conducted in normal rabbits was repeated in diabetic rats.

Statistical Analysis:

Data were summarized as means ± SEM. One way analysis of variance (ANOVA) followed by Dunnet's multiple comparison test was used to conduct the significance of association using SPSS program version 21. Differences were considered significant at P values of less than 0.05.

RESULT

Normal Rabbits Study:

Gliclazide was produced a significant serum glucose reduction at 8, 12, 16, and 20 hour when compared to control group. In serum insulin levels, a significant change

also shown at 8 and 12 hours (22.0±2.13) and (21.8±3.18) respectively when compared to control group. In single and multiple doses treatments, gliclazide has no exhibited significant reduction in serum glucose levels except at 4, 12, and 16 h in multiple doses of lisinopril 1mg/kg along 24 h of experimental study. Likewise, a significant increase in serum insulin levels was seen only at 4 h of multiple doses (table 1) (figure1, 2).

Table 1: The effect of gliclazide and lisinopril (single and multiple doses) on (mean ±SEM) blood glucose level (mg/dl) in normal rabbits (n=6)

Hour	**Control**	**gliclazide 3.7mg/kg**	**gliclazide3.7mg/kg+ lisinopril 1mg single dose**	**gliclazide3.7 mg/kg +lisinopril 1mg multiple doses**
0	118.8±3.63	110.8±2.21	109.4±2.79	112.0±3.20
4	109.6±2.66	104.6±4.43†	101.6±2.80	88.8±4.12**†
8	106.3±2.21	93.8±4.66*†	96.0±3.49†	93.8±3.57†
12	110.0±2.33	94.0±1.15*†	91.0±2.42†	87.2±2.18**†
16	107.6±1.66	99.3±1.92*†	93.2±4.72†	91.6±1.21**†
20	113.7±3.50	102.3±2.03*	102.8±2.27	104.8±1.75
24	107.5±2.21	104.3±4.63	103.8±3.82	101.3±1.60†

*Significant compared to control at P-value < 0.05, ** Significant compared to gliclazide group. at P-value < 0.05, † Significant compared to zero time to same group. at P-value < 0.05

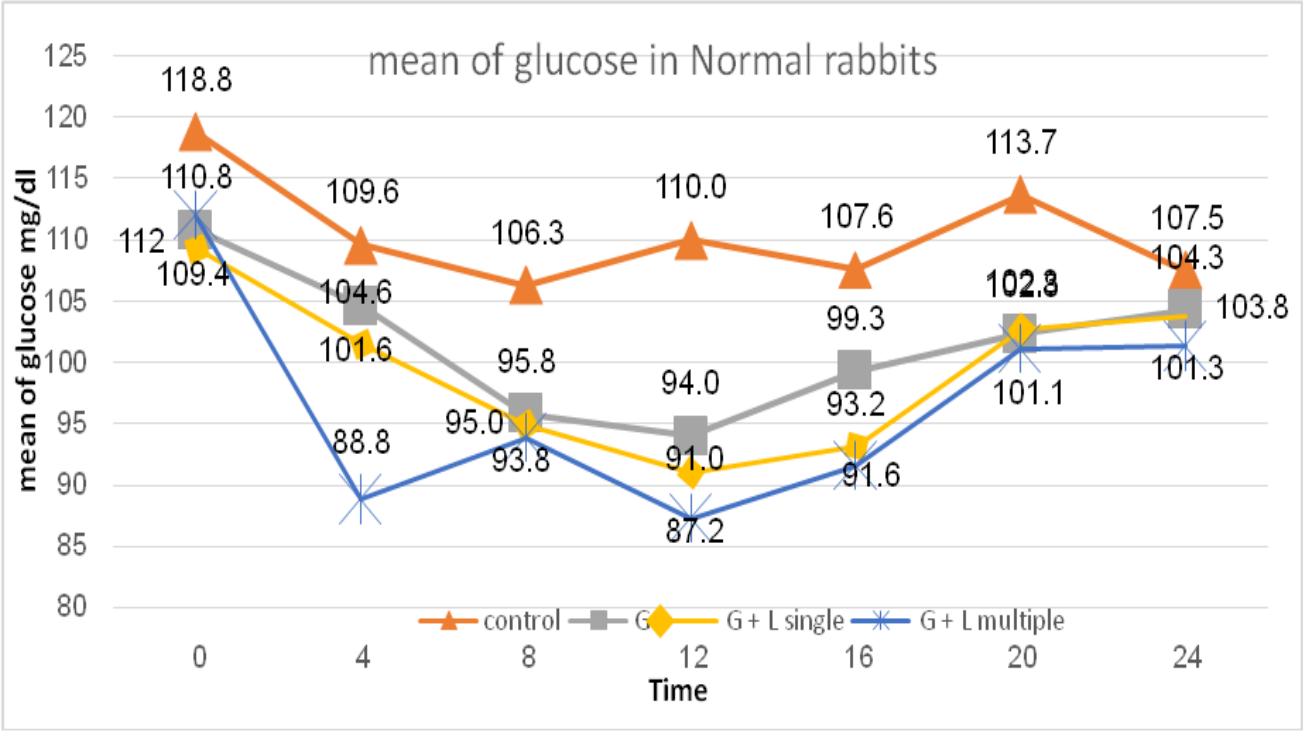

Figure 1: The effect of gliclazide and lisinopril (single and multiple doses) on (mean ±SEM) blood glucose level (mg/dl) in normal rabbits (n=6). *Significant compared to control at P-value < 0.05, ** Significant compared to gliclazide group. at P-value < 0.05, † Significant compared to zero time to same group. at P-value < 0.05.

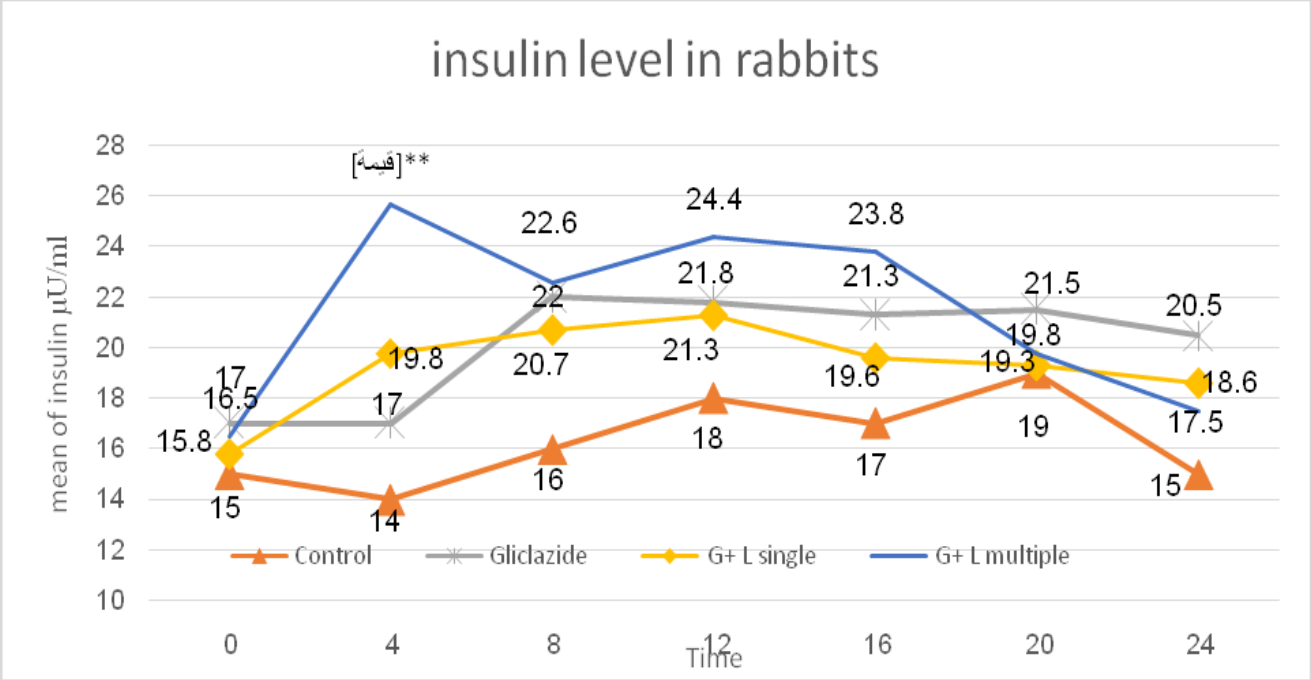

Figure 2: The effect of gliclazide and lisinopril (single and multiple doses) on (mean ±SEM) serum insulin level (µU/ml)in normal rats (n=6). *Significant compared to control at P-value < 0.05, ** Significant compared to gliclazide group. at P-value < 0.05, † Significant compared to zero time to same group. at P-value < 0.05.

Normal Rat Study:

In normal rats treatments, gliclazide was produced a significant serum glucose reduction at 8, 12, 16, and 20 hours (135.5±4.33), (135.7±5.11), (132.3±5.17) and (135.3±5.36) respectively, as compared to control group. Significant changes in serum insulin levels were at all-time intervals along 24 hours of experimental study. Single dose treatment, exhibited a significant effect only at 8 hours in both glucose and insulin serum levels (117.8±4.46) and (26.7±2.33) respectively, when compared to gliclazide group.

Table 2: The effect of gliclazide and lisinopril (single and multiple doses) on (mean ±SEM) blood glucose level (mg/dl) in normal rats (n=6)

Hour	Control	gliclazide 3.7mg/kg	gliclazide 3.7 mg/kg+lisinopril 1mg single dose	gliclazide3.7mg/kg+lisinopril1mg/kg multiple doses
0	154.0±1.37	153.4±4.24	148.6±2.08	144.6±2.8
4	151.1±3.46	135.5±4.33*†	133.5±3.95†	105.8±3.73**†
8	156.3±2.59	135.7±5.11*†	117.8±4.46**†	100.3±1.20**†
12	152.9±2.78	132.3±5.17*†	120.5±5.55†	103.3±1.70**†
16	157.0±2.60	135.3±5.36*†	124.5±4.05†	106.5±4.50**†
20	148.4±3.16	137.5±8.50†	133.0±3.16 †	102.5±2.22**†
24	143.0±2.48	141.3±5.17	136.0±4.90	109.3±1.97**†

* Significant compared to control at P-value < 0.05.** Significant compared to gliclazide group. at P-value < 0.05.† Significant compared to zero time to same group. at P-value < 0.05.

In multiple doses treatment, when compared to hypoglycemic activity of gliclazide group, treated with 1 mg of lisinopril followed by 3.7 mg/kg of gliclazide 30 min later once daily for 7 days showed significant changes in serum glucose and insulin levels at all-time intervals, along the experimental study. (table2)(figure3, 4)

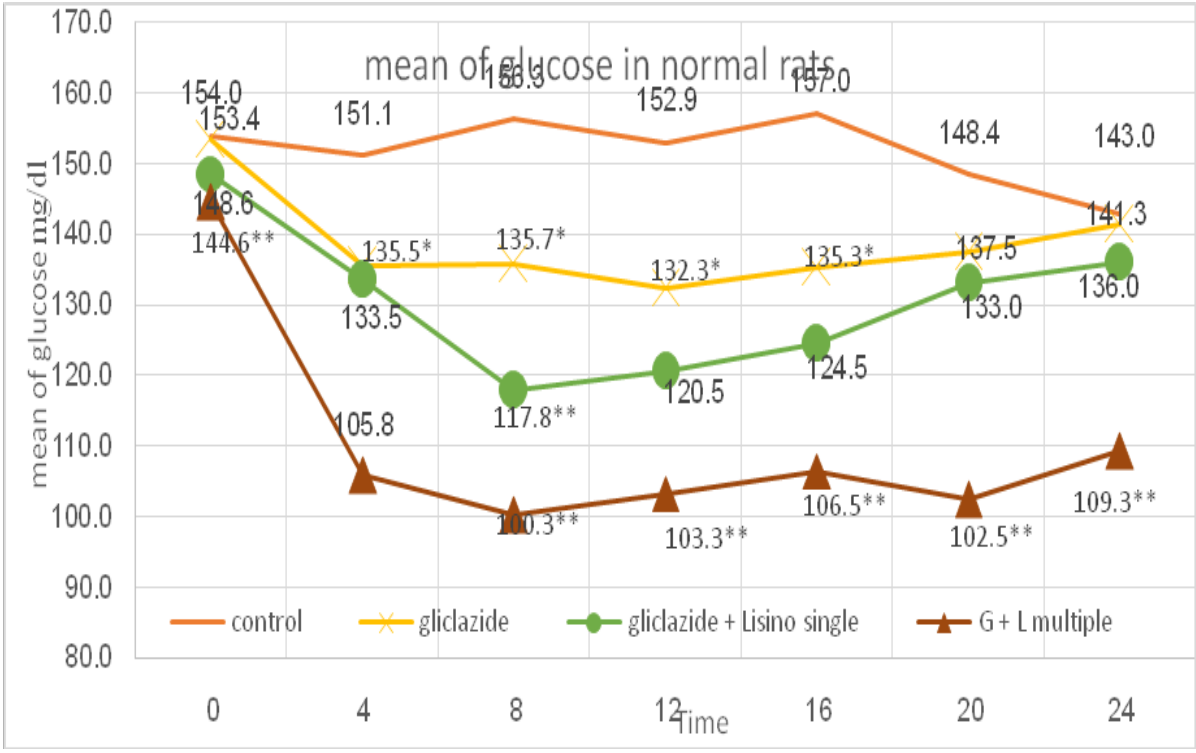

Figure 3: The effect of gliclazide and lisinopril (single and multiple doses) on (mean ±SEM) blood glucose level (mg/dl) in normal rats (n=6). *Significant compared to control at P-value < 0.05, ** Significant compared to gliclazide group. at P-value < 0.05, † Significant compared to zero time to same group. at P-value < 0.05.

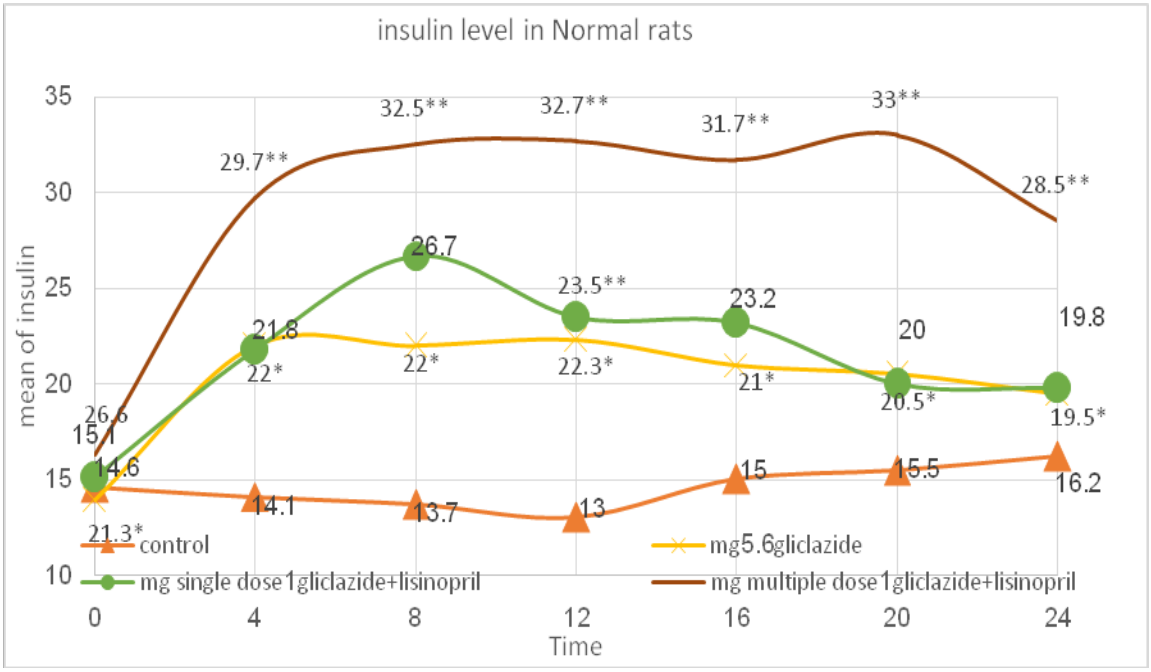

Figure 4: The effect of gliclazide and lisinopril (single and multiple doses) on (mean ±SEM) serum insulin level (µU/ml)in normal rats (n=6). *Significant compared to control at P-value < 0.05, ** Significant compared to gliclazide group. at P-value < 0.05, † Significant compared to zero time to same group. at P-value < 0.05.

Diabetic Rat Study:

Gliclazide was produced a significant serum glucose reduction at all prefixed time intervals throughout 24 h of the experimental study when compared to control group. A similar effect was seen in serum insulin levels, a significant increase observed at all prefixed time intervals except at 20 and 24 h of the experimental study.

The group was treated with lisinopril 1mg/kg followed by gliclazide 3.7 mg/kg 30 min later single dose showed a significant blood glucose reduction along time intervals during the experimental study except at 16 h when compared to gliclazide group. Same effect was produced in multiple doses, a significant effect of serum glucose reduction was seen at all intervals of time throughout of experimental study. In serum insulin levels, significant increases were seen at all time intervals of the experimental study when we compared to gliclazide group. (table3)(figure5, 6)

Table 3: The effect of gliclazide and lisinopril (single and multiple doses) on (mean ±SEM) blood glucose level (mg/dl) in diabetic rats (n=6)

Hour	Control	gliclazide 3.7mg/kg	gliclazide3.7 mg/kg+ lisinopril 1mg single dose	gliclazide3.7 mg/kg +lisinopril 1mg multiple doses
0	359.4±6.46	339.6±4.37†	334.0±4.85	344.5±2.60
4	350.0±8.24	273.8±3.57*†	249.3±2.91**†	205.7±2.96**†
8	368.6±7.45	262.3±2.96*†	222.7±7.26**†	204.5±1.50**†
12	345.0±8.32	251.0±5.51*†	219.5±3.50**†	203.3±1.20 **†
16	336.5±7.47	261.5±3.50 *†	250.3±8.74 †	203.7±4.37**†
20	329.0±5.33	284.3±4.41*†	250.0±1.00**†	214.5±5.11**†
24	354.0±6.29	286.7±9.56*†	264.3±1.20**†	226.0±2.65**†

*Significant compared to control at P-value < 0.05, ** Significant compared to gliclazide group. at P-value < 0.05, † Significant compared to zero time to same group. at P-value < 0.05

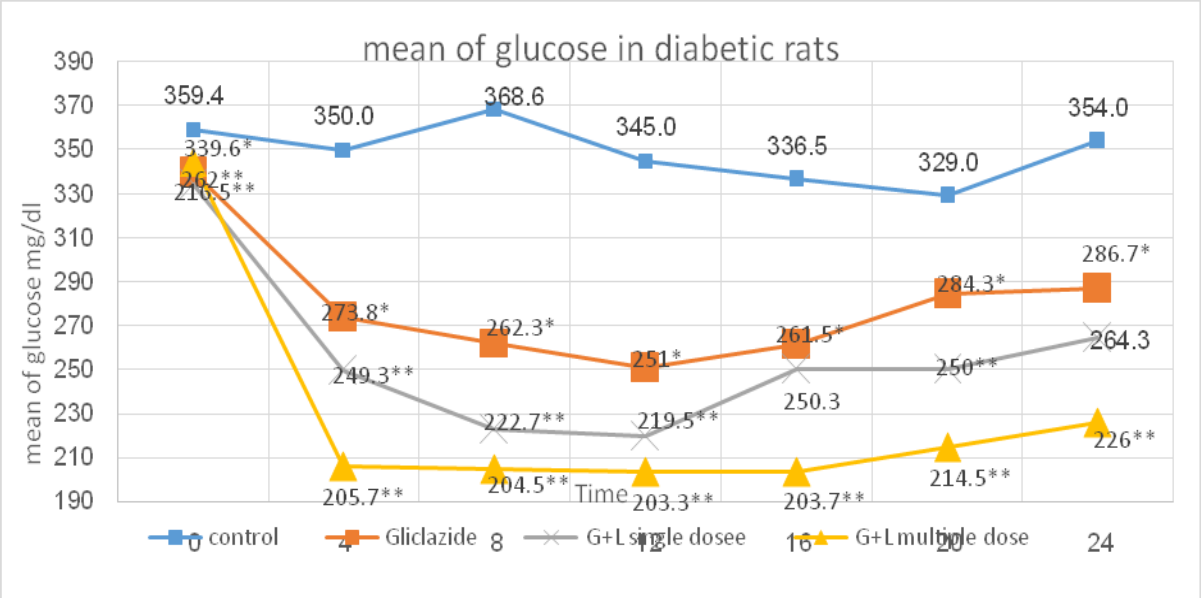

Figure 5: The effect of gliclazide and lisinopril (single and multiple doses) on (mean ±SEM) blood glucose level (mg/dl) in diabetic rats (n=6). *Significant compared to control at P-value < 0.05, ** Significant compared to gliclazide group. at P-value < 0.05, † Significant compared to zero time to same group. at P-value < 0.05.

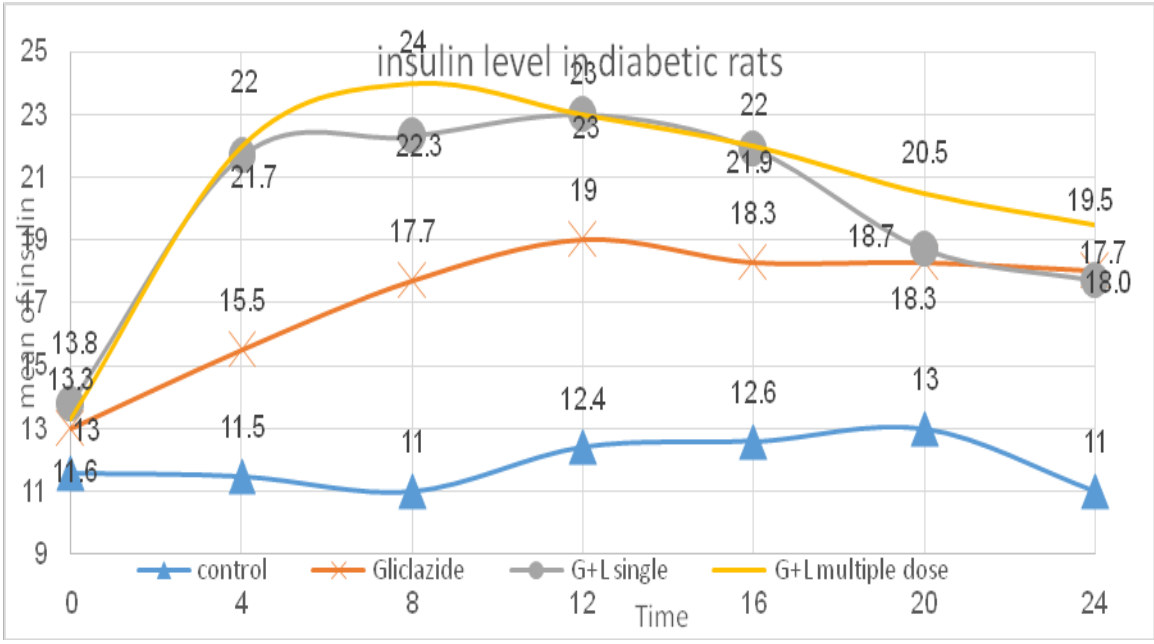

Figure 6: The effect of gliclazide and lisinopril (single and multiple doses) on (mean ±SEM) serum insulin level (µU/ml)in diabetic rats (n=6). *Significant compared to control at P-value < 0.05, ** Significant compared to gliclazide group. at P-value < 0.05, † Significant compared to zero time to same group. at P-value < 0.05.

DISCUSSION

The present study was aimed to detect the presence of any drug-drug interaction features between single and multiple dose treatment of lisinopril (1mg/kg) an ACE inhibitor on antidiabetic activity of gliclazide (3.7 mg/kg) in both normal, diabetic rats and in normal rabbits when they are used concomitantly.

The using of Rat animal model served to quick detect the interaction either in normal or in diabetic. Over that, diabetic animal serve to validate the same response in the actually used condition of the drug. The rabbit model is additional different species to endorse the incident of the interaction. Significant effect was seen in multiple doses treatment of lisinopril and gliclazide in normal and diabetic rats, whereas, single dose treatment was seen very broadly in diabetic rats.

In the current work, gliclazide group showed significant (P <0.05) reduction of serum glucose levels and increase in serum insulin levels. When compared to control group in all time of the intervals of the experimental study, especially around $t_{1/2}$ of gliclazide (11 h). Gliclazide is known to produce a hypoglycemic effect by two mechanisms, insulin secretion by K+ channel inhibition the β cells in the pancreas and extra pancreatic by increasing muscles and tissue uptake of glucose. The target for sulphonylureas activity is ATP sensitive K+ channels (K+ATP channels). The gliclazide (sulphonylurea) drug used in type II diabetes encourages insulin secretion by closing K+ATP channels in pancreatic β cells. [10]These results are also consistent with the previous study. [11,12,13] Furthermore, gliclazide raises the sensitivity of pancreatic ß cells to blood glucose. Gliclazide might have an extra pancreatic effect which reestablishes peripheral insulin sensitivity, such as decreasing hepatic glucose production (Glycogenolysis and gluconeogenesis), and increasing blood glucose elimination and skeletal muscle glycogen synthesis. [14]

Our study found that, the effect of a single dose of lisinopril on gliclazide was shown a significant change in serum glucose and insulin levels in diabetic rats, while in normal rats and rabbit, neither significant effect appeared on serum blood glucose nor insulin levels.it is suggest that lisinopril may have improve insulin sensitivity, that it may possess potential effect in type II diabetes but not in normoglycemic rats and rabbits, this confirm with the available literature. [15, 16]

Another reason for this effect, may be referred to the elevation of bradykinins levels associated with ACE inhibitor use may lead to improve on insulin sensitivity. [17, 18] One study has demonstrated that ACE inhibitor have no impact on hepatic or peripheral insulin sensitivity. [19] So our findings contradictory with this study.

Moreover, co-administration of lisinopril 1mg/kg followed by gliclazide 3.7mg/kg 30 min. later for 7 days once daily dose, showed significant decreased in serum glucose and increased in insulin levels in normal rats,

diabetic rats and insignificant effect in normal rabbits. Multiple doses of lisinopril showed more impact on the increase antidiabetic activity of gliclazide in the normal and diabetic rats. These effects probably due to the ability of lisinopril to improve insulin sensitivity as mentioned previously, but in this current work, it suggested that insulin sensitivity improvement of lisinopril also happens in normal rats when treated with multiple doses but not in single dose.[15, 16] Our finding is a compatible with another study that suggested that insulin sensitivity of ACE inhibitors occurred in type II diabetic mellitus but not in normoglycemic rats. Additionally, another hypothesis for this effect that increase concentrations of bradykinin during uses of ACE inhibitors are responsible for the increase glucose metabolism and improving glucose sensitivity. [20]Additionally, the most reduction of blood glucose effect was seen within 4 to 8 hours, these effects increased to 12 hours, even to 16 hours in multiple doses treatment in all experiment's parts. These effects, probable that the plasma peak concentration (Cmax) reached within 2 to 8 hours after a single dose of gliclazide by oral route. Tmax and Cmax are increased after repeated gliclazide administration.[21]

Variations of response to treatment in normal rabbits noticeably seen during experimental parts, there are known possible limitations with these variations. First the preclinical results may not be connected directly to human existences considering inter-species metabolic variations. Second, lisinopril impact was studied in normal rabbit models rather than diabetic models Third, the gliclazide produced a biphasic response in rat model may be due to its enterohepatic circulation in rats [22, 23] and humans.[24]Such effect does not happen in rabbit models. Finally, rat models have more than 95% gene mapping with human which consider the most animal models relevant to human beings.[25]

CONCLUSIONS

In conclusion, based on available evidence, the effect of lisinopril on hypoglycemic of gliclazide in single dose in normal rats was not obviously detected, while a significant effect of lisinopril on the hypoglycemic of gliclazide was observed during multiple co-administration of lisinopril and gliclazide. Hence, concomitantly administration of lisinopril and gliclazide is not preferred according to this study outcomes. Otherwise, they should be used with caution in clinical condition, to avoid adverse hypoglycemic incidents in diabetic individuals. Further studies in large sample size with highly sensitive modern scientific is necessary to validate the above observation or analyze the precise mechanism of action involved in the antihyperglycemic activity of Lisinopril.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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