

EFFECT OF CARVEDILOL ON THE PHARMACOKINETICS AND PHARMACODYNAMICS OF GLIPIZIDE

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ABSTRACT

The present work studied the effect of carvedilol on the pharmacokinetics (PK) and Pharmacodynamic (PD) of glipizide in normal and diabetic rat models to evaluate the safety and effectiveness of the combination. The study was conducted on Streptozotocin (STZ) induced diabetic rats. Diabetes was induced by a single intraperitoneal injection of STZ (45 mg/kg), then glipizide and carvedilol were administered orally for 7 days. On 8th day the blood samples were collected for 8 hrs at regular time intervals and the pharmacodynamic and pharmacokinetic parameters like K_a , $t_{1/2}$, AUC, t_{max} , Clearance, V_d , C_{max} of glipizide, before and after carvedilol treatment were determined. The blood glucose levels were estimated by Glucose Oxidase-Peroxidase (GOD-POD) method on 1st, 4th and 8th day. Carvedilol treatment has shown maximum hypoglycemic effect at 4th hour after oral administration. In diabetic rats, carvedilol pre-treatment has shown significant increase in serum glipizide levels. Carvedilol pre-treatment in combination with glipizide has shown significant decrease in blood glucose when compared to rats treated with glipizide alone. Present study results suggest that, carvedilol significantly inhibits P-gp and it increases the concentration and AUC of glipizide. Hence the present study indicated that carvedilol has effect on pharmacokinetics and pharmacodynamics of glipizide.

Keywords: Streptozotocin, Glipizide, Carvedilol, Diabetic cardiomyopathy, Beta blockers, Type 2 Diabetes mellitus, GOD-POD.

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia, resulting from defects in insulin secretion, insulin action or both. Patients with diabetes mellitus are more susceptible to the vascular complications of hypertension and heart failure. Diabetes is the largest morbidity of patients with heart failure and being a major risk factor for cardiovascular disease¹. Cardiovascular disease is responsible for 80% of deaths among diabetic patients much of which has been attributed to CAD (coronary artery disease). However, there is an increasing recognition that diabetic patients suffer from an additional cardiac insult termed 'diabetic cardiomyopathy'. This entity was originally described in 1972 on the basis of observations in four diabetic patients who presented with HF (heart failure) without evidence of hypertension, CAD, valvular or congenital heart disease².

Oxidative stress has been associated with the pathogenesis of chronic diabetic complications including cardiomyopathy³. Hyperglycemia may mediate its damaging effects through a series of secondary transducers. An increase in ROS decreases NO levels, which leads to myocardial inflammation and endothelial dysfunction via PARP [poly (ADP-ribose) polymerase], inhibition of which has been shown to reverse diabetic endothelial dysfunction⁴. Endothelial dysfunction is a precursor to and an effect of atherosclerosis. Anatomical and functional abnormalities of the vascular endothelium are commonly associated with diabetes⁵.

Glipizide is an oral hypoglycemic agent, which is a commonly prescribed drug for the treatment of patients with type II diabetes mellitus who have failed diet and exercise therapy and it appears to be the most effective insulin secretagogue both in first phase insulin secretion and in sustained stimulatory response during long term administration⁶. The dosage of glipizide is between 2.5 mg and 15 mg once daily. It is recommended that if the daily dose exceeds 15 mg (maximum 20 mg) it should be given in 2 or 3 doses. Glipizide is completely absorbed from the gastrointestinal tract. Its peak plasma concentration is 200 ng/mL after the oral administration of 2.5 mg glipizide, and is generally reached within 1 to 2 hours^{7, 8}. Like glibenclamide, glipizide is also highly bound to plasma protein (92% to 99%)^{8, 9, 10} and has a low volume of distribution (about 10 liters).

Glipizide is extensively metabolized in the liver, and its main metabolites are 4-trans-hydroxyglipizide, 3-cis-hydroxyglipizide, and N-(2-acetyl-amino-ethyl-phenyl-sulfonyl) N-cyclo-hexylurea (DCAA) (Wensing 1989). 2C9 is responsible for metabolism of a large number of commonly utilized drugs. Greater than 100 substrates have been discovered¹¹. CYP2C9 is probably the main

CYP enzyme involved in the oxidative metabolism of glipizide, because in an individual homozygous for the defective CYP2C9*3 allele, the oral clearance of glipizide was drastically lower than in other¹². The metabolites of glipizide are pharmacologically inactive and are excreted primarily in the urine^{9, 10}; its $t_{1/2}$ is between 2 and 4 hours^{8, 9}; glipizide pharmacokinetics are unaffected by aging¹³.

Carvedilol is a non selective β - adrenoceptor and selective α_1 -adrenoceptor blocker. So far, it has been widely used in the treatment of heart failure, hypertension with or without diabetes³. Carvedilol is used as a racemic mixture clinically; S-carvedilol has greater affinity for β -adrenoceptors than R-carvedilol, although S- and R-carvedilol have equal affinity for α -adrenoceptors. Both the R(+) and the S(-) enantiomers of carvedilol were metabolized in human liver microsomes primarily to 4'- (4OHC) and 5'-(5OHC) hydroxyphenyl, 8-hydroxy carbazoyl (8OHC) and O-desmethyl (ODMC) derivatives. The S(-) enantiomer was metabolized faster than the R(+) enantiomer although the same P450 enzymes seemed to be involved in each case.

β - blockers have been always reluctant to be used on the treatment of diabetes, because they often brought negative effects on the glucose and lipid metabolisms. But it has been reported carvedilol had no negative effects including insulin sensitivity besides glucose and lipid metabolisms^{14, 15}. Carvedilol has antioxidant and free radical scavenger properties. In physicochemical, biochemical, and cellular assays carvedilol inhibited the formation of reactive oxygen radicals and lipid peroxidation, scavenged oxygen free radicals, and prevented the depletion of endogenous anti-oxidants¹⁶.

In the U.S. Carvedilol Program in patients with heart failure of diverse severity, the beta-adrenergic blocker carvedilol has been demonstrated to be well tolerated and to produce a significant reduction of overall mortality¹⁷. Carvedilol also increases insulin sensitivity (metoprolol has the opposite effect), and has unique antioxidant properties that have been hypothesized to improve endothelial dysfunction and to prevent apoptosis¹⁸. However, whether carvedilol can improve cardiac function in diabetic animal model and this protection via antioxidant pathway is not clear.

MATERIALS AND METHODS

Drugs and chemicals

Glipizide and Carvedilol were obtained as gift samples from Matrix Pvt. Ltd, Hyderabad. Verapamil was obtained as a gift sample from Kakatiya University, Warangal. Glipizide were obtained from Dr. Reddy's laboratories, Hyderabad, India as a gift samples.

Streptozotocin (STZ) was purchased from Sigma Chemical Co. (St. Louis, MO). The glucose estimation (GOD- POD) kit (excel diagnostic, Hyderabad) was procured from drug store. All HPLC grade solvents (methanol and water) were procured from drug store. All other chemicals including potassium dihydrogen phosphate, sodium citrate, sodium carboxy methyl cellulose, phosphoric acid, DMSO, glucose, sodium chloride, sodium bicarbonate, magnesium sulphate, potassium chloride, calcium chloride, diethyl ether were procured from finar chemicals Ltd., Ahmadabad. All chemicals used were analytical grade or otherwise specified.

Experimental animals

Experiments were performed with male wistar rats procured from Mahaveera enterprises (Hyderabad, A.P., India), weighing between 180 - 220 g. The animals were housed in individual polypropylene cages under standard laboratory conditions of light, temperature ($22 \pm 1^\circ\text{C}$) and relative humidity for at least one week before the beginning of experiment, to adjust to the new environment and to overcome stress possibly incurred during transit. Animals were given standard rat pellets and drinking water *ad libitum*. The animals were fasted 12 hours before the conduct of experiment and during the experiment they were withdrawn from food and water. The experiments were planned after the approval of Institutional Animal Ethical Committee (IEAC) (Code no: 2010/8/1/1- 4).

Selection of doses and preparation of oral test solution/suspension

In clinical practice, glipizide and carvedilol in a therapeutic dose will be administered orally as antidiabetic and hypertension and congestive heart failure therapy, respectively. Human oral therapeutic doses of the respective drugs were extrapolated to rat/rabbit based on body surface area¹⁹. But the dose of glipizide for rat experiments was selected as 5 mg/kg bd. wt.²⁰ based on the influence of dose effect-relationship of gliclazide on blood glucose in normal rats. Carvedilol and glipizide were suspended in 2% sodium CMC for oral administration. All the drugs were administered to the respective groups by oral gavage.

Experimental induction of diabetes

Diabetes was induced by single intra peritoneal injection of freshly prepared solution of STZ at the dose of 60mgkg^{-1} in 0.1M citrate buffer (pH 4.5) to the rats fasted overnight. After 3 days of STZ induction, blood glucose levels between 300–400 mg/dL were selected for the study.

Pharmacokinetic and pharmacodynamic interaction study in normal and diabetic rats

Wistar rats were randomly distributed into four groups of six animals in each group; they were housed in well ventilated polypropylene cages and maintained on uniform diet and temperature with 12 h light and dark cycle. Before experiment all animals were fasted for 18 hours and water *ad libitum*, water was withdrawn during experiment. After collection of initial blood samples, drugs were administered in the following order. Group I—Control (0.2 mL of 0.5% CMC Sodium; p.o.); Group II—Glipizide (5 mg/kg; p.o.); Group III ---Carvedilol (2 mg/kg; p.o.) and Group IV—Administration of carvedilol (2 mg/kg) followed by glipizide (5 mg/kg) after 30 minutes.

Diabetic rats were randomly distributed into four groups of six animals in each group; they were housed in well ventilated polypropylene cages and maintained on uniform diet and temperature with 12 h light and dark cycle. Before experiment all animals were fasted for 18 hours and water *ad libitum*, water was withdrawn during experiment. After collection of initial blood samples, drugs were administered in the following order daily for 8 days. Group I — Control (0.2 mL of 0.5% CMC Sodium; p.o.); Group II — Glipizide (5 mg/kg; p.o.); Group III ---Carvedilol (2 mg/kg; p.o.) and Group IV—Administration of carvedilol (2 mg/kg) followed by glipizide (5 mg/kg) after 30 minutes.

In this study, Blood samples were withdrawn from retro orbital plexus²¹ of each rat at 0, 0.5, 2, 3, 4, 6 and 8 h. in 2 mL eppendorfs tubes containing sodium citrate as anticoagulant. Plasma was separated by centrifugation at 5000 rpm/10 min and stored at -20°C . These samples were used to analyze for both blood glucose levels estimated by GOD-POD method on 1st, 4th and 8th day and plasma concentration of glipizide was estimated by a sensitive RP-HPLC method on 8th day.

HPLC conditions

HPLC (A Shimadzu Class VP series HPLC system with two LC- 10AT pumps, a SPD-10A variable wavelength programmable UV/Vis detector, a SCL-10A system controller and a RPC-18 column (Thermo, USA; 150mm×4.6mm, 5µm) was used. The system was equipped with ClassVP series version 6.12. software. The mobile phase consisted of methanol-0.01 mol/L phosphate buffer (70:30, v/v). The pH of phosphate buffer was adjusted to 3.0 with phosphoric acid. The injection volume was 10 µL and the mobile phase was delivered at 1.0 mL/min. The mobile phase was filtered through 0.22 µm membrane filter. The flow rate was adjusted to 1 mL/min and the effluent was monitored at 223 nm. The total run time of the method was set at 8 min. Retention times of glipizide and gliclazide were 2.89 and 3.64 min respectively.

Statistical analysis

The results were expressed as mean±SEM. The significance was determined by applying Student's paired't' test and one way ANOVA.

RESULTS

Pharmacodynamic interaction study in normal and diabetic rats

Glipizide produced hypoglycemic activity with maximum reduction of $38.2 \pm 0.94\%$ in normal rats and $33.74 \pm 0.43\%$ in diabetic rats at 4 hr. Carvedilol has significant effect on the blood glucose levels with reduction of $8.27 \pm 0.87\%$ in normal rats and $17.67 \pm 0.67\%$ in diabetic rats at 4th hr. In combination, carvedilol produced enhanced hypoglycemic effect of glipizide with maximum blood glucose reduction of $58.64 \pm 0.47\%$ and $61.23 \pm 1.67\%$ at 4 h, following single dose and multiple dose administration of carvedilol, respectively, in normal rats (Table 1). In combination, carvedilol produced enhanced hypoglycemic effect of glipizide with maximum blood glucose reduction of $64.41 \pm 0.65\%$ and $65.141 \pm 1.65\%$ at 4 h, following single dose and multiple dose administration of carvedilol, respectively, in diabetic rats (Table 2). The enhancement in glipizide effect is more with the multiple dose treatment of carvedilol than single dose treatment.

Table 1: Mean percent blood glucose reduction in normal rats (N = 6)

Time (hrs)	Glipizide	Carvedilol	Glipizide + Carvedilol (SDT)	Glipizide + Carvedilol (MDT)
0	0 ± 0	0 ± 0	0 ± 0	
0.5	13.27 ± 1.20	1.22 ± 1.85	24.0 ± 0.54***	25.83 ± 0.67***
1	16.61 ± 2.03	3.39 ± 0.45	30.14 ± 1.21***	32.42 ± 0.12***
2	27.22 ± 0.25	6.48 ± 0.54*	37.72 ± 1.32***	38.61 ± 0.59***
3	33.22 ± 1.31	7.67 ± 2.01	50.73 ± 1.01***	52.04 ± 1.04***
4	38.2 ± 0.94	8.27 ± 0.87*	58.64 ± 0.47***	61.23 ± 1.67***
6	14.99 ± 0.54	4.01 ± 0.64*	53.48 ± 0.54***	54.86 ± 1.58***
8	9.31 ± 1.25	3.23 ± 0.25	13.30 ± 0.72***	15.45 ± 0.95***

***Significant at P < 0.001 compared to gliclazide control (normal rats).

**Significant at P < 0.05 compared to gliclazide control (diabetic rats).

Table 2: Mean percent blood glucose reduction in diabetic rats (N = 6)

Time (hrs)	Glipizide	Carvedilol	Glipizide + Carvedilol (SDT)	Glipizide + Carvedilol (MDT)
0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
0.5	6.12 ± 0.51	2.61 ± 1.08	18.75 ± 0.86**	19.25 ± 0.58**
1	12.83 ± 1.0	8.07 ± 1.15*	22.83 ± 1.57**	25.21 ± 0.69**
2	18.66 ± 0.81	10.38 ± 0.82*	34.13 ± 1.05**	36.18 ± 1.57**
3	24.56 ± 0.65	13.27 ± 0.93*	53.47 ± 0.95**	55.67 ± 2.5**
4	33.74 ± 0.43	17.67 ± 0.67*	64.41 ± 0.65**	65.141 ± 1.65**
6	19.07 ± 0.78	15.66 ± 0.15*	36.83 ± 0.27**	38.08 ± 1.57**
8	10.69 ± 0.94	9.21 ± 0.58*	12.10 ± 0.65**	14.43 ± 0.51**

*Significant at P < 0.05 compared to gliclazide control (normal rats).

**Significant at P < 0.05 compared to gliclazide control (diabetic rats).

Table 3: Mean pharmacokinetic parameters of glipizide before and after carvedilol administration in healthy rats.

Parameter	Glipizide	Glipizide +Carvedilol (SDT)	Glipizide +Carvedilol (MDT)
C _{max} (ng/mL)	7.8 ± 1.15	8.046 ± 1.61*	12.54 ± 1.24*
T _{max} (h)	4 ± 0.0	4 ± 0.0	4 ± 0.0
AUC(ng/mL/h)	24.70 ± 6.12	34.92 ± 8.51**	56.47 ± 8.62**
t _{1/2} (h)	1.25 ± 0.95	1.511 ± 0.18*	2.43 ± 0.32*
CL _F (mL/h)	35.09 ± 1.12	25.84 ± 0.95**	18.67 ± 0.58**
V _F (mL)	63.59 ± 2.15	56.39 ± 1.65*	50.21 ± 1.86*

*Significant at P < 0.05 compared to gliclazide control (normal rats).

**Significant at P < 0.05 compared to gliclazide control (diabetic rats).

Table 4: Mean pharmacokinetic parameters of glipizide before and after carvedilol administration in diabetic rats.

Parameter	Glipizide	Glipizide +Carvedilol (SDT)	Glipizide +Carvedilol (MDT)
C _{max} (ng/mL)	7.90 ± 1.13	8.82 ± 1.03*	11.62 ± 1.58*
T _{max} (h)	4 ± 0.0	4 ± 0.0*	4 ± 0.0*
AUC(ng/mL/h)	35.51 ± 5.29	42.01 ± 8.13**	61.53 ± 6.2**
t _{1/2} (h)	2.10 ± 0.02	2.58 ± 0.02*	3.54 ± 0.54*
CL _F (mL/h)	20.95 ± 0.70	18.01 ± 1.08**	16.61 ± 0.23**
V _F (mL)	69.78 ± 1.89	67.20 ± 1.99*	53.67 ± 0.12*

*Significant at P < 0.05 compared to gliclazide control (normal rats).

**Significant at P < 0.05 compared to gliclazide control (diabetic rats).

Pharmacokinetic interaction study in normal and diabetic rats

The serum glipizide levels were increased and pharmacokinetic parameters of gliclazide like C_{max}, T_{max}, AUC, K_{el} and T_{1/2} were altered significantly with single and multiple dose treatments of carvedilol in normal and diabetic rats (Table 3 & 4). The percent increase of serum gliclazide level is 23.75% and 28.61% following single dose and multiple dose administration of carvedilol, respectively.

DISCUSSION

Drug interactions are usually seen in clinical practice, and the mechanisms of interactions are evaluated usually in animal

models. We studied the effect of carvedilol on the Pharmacodynamic and pharmacokinetics of glipizide in rats.

The normal rat model served to quickly identify the interaction and the diabetic rat model served to validate the same response in the actually used condition of the drug. In this study, carvedilol produce a significant activity on blood glucose levels of normal (fig. 1) and diabetic rats (fig. 2), and the results were consistent with earlier studies on the effects of atenolol on glucose homeostasis in normal and diabetic human subjects suggest that atenolol has no significant hypoglycemic effect in humans²² but atenolol has a hypoglycemic action in the rat.

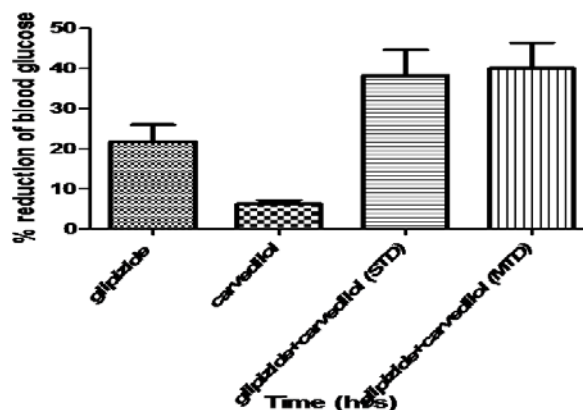


Fig. 1: Effect of carvedilol and glipizide and their combination on % reduction of blood glucose levels in healthy rats (N = 6).

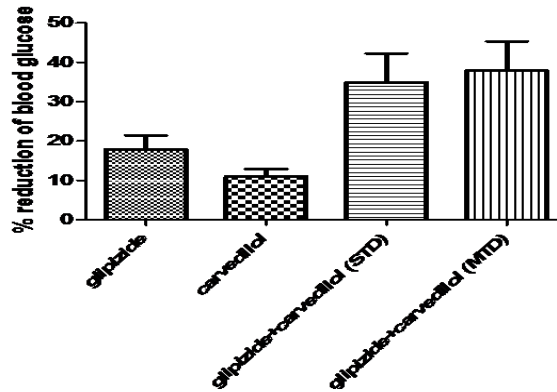


Fig. 2: Effect of carvedilol and glipizide and their combination on % reduction of blood glucose levels in diabetic rats (N = 6).

Atenolol reduces plasma glucose and raises plasma insulin levels in fasted rats, as well as in alloxan diabetic rats upon i.v. application of doses (1 and 4 mg/kg, respectively) that are comparable to human therapeutic ones²³. This is suggested that β -blocking drugs have a significant impact on glucose homeostasis. Interestingly, however, the glipizide hypoglycemic and antidiabetic activity was significantly enhanced by carvedilol, following a single and multiple dose treatment in rats, and this may be evidence the presence of potential interaction between glipizide and carvedilol.

There was a significant rise in serum glipizide levels and an alteration in pharmacokinetic parameters like C_{max} , T_{max} , AUC, K_{el} and $T_{1/2}$ of glipizide with single and multiple dose treatments of carvedilol. The increase in AUC and AUMC indicates improved availability of glipizide in presence of carvedilol both normal (fig. 3) and diabetic rats (fig. 4). The same results were observed in case of doxorubicin with carvedilol²⁴. This might be an interaction at absorption level, because carvedilol is a P-gp inhibitor, it increases the cytotoxicity of doxorubicin²⁴. Glibenclamide is a P-gp substrate and inhibitor²⁵.

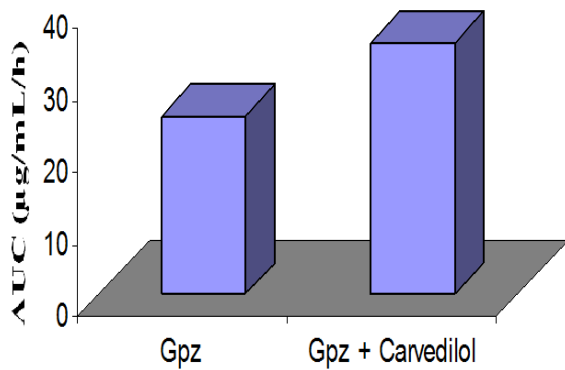


Fig. 3: AUC of glipizide in combination with carvedilol and glipizide alone in healthy rats

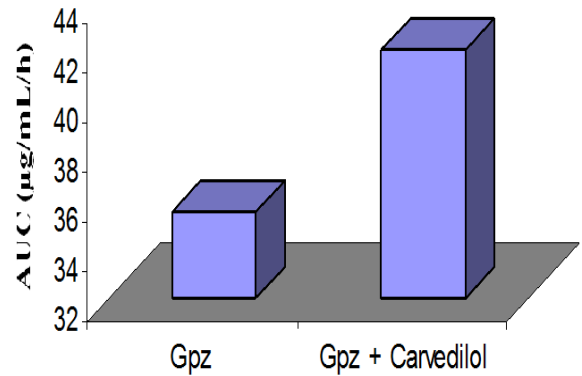


Fig. 4: AUC of glipizide in combination with carvedilol and glipizide alone in diabetic rats

In this study carvedilol increases the bioavailability of glipizide by inhibiting P-gp. These results revealed that glipizide may be a P-gp substrate. The altered K_{el} (fig. 5 and 6) and $T_{1/2}$ (fig. 7 and 8) in normal and diabetic rats indicated alteration either in metabolism or the excretion process.

Carvedilol is not CYP2C9 and CYP3A4 inhibitor, so it is not inhibit the metabolism of glipizide. The major elimination pathway of glipizide is the biliary route. Hence, the interaction at absorption level with increased glipizide absorption by carvedilol, leading to raised serum levels, remains possible.

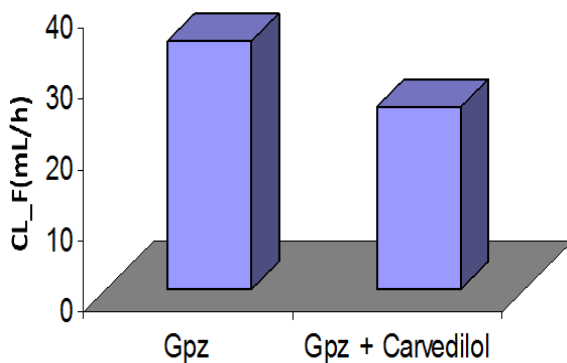


Fig. 5: Clearance (mL/hr) of glipizide in combination with

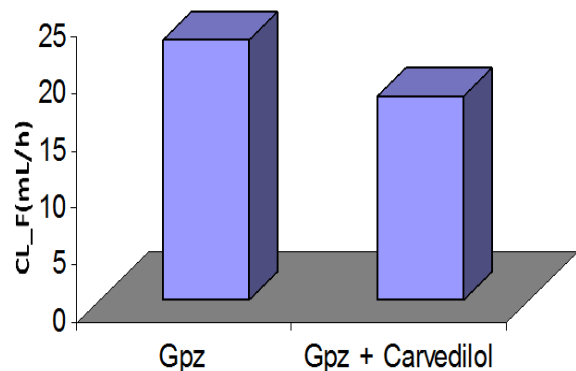


Fig. 6: Clearance (mL/hr) of glipizide in combination with

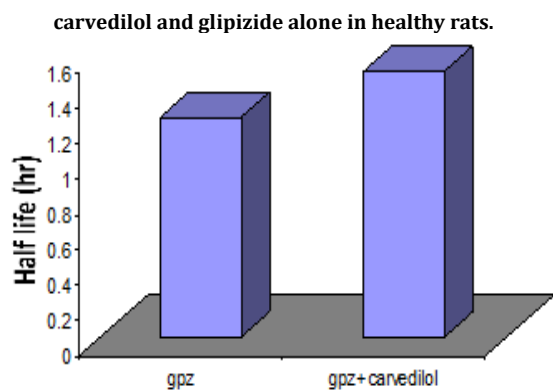


Fig. 7: Half life of glipizide in combination with carvedilol and glipizide alone in healthy rats.

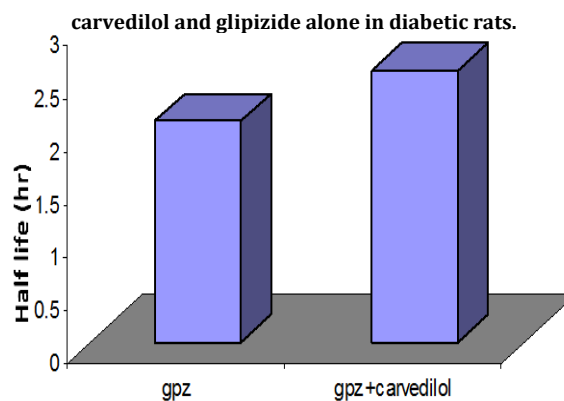


Fig. 8: Half life of glipizide in combination with carvedilol and glipizide alone in diabetic rats.

Further, rat everted intestinal sacs were used to study the effect of carvedilol on the intestinal transport of glipizide. The transport of glipizide from serosal side to mucosal side was decreased from 53.12 ± 1.27 to 5.15 ± 0.45 $\mu\text{g}/\text{mL}$ in the presence of carvedilol and 53.12 ± 1.27 to 6.68 ± 0.31 $\mu\text{g}/\text{mL}$ in the presence of (fig. 9) Verapamil (Standard P-gp inhibitor). The efflux function of intestinal P-gp is further supported by the observations that a significant amount of paclitaxel was excreted directly from the blood circulation into the intestinal lumen after intravenous dosing in mice. The P-gp-

mediated intestinal excretion in mice was also reported for digoxin. The bioavailability of docetaxel in cancer patients increased from 8% to 88% in combination with cyclosporine A. Because cyclosporine A is a potent P-gp inhibitor, these results suggest the involvement of P-gp as an intestinal barrier in limiting the absorption of paclitaxel and docetaxel. However, cyclosporine A is also known as an inhibitor of CYP3A4 and other CYP enzymes. These results suggested that carvedilol is a P-gp inhibitor, So eventually it decreases the glipizide transport.

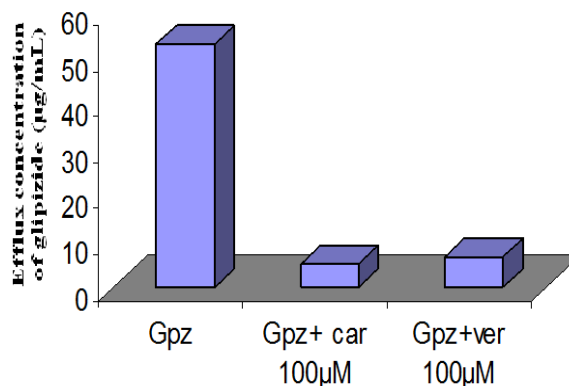


Fig. 9: The efflux concentration of glipizide in combination with carvedilol and verapamil and glipizide alone.

The above results suggested that, carvedilol produces synergistic effect with glipizide, because it is having hypoglycemic effect in both healthy rats and diabetic rats. Carvedilol is a P-gp inhibitor, it interacts with glipizide at absorption level and increases the plasma concentration. Further studies are needed for the conformation of glipizide as a P-gp substrate.

CONCLUSION

The present study results suggest that, the combination of carvedilol and glipizide enhanced the bioavailability there by increasing the hypoglycemic activity of glipizide. Carvedilol also produce little hypoglycemic effect, so the combination of these drugs produces synergistic effect. This combination used in the treatment of diabetic cardiomyopathy. The simultaneous administration of carvedilol significantly increases the intestinal absorption and decreases the efflux of glipizide. The observed effect may be beneficial to develop oral glipizide dosage forms using safe p-gp inhibitor (carvedilol) to improve its oral bioavailability. Clinically concomitant carvedilol and glipizide treatment would allow dose reduction, more importantly the risk of intestinal toxicity could be substantially reduced because of reduced dose and lowered accumulation. Continuous blood glucose monitoring is required through out treatment because of

severe hypoglycemia. Well designed controlled studies in human beings are needed to confirm the interaction.

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