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Influence of fenofibrate on the pharmacodynamics and pharmacokinetics of gliclazide in rats and rabbits

Chandrashekhar M Sultanpur[1], S.Satyanarayana[2], N Sreenivasa Reddy[1], Vasanth Kumar[1]

[1]Government college of Pharmacy, Bangalore, Karnataka, India

[2]University College of Pharmaceutical Sciences,Andhra University, Visakhapatnam-530003, India.

ABSTRACT

Atherosclerosis and dyslipidemia are associated with chronic diabetes, since diabetes disorder is supplementation with antihyperlipidemic drugs (fabric acid derivatives) may improve the condition. The purpose of this study is to know the effect of oral administration of fenofibrate on blood glucose & its influence on gliclazide induced hypoglycaemia in normal and alloxan induced diabetic rats. Studies were conducted in normal rats, alloxan induced rats and normal rabbits with oral doses of gliclazide (1/2 TD), fenofibrate (TD) and their combinations with adequate washout periods in between treatments. Blood samples were collected in rats from retro orbital puncture at 0, 1, 2, 3, 4, 6, 8, 10 and 12 h and by marginal ear vein puncture in rabbits at 0, 1, 2, 3, 4, 6, 8, 12, 18 and 24 h. All the blood samples analysed for glucose by GOD/POD method. The blood samples of rabbits were analysed by HPLC for gliclazide. Gliclazide (½ TD) produced hypoglycaemic activity in normal, diabetic rats and normal rabbits. Fenofibrate (TD) alone had no hypoglycaemia in normal rats / diabetic rats / normal rabbits. It increased the hypoglycaemic effect of gliclazide when administered together. The serum gliclazide levels and pharmacokinetic parameters of gliclazide were altered significantly.

Key words: Diabetes, gliclazide, fenofibrate, pharmacodynamics, pharmacokinetics.

Corresponding author
Email: shekarsultan@yahoo.co.in

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INTRODUCTION

Diabetes mellitus is a metabolic disorder resulting from deficiency of insulin leading to complications involving many organs. According to WHO, in near future, the maximum increase in diabetes would occur in India. Studies in various urban areas of India revealed several fold increase in the prevalence of type 2 diabetes in the last two decades [1]. Diabetes requires lifelong treatment with drugs, diet control and exercise. Insulin is the drug of choice in type 1 diabetes and sulfonylureas are the drugs of choice in type 2 diabetes [2]. Type 2 diabetes is more common than type 1. Sulfonylureas are most widely used drugs in type 2. Gliclazide is taken as a prototype drug of sulfonylureas in our study. There are several reports that chronic diabetes associated with atherosclerosis and dyslipidemia. In such a situation there is every possibility for the concomitant use of drugs used for the treatment of the above disorders with gliclazide, which may lead to drug interaction problems. So in the present study the interaction of selected antihyperlipidemic drugs fibrates like fenofibrate with gliclazide was taken up to establish the safety of the drug combination in diabetes in animal model. Once the mechanisms of interactions are established it is possible to improve the effectiveness and safety of drug therapy for the patients by choosing appropriate time schedules, dosage adjustments or alternative combinations. Since concomitant administration of gliclazide with fenofibrate in diabetes associated with atherosclerosis, there is every possibility for drug-drug interaction with enhanced / decreased gliclazide activity, which is unwanted.

Fenofibrate is mild to moderate inhibitor of CYP 450 CYP 2C9 enzymes in the liver [3] and gliclazide, which is also metabolized by both CYP 2C9 and CYP 3A4 [4]. Hence there is possibility of fenofibrate for decreasing the metabolism of gliclazide.

The safety of the above drug combinations with respect to blood glucose is not known and needs to be established by preclinical and clinical studies. This study is planned to establish the safety of the drug combinations in two dissimilar species of animal models namely rats and rabbits with respect to blood glucose level and find out the mechanisms responsible for the interaction if any.

MATERIALS AND METHODS

Chemicals and drugs

Gliclazide and fenofibrate gift samples were obtained from Micro Labs & Biocon Ltd, Bangalore respectively. Alloxan monohydrate was purchased from Sigma Chemical Co. Glucose test kit (Enzymatic, GOD-POD Method) was purchased from span diagnostic Ltd. India.

Biochemical analysis

All the blood samples for glucose levels were analysed by GOD/POD method using autoanalyser. The drug concentration in blood samples of rabbits were analysed by HPLC method.

Animals

Albino rats of either sex, weighing between 160-280 g procured from Drugs Testing Lab, Bangalore were used in the study. Albino rabbits of either sex, weighing between 1.35-1.72 kg were procured from Drugs Testing Lab, Bangalore were used in the study.

Experimental procedure

Animals were maintained under standard laboratory conditions at ambient temp of 25± 2º C with 12 h light/12 h dark cycle. They were fed with standard pellet diet (Venkateshwar enterprises Pvt. Ltd, Bangalore) and water *ad libitum*. Animals were fasted for 18 h before experiment and during the experiment they were withdrawn from food and water.

Pharmacodynamic study in normal / diabetic rats

A group of six rats were administered with 0.72 mg/ 200 g of bd wt of gliclazide (½ TD), orally. The same group was administered with fenofibrate (TD) 3.6 mg / 200 g bd wt orally and combination of fenofibrate and gliclazide. One week washout period was maintained between treatments. The same treatment was repeated in a group of six alloxan induced diabetic rats. Blood samples were withdrawn by retro orbital puncture (5) at 0, 1, 2, 3, 4, 6, 8, 10 and 12 h and were analysed for blood glucose by GOD/POD method (6) using commercial glucose kits (Span diagnostics).

Induction of diabetes

Diabetes was induced in albino rats by the administration of aqueous alloxan monohydrate at dose of 100 mg / kg bd wt intraperitoneally. Blood glucose was measured after 18hr of alloxanisation. Another 50mg/kg was given through IP route, after 72 hr to some rats in which there was no induction of diabetes was seen. Rats showing fasting blood glucose levels above 250mg/dl were selected for the study [11].

Pharmacokinetic and pharmacodynamic study in rabbits

A group of five rabbits were administered with 2.8 mg/1.5 kg bd wt of gliclazide (½ TD), orally. The same group was administered with 13.5 mg/ 1.5kg bd wt of fenofibrate (TD), orally after a washout period of one week. After a further washout period the same group was administered with the combination of gemfibrozil (TD) and gliclazide (½ TD). After interaction study the same group was continued with the daily treatment of interacting drug (gemfibrozil) for the next eight days with regular feeding. Later after 18 h fast they were again given the combined treatment on the ninth day. Blood samples were withdrawn from the marginal ear vein of each rabbit at 0, 1, 2, 3, 4, 6, 8, 12, 18 and 24 h. They were analysed for glucose by GOD/POD [6] and for gliclazide by HPLC.

The animal experiments conducted by approved by our Institutional Animal Ethics committee and by the Government regulatory body for animal research. (Regd. No. GCP/CPCSEA/04/2005-06).

Data and statistical analysis

Data were express as mean ± standard error of mean (SEM). The significance was determined by applying Student's paired't' test.

RESULTS

Gliclazide produced biphasic hypoglycaemic activity with maximum reduction of 35.89± 1.55 and 38.26± 2.45 at 2 h and 8 h respectively in normal rats and hypoglycaemic activity with maximum reduction of 37.44± 1.51 and 35.03± 0.57 at 2 h and 8 h in diabetic rats respectively. It produced peak activity of 30.85± 1.41 reductions at 3 h in normal rabbits. Fenofibrate alone produced 3.67± 3.61 & 7.35± 5.00 and 2.78± 1.00 & 5.28± 1.00 decrease in the blood glucose in normal and diabetic rats at 2 h & 8 h respectively and 0.96± 1.27 in normal rabbits at 3 h. When gliclazide given in combination with fenofibrate produced increased hypoglycaemic effect with maximum reduction of 42.41± 2.93 & 40.43± 3.06 and 41.75± 0.89 & 41.88± 1.11 in the blood glucose in normal and diabetic rats at 2 h & 8 h and 37.19± 2.83 in normal rabbits at 3 h respectively. The serum gliclazide levels and pharmacokinetic parameters of gliclazide were altered significantly with single and multiple dose treatments of fenofibrate in rabbits. The serum insulin levels were increased with fenofibrate treatment in rabbits.

DISCUSSION

Drug interactions are usually seen in clinical practice and the mechanisms of interactions are evaluated usually in animal models. We studied the influence of gemfibrozil on the pharmacodynamics and pharmacokinetics of gliclazide in normal & diabetic rats and in rabbits. The normal rat model served to quickly identify the interaction and diabetic rat model served to validate the same response in the actually used condition of the drug. The rabbit model is another dissimilar species to validate the occurrence of the interaction.

Gliclazide produced biphasic response in rat model when administered alone, which may due to its biliary excretion and enterohepatic cycling [8]. Such effect is not seen in rabbit model. Gliclazide is known to produce hypoglycaemic activity by pancreatic [9-10] (stimulating insulin secretion by blocking K^+ channels in the pancreatic β cells) and extra pancreatic [11-12] (increasing tissue uptake of glucose) mechanisms.

Fenofibrate did not produce antihyperglycaemic action when administered alone in normal and diabetic rats. It enhanced hypoglycaemic action produced by gliclazide when administered in combination. Since fenofibrate is known to be metabolized partly by CYP 450 2C9 by which gliclazide is also metabolized, the interaction might be at the level of their

metabolism. Fenofibrate might compete with gliclazide metabolism and delay the metabolism of gliclazide leading to its enhanced effect.

Fenofibrate is another antihyperlipidemic agent. It has no effect on blood glucose levels in rabbits when administered alone and produced enhanced hypoglycaemic effect of gliclazide when administered together. Fenofibrate altered significantly the serum gliclazide levels and pharmacokinetic parameters of gliclazide with single and multiple dose treatments of fenofibrate. The absorption half life (T ½ Ka), elimination half life (T ½ Kel) and clearance were not altered in the presence of fenofibrate indicating that the enhanced serum levels might be due to either inhibition of metabolism or alteration in the distribution of gliclazide in the presence of fenofibrate. In the presence of fenofibrate, the peak serum gliclazide levels were observed at 3 h. The study indicates that, interaction observed was pharmacokinetic interaction. Drug profile of fenofibrate show that it is a highly protein bound drug and about 99% or greater was bound to plasma proteins, further gliclazide was reported to interact highly protein bound drugs. Since both gliclazide and fenofibrate are highly protein bound drugs fenofibrate might displace another from proteins binding sites leading to its enhanced response [13-16].

Fenofibrate is mild to moderate inhibitor of CYP 450 2C9 by which gliclazide also is metabolized, the interaction might be at the level of their metabolism also in addition to distribution.

Table 1. Mean percent blood glucose change after oral administration of gliclazide, fenofibrate and their combination in normal rats (n=6).

Time (h)	Gliclazide (½ TD)	Fenofibrate (TD)	Gliclazide (½ TD)+ fenofibrate (TD)
0	-	-	-
1	-28.85 ± 2.33	-1.895 ± 4.64	-27.94 ± 2.32
2	-35.89 ± 1.55	-3.678 ± 3.61	-42.41 ± 2.93
3	-21.42 ± 2.58	-4.347 ± 5.12	-29.70 ± 6.40
4	-15.40 ± 2.70	-5.351 ± 5.64	-21.23 ± 6.83
6	-30.24 ± 2.89	-6.8 ± 4.56	-26.07 ± 4.09
8	-38.26 ± 2.45	-7.357 ± 5.00	-40.43 ± 3.06
10	-28.72 ± 2.84	-8.695 ± 2.12	-24.42 ± 2.50
12	-19.77 ± 2.74	-9.698 ± 3.50	-16.06 ± 2.03

**Significant at P<0.01, *Significant at P<0.05 compared to gliclazide control

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Table 2. Mean percent blood glucose change after oral administration of gliclazide, fenofibrate and their combination in diabetic rats (n=6).

Time (h)	Gliclazide (½ TD)	Fenofibrate (TD)	Gliclazide (½ TD) + fenofibrate (TD)
0	-	-	-
1	-34.32 ± 1.10	-1.539 ± 0.89	-30.25 ± 1.11
2	-37.44 ± 1.51	-2.787 ± 1.00	-41.75 ± 0.89 *
3	-30.29 ± 0.80	-3.640 ± 0.95	-37.79 ± 0.91 ***
4	-26.67 ± 0.97	-4.784 ± 0.70	-34.24 ± 1.23 **
6	-30.56 ± 1.31	-4.909 ± 0.68	-37.02 ± 0.74 *
8	-35.03 ± 0.57	-5.283 ± 1.00	-41.88 ± 1.11 **
10	-29.07 ± 1.99	-5.990 ± 0.80	-33.32 ± 1.39 *
12	-22.14 ± 0.23	-7.529 ± 0.57	-29.74 ± 0.91 ***

***Significant at P<0.001;**Significant at P<0.01;*Significant at P<0.05 compared to gliclazide control

Table 3. Mean percent blood glucose change after oral administration of gliclazide, fenofibrate and their combination in normal rabbits (n=5).

Time (h)	Gliclazide (½ TD)	Fenofibrate (TD)	Gliclazide (½ TD) + fenofibrate (TD) (Acute)	Gliclazide + fenofibrate (Chronic)
0	-	-	-	-
1	-24.99±2.60	-1.661±1.40	-21.66± 3.42	-19.60±3.37 **
2	-27.17±1.92	-3.190±1.62	-28.20±3.67 *	-29.97±3.41 *
3	-30.85±1.41	-0.961±1.27	-37.19±2.83 *	-38.61±2.54 *
4	-28.07±2.15	-4.973±1.98	-29.88±2.28 *	-32.67±2.15 **
6	-26.20±2.15	-6.948±2.28	-28.27±3.34 *	-28.05±2.77
8	-24.75±1.28	-6.693±2.75	-22.63±4.14	-24.42±2.19
12	-15.56±3.26	-5.483±2.16	-16.58±3.54	18.15±2.18 ***
18	-08.43±1.98	-8.922±1.89	-9.681±2.69 *	-12.87±3.23 **
24	-5.105±2.17	-8.285±2.28	-5.294±3.37	-6.269±2.14

***Significant at P<0.001;**Significant at P<0.01, *Significant at P<0.05 compared to gliclazide control

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Table 4. Mean serum gliclazide concentration (ng/ml) before and after oral administration with fenofibrate in normal rabbits (n=5)

Time (h)	Gliclazide (½ TD)	Gliclazide (½ TD) + fenofibrate (TD) (Acute)	Gliclazide + fenofibrate (Chronic)
0	0	0	0
1	78.56±2.16	101.7±03.18 ***	108.5±05.20 **
2	213.92±5.00	236.2±07.40 *	232.1±11.87
3	338.75±6.99	376.7±11.99 *	379.7±10.62 *
4	280.80±5.90	304.5±08.70 *	305.5±08.23 *
6	247.34±5.12	266.5±11.23 *	270.9±12.80
8	184.65±4.66	216.9±09.83 *	225.7±07.41 **
12	108.27±5.64	120.4±08.50 *	123.2±05.79 **
18	80.19±2.86	98.84±04.69	99.24±06.39 *
24	68.47±2.97	84.05±04.83 *	86.36±04.68 *

***Significant at P<0.001;**Significant at P<0.01;*Significant at P<0.05 compared to gliclazide control

Table V. Mean pharmacokinetic parameters of gliclazide before and after oral administration with fenofibrate in normal rabbits (n=5)

Kinetic Parameters	Gliclazide **(½ TD)**	**Gliclazide (½TD) + fenofibrate (TD) (Acute)**	Gliclazide + fenofibrate (Chronic)
AUC $_{0-24}$ (ng/ml/h)	3322±52.18	3790±52.69 ***	3885±16.35 ***
AUMC$_{0-24}$ (n(ng/ml/h*h)	40196±818.30	49205±2274 *	49134±1732 **
Kel (h^{-1})	0.07±0.02	0.06±0.03	0.06±0.02 *
AUC $_{0-\infty}$ (ng/ml/h)	4249±64.89	5063±136.50 **	5104±80.14 **
AUMC$_{0--\infty}$ (n(ng/ml/h*h)	75072±3451	99292±8871	96093±5554 **
T1/2(h)	9.36±0.25	10.37±0.57	9.97±0.30 *
Ka (h^{-1})	1.15±0.00	1.15±0.00	1.15±0.00
Clearance (ml/h)	754±35.85	3072±170.80 ***	2971±147.40 ***
Clearance (ml/h/kg)	438.30±6.68	1783±47.75 ***	1765±27.40 ***
Vdss(ml)	12586±349.60	56575±2557 ***	52775±1090 ***
Vdss(ml/kg)	7351±266.50	32999±1546 ***	31520±891.60 ***
Vdarea(ml)	10137±296.40	45559±1725 ***	42551±1243 ***
Vdarea(ml/kg)	5911±151.20	26566±976.20 ***	25366±427.30 ***
MRT(h)	17.66±0.64	19.49±1.26	18.77±0.78 *
C_{max} (ng/ml)	338.75±6.99	376.70±11.99 *	379.70±10.62 *
T_{max} (h)	3.00±0.00	3.00±0.00	3.00±0.00

***Significant at P<0.0001;**Significant at P<0.001;*Significant at P<0.05 compared to gliclazide control.

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Fig I. The percent blood glucose change with gliclazide alone, fenofibrate alone and in combination in normal rats. (n=6)

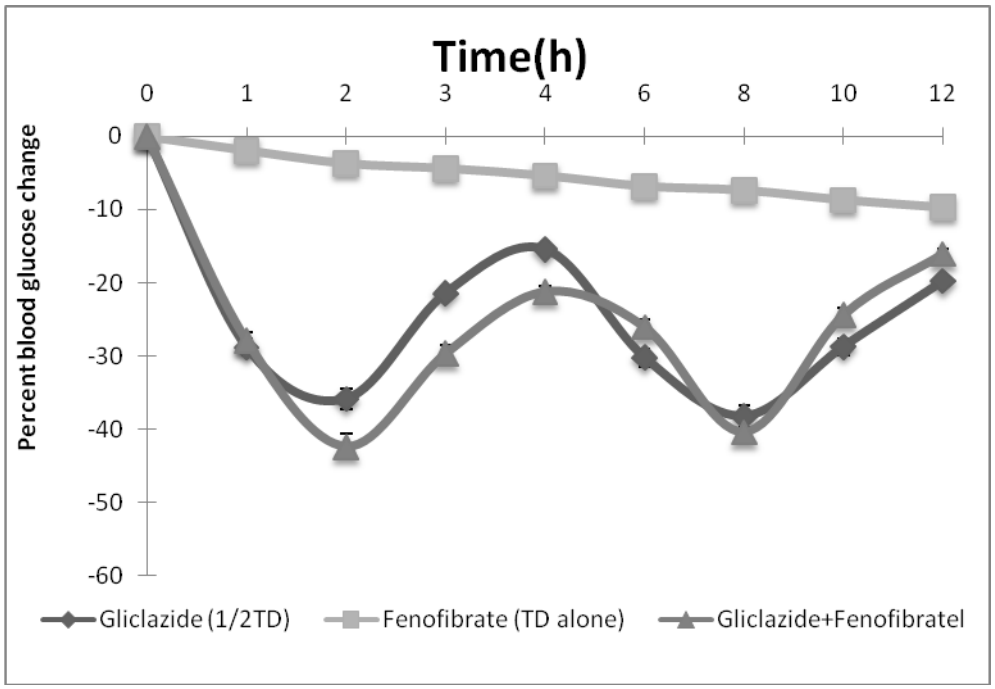

Fig II. Mean percent blood glucose change with gliclazide alone, fenofibrate alone and in combination in diabetic rats. (n=6)

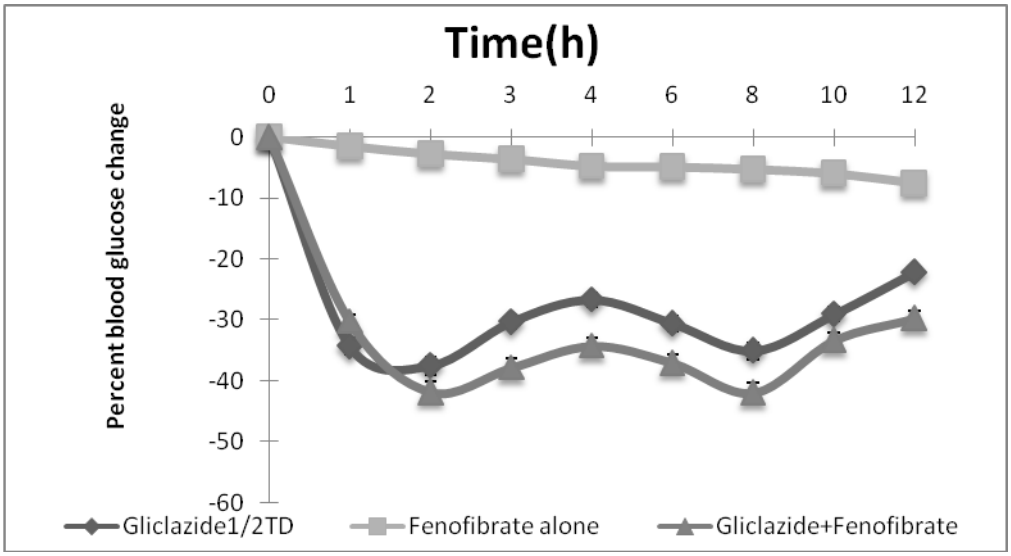

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Fig III. Effect of acute and chronic administration of fenofibrate on the hypoglycaemic activity of gliclazide in normal rabbits (n=5)

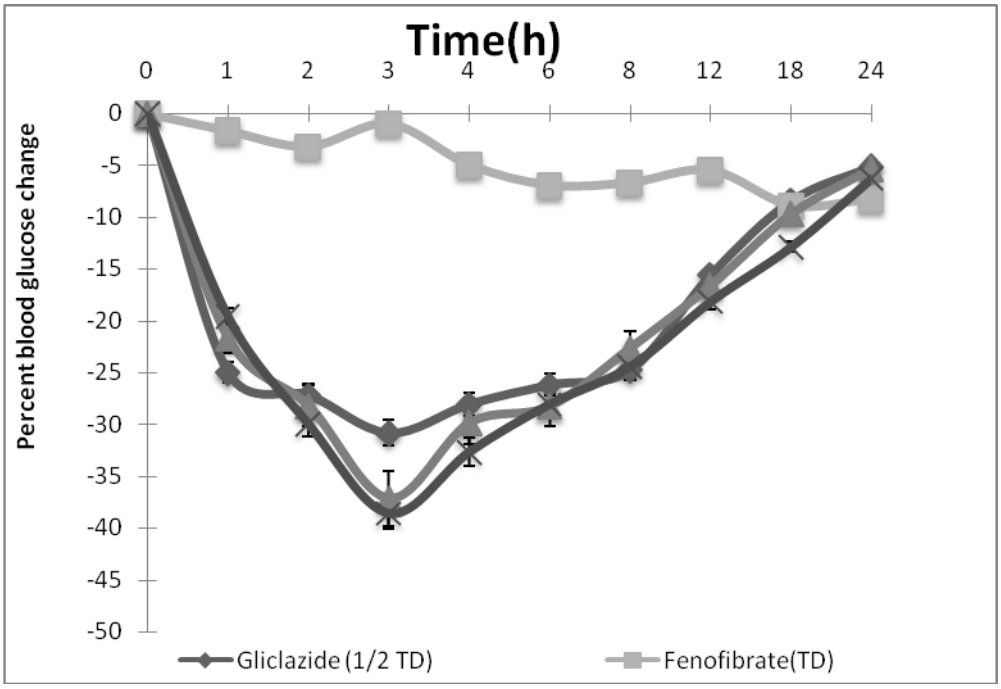
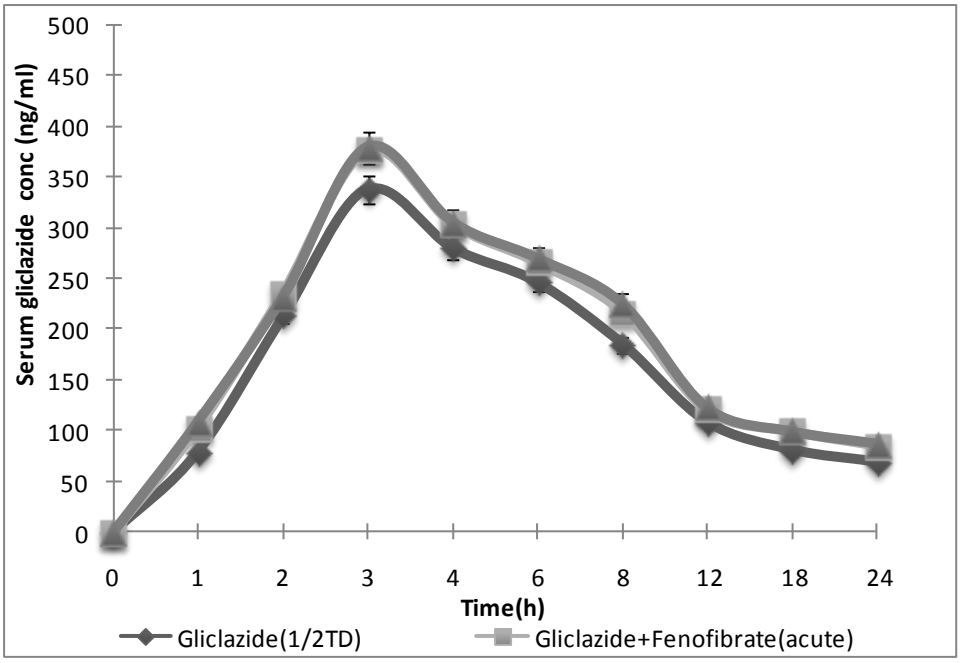


Fig IV. Serum gliclazide concentration vs time in normal rabbits treated with fenofibrate (n=5)



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CONCLUSION

The interaction was observed in two dissimilar species, it is likely to occur in humans also. Hence, the combination gliclazide (½ TD) + fenofibrate (TD) should be contraindicated / used with caution in a clinical situation.

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