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Original Article

Pharmacodynamic and Pharmacokinetic Interactions of Piperine on Gliclazide in Animal Models

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ABSTRACT

Back Ground: The objective of the present study was to find out the pharmacodynamic and pharmacokinetic interactions of piperine on gliclazide in rats and rabbits. **Methods:** Influence of piperine on the activity of gliclazide was determined by conducting single- and multiple-dose interaction studies in rats (normal and diabetic) and diabetic rabbits. Blood samples collected at predetermined time intervals from experimental animals were used for the estimation of glucose and insulin levels by using automated clinical chemistry analyzer and radioimmunoassay method, respectively. β-cell function was determined by homeostasis model assessment. Additionally, serum gliclazide levels in rabbits were analyzed by high-performance liquid chromatography. **Results:** Gliclazide showed significant reduction in blood glucose levels in diabetic rats and rabbits. Similarly, piperine also showed significant reduction in blood glucose levels in animals. Additionally, , samples analyzed from all time points in combination with piperine showed peak reduction in blood glucose in diabetic rats and rabbits. The pharmacokinetics of gliclazide was also altered by single- or multiple-dose piperine treatments in rabbits. **Conclusion:** The interaction of piperine with gliclazide upon single and multiple-dose treatment was pharmacodynamic and pharmacokinetic in nature, indicating the need for periodic monitoring of glucose levels and dose adjustment as necessary when this combination is prescribed to diabetic patients.

Key words: Diabetes, Drug interaction, Gliclazide, Piperine.

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INTRODUCTION

Diabetes mellitus is the most severe metabolic disorder characterized by absolute or relative insufficiency in insulin secretion and/or its action.[1] Gliclazide (second generation sulfonylurea derivative) is the preferred choice of drug.[2] Piperine is an alkaloidal compound and is an active constituent of black and long peppers. It has been found to have anti-diabetic activity per se. Piperine can improve the bioavailability of many drugs and decrease the elimination of the drugs and finally improves the biological effectiveness. Piperine is known to inhibit human CYP2C9, CYP3A4 and P-glycoprotein.[3,4] But the influence of piperine on diabetic patients who are under the treatment with Gliclazide is not proved yet. Hence, the present study was designed to find out the pharmacodynamic and pharmacokinetic interactions of piperine on gliclazide in rats and rabbits.

MATERIALS AND METHODS

Drugs and chemicals

Gliclazide was obtained as a gift sample from Dr Reddy's Laboratories (Bachupally, Hyderabad, Telangana, India). Piperine was purchased from HiMedia Laboratories private limited, Mumbai. Alloxan monohydrate was purchased from Loba Chemie (Mumbai, Maharashtra, India). All reagents and chemicals used in the study were of analytical grade.

Gliclazide solution Gliclazide solution was prepared by dissolving in few drops of 0.1 N sodium hydroxide and the final volume was made with water.[5]

Preparation of Piperine solution

Piperine solution was prepared in 2% Gum acacia solution.

Preparation of alloxan solution

Alloxan monohydrate 110 mg/Kg was dissolved in sterile saline and injected by subcutaneous route immediately within five min to avoid degradation.[6]

Animals

Eight to 9-week-old male albino rats weighing between 170 and 250 g and 3-month-old male albino rabbits weighing between 1 and 1.5 kg were procured from M/s Mahavir Enterprises, Hyderabad. They were maintained under controlled room temperature (24±2^{0}C; relative humidity 60-70%) in a 12h light – dark cycle. The animals were given a standard laboratory diet and water *ad libitum*. The animals were acclimatized before the study.

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Experimental study design

Male albino rats/rabbits were divided into five groups each consisting of six animals. From the results of gliclazide dose–effect relationship study conducted in normal rats and rabbits, the doses of 2 and 4 mg/kg body weight were selected, respectively, for administration in animals. The design of the study is as follows:

Group I: Normal control

Group II: Diabetic control

Group III: Gliclazide (2 mg/kg for rats/4 mg/kg for rabbits) body weight, p.o.

Group IV: Piperine (20 mg/kg) body weight, p.o.

Group V: Piperine (20 mg/kg) + Gliclazide (2 mg/kg for rats/4 mg/kg for rabbits) body weight, p.o.

Stage 1: Pharmacodynamic interaction in normal rats.

Stage 2: Pharmacodynamic interaction in diabetic rats.

Stage 3: Pharmacodynamic and pharmacokinetic interaction in diabetic rabbits.

Pharmacodynamic interaction in normal rats

Group I animals considered as normal rats and treated with vehicle only. Group III rats were given gliclazide via the oral route at 2 mg/kg body weight, and their blood samples were collected at predetermined time points. Similar procedure was performed with either orally administered Piperine 20 mg/kg, p.o. (Group IV) only or combination treatment with both Piperine and gliclazide (Group V) at the previously mentioned doses. After these single-dose interaction studies, the same group of animals were considered as multiple dose interaction study. Blood samples were collected at predetermined time intervals after each treatment with gliclazide alone, Piperine alone, or combination treatments (single and multiple).[7]

Pharmacodynamic interaction in diabetic rats

Male albino rats weighing (170-250 g) were fasted for overnight before challenging with single subcutaneous route (s.c) of alloxan monohydrate, freshly prepared and injected within 5 min of preparation to prevent degradation at a dose of 110 mg/kg. After administration of alloxan monohydrate 5% glucose solution was given for 72 h to prevent hypoglycemic shock. Animals had access to feed and water. The development of hyperglycemia in rats was confirmed by fasting serum glucose estimation 72 h post alloxan monohydrate injection where in the animals were fasted again for 14 h before blood collection from retro orbital plexus. The rats with fasting serum glucose level of above 200 mg/dl at 72 h were considered as diabetic and are included in the study. Similar procedure followed for dosing and blood sample collection as per discussed in pharmacodynamic study in normal rats experiment. Group II considered as diabetic control. Blood glucose levels were estimated on initial, 1st, 3rd, 7th, 14th, and 21st day of the treatment.[8,9]

Pharmacodynamic and pharmacokinetic interaction in diabetic rabbits

Six rabbits were selected for each group. Diabetes induced by using alloxan monohydrate treatment Group III rabbits were given gliclazide via the oral route at 4 mg/kg body weight, and their blood was collected at predetermined time points. Similar procedure was performed with either orally administered Piperine only (Group IV) or combination treatment with both Piperine and gliclazide (Group V) at the previously mentioned doses. After this single-dose interaction study, the same animals were considered for multiple dose interaction study. Blood samples were collected at predetermined time intervals after each treatment of gliclazide, Piperine, or combination treatments (single and multiple).[10]

Collection of serum samples

The blood was drawn from the retro orbital plexus of the rats (fasted for 14 h) under light ether anaesthesia on different occasions i.e., day 0, day 1, day 3, day 7, day 14 and day 21. On day 0 (SDT) and day 21st (MDT) blood samples collected at different time intervals as 0hr, 1hr, 2hr, 4hr, 8hr, 10hr and 12hr for pharmacokinetic study experiment. Blood samples were withdrawn from the marginal ear vein of each rabbit. Blood samples collected at predetermined time intervals from experimental animals were used for the estimation of glucose and insulin levels by using automated clinical chemistry analyzer and radioimmunoassay method, respectively. β-cell function was determined by homeostasis model assessment. Additionally, serum gliclazide levels in rabbits were analyzed by high-performance liquid chromatography.

Determination of β-cell function

β-cell function was assessed by the Homeostatic Model Assessment protocol and was calculated as follows.[5,11,12]

β-cell function = (20× FSI)/ (FSG – 3.5) ×100

Where fasting serum insulin (FSI) is expressed in μIU/mL and

Fasting serum glucose (FSG) in mg/dL.

Pharmacokinetic analysis

Pharmacokinetic parameters of gliclazide in rabbit serum such as peak serum concentration, peak time, area under the concentration time curve, area under first moment curve, terminal half-life, elimination rate constant, mean resident time, and clearance were estimated by using Kinetica 5.0 software.

Data and statistical analysis

The data was analyzed using one-way analysis of variance (ANOVA), followed by Dunnett's test and $p<0.05$ was considered as statistically significant. The data was expressed as mean ± Standard deviation (SD).

RESULTS

Pharmacodynamic interaction between Piperine and Gliclazide

Gliclazide produced significant hypoglycemic activity in normal rats with maximum percent blood glucose reduction of 45.5% (Table 1) and antihyperglycemic activity in diabetic rats and rabbits with peak percent blood glucose reduction of 56.4% and 47.5%, respectively (Table 2 and 5). Piperine also produced significant hypoglycemic activity in normal rats with maximum percent blood glucose reduction of 35.5% (Table 1) and antihyperglycemic activity in diabetic rats and rabbits with peak percent blood glucose reduction of 48.6% and 37.6%, respectively (Table 2 and 5). The combination of Gliclazide with Piperine produced significant hypoglycemic activity in normal rats with maximum percent blood glucose reduction of 49.6% (Table 1) and antihyperglycemic activity in diabetic rats and rabbits with peak percent blood glucose reduction of 68.8% and 62.0%, respectively (Table 2 and 5). Single and multiple dose combination of Piperine with gliclazide induced significant changes in percent blood glucose reduction, insulin levels and β-cell function in animal models. However, multiple-dose combination of Piperine with gliclazide produced significantly greater reduction in percent blood glucose reduction after treatment in diabetic rats and rabbits when compared with diabetic control. Piperine exhibited additive effect by increasing the activity of gliclazide. Significant changes were observed in insulin levels and β-cell function (Tables 3,4,6 and 7) in both the animal models.

Pharmacokinetic interaction between piperine and gliclazide

The pharmacokinetic parameters of gliclazide alone and in the presence of piperine following single- and multiple-dose administrations were

Table 1: Mean percent blood glucose reduction of gliclazide in presence and absence of Piperine in single and multi-dose study for normal rats (n=6).

Treatment	Mean percent blood glucose reduction					
	0 day	1st day	3rd day	7th day	14th day	21st day
Gliclazide (2 mg/kg)	40.7**	41.8**	42.1**	43.3**	44.2**	45.5**
Piperine (20 mg/kg)	30.1**	31.1**	32.2**	33.3**	34.2**	35.5**
Piperine (20 mg/kg) + Gliclazide (2 mg/kg)	42.3**	43.4**	44.6**	45.8**	47.5**	49.6**

Notes: Data expressed as mean ± standard deviation. ** (*p<0.01)* statistically significant when compared with normal control.

Table 2: Mean percent blood glucose reduction of gliclazide in presence and absence of Piperine in single and multi-dose study for diabetic rats (n=6).

Group	Treatment	Mean percent blood glucose reduction					
		0 day	1st day	3rd day	7th day	14th day	21st day
III	Gliclazide (2 mg/kg)	43.8**	45.7**	47.4**	50.6**	53.8**	56.4**
IV	Piperine (20 mg/kg)	31.3**	33.2**	36.1**	39.1**	44.6**	48.6**
V	Piperine (20 mg/kg) + Gliclazide (2 mg/kg)	53.1**	55.0**	57.9**	61.9**	64.6**	68.8**

Notes: Data expressed as mean ± standard deviation. ** (*p<0.01)* statistically significant when compared with diabetic control.

Table 3: Effect of Piperine on insulin levels in diabetic rats (n=6).

Group	Treatment	Insulin (μIU/mL)					
		0 day	1st day	3rd day	7th day	14th day	21st day
III	Gliclazide (2 mg/kg)	15.43±0.15	16.93±0.24	17.62±0.28	19.53±0.21	22.86±0.01	25.11±0.21
IV	Piperine (20 mg/kg)	11.15±0.12	12.01±0.10	13.25±0.02	15.56±0.25	17.99±0.14	20.25±0.15
V	Piperine (20 mg/kg)+ Gliclazide (2 mg/kg)	16.19±0.22	17.62±0.11	19.56±0.18	21.81±0.32	24.39±0.12	27.98±0.36

Notes: Data expressed as mean ± standard deviation.

Table 4: Effect of Piperine on β-cell function in diabetic rats (n=6).

Treatment	β-cell function					
	0 day	1st day	3rd day	7th day	14th day	21st day
Gliclazide (2 mg/kg)	174.84±1.00	197.43±0.88	212.93±1.22	252.82±0.30	312.08±0.48	367.91±0.71
Piperine (20 mg/kg)	103.00±0.29	113.57±0.71	131.51±0.66	162.51±0.53	203.85±0.79	250.77±1.51
Piperine (20 mg/kg)+ Gliclazide (2 mg/kg)	221.02±0.55	249.05±0.69	297.49±0.50	368.10±0.52	437.49±0.74	579.90±0.73

Notes: Data expressed as mean ± standard deviation. Calculated by homeostasis model assessment

Table 5: Mean percent blood glucose reduction of gliclazide in presence and absence of Piperine in single and multi-dose study for diabetic rabbits (n=6).

Group	Treatment	Mean percent blood glucose reduction					
		0 day	1st day	3rd day	7th day	14th day	21st day
III	Gliclazide (4 mg/kg)	37.9**	39.0**	41.9**	44.8**	47.1**	47.5**
IV	Piperine (20 mg/kg)	30.7**	31.9**	32.6**	33.8**	35.5**	37.6**
V	Piperine (20 mg/kg) + Gliclazide (4 mg/kg)	42.4**	46.1**	49.5**	52.1**	57.0**	62.0**

Notes: Data expressed as mean ± standard deviation. ** (*p<0.01)* statistically significant when compared with diabetic control.

Table 6: Effect of Piperine on insulin levels in diabetic rabbits (n=6).

Group	Treatment	Insulin (µIU/mL)					
		0 day	1st day	3rd day	7th day	14th day	21st day
III	Gliclazide (4 mg/kg)	20.13±0.21	20.89±0.31	22.23±0.25	23.91±0.19	26.28±0.36	28.35±0.53
IV	Piperine (20 mg/kg)	15.45±0.22	16.01±0.25	17.15±0.39	18.56±0.25	20.69±0.54	22.25±0.55
V	Piperine (20 mg/kg)+ Gliclazide (4 mg/kg)	24.09±0.52	25.12±0.41	26.01±0.38	27.18±0.62	29.79±0.72	31.08±0.66

Notes: Data expressed as mean ± standard deviation.

Table 7: Effect of Piperine on β-cell function in diabetic rabbits (n=6).

Treatment	β-cell function					
	0 day	1st day	3rd day	7th day	14th day	21st day
Gliclazide (2 mg/kg)	215.87±1.00	236.71±0.68	268.64±0.22	305.56±0.43	346.93±0.48	374.26±0.51
Piperine (20 mg/kg)	156.46±0.59	162.13±0.41	178.18±0.66	196.92±0.35	223.07±0.29	246.54±0.51
Piperine (20 mg/kg)+ Gliclazide (2 mg/kg)	294.68±0.15	323.09±0.49	362.51±0.58	401.18±0.62	486.37±0.67	572.90±0.47

Notes: Data expressed as mean ± standard deviation. Calculated by homeostasis model assessment

Table 8: Mean pharmacokinetic parameters of gliclazide in presence and absence of Piperine in rabbits (n=6).

Pharmacokinetic parameter	Gliclazide (4mg/kg)	Piperine (20 mg/kg)+ Gliclazide (4 mg/kg) (SDT)	Piperine (20 mg/kg)+ Gliclazide (4 mg/kg) (MDT)
C_{max} (ng/mL)	365.19±8.42	369.57±4.32	405.19±8.42
T_{max} (h)	3±0.00	3±0.00	3±0.00
AUC (h ng/mL)	3968.68±34.29	4067.64±26.14	4893.62±39.23
AUMC (h ng/mL)	38206.86±302.35	39892.15±282.32	48937.48±302.35
$T_{1/2}$	10.39±0.54	10.41±0.22	10.59±0.54
K_{el} (l/h)	0.066±0.01	0.066±0.00	0.065±0.01
MRT (h)	9.62±0.01	9.80±0.00	10.01±0.01
CL (L/h)	0.08±0.00	0.07±0.00	0.06±0.00

Notes: Data expressed as mean ± standard deviation.

given in (Table 8). Piperine was found to alter the pharmacokinetics of gliclazide in rabbits.

DISCUSSION

Diabetes mellitus is the most severe metabolic disorder characterized by absolute or relative insufficiency in insulin secretion and/or its action.[1] Gliclazide (second generation sulfonylurea derivative) is the preferred choice of drug.[2] Gliclazide is primarily metabolized by CYP2C9 and partly by CYP3A4.[7] Piperine is an alkaloidal compound and is an active constituent of black and long peppers.[3,4] It has been found to have anti diabetic activity per se.[13] Piperine can improve the bioavailability of many drugs and decrease the elimination of the drugs and finally improves the biological effectiveness. Piperine is known to inhibit human CYP2C9, CYP3A4 and P-glycoprotein.[3,4] The present study was designed to assess the pharmacodynamic and pharmacokinetic interactions of piperine on gliclazide in animal models. The study revealed that piperine exhibited significant hypoglycemic and antihyperglycemic activity. It also enhanced the activity of Gliclazide significantly and showed additive effect. Piperine increased the insulin levels in diabetic rats and rabbits significantly and enhanced the β-cell function. The possible mechanisms of hypoglycaemic action may be by increasing either the pancreatic secretion of insulin from β-cell of islet of Langerhans or its release from pro-insulin form.[14] Piperine also altered the pharmacokinetic parameters of Gliclazide which might be due to inhibition of human CYP 2C9.

CONCLUSION

The interaction of piperine with gliclazide up on single and multiple-dose treatment was pharmacodynamic and pharmacokinetic in nature, indicating the need for periodic monitoring of glucose levels and dose adjustment as necessary when this combination is prescribed to diabetic patients.

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ABBREVIATIONS USED

CYP: Cytochrome P450; **g:** gram; **kg:** kilo gram; **◦C:** degree celsius; **% :** percentage; **h:** hours; **p.o. :** per oral; **dL:** deciliter; **µ:** micro; **IU:** International units; **mL:** milliliter; **C_{max} :** maximum concentration; **T_{max} :** time to maximum; **AUC :** area under the curve; **AUMC :** Area under the first moment curve; **t1/2 :** elimination half-life; **kel :** elimination rate constant; **MRT :** mean residence time; **Cl :** clearance; **ng :** nano gram; **mg :** microgram.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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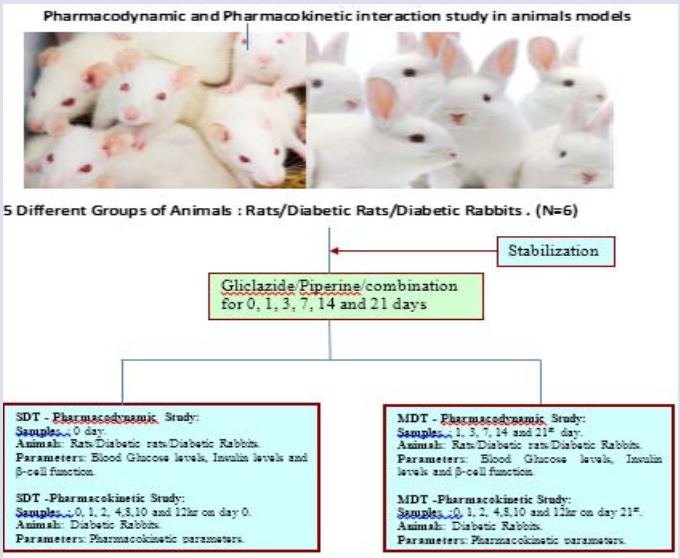
GRAPHICAL ABSTRACT



SUMMARY

- Nevertheless the adequate information on the safety of phytochemical constituents not well established, their use as alternative and/or complementary medicine is globally popular. Piperine, a richest source from pepper is a natural bioenhancer, confirmed for hypoglycemic activity in normal animals and exhibits significant anti hyperglycemic activity in diabetic models. The combination of gliclazide and piperine was confirmed to be a pharmacodynamics and pharmacokinetic drug interaction category and the antidiabetic activity was more significant in combination compared with each individual drug results.

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