Original Research Article (Experimental)

Interaction of p-synephrine on the pharmacodynamics and pharmacokinetics of gliclazide in animal models

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A B S T R A C T

Background: Type 2 diabetes is frequently seen in patients suffering from obesity. p-synephrine and gliclazide are widely used medicines for the treatment of obesity and diabetes, respectively.
Objectives: The present study was undertaken to determine the potential for interactions between p-synephrine and gliclazide, based on the relationship between obesity and diabetes.
Methods: Influence of p-synephrine on the activity of gliclazide was determined by conducting single and multiple dose interaction studies in animal models. Blood samples collected at pre-determined time intervals from experimental animals were used for the estimation of glucose and insulin levels. The insulin resistance and β-cell function were determined by homeostasis model assessment. Additionally, serum gliclazide levels in rabbits were analyzed by high-performance liquid chromatography (HPLC).
Results: Gliclazide alone showed peak reduction in blood glucose levels at 2 and 8 h after administration in rats and after 3 h in rabbits. The activity of gliclazide was not altered by a single dose treatment with p-synephrine. However, in multiple dose interaction studies, samples from all the time points analyzed showed significant changes in percent blood glucose reduction ranging from 19.73 to 44.18% in normal rats, 23.76–46.43% in diabetic rats and 16.36–38.34% in normal rabbits. The homeostasis model assessment parameters were also significantly altered in multiple dose interaction studies. The pharmacokinetics of gliclazide was not altered by either single or multiple dose p-synephrine treatments in rabbits.
Conclusion: The effect of multiple dose p-synephrine treatments upon gliclazide appeared to be pharmacodynamic in nature, indicating the need for periodic monitoring of glucose levels and dose adjustment as necessary when this combination is prescribed to obese patients.
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1. Introduction

Herbal medicines are widely used today either alone or in combination with modern pharmaceuticals. Combinations may be therapeutic or toxic at prescribed doses. The possible adverse effects from herb—drug interactions remain to be explained. Fluctuations in the pharmacological or toxicological effects of either components may be seen when herbal active constituents and drugs are administered in combination. Therefore, it is important to study the interactions between herbs and drugs [1]. The use of combinations of drugs and/or herbal active constituents for prolonged durations is required in disease conditions such as diabetes and obesity. Therefore, there is a likelihood for potential herb—drug interactions in patients with obesity and diabetics. Patients with obesity are prone to develop diabetes, and maintenance of normal blood glucose level is very essential for the prevention of undesirable complications associated with both hyperglycemia and hypoglycemia. Gliclazide, a derivative of sulfonylurea is an oral hypoglycemic agent and the preferred drug for the treatment of type 2 diabetics. It acts by selectively inhibiting pancreatic K^+ ATPase channels [2]. Further, gliclazide was reported to exhibit antioxidant properties, low incidence of severe hypoglycemia and other hemobiological effects [3]. Gliclazide is primarily metabolized by hepatic microsomal enzymes CYP2C9 and partly by CYP3A4 [2].

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p-Synephrine, a protoalkaloid present in *Citrus aurantium* (bitter orange) and other citrus species [4] where the highest levels of p-synephrine is found in the peel of unripe fruits [5]. Historically, *C. aurantium* has been used for various digestive and respiratory problems in traditional Chinese medicine. Bitter orange extract and p-synephrine are widely used in weight management and sports performance products. p-Synephrine is consumed daily in various foods and juices derived from citrus species apart from their use in dietary supplements [6]. Despite wide spread consumption, the safety of p-synephrine is frequently questioned. p-Synephrine is structurally related to ephedrine and is reported to have affinity for adrenergic receptors [7]. Activation of β-3 receptors resulted in increased lipolysis in adipose tissues, decreased food consumption, improved insulin resistance and glycemic control in rats [8,9]. It is also reported that intake of p-synephrine inhibits enzyme CYP3A4 thereby boosting the drugs levels in the blood and thus their activity. p-Synephrine significantly increased the peak serum concentration (C_{max}) of amiodarone upon repeated dose administration in rats [10]. The potential interactions of p-synephrine with other drugs have not been systematically studied. Based on the background information, the present study was designed and undertaken with the hypothesis that pre-treatment with p-synephrine will influence the pharmacodynamics and pharmacokinetics of gliclazide in animal models.

2. Material and methods

2.1. Drugs and chemicals

Gliclazide was obtained as gift sample from Dr. Reddy's Laboratories, Bachupally, Hyderabad.

p-Synephrine ready to use formula was obtained from SB Natural Products, USA. Each capsule contains 30% active p-synephrine. Alloxan monohydrate was purchased from LOBA Chemie, Mumbai, India. All reagents and chemicals used in the study were of analytical grade.

2.2. Animals and husbandry

8 to 9 week old male albino rats weighing between 170 and 250 g were procured from Vivo Biotech, Hyderabad, India and 3 month old male albino rabbits weighing between 1 and 1.5 kg were procured from Rabiroof, Hyderabad, India. They were maintained under standard laboratory husbandry conditions at 25 ± 2 °C and 50 ± 15% relative humidity with a 12 h light/dark cycle. Animals were fed with a commercially available pellet diet (Rayan's Biotechnologies Pvt Ltd, Hyderabad, India) and drinking water was provided *ad libitum*. Animals were fasted for 10 h prior to the

experiment and during the experiment they were withdrawn from food. The animal experiments were performed following approval of study protocol by the Institutional Animal Ethics Committee (DLL/IAEC/2013/02/04). The study was also conducted in accordance with the guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

2.3. Study design

100 and 50 mg/kg doses of p-synephrine were calculated from human oral therapeutic doses based on body surface area for rats and rabbits, respectively [11]. 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 [12]. Oral dose formulation for p-synephrine was prepared by suspending in 0.5% carboxymethylcellulose sodium (CMC Na). Gliclazide solution was prepared by initially dissolving in few drops of 0.1 N sodium hydroxide and the final volume was made with water [12]. The study was designed as follows:

Stage-1: Pharmacodynamic interaction study in normal rats [12].
Stage-2: Pharmacodynamic interaction study in diabetic rats [12].
Stage-3: Pharmacodynamic and pharmacokinetic interaction study in normal rabbits [12].

2.4. Stage 1: Pharmacodynamic interaction study in normal rats

Six rats were selected for stage 1 experiment. These rats were given gliclazide via the oral route at 2 mg/kg body weight, and their blood was collected at pre-determined time points. Similar procedure was performed with either orally administered p-synephrine only or combination treatment with both p-synephrine and gliclazide at the previously mentioned doses with washout period between each experiment being a week. After the single dose interaction studies, the same group of animals were given daily treatments with p-synephrine for the next 20 days with regular feeding. On day 21, animals were fasted for 10 h before administering p-synephrine. 30 min later, these animals were given gliclazide at 2 mg/kg body weight. Blood samples were collected at pre-determined time intervals after each treatment with gliclazide alone, p-synephrine alone or combination treatments (single and multiple).

2.5. Stage 2: Pharmacodynamic interaction study in diabetic rats

For stage 2 experiments, diabetes was induced in rats as previously described [13]. Briefly, diabetes was induced in rats by the

Table 1
Mean blood glucose levels and % blood glucose reduction with gliclazide in presence and absence of p-synephrine in normal rats.

Time (h)	Gliclazide		p-Synephrine		Gliclazide + p-Synephrine (SDT)		Gliclazide + p-Synephrine (MDT)	
	Mean blood glucose level (mg/dL)	% Blood glucose reduction	Mean blood glucose level (mg/dL)	% Blood glucose reduction	Mean blood glucose level (mg/dL)	% Blood glucose reduction	Mean blood glucose level (mg/dL)	% Blood glucose reduction
0	103.50 ± 2.43	–	99.83 ± 1.60	–	99.00 ± 1.41	–	96.17 ± 1.60	–
1	73.50 ± 2.43	28.99 ± 1.22	101.50 ± 2.26	−1.67 ± 1.85	69.33 ± 1.21	29.96 ± 1.29	64.83 ± 1.47	32.57 ± 1.73*
2	64.83 ± 1.83	37.35 ± 1.69	96.50 ± 1.64	3.33 ± 1.50	61.67 ± 1.63	37.72 ± 0.84	53.67 ± 1.21	44.18 ± 1.46*
3	75.33 ± 1.63	27.21 ± 1.04	95.83 ± 0.75	3.99 ± 1.20	72.17 ± 0.75	27.09 ± 1.40	65.67 ± 1.21	31.70 ± 1.85*
4	81.33 ± 1.37	21.40 ± 1.15	92.83 ± 1.17	6.99 ± 1.91	77.50 ± 0.84	21.71 ± 0.83	70.50 ± 1.38	26.69 ± 1.08*
6	76.33 ± 1.63	26.24 ± 1.27	95.17 ± 1.47	4.67 ± 1.01	73.00 ± 1.79	26.27 ± 1.04	66.33 ± 0.82	31.01 ± 1.35*
8	66.83 ± 1.47	35.41 ± 1.65	94.67 ± 1.03	5.16 ± 1.54	64.17 ± 1.94	35.19 ± 1.23	58.33 ± 1.21	39.34 ± 1.17*
10	79.33 ± 1.75	23.34 ± 0.83	94.67 ± 0.82	5.16 ± 1.40	75.33 ± 1.03	23.90 ± 0.63	70.67 ± 1.21	26.50 ± 1.92*
12	85.83 ± 1.33	17.05 ± 1.61	92.50 ± 1.38	7.31 ± 2.61	81.67 ± 1.51	17.51 ± 1.16	77.17 ± 0.75	19.73 ± 1.86*

Data expressed as Mean ± SD, $n = 6$; *Significant when compared to gliclazide control; SDT: single dose treatment; MDT: multiple dose treatment.

Table 2
Mean blood glucose levels and % blood glucose reduction with gliclazide in presence and absence of p-synephrine in diabetic rats.

Time (h)	Gliclazide		p-Synephrine		Gliclazide + p-Synephrine (SDT)		Gliclazide + p-Synephrine (MDT)	
	Mean blood glucose level (mg/dL)	% Blood glucose reduction	Mean blood glucose level (mg/dL)	% Blood glucose reduction	Mean blood glucose level (mg/dL)	% Blood glucose reduction	Mean blood glucose level (mg/dL)	% Blood glucose reduction
0	257.67 ± 4.72	–	258.33 ± 4.41	–	268.17 ± 2.93	–	273.50 ± 3.27	–
1	177.00 ± 2.45	31.29 ± 1.49	257.50 ± 3.83	0.32 ± 0.38	184.83 ± 0.98	31.07 ± 0.76	179.83 ± 1.33	34.24 ± 0.49*
2	149.83 ± 2.23	41.84 ± 0.57	254.50 ± 7.45	1.50 ± 1.45	157.50 ± 4.59	41.27 ± 1.53	146.50 ± 2.74	46.43 ± 1.26*
3	178.50 ± 1.52	30.70 ± 1.43	252.50 ± 7.23	2.27 ± 1.48	185.67 ± 1.75	30.76 ± 0.74	169.83 ± 2.14	37.90 ± 0.99*
4	196.00 ± 3.35	23.92 ± 1.48	249.50 ± 4.68	3.42 ± 0.87	204.33 ± 2.07	23.79 ± 1.38	192.33 ± 2.88	29.67 ± 0.95*
6	178.83 ± 2.14	30.57 ± 2.01	248.17 ± 4.26	3.93 ± 0.56	182.83 ± 2.79	31.82 ± 0.90	177.17 ± 1.83	35.21 ± 1.17*
8	155.00 ± 1.90	39.83 ± 1.11	245.83 ± 4.07	4.83 ± 1.13	163.17 ± 3.31	39.14 ± 1.48	152.67 ± 2.16	44.17 ± 1.16*
10	186.17 ± 3.31	27.75 ± 0.59	245.33 ± 3.98	5.02 ± 1.26	198.33 ± 2.16	26.03 ± 1.26	185.17 ± 1.47	32.29 ± 0.77*
12	210.17 ± 2.40	18.42 ± 1.20	242.67 ± 3.93	6.06 ± 0.90	214.67 ± 3.56	19.94 ± 1.82	208.50 ± 1.64	23.76 ± 0.63*

Data expressed as Mean ± SD, $n = 6$; *Significant when compared to gliclazide control; SDT: single dose treatment; MDT: multiple dose treatment.

administration of alloxan monohydrate in two divided doses, i.e. 100 mg/kg and 50 mg/kg body weight intraperitoneally on two consecutive days. After 72 h, blood samples were collected from surviving rats by retro-orbital puncture, and blood glucose levels were measured using automated clinical chemistry analyzer. Six rats with blood glucose levels ≥ 200 mg/dL were considered diabetic and selected for the study. The same treatment procedures as described in stage 1 were tested in diabetic rats.

2.6. Stage 3: Pharmacodynamic and pharmacokinetic study in normal rabbits

Six rabbits were selected for stage 3 experiment. These rabbits were given gliclazide via the oral route at 4 mg/kg body weight, and their blood was collected at pre-determined time points. Similar procedure was performed with either orally administered p-synephrine only or combination treatment with both p-synephrine and gliclazide at the previously mentioned doses with washout period between each experiment being a week. After this single dose interaction study, the same animals were given daily treatments with p-synephrine for the next 20 days with regular feeding. On day 21, animals were fasted for 10 h before administering p-synephrine. 30 min later, these animals received gliclazide at 4 mg/kg body weight. Blood samples were collected at pre-determined time intervals after each treatment with gliclazide alone, p-synephrine alone or combination treatments (single and multiple).

2.7. Collection of blood samples

Blood samples were collected from retro-orbital plexus of each rat at 0, 1, 2, 3, 4, 6, 8, 10 and 12 h. Blood samples were withdrawn from the marginal ear vein of each rabbit at 0, 1, 2, 3, 4, 6, 8, 10, 12, 16 and 24 h. The blood samples collected at all the intervals (except for

16 and 24 h in rabbits) were tested for blood glucose. Blood samples collected at 2 h and 8 h time intervals in rats (normal and diabetic) and at 3 h from rabbits were also used for the estimation of serum insulin [12]. Additionally, blood samples collected from rabbits were used for the estimation of gliclazide concentration in rabbit serum [12].

2.8. Determination of insulin resistance index and β-cell function

The insulin resistance index and β-cell function were assessed by homeostatic model assessment protocol and were calculated as follows [12,14,15].

$$Insulin\ resistance = (FSI*FSG)/22.5 \ \ and \ \ \beta - cell\ function$$
$$= (20*FSI)/(FSG - 3.5)*100$$

where FSI is fasting serum insulin (μIU/mL) and FSG is fasting serum glucose (mg/dL).

2.9. Determination of glucose and insulin levels

Blood samples collected from animals at various time intervals were centrifuged at 5000 rpm at 2—8 °C and serum was separated. Glucose estimation was done by using automated clinical chemistry analyzer (Siemens Dimension clinical chemistry system). The samples were loaded into sample container where sampling, reagent delivery, mixing, processing and printing of results were automatically performed by the system. The serum insulin levels in rats (normal and diabetic) were estimated by Vimta Labs Limited, Hyderabad. The assay was done according to the procedure of commercially available radioimmunoassay kit (Millipore, USA). The insulin levels were estimated as per the specifications/instructions of the kit manufacturer.

Table 3
Mean blood glucose levels and % blood glucose reduction with gliclazide in presence and absence of p-synephrine in rabbits.

Time (h)	Gliclazide		p-Synephrine		Gliclazide + p-Synephrine (SDT)		Gliclazide + p-Synephrine (MDT)	
	Mean blood glucose level (mg/dL)	% Blood glucose reduction	Mean blood glucose level (mg/dL)	% Blood glucose reduction	Mean blood glucose level (mg/dL)	% Blood glucose reduction	Mean blood glucose level (mg/dL)	% Blood glucose reduction
0	98.67 ± 1.37	–	98.67 ± 2.16	–	96.67 ± 2.34	–	100.83 ± 1.72	–
1	79.17 ± 1.17	19.76 ± 0.94	98.83 ± 1.72	−0.20 ± 2.50	78.33 ± 1.97	18.96 ± 0.69	79.00 ± 2.00	21.65 ± 1.42*
2	69.83 ± 0.75	29.22 ± 0.70	95.00 ± 5.02	3.73 ± 4.22	68.67 ± 1.37	28.96 ± 0.54	69.83 ± 1.47	30.74 ± 0.92*
3	63.67 ± 2.16	35.48 ± 1.91	95.83 ± 1.47	2.85 ± 1.71	63.00 ± 1.41	34.82 ± 0.67	62.17 ± 0.98	38.34 ± 0.75*
4	72.17 ± 1.17	26.84 ± 1.78	93.33 ± 1.51	5.38 ± 1.97	71.50 ± 2.59	26.03 ± 1.99	71.17 ± 1.72	29.42 ± 1.24*
6	75.50 ± 1.22	23.47 ± 1.32	93.67 ± 0.52	5.03 ± 2.11	75.17 ± 1.83	22.24 ± 0.84	73.33 ± 1.63	27.26 ± 1.78*
8	79.50 ± 1.05	19.42 ± 1.31	94.67 ± 1.37	4.04 ± 0.82	78.17 ± 1.83	19.14 ± 0.21	78.33 ± 2.07	22.31 ± 1.58*
10	82.83 ± 1.17	16.03 ± 1.70	93.67 ± 1.21	5.03 ± 2.40	80.67 ± 2.42	16.56 ± 0.98	80.33 ± 1.03	20.31 ± 1.69*
12	87.17 ± 2.32	11.64 ± 2.52	92.33 ± 1.37	6.41 ± 0.92	85.67 ± 1.86	11.37 ± 0.46	84.33 ± 1.21	16.36 ± 0.86*

Data expressed as Mean ± SD, $n = 6$; *Significant when compared to gliclazide control; SDT: single dose treatment; MDT: multiple dose treatment.

Table 4
Effect of p-synephrine on glucose insulin homeostasis of gliclazide in normal rats.

Parameter	Insulin (μIU/mL)		Insulin resistance		β-cell function	
	2 h	8 h	2 h	8 h	2 h	8 h
Gliclazide	11.94 ± 0.89	12.10 ± 0.51	34.40 ± 2.83	35.95 ± 1.92	389.57 ± 30.94	382.25 ± 16.21
p-Synephrine	7.10 ± 0.33	6.73 ± 0.62	30.44 ± 1.40	28.30 ± 2.49	152.72 ± 8.30	147.67 ± 14.51
Gliclazide + p-Synephrine (SDT)	12.13 ± 0.62	11.70 ± 0.52	33.23 ± 1.63	33.38 ± 2.14	417.48 ± 27.79	385.84 ± 16.05
Gliclazide + p-Synephrine (MDT)	14.82 ± 0.56*	14.23 ± 0.63*	35.34 ± 1.54	36.89 ± 1.65	590.92 ± 26.75*	519.33 ± 27.71*

Data expressed as Mean ± SD, $n = 6$; *Significant when compared to gliclazide control; SDT: single dose treatment; MDT: multiple dose treatment.

2.10. Chromatography

The serum gliclazide concentrations were determined by high performance liquid chromatography (HPLC) method. Briefly, HPLC equipped with automated Waters, 2695 pump model, variable wavelength programmable DAD or UV detector no: 2487, xttera C8 column (100 mm length × 4.6 mm diameter), particle size: 5 μm was used. The HPLC system was equipped with the software "Empower 2 (Waters)". Metformin was used as internal standard (IS) and the mobile phase (100%) consisted of phosphate buffer and acetonitrile in the ratio of 60:40 using isocratic method. The flow rate of mobile phase was set to 1.2 mL/min and the effluent was monitored at a wavelength of 229 nm. The ratio of peak area of gliclazide to that of IS was used for the quantification of gliclazide in serum samples. The HPLC method was validated in terms of linearity, precision, accuracy and recovery and then applied for the estimation of serum gliclazide in rabbits.

2.11. Pharmacokinetic analysis

Non-compartmental open model was used for estimation of pharmacokinetic parameters by using Kinetica 5.0 software. Pharmacokinetic parameters of gliclazide in rabbit serum such as C_{max}, time to reach C_{max} (T_{max}), area under the concentration time curve (AUC), area under first moment curve (AUMC), terminal half-life ($T_{1/2}$), elimination rate constant (K_{el}), mean residence time (MRT) and clearance (CL) were estimated.

2.12. Data and statistical analysis

Data are expressed as mean ± SD. Student's paired *t*-test was used and $p < 0.05$ was considered as statistically significant.

3. Results

3.1. Pharmacodynamic interaction study between p-synephrine and gliclazide

Gliclazide produced hypoglycemic activity in normal rats with maximum biphasic reduction of 37.35 ± 1.69% and 35.41 ± 1.65%

(Table 1) and antihyperglycemic activity in diabetic rats with peak biphasic reduction of 41.84 ± 0.57% and 39.83 ± 1.11% at 2 h and 8 h after administration, respectively (Table 2). Peak hypoglycemic activity was observed with maximum reduction of 35.48 ± 1.91% at 3 h after gliclazide administration in normal rabbits (Table 3). p-Synephrine alone or in single dose combination with gliclazide did not induce any significant changes in percent blood glucose reduction, insulin levels, insulin resistance and β-cell function in rats (normal and diabetic) and in normal rabbits. However, multiple dose combination of p-synephrine with gliclazide produced significant increase in percent blood glucose reduction, insulin levels and β-cell function at all-time intervals in rats (normal and diabetic) and rabbits (Tables 4—6) when compared to gliclazide control.

3.2. Chromatography

The calibration curve in the rabbit serum for gliclazide was linear with the concentration range of 20—800 ng/mL (Fig. 1). The intra-day and inter-day precision was determined by analyzing three spiked serum replicates of different concentrations of gliclazide prepared on the same day and next day, respectively. The values obtained were in the limits of ≤15% relative standard deviation (RSD) specified for both inter-day and intra-day precision (Table 7). The recovery of gliclazide spiked in serum along with IS was estimated in triplicates at 20, 50, 100, 200, 400 and 800 ng/mL concentrations. These sample areas were compared with those of corresponding standards containing the respective concentrations of gliclazide standard and IS in mobile phase. Percent recovery was calculated by comparing the mean peak area obtained by direct injection of pure gliclazide in mobile phase with that of area obtained from the extracted serum samples spiked with same concentrations of gliclazide (Table 8). Limit of quantification (LOQ) of gliclazide was 20 ng/mL. Chromatogram of blank and standard are provided in Figs. 2 and 3, respectively.

3.3. Extraction procedure

To 0.3 mL of serum (either unknown serum samples or serum spiked with known amount of gliclazide standard and IS) in 1.5 mL

Table 5
Effect of p-synephrine on glucose insulin homeostasis of gliclazide in diabetic rats.

Parameter	Insulin (μIU/mL)		Insulin resistance		β-cell function	
	2 h	8 h	2 h	8 h	2 h	8 h
Gliclazide	13.19 ± 0.75	12.35 ± 0.76	87.82 ± 5.19	85.06 ± 5.59	180.28 ± 10.57	162.96 ± 9.68
p-Synephrine	7.06 ± 0.60	6.93 ± 0.50	79.82 ± 7.19	75.71 ± 5.10	56.27 ± 4.94	57.24 ± 4.58
Gliclazide + p-Synephrine (SDT)	12.23 ± 0.25	12.08 ± 0.55	85.66 ± 4.03	87.60 ± 4.13	158.91 ± 2.95	151.41 ± 8.07
Gliclazide + p-Synephrine (MDT)	14.35 ± 0.30*	14.31 ± 0.37*	93.41 ± 2.70	97.09 ± 3.13*	200.71 ± 5.46*	191.86 ± 5.04*

Data expressed as Mean ± SD, $n = 6$; *Significant when compared to gliclazide control; SDT: single dose treatment; MDT: multiple dose treatment.

Table 6
Effect of p-synephrine on glucose insulin homeostasis of gliclazide in rabbits.

Parameter	Insulin (μIU/mL)	Insulin resistance	β-cell function
	3 h	3 h	3 h
Gliclazide	20.76 ± 0.93	58.79 ± 4.36	690.07 ± 20.48
p-Synephrine	8.11 ± 0.37	34.54 ± 1.29	175.81 ± 9.97
Gliclazide + P-Synephrine (SDT)	19.88 ± 0.39	55.65 ± 1.36	668.58 ± 23.73
Gliclazide + P-Synephrine (MDT)	22.55 ± 0.58*	62.31 ± 1.71	769.09 ± 25.63*

Data expressed as Mean ± SD, $n = 6$; *Significant when compared to gliclazide control; SDT: single dose treatment; MDT: multiple dose treatment.

Eppendorf tube, 0.3 mL of acetonitrile was added to precipitate proteins and for extraction of gliclazide and IS. The tubes were vortexed for 3 min and centrifuged at 3000 rpm for 5 min. The supernatant was separated and filtered through 0.45 μm nylon membrane filter. About 20 μL of filtrate was injected into the system and elute was detected for gliclazide at 229 nm.

3.4. Pharmacokinetic interaction study between p-synephrine and gliclazide

The pharmacokinetic parameters of gliclazide alone, and in the presence of p-synephrine following single and multiple dose administrations are given in Table 9. p-Synephrine did not alter the pharmacokinetics of gliclazide in rabbits both in single and multiple dose treatments. The concentration versus time profile of gliclazide in presence of p-synephrine (both single and multiple dose treatments) is shown in Fig. 4.

4. Discussion

Drug interaction studies are an important aspect of pharmacology research, and such interactions are evaluated in animal models (rodents and non-rodents). Although animal models can never replace the need for comprehensive studies in human subjects, their use can provide important information for understanding the mechanisms of drug interactions. The present study is designed to evaluate the effect of p-synephrine on the activity of gliclazide in rats (normal and diabetic) and in rabbits. The normal rat model serves to quickly identify any potential drug interactions and the diabetic model aids to validate the interaction in the disease conditions where these drug combinations typically used. The rabbit model was used to further validate the same in a dissimilar species [12]. Diabetes was induced with alloxan monohydrate, since it is more economical and most widely used toxicant to induce diabetes in animal models.

Rats are known to be more sensitive to gliclazide response. Consistent with previous literature [2,12,16] gliclazide administered alone produced a biphasic response in terms of percent blood glucose reduction at 2 h and 8 h time points in rat model, perhaps due to biliary excretion and enterohepatic recycling. The biphasic effect was not seen in rabbits and the maximum reduction in blood glucose level was observed at 3 h time point. This might be due to absence of enterohepatic recycling in rabbits. Clinical studies [17] have shown that enterohepatic recirculation of gliclazide occurs in humans and therefore substantiating the correlation between our preclinical data to that seen in humans. Gliclazide is known for its hypoglycemic activity in rat models by blocking K^+ channels in the pancreatic β-cells. The resultant antihyperglycemic activity in diabetic rats is mediated by increasing both insulin secretion and tissue uptake of glucose [18,19]. Insulin levels were estimated at the time points where peak reduction in percent blood glucose levels was observed both in rats (2 h and 8 h intervals) and in rabbits (3 h interval) [12].

The study revealed the influence of p-synephrine on the pharmacodynamic activity of gliclazide alone and in combination using single and multiple dose treatments in rats and rabbits. The end points were evaluated in terms of glucose levels (% reduction), insulin levels, β-cell function and insulin resistance using homeostatic model assessment and pharmacokinetics of gliclazide in rabbits. In the present study, no significant changes were observed in the pharmacodynamics and pharmacokinetics of gliclazide following single dose administration with p-synephrine. Multiple dose treatments of p-synephrine in presence of gliclazide resulted in significant increase in percent blood glucose reduction, insulin
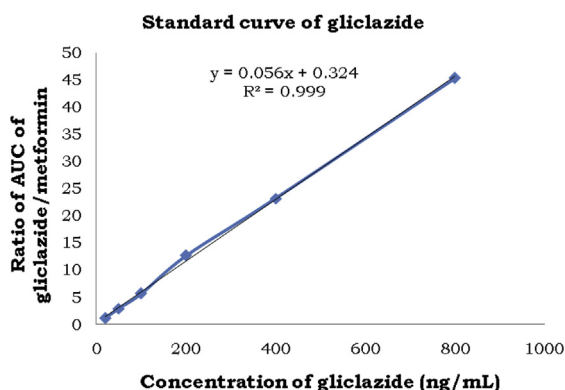

Fig. 1. Standard curve of gliclazide.

Table 7
Intra-day and inter-day precision for determination of gliclazide in rabbit serum.

Concentration of gliclazide (ng/mL)	Mean peak area of gliclazide			
	Intra-day precision		Inter-day precision	
	Mean ± SD	%RSD	Mean ± SD	%RSD
20	2245.67 ± 11.06	0.49	2109.00 ± 11.53	0.55
50	5317.67 ± 68.06	1.28	5322.67 ± 61.45	1.15
100	10,861.33 ± 35.70	0.33	10,242.33 ± 42.67	0.42
200	23,112.33 ± 31.37	0.14	23,245.67 ± 55.54	0.24
400	43,217.00 ± 33.42	0.08	44,296.67 ± 35.91	0.08
800	84,480.00 ± 94.41	0.11	84,769.00 ± 93.00	0.11

SD: Standard Deviation; RSD: Relative Standard Deviation.

Table 8
Accuracy of gliclazide in mobile phase and in rabbit serum.

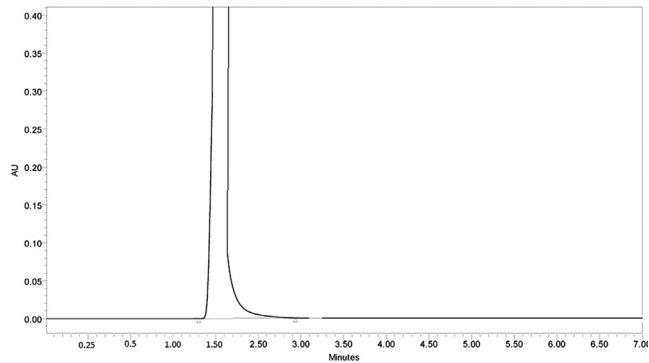| Concentration of gliclazide in mobile phase and spiked serum (ng/mL)	Mean peak area of gliclazide in mobile phase	Mean peak area of gliclazide in spiked serum	Accuracy (% recovery)
20	2345	2318	98.84
100	10,412	10,289	98.82
400	44,874	44,798	99.81
800	83,578	83,475	99.76

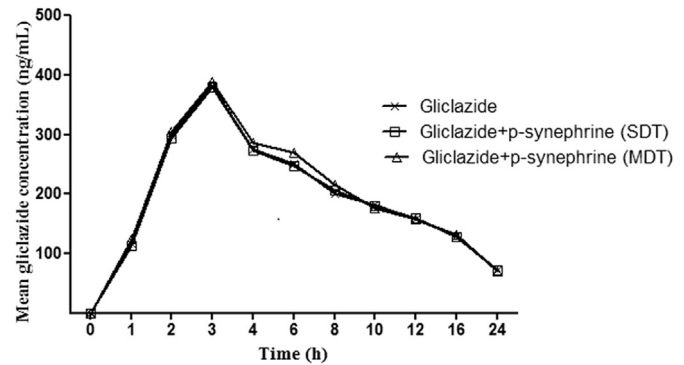**Fig. 2.** Chromatogram of blank serum.

Fig. 4. Concentration vs time profile of gliclazide before and after p-synephrine treatment in rabbits.

levels and β-cell function in rats (normal and diabetic) and in rabbits. These changes observed might be due to the agonistic activity on β-3 adrenoreceptors, resulting in increased metabolic rate as well as exhibiting hypoglycemic and insulin stimulatory properties [20]. It is reported that p-synephrine stimulated the translocation of glucose transporter-4 from the cytoplasm to the plasma membrane resulting in decreased insulin resistance [21]. Increased insulin levels in rats (normal and diabetic) and rabbits are supported by significant increase in β-cell function. The elevated insulin levels accompanied by high blood glucose levels suggest an insulin resistant state. However, in the present study there was no such significant change observed in insulin resistance index, which can be attributed due to the combined effect of p-synephrine and gliclazide.
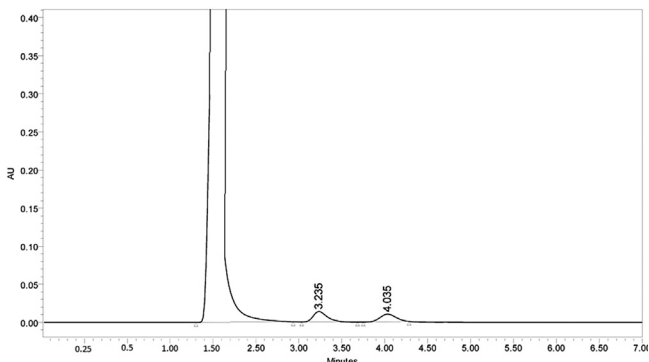

Fig. 3. Chromatogram of gliclazide in rabbit serum; retention time of 3.235 and 4.035 min represents the IS (metformin) and gliclazide, respectively.

Several findings have confirmed the functional similarity of CYP450 in rabbits and humans. Rabbits are valuable *in vivo* models for the assessment of pharmacokinetic interactions because of the convenience of serial blood sampling [22,23]. Additionally, rabbits and humans share similarities in metabolizing drugs via CYP450 making it an effective model to assess the pharmacokinetics of drugs [24]. In the present study, the pharmacokinetics of gliclazide after single oral dose was examined in rabbits with and without prior exposure to p-synephrine. Consistent with literature [12], C_{max} of gliclazide obtained at 3 h time point can be correlated with peak glucose reduction and maximum insulin level thereby establishing a link between the pharmacokinetic data and the pharmacodynamic results in rabbits. This consistency was not altered by p-synephrine either in single or multiple dose treatments.

Any drug/herbal active constituent which is potential inducer or inhibitor of microsomal metabolizing enzyme may alter the pharmacokinetic and pharmacodynamic activity of the substrate [12]. Non-compartmental compartment open model was used to evaluate the pharmacokinetic parameters. The serum gliclazide levels obtained in the study were comparable with previous reports in rabbits [12]. p-Synephrine is reported to supress the drug metabolizing enzymes CYP3A4 in the liver and intestine both *in vitro* and *in vivo* [25]. However, from the pharmacokinetic results in the present study, it can be ascertained that p-synephrine may not have any significant effect on the metabolism of gliclazide, which is primarily metabolized by CYP2C9 and partly by CYP3A4. This could probably explain why p-synephrine in both single and multiple dose treatments did not alter the pharmacokinetics of gliclazide.

Table 9
Mean pharmacokinetic parameters of gliclazide before and after administration of p-synephrine in rabbits.

Pharmacokinetic parameters	Gliclazide	Gliclazide + p-Synephrine (SDT)	Gliclazide + p-Synephrine (MDT)
C_{max} (ng/mL)	382.29 ± 8.28	381.98 ± 7.12	389.01 ± 6.42
T_{max} (h)	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00
AUC_{last} (h ng/mL)	4010.77 ± 17.64	4023.45 ± 23.52	4070.92 ± 57.32
AUC_{inf} (h ng/mL)	5080.01 ± 63.03	5096.49 ± 113.24	4920.53 ± 432.96
$AUMC_{last}$ (h h ng/mL)	38,286.32 ± 312.86	38,437.27 ± 328.94	38,489.21 ± 563.62
$AUMC_{inf}$ (h h ng/mL)	79,973.08 ± 2722.13	80,412.74 ± 5192.61	76,995.64 ± 6714.37
$T_{1/2}$ (h)	10.38 ± 0.25	10.44 ± 0.55	9.90 ± 0.74
K_{el} (1/h)	0.07 ± 0.00	0.07 ± 0.00	0.07 ± 0.01
MRT (h)	9.55 ± 0.05	9.55 ± 0.05	9.40 ± 0.07
CL (L/h)	0.07 ± 0.00	0.07 ± 0.00	0.07 ± 0.01

Data expressed as Mean ± SD, $n = 6$; SDT: single dose treatment; MDT: multiple dose treatment; AUC_{last}: AUC from time zero to last time point collected; AUC_{inf}: AUC from time zero to infinite.

5. Conclusion

The study confirmed that the interaction of p-synephrine with gliclazide is pharmacodynamic in nature upon multiple dose treatments. Since the interaction is observed in two dissimilar species, it is also likely to occur in humans. Hence, this combination needs monitoring of glucose levels periodically when administered for their clinical use. However, further studies are necessary to determine the possibility of these interactions in humans and also to know the exact mechanism of action behind this interaction, if any.

References

[1] Chien CF, Wu YT, Lee WC, Lin LC, Tsail TH. Herb–drug interaction of *Andrographis paniculata* extract and andrographolide on the pharmacokinetics of theophylline in rats. Chem Biol Interact 2010;184(3):458–65.

[2] Satyanarayana S, Kilari EK. Influence of nicorandil on the pharmacodynamics and pharmacokinetics of gliclazide in rats and rabbits. Mol Cell Biochem 2006;291:101–5.

[3] Brien RC, Luo M, Balaza N. *In vitro* and *in vivo* antioxidant properties of gliclazide. J Diabetes Complications 2000;14(4):201–6.

[4] Andrade AS, Schmitt GC. Gas chromatographic method for analysis of p-synephrine in *Citrus aurantium*. Chromatographia 2009:S225–9.

[5] Avula B, Upparapalli SK. Simultaneous quantification of adrenergic amines and flavonoids in *Citrus aurantium*, various citrus species and dietary supplements by liquid chromatography. J AOAC Int 2005;88:1593–606.

[6] Dragull K, Breksa AP, Cain B. Synephrine content of juice from Satsma mandarins (*Citrus unshiu* Marcovitch). J Agric Food Chem 2008;59:8874–8.

[7] Fugh-Berman A, Myers A. *Citrus aurantium*, an ingredient of dietary supplements marketed for weight loss: current status of clinical and basic research. Exp Biol Med 2004;229(8):698–704.

[8] Arch JRS. β3-adrenoceptor agonists: potential, pitfalls and progress. Eur J Pharmacol 2002;440:99–107.

[9] Hamilton BS, Doods HN. Identification of potent agonists acting as an endogenous atypical β_3-adrenoceptor state that modulates lipolysis in rodent fat cells. Eur J Pharmacol 2008;580:55–62.

[10] Rodrigues M, Alves G, Falcão A. Investigating herb–drug interactions: the effect of *Citrus aurantium* fruit extract on the pharmacokinetics of amiodarone in rats. Food Chem Toxicol 2013;60:153–9.

[11] Shaw SR, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. FASAB J 2007;22(3):659–61.

[12] Vatsavai LK, Kilari EK. Influence of curcumin on the pharmacodynamics and pharmacokinetics of gliclazide in animal models. J Exp Pharmacol 2016;8: 69–76.

[13] Heikkila RE. The prevention of alloxan-induced diabetes in mice by dimethyl sulfoxide. Eur J Pharmacol 1977;44(2):191–3.

[14] Bonora E, Targher G, Alberiche M, Bonadonna RC, Saggiani F, Zenere MB, et al. Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subject with various degrees of glucose tolerance and insulin sensitivity. Diabetes Care 2000;23(1): 57–63.

[15] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28(7):412–9.

[16] Mastan SK, Eswar Kumar K. Effect of ritonavir on the pharmacodynamics of gliclazide in animal models. Diabetologia Croatica 2009;38(4):105–13.

[17] Davis TME, Daly F, Walsh JP, Ilett KF, Beilby JP, Dusci LJ, et al. Pharmacokinetics and pharmacodynamics of gliclazide in Caucasians and Australian Aborigines with type 2 diabetes. Br J Clin Pharmacol 2000;49(3):223–30.

[18] Ma A, Kamp M, Bird B. The effects of long term gliclazide administration on insulin secretion and insulin sensitivity. Aust N Z J Med 1989;19(1): 14–49.

[19] Vanhaeften TW, Vaneman TF, Gerich JE. Influence of gliclazide on glucose stimulated insulin release in man. Metabolism 1991;40(7):751–5.

[20] Parmer HS, Kar A. Medicinal values of fruit peels from *Citrus sisensis, Punica granatum* and *Musa paradisiacal* with respect to alterations in tissue lipid peroxidation and serum concentration of glucose, insulin and thyroid hormones. J Med Food 2008;11:376–81.

[21] Hong NY, Cui ZG, Kang HK, Lee YK, Park DB. p-Synephrine stimulates glucose consumption via AMPK in L6 skeletal muscle cells. Biochem Biophys Res Commun 2012;418:720–4.

[22] Fang HM, Xu JM, Mei Q, Diao L, Chen ML, Jin J, et al. Involvement of cytochrome P4503A4 and P-glycoprotein in first pass intestinal extraction of omeprazole in rabbits. Acta Pharmacol Sin 2009;30(11):1566–72.

[23] Nakamura T, Okada K, Nagata K, Yamaxoe T. Intestinal cytochrome P450 and response of rifampicin in rabbits. Jpn J Pharmacol 2000;82(3): 232–9.

[24] Pichard L, Gillet G, Fabre I, Maurel P. Identification of the rabbit and human cytochrome P-450 IIIA as the major enzymes involved in the N-demethylation of diltiazem. Drug Metab Dispos 1990;18:711–9.

[25] Suzuki O, Matsumoto T, Oya M, Katsumata Y. Oxidation of synephrine by type A and type B monoamine oxidase. Experientia 1979;35(10):1283–4.