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INFLUENCE OF FORSKOLIN ON THE PHARMACODYNAMICS AND PHARMACOKINETICS OF GLICLAZIDE IN ANIMAL MODELS

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ABSTRACT

Objective: Type 2 diabetes may occur in patients suffering from obesity. Gliclazide is the most commonly used drug of choice for the treatment of type 2 diabetes and forskolin is widely used for the management of obesity. As these two drugs intersect at a point, it is essential to investigate the effect of oral administration of forskolin on the pharmacodynamics and pharmacokinetics of gliclazide in animal models. **Methods:** Influence of forskolin on the activity of gliclazide was determined by conducting single and multiple dose interaction studies in rats (normal and diabetic) and rabbits. Blood samples collected at predetermined time intervals were used for the estimation of glucose and insulin levels by using automated clinical chemistry analyzer and radio immune assay methods, respectively. The insulin resistance and β-cell function were determined by homeostasis model assessment. Additionally, serum gliclazide levels in rabbits were analyzed by validated method using high performance liquid chromatography. **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 forskolin. However, in multiple dose interaction studies, samples from all the time points analyzed showed significant changes in percent blood glucose reduction ranging from 11.17 to 38.39% in normal rats, 14.08 to 35.93% in diabetic rats and 5.21 to 24.98% in normal rabbits. This observation was coupled with subtle and significant decrease in insulin levels in rats and rabbits, respectively and significant reduction in β-cell function as well. The pharmacokinetics of gliclazide was significantly altered by multiple dose treatments of forskolin and the percent decrease in serum gliclazide concentration level was found to be 18.14 % in rabbits when compared to gliclazide control. **Conclusion:** The effect of multiple dose forskolin treatments upon gliclazide appeared to be pharmacokinetic in nature. This combination when prescribed/taken for clinical use in obese patients requires dose adjustments and periodic monitoring of blood glucose levels.

INTRODUCTION:

Herbal medicines are widely used today either alone or in combination with modern pharmaceuticals. This therapeutic combination may be toxic at prescribed doses and the possible adverse effects of herb-drug interactions remain to be explained. Increase or decrease in the pharmacological or toxicological effects of either component may be seen when herbal medicine and drugs are administered in combination [1]. Therefore, it is important to study the interactions between herbal medicines and drugs. Patients with obesity are prone to diabetes and such complications need treatment for prolonged durations where combinations of drugs and/ or herbal medicines are used. Maintenance of normal blood glucose level is very important in this condition since both hyperglycemia and hypoglycemia are undesirable. 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 have 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].

Forskolin is a diterpene derived from plant *Coleus forskohlii* [4]. It is native to India and has been used for centuries in Ayurvedic medicine to treat various diseases [5]. Forskolin is a popular active herbal ingredient used as commercial weight loss dietary supplements. It is reported that forskolin increases cAMP levels via activation of enzyme adenylate cyclase which results in increased lipolysis leading to fat degeneration and weight loss [6, 7]. Forskolin metabolism and its interactions with drugs have been studied *in vitro* and is reported that it significantly induced hepatic metabolic enzymes [8]. It is also reported that administration of 10% forskolin obviously induced dose dependant CYP450 enzymes [9]. Although, *in vitro* investigations on forskolin suggests that it might be an inducer of CYP enzymes, correlation of the same to its *in vivo* effects is hypothetical and opens an avenue for further investigation. There was no much *in vivo* data available for the potential interaction of forskolin with drugs. Thus, the present study was designed and undertaken with the hypothesis that pre-treatment of forskolin with gliclazide might lead to significant herb-drug interactions in animal models.

MATERIALS AND METHODS

Drugs and Chemicals

Gliclazide was obtained as gift sample from Dr. Reddy's Labs (Bachupally, Hyderabad, India). Forskolin ready to use formula was obtained from All Natural Pharmaceuticals (California, USA). Each capsule contains 20 % of active forskolin. Alloxan monohydrate was purchased from LOBA Chemie (Mumbai, India). All reagents and chemicals used in the study were of analytical grade.

Animals and Husbandry

8 to 9 week old male albino rats weighing between 170–250 g were procured from Vivo Biotech, Hyderabad, India and 3 month old male albino rabbits weighing between 1–1.5 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 Bio-technologies 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).

Study Design

Forskolin doses of 50 mg/kg and 25 mg/kg was calculated from human oral therapeutic dose based on body surface area for rats and rabbits, respectively [10]. From the results of gliclazide dose-effect relationship study conducted in normal rats and rabbits, the dose of 2 mg/kg and 4 mg/kg body weight were selected, respectively, for administration in animals [11, 12]. Oral dose formulation of forskolin was prepared by suspending in 0.5% Carboxymethylcellulose sodium. Gliclazide solution was prepared by dissolving in few drops of 0.1N Sodium hydroxide and final volume was made with water [13]. The study was designed as follows.

Stage-1: Pharmacodynamic interaction in normal rats [11, 12]

Stage-2: Pharmacodynamic interaction in diabetic rats [11, 12]

Stage-3: Pharmacodynamic and pharmacokinetic interaction in normal rabbits [11-13]

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 predetermined time points. With a week washout period between each experiment, similar procedure was performed with either orally administered forskolin only or combination treatment with both forskolin and gliclazide at the previously mentioned doses. After the single dose interaction studies, the same group of animals were given daily treatments with forskolin for the next 20 days with regular feeding. On day 21, animals were fasted for 10 h before administering forskolin. 30 minutes later, these animals were given gliclazide at 2 mg/kg body weight. Blood samples were collected at predetermined time intervals after each treatment with gliclazide alone, forskolin alone or combination treatments (single and multiple).

Pharmacodynamic Interaction Study in Diabetic Rats

For stage 2 experiments, diabetes was induced in rats as previously described [14]. Briefly, diabetes was induced in rats by the 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.

Pharmacodynamic and Pharmacokinetic Interaction Study in 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 predetermined time points. With a week washout period between each experiment, similar procedure was performed with either orally administered forskolin only or combination treatment with both forskolin and gliclazide at the previously mentioned doses. After this single dose interaction study, the same animals were given daily treatments with forskolin for the next 20 days with regular feeding. On day 21, animals were fasted for 10 h before administering forskolin. 30 minutes later, these animals received gliclazide at 4 mg/kg body weight. Blood samples were collected at predetermined time intervals after each treatment with gliclazide alone,

forskolin alone or combination treatments (single and multiple).

Collection of Blood Samples and Estimation

Blood samples were collected from retro orbital plexus of each rat at 0, 1, 2, 3, 4, 6, 8, 10 and 12 h [15]. 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 [11, 12]. Additionally, blood samples collected from rabbits were used for the estimation of gliclazide concentration in rabbit serum [11, 12].

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 was calculated as follows (13, 16, 17).

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

Where FSI is fasting serum insulin (µu/mL) and FSG is fasting serum glucose (mg/dL).

Determination of Glucose and Insulin Levels

Blood samples collected from animals at various time intervals were centrifuged at 5000 rpm at 2–8^{0}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.

Pharmacokinetic Analysis

One 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}, T_{max}, AUC, AUMC, $T_{1/2}$, K_{el}, MRT and CL were estimated.

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.

RESULTS

Pharmacodynamic Interaction Study between Forskolin and Gliclazide

Gliclazide produced hypoglycemic activity in normal rats with maximum biphasic reduction of 40.68 ± 0.99 % and 38.97 ± 0.71 % (Table 1) and antihyperglycemic activity in diabetic rats with peak biphasic reduction of 40.62 ± 1.12 % and 39.46 ± 0.61 % (Table 2) at 2 h and 8 h intervals after administration, respectively. Peak hypoglycemic activity was observed with maximum reduction of 34.80 ± 1.60 % at 3 h after gliclazide administration in normal rabbits (Table 3). Forskolin 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 rabbits. However, multiple dose combination of forskolin with gliclazide produced significant decrease in percent blood glucose reduction at 2, 3, 4, 6, 8, 10 and 12 hours and β-cell function (Table 4 - 6) in rats (normal and diabetic) and rabbits when compared to gliclazide control.

Pharmacokinetic Interaction Study between Forskolin and Gliclazide

The pharmacokinetic parameters of gliclazide alone, and in the presence of forskolin following single and multiple

dose administrations are given in Table 7. However, the pharmacokinetic parameters of gliclazide were significantly altered by forskolin upon multiple dose treatments. The concentration versus time profile of gliclazide in presence of forskolin (both single and multiple dose treatments) is shown in Fig. 1.

DISCUSSION:

Drug interactions are usually seen in clinical practice and the mechanism of interactions are evaluated in animal models (18). 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 evaluates the influence of forskolin on the activity of gliclazide in animal models. 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 (19). 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 (11-13, 19), 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 rabbit model where maximum reduction in blood glucose level was observed at 3 h time interval. Gliclazide is known for its hypoglycemic activity by blocking K+ channels in the pancreatic β-cells thereby stimulating insulin secretion and antihyperglycemic activity by increasing tissue uptake of glucose in normal and diabetic rats respectively (20, 21). Insulin levels were estimated at time intervals 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).

The study revealed the influence of forskolin 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 forskolin. Multiple dose treatments of forskolin in presence of gliclazide showed significant decrease in percent blood glucose reduction and β-cell function in rats (normal and diabetic) and in rabbits. These changes observed might be due to stimulatory effect of forskolin on A cells of pancreas which resulted in increased glucagon as reported earlier (22) and also might be due to induction of hepatic microsomal enzymes (9) where the concentration of gliclazide in rabbit serum was significantly reduced. Decrease in insulin levels in rats (normal and diabetic) and rabbits resulted in significant decrease in β-cell function. Significant decrease in insulin levels in rabbits might be due to reduced serum levels of gliclazide which was also substantiated with the results of pharmacokinetic interaction study in rabbits. Enzyme induction has important clinical implications when enhanced drug metabolism results in lower drug concentrations, which leads to a decrease in substrate efficacy. Forskolin upon repeated administration altered the pharmacokinetic of gliclazide in rabbits. Significant reduction of serum concentration of gliclazide along with exposure was observed upon multiple dose administration of forskolin in rabbits.

Table 1: Mean percent blood glucose reduction with gliclazide in presence and absence of forskolin in normal rats (N=6)

Time (h)	Gliclazide	Forskolin	Gliclazide + Forskolin (SDT)	Gliclazide + Forskolin (MDT)
1	30.52 ± 0.97	0.13 ± 1.67	30.49 ± 1.22	30.20 ± 1.50
2	40.68 ± 0.99	3.22 ± 2.37	40.68 ± 1.13	38.39 ± 1.22*
3	28.45 ± 1.34	3.22 ± 2.31	28.65 ± 1.51	25.70 ± 1.77*
4	22.59 ± 0.70	4.18 ± 3.15	23.64 ± 1.33	20.18 ± 1.66*
6	31.21 ± 0.55	3.34 ± 3.96	31.47 ± 2.67	27.37 ± 1.73*
8	38.97 ± 0.71	3.05 ± 2.21	38.34 ± 1.71	30.18 ± 2.48*
10	25.50 ± 1.81	3.68 ± 3.73	25.78 ± 1.48	22.05 ± 1.63*
12	16.54 ± 1.42	3.08 ± 0.90	15.88 ± 1.80	11.17 ± 1.09*

Data expressed as Mean ± SD; *Significant when compared to gliclazide control; SDT: Single Dose Treatment; MDT: Multiple Dose Treatment.

Table 2: Mean percent blood glucose reduction with gliclazide in presence and absence of forskolin in diabetic rats (N=6)

Time (h)	Gliclazide	Forskolin	Gliclazide + Forskolin (SDT)	Gliclazide + Forskolin (MDT)
1	31.51 ± 1.60	0.40 ± 1.96	30.63 ± 0.70	32.70 ± 1.04
2	40.62 ± 1.12	1.31 ± 2.60	40.74 ± 1.15	35.93 ± 1.25*
3	29.63 ± 1.52	4.00 ± 1.33	29.97 ± 1.00	25.98 ± 1.57*
4	22.74 ± 1.25	4.01 ± 0.89	22.54 ± 1.18	19.45 ± 0.73*
6	30.09 ± 1.58	4.15 ± 0.44	30.99 ± 1.10	26.30 ± 1.06*
8	39.46 ± 0.61	4.44 ± 1.10	39.21 ± 0.47	34.03 ± 1.39*
10	26.14 ± 0.94	3.52 ± 0,53	27.06 ± 1.38	23.85 ± 1.31*
12	16.71 ± 0.86	3.10 ± 0.39	16.83 ± 1.91	14.08 ± 1.84*

Data expressed as Mean ± SD; *Significant when compared to gliclazide control; SDT: Single Dose Treatment; MDT: Multiple Dose Treatment.

Table 3: Mean percent blood glucose reduction with gliclazide in presence and absence of forskolin in rabbits (N=6)

Time (h)	Gliclazide	Forskolin	Gliclazide + Forskolin (SDT)	Gliclazide + Forskolin (MDT)
1	18.01 ± 1.32	1.95 ± 2.05	18.65 ± 0.70	18.91 ± 1.94
2	28.97 ± 1.94	3.44 ± 1.31	28.64 ± 0.96	24.82 ±1.02*
3	34.80 ± 1.60	5.74 ± 1.16	34.91 ± 1.14	24.98 ± 1.02*
4	27.10 ± 1.55	4.92 ± 1.44	27.62 ± 1.11	25.00 ± 0.99*
6	23.85 ± 1.74	4.92 ± 1.84	24.39 ± 1.43	18.93 ± 2.59*
8	18.18 ± 1.47	5.56 ± 2.53	18.99 ± 0.50	13.19 ± 1.34*
10	14.91 ± 1.81	4.72 ± 2.66	15.76 ± 0.32	9.90 ± 1.34*
12	11.83 ± 1.28	6.05 ± 2.45	12.00 ± 2.28	5.21 ± 0.94*

Data expressed as Mean ± SD; *Significant when compared to gliclazide control; SDT: Single Dose Treatment; MDT: Multiple Dose Treatment.

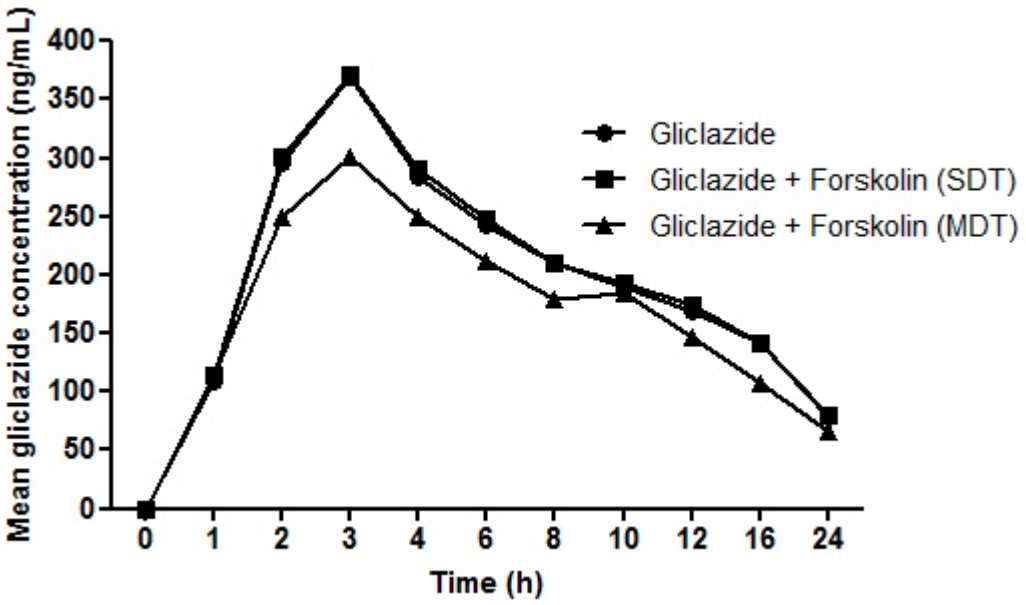

Figure 1: Mean serum gliclazide concentration (ng/mL) before and after treatment with forskolin in rabbits. SDT: Single Dose Treatment; MDT: Multiple Dose Treatment

Table 4: Effect of forskolin on homeostasis of gliclazide in normal rats (N=6)

Parameter	Insulin		Insulin Resistance[#]		β-Cell Function[#]	
	2 h	8 h	2 h	8 h	2 h	8 h
Gliclazide	12.03 ± 0.56	11.63 ± 0.31	30.65 ± 1.34	30.50 ± 1.07	447.19 ± 25.55	419.53 ± 17.31
Forskolin	7.03 ± 0.39	7.46 ± 0.43	30.92 ± 1.66	32.88 ± 1.89	147.23 ± 8.68	155.99 ± 9.38
Gliclazide + Forskolin (SDT)	12.04 ± 0.67	11.49 ± 0.43	31.58 ± 2.05	31.32 ± 1.27	433.66 ± 20.86	397.77 ± 20.76
Gliclazide + Forskolin (MDT)	11.27 ± 0.82	10.04 ± 0.65	30.81 ± 2.21	31.05 ± 1.54	389.05 ± 32.00*	303.83 ± 25.66*

Data expressed as Mean ± SD; *Significant when compared to gliclazide control; #Calculated by homeostasis model assessment; SDT: Single Dose Treatment; MDT: Multiple Dose Treatment.

Table 5: Effect of forskolin on homeostasis of gliclazide in diabetic rats (N=6)

Parameter	Insulin		Insulin Resistance[#]		β-Cell Function[#]	
	2 h	8 h	2 h	8 h	2 h	8 h
Gliclazide	12.10 ± 0.42	12.30 ± 0.32	81.85 ± 2.91	84.84 ± 2.10	162.85 ± 6.19	162.27 ± 5.03
Forskolin	7.21 ± 0.30	6.91 ± 0.29	85.09 ± 4.37	79.01 ± 3.79	54.97 ± 2.14	54.45 ± 2.58
Gliclazide + Forskolin SDT)	12.08 ± 0.57	12.05 ± 0.62	87.70 ± 4.12	89.76 ± 4.52	151.17 ± 10.21	146.79 ± 8.08
Gliclazide + Forskolin (MDT)	11.98 ± 0.54	10.03 ± 0.56	93.20 ± 5.73	80.31 ± 4.73	139.65 ± 4.94*	113.55 ± 6.43*

Data expressed as Mean ± SD; *Significant when compared to gliclazide control; #Calculated by homeostasis model assessment; SDT: Single Dose Treatment; MDT: Multiple Dose Treatment.

Table 6: Effect of forskolin on homeostasis of gliclazide in rabbits (N=6)

Parameter	Insulin	Insulin Resistance[#]	β-Cell Function[#]
	3 h	3h	3 h
Gliclazide	20.42 ± 0.53	57.47 ± 1.86	682.53 ± 19.91
Forskolin	8.91 ± 0.38	37.75 ± 1.79	194.05 ± 8.54
Gliclazide + Forskolin (SDT)	19.95 ± 0.66	56.79 ± 3.21	659.48 ± 8.13
Gliclazide + Forskolin (MDT)	18.76 ± 0.58*	50.06 ± 3.32	547.82 ± 13.17*

Data expressed as Mean ± SD; *Significant when compared to gliclazide control; #Calculated by homeostasis model assessment; SDT: Single Dose Treatment; MDT: Multiple Dose Treatment.

Table 7: Mean pharmacokinetic parameters of gliclazide before and after administration of forskolin in rabbits (N=6)

Pharmacokinetic Parameter	Gliclazide	Gliclazide + Forskolin (SDT)	Gliclazide + Forskolin (MDT)
C_{max} (ng/mL)	368.61 ± 6.19	371.99 ± 4.60	301.75 ± 9.08*
T_{max} (h)	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00
AUC_{last} (h*ng/mL)	4150.88 ± 15.17	4196.34 ± 46.77	3534.11 ± 64.62*
AUC_{inf} (h*ng/mL)	5426.89 ± 41.86	5484.45 ± 146.08	4522.93 ± 9.83*
$AUMC_{last}$ (h*h*ng/mL)	40748.60 ± 130.47	41057.58 ± 658.64	33963.38 ± 828.17*
$AUMC_{inf}$ (h*h*ng/mL)	92029.19 ± 2261.89	91300.61 ± 6735.43	72620.63 ± 4236.49*
$T_{1/2}$ (h)	11.22 ± 0.18	10.84 ± 0.78	10.43 ± 0.48*
K_{el} (1/h)	0.06 ± 0.18	0.06 ± 0.00	0.07 ± 0.00
MRT (h)	9.82 ± 0.02	9.80 ± 0.06	9.61 ± 0.09*
CL (L/h)	0.06 ± 0.00	0.06 ± 0.00	0.07 ± 0.00*

Data expressed as Mean ± SD; *Significant when compared to gliclazide control; SDT: Single Dose Treatment; MDT: Multiple Dose Treatment.

It did not alter the onset of action (T_{max}) of gliclazide but significantly decreased the overall plasma exposure (AUC and AUMC) and peak concentration of gliclazide indicating decreased availability of gliclazide. This indicates that there might not be interaction at absorption levels as the T_{max} of gliclazide was not altered by repeated dose administration of forskolin. There was also significant decrease in MRT and increase in clearance of gliclazide suggesting that there might be potential interaction either in metabolism or excretion process. Results from the pharmacokinetic study suggest that forskolin might induce drug metabolising enzymes that are responsible for the metabolism of gliclazide thereby decreasing the serum levels of gliclazide. The changes in blood glucose levels, insulin levels in rats (normal and diabetic) and in rabbits might be due to combined effects of gliclazide and forskolin.

CONCLUSION

The study confirmed that the interaction of forskolin with gliclazide is pharmacokinetic in nature upon multiple dose treatments. Since the interaction was observed in two dissimilar species, it is likely to occur in humans resulting in decrease activity of gliclazide. Hence this combination needs dose adjustment and monitoring of glucose levels periodically when administered for their clinical benefits in obese/diabetic patients. However, further studies are necessary to determine the possibility of these interactions in clinic and also to determine the exact mechanism of action of such interactions.

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