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Influence of Mangiferin on the Pharmacodynamics and Pharmacokinetics of Gliclazide in Normal Rabbits

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A B S T R A C T

The drug interaction studies between Mangiferin and Gliclazide were conducted in normal rabbits by administering them individually and in combination. A dose of Gliclazide which produced 30-40 % reduction in blood glucose and Mangiferin given as single and multiple doses (10 doses) was chosen for the combination study. At designated time intervals, blood samples were collected and the serum glucose was estimated by GOD/POD method and serum Gliclazide was measured by High Performance Liquid chromatography. We observed significant interaction between Mangiferin (single dose or multiple doses) and Gliclazide in normal rabbits reflected through the elevated serum concentrations of Gliclazide and enhanced reduction in serum glucose. Hence, it is evident that Mangiferin alters the pharmacokinetics as well as pharmacodynamics of Gliclazide and caution must be exercised if combination therapy is used.

***Key words*:** Mangiferin, Gliclazide, Pharmacokinetics, Pharmacodynamics

Type II diabetes mellitus (T2DM) is a chronic disease characterized by persistent hyperglycemia, due to inefficient release or functioning of pancreatic hormone insulin and/or abnormal resistance to insulin. The treatment is aimed at bringing the blood glucose levels under control in order to avoid death and other long-term consequences such retinopathy, neuropathy, renal dysfunction, and non-traumatic limb amputations. As this disease is chronic in nature, it necessitate multiple drugs, which might lead to drug interactions.The use of multiple medications for treatment of single or multiple ailments is termed Polypharmacy, a prevalent practice across the globe. In this case, one drug may interact with another, resulting in issues with drug interactions.

Many medications (be it herbal or allopathic) can enhance or reduce the activity of various Cytochrome P450 (CYP) isozymes by inducing the biosynthesis of the isozyme (enzyme induction) or directly inhibiting the CYP activity (enzyme inhibition). Variations in CYP enzyme activity can alter the metabolism and clearance of many medicines, which makes it a major source for harmful drug interactions. For example, if one medication (herbal or allopathic) inhibits another drug's CYP-mediated metabolism, the second drug may build up to hazardous levels in the body. As a result of these drug interactions, dose changes or the use of therapeutic agents that do not interact with the CYP system may be necessary. Such medication interactions are especially important to consider when utilizing drugs which are critical to the patient's health, drugs with significant adverse effects, and drugs with narrow therapeutic windows, but any drug can have its plasma concentration altered due to changes in drug metabolism.

Herbal medicines are widely used as treatments for chronic disorders, and they play an important role in the health care of the great majority of the world's people. According to the World Health Organization, 4 billion people (or 80% of the world's population) rely on traditional medicine, which is primarily based on plant material (WHO, 1993). A total of 21,000 plants are utilized for therapeutic reasons around the world, according to the World Health Organization (WHO). There are around 400 plants available for the treatment of diabetes among them. The anti-diabetic properties of medicinal plants are due to the presence of phenolic chemicals, flavonoids, terpenoids, and coumarins. These components have been demonstrated to lower blood glucose levels. Pycnogenol, acarbose, miglitol, and voglibose are some examples of commercially available antidiabetic medicines derived from natural sources [1].

Although herbal remedies are beneficial in the treatment of a variety of ailments, they are frequently exploited and/or misused without scientific backing. As a result, in light of modern science, these plant medications warrant in-depth research.

As the use of plants becomes more widespread, interactions between medicines and allopathic pharmaceuticals must be addressed and thoroughly examined. When taken with

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prescription medicines, several herbal supplements can have potentially hazardous adverse effects. Alternative therapy is frequently not overseen by physicians, resulting in greater risk to patients, particularly when herbal and pharmaceutical medications with latent interactions are used. These Herbal medicines interact with conventional medicines in two ways: pharmacokinetically and pharmacodynamically. Absorption, distribution, metabolism, and elimination of the drug/natural medicine are all affected by pharmacokinetic interactions. These interactions change the amount of drug available to have an effect, either increasing or decreasing the amount of drug available. The interfering substance could serve as an inducer, inhibitor, or substrate of the cytochrome P-450 enzyme, which is responsible for drug metabolism. This is the most critical mechanism for herbal treatments and antiretroviral medicines to interact. Pharmacodynamic interactions have a qualitative impact on a drug's activity, either by increasing (synergistic or additive actions) or antagonizing (antagonistic) effects. Mangiferin, owing to its numerous biological activities, has the potential of being a candidate in nutraceutical and food supplement industries. It has proven merits in diseases like diabetes, cancer, cardiovascular disorders etc. due to its properties like immune boosting, anti-oxidant, neuroprotection etc. [2-4]. Due to its glucose lowering activity, its potential as an add-on therapy in diabetes for conventional anti-diabetic drugs can be investigated. Hence it becomes extremely important to prove the safety or drug-herb interaction potential for actual usage. In this study, the commonly used herbal isolate Mangiferin, was investigated for its interaction with gliclazide, a routinely prescribed second generation sulphonylurea derivative, used in diabetes mellitus.

MATERIALS AND METHODS

Gliclazide (Dr. Reddy's Labs, Hyderabad, Telangana), Diltiazem (Sun pharmaceuticals, Mumbai), Mangiferin (Laila Impex, Vijayawada, Andhra Pradesh), Acetonitrile (HPLC grade, Qualigens chemicals, Mumbai, India), Orthophosphoric acid (AR grade, SD fine chemicals, Mumbai).

Albino rabbits of either sex procured from Mahaveer Enterprises, Hyderabad, India were used in the study. They were maintained under standard laboratory conditions at ambient temperature of 25±2°C and 50±15% relative humidity with 12 hours light/12 hours dark cycle. Rabbits were fed with commercial pellet dietn (Rayan's Biotechnologies Pvt. Ltd, Hyderabad, India) and water ad libitum. The experimental protocol has been approved by the Institutional Animal Ethics Committee and by the regulatory body of the government. (Regd No.516/01/A/CPCSEA). Rabbits were fasted for 18 hours prior to the experiment, allowing access to water and during the experiment food and water were withdrawn.

Experimental design

Normal albino rabbits of either sex weighing between 1.35 to 1.55 kg were used in the study. The experiment was conducted in two stages.

Stage-I: Selection of optimal dose of Gliclazide

Rabbits were administered with vehicle control (water) and standard Gliclazide at three different doses (2.8, 5.6 and 11.2 mg/1.5kg bd.wt) and blood samples were collected at different time intervals and were analyzed for blood glucose.

Stage-II

The dose of Mangiferin that produced optimal reduction in blood glucose levels were evaluated for its influence on the pharmacodynamics (glucose levels) and pharmacokinetics of gliclazide.

Experimental design (Stage-I)

Group-1 (N=6): Water (Vehicle control)
Group-2 (N=6): Gliclazide 2.8 mg/1.5 kg bd.wt
Group-3 (N=6): Gliclazide 5.6 mg/1.5 kg bd.wt
Group-4 (N=6): Gliclazide 11.2 mg/1.5 kg bd.wt

After washout period of 7 days, stage-II experiments were performed using the same rabbits

Experimental design (Stage-II)

Group-1 (N=6): Mangiferin 100 mg/1.5 kg bd.wt

Group-2 (N=6): Gliclazide 5.6 mg/1.5 kg bd.wt

Group-3(N=6): Mangiferin 100 mg/1.5 kg bd.wt (Single dose: SD) +Gliclazide 5.6 mg/1.5 kg bd.wt

Group-4 (N=6): Mangiferin 100 mg/1.5 kg bd.wt (Multiple dose: MD) +Gliclazide 5.6 mg/1.5 kg bd.wt

Dosing and blood sampling

Gliclazide and/or Mangiferin were administered orally to respective groups. The blood samples from rabbits were collected at 0, 1, 2, 3, 4, 6, 8, 12, 16, 20, and 24 hour intervals from the marginal ear vein. The blood samples were centrifuged (4000 rpm for 12min) for serum separation. The glucose levels in blood samples were analyzed by GOD/POD method in semi-auto analyzer by the help of commercial kit (Coral Pvt. Ltd) [5]. The serum gliclazide concentrations were estimated by HPLC method [6]. Pharmacokinetic parameters were calculated using *Ramkin* software.

Data and statistical significance

Data was expressed as Mean± Standard Error Mean (SEM). The significance of the observed differences in pharmacokinetic parameters and percent blood glucose reduction of gliclazide between the drug treated rabbits (percent glucose reduction and pharmacokinetic parameters in normal rabbits) were assessed by student's paired t-test. Value of $P < 0.05$ is considered for statistical significance to find out the difference between the parameters of comparison.

RESULTS AND DISCUSSION

The study in normal rabbits was conducted to validate the existence/absence of interaction between selected herbal isolate, mangiferin and gliclazide in a non-rodent model. Rabbits were selected for the study because they are one of the official models for bioassay of insulin [7], can be easily maintained in the laboratory and sufficient quantities of blood samples can be collected by puncturing the marginal ear vein for estimating the blood glucose, and the standard drug at several intervals of time.

Gliclazide produced a dose dependent reduction in blood glucose levels with 2.8, 5.6 and 11.2 mg/1.5 Kg bd.wt. An optimal reduction (30-40%) in blood glucose was obtained with 5.6 mg/1.5 Kg bd. Wt. of gliclazide in normal rabbits and the same dose was selected for the interaction study. The mean percentage reduction in blood glucose at 2.8, 5.6 and 11.2 mg/1.5 Kg body weight was given in (Table 1, Fig 1).

Table 1 Dose effect relationship of gliclazide (2.8 mg, 5.6 mg and 11.2 mg/kg bd.wt.) in normal rabbits (N=6)

Time (h)	Mean Percentage reduction of blood glucose levels (%)		
	Gliclazide (2.8 mg/kg bd.wt.)	Gliclazide (5.6 mg/kg bd.wt.)	Gliclazide (11.2 mg/kg bd.wt.)
0	0.00±0.00	0.00±0.00	0.00±0.00
1	18.34±0.86	30.12±1.17	32.43±0.65
2	23.65±0.83	36.16±1.10	42.16±1.00
4	29.69±0.62	40.02±0.73	48.39±0.36
6	25.42±1.18	34.92±0.47	43.76±0.44
8	21.60±1.48	30.60±0.75	37.52±1.31
12	18.32±1.31	26.07±0.75	33.23±1.09
18	14.03±1.17	21.46±0.58	28.86±0.86
24	8.93±0.88	16.80±0.66	24.55±1.40

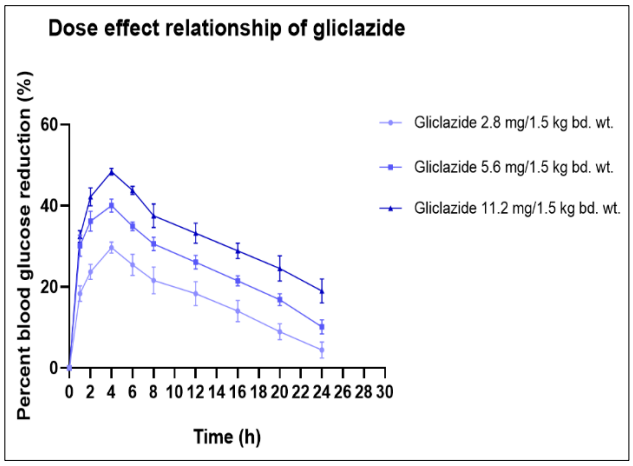

Fig 1 Dose effect relationship of gliclazide (N=6) in normal rabbits

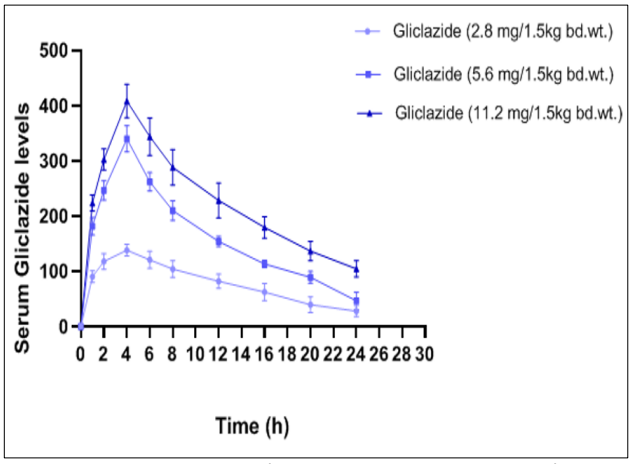

Fig 2 Serum Gliclazide (ng/mL) with (2.8, 5.6 & 11.2 mg/kg bd. Wt.) in normal rabbits

Table 2 Mean Serum gliclazide levels (ng/mL) with 2.8, 5.6 and 11.2 mg/1.5 kg body weight in normal rabbits

Time (h)	Mean serum gliclazide levels (Mean ± SEM)		
	2.8 mg/1.5 kg bd.wt.	5.6 mg/1.5 kg bd.wt.	11.2 mg/1.5 kg bd.wt.
0	0.00±0.00	0.00±0.00	0.00±0.00
1	90.18±4.63	181.53±7.07*	223.47±6.60*
2	117.78±6.37	246.50±7.88*	302.17±8.61*
4	137.94±4.72	339.79±10.63*	408.20±13.72*
6	120.78±6.99	262.05±7.48*	343.53±15.10*
8	104.08±6.74	209.93±7.91*	288.24±14.35*
12	82.07±5.80	153.81±4.25*	228.15±14.17*
16	62.16±6.86	113.09±3.08*	179.49±8.77*
20	39.24±6.45	88.94±4.84*	136.50±7.86*
24	27.68±4.57	46.64±7.10*	104.30±6.56*

p<0.05*Significance followed by student t test followed by Dunnet's multiple comparison test when compared with gliclazide alone group

Similarly, the mean serum Gliclazide levels in rabbits at 2.8, 5.6 and 11.2 mg/1.5 Kg body weight were given in (Table 2, Fig 2).

The selected dose of gliclazide (5.6 mg/1.5 Kg bd. wt) produced significant reduction in blood glucose levels and the peak serum gliclazide was found to be at 4 hr. Gliclazide was selected as prototype of sulphonylureas in the present study since it is widely used drug clinically. The dose of gliclazide human therapeutic dose extrapolated to rabbits basing on body surface area formula [8-9] and selected herbal isolate for interaction study (Mangiferin) was fixed based on the hypoglycaemic study conducted in normal rats.

In normal rabbits selected dose of mangiferin produced significant reduction in blood glucose levels, which might be due to its effect on the insulin sensitizing activity and also its effect on the glucose and fat metabolism [10-11]. When administered in combination the selected dose of mangiferin significantly enhanced the hypoglycemic activity of gliclazide from 1hr to 24 hr intervals with single and multiple dose treatments. The results are shown in (Table 3, Fig 3).

Table 3 Effect of single and multiple doses of Mangiferin on the hypoglycaemic activity of gliclazide in normal rabbits (N=6)

Time (h)	Mean Percentage blood glucose levels (%)			
	Mangiferin (100 mg/1.5 kg bd.wt.)	Gliclazide (5.6 mg/1.5 kg bd.wt.)	Gliclazide + Mangiferin (SD)	Gliclazide + Mangiferin (MD)
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
1	18.03±0.71	27.74±0.83	36.31±0.77*	39.77±0.53*
2	24.07±0.44	33.78±1.06	45.60±0.84*	50.25±0.35*
4	31.06±0.55	38.77±0.45	50.08±0.56*	53.42±0.63*
6	26.24±0.61	34.40±0.87	48.95±0.73*	55.26±0.55*
8	21.36±0.62	30.88±0.52	45.60±1.02*	54.57±0.74*
12	17.06±0.65	26.85±0.77	40.49±1.41*	53.02±1.22*
18	12.69±0.57	21.85±0.94	35.32±1.84*	48.85±1.66*
24	9.65±0.59	17.36±0.96	30.55±1.68*	43.68±1.77*

p<0.05* Significance followed by student t test followed by Dunnet's multiple comparison test when compared with gliclazide alone group

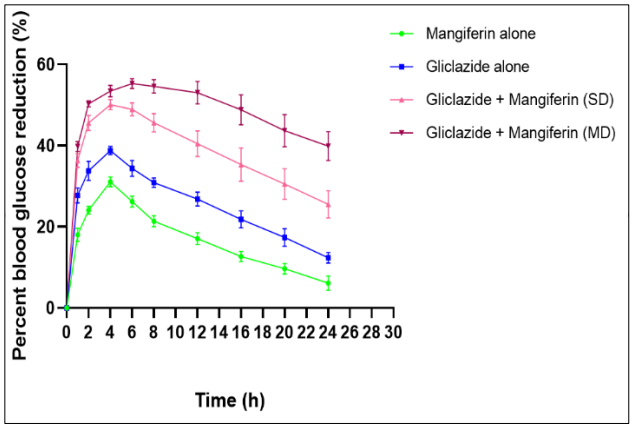

Fig 3 Effect of single and multiple doses of mangiferin on the hypoglycaemic effect of Gliclazide

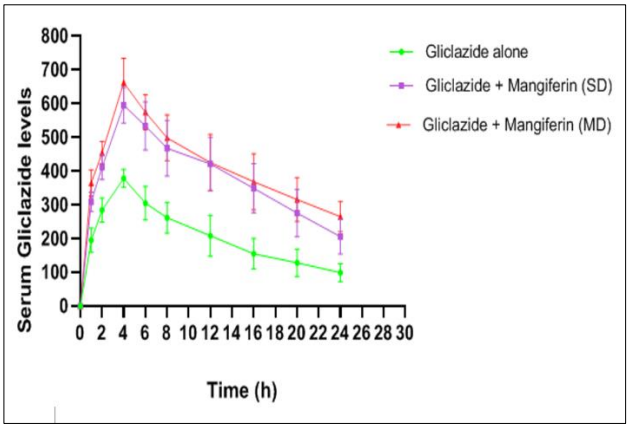

Fig 4 Serum gliclazide levels (ng/mL) with gliclazide and in combination with single and multiple doses of mangiferin in normal rabbits

The serum gliclazide levels were found to be enhanced significantly with single and multiple dose treatments of mangiferin from 2 hr to 24 hr. The results are shown in (Table 4, Fig 4).

Table 4 Mean serum gliclazide levels (ng/mL) with gliclazide alone and in combination with single and multiple doses of mangiferin in normal rabbits

Time (h)	Mean serum gliclazide levels (Mean ± SEM)		
	Gliclazide alone	Gliclazide+ mangiferin (SD)	Gliclazide+ mangiferin (MD)
0	0.00±0.00	0.00±0.00	0.00±0.00
1	195.23±16.11	308.70±13.01*	364.41±17.54*
2	284.64±16.31	411.56±16.32*	454.46±14.70*
4	378.59±12.13	595.03±23.46*	661.80±32.45*
6	304.83±22.45	533.57±31.90*	574.33±23.39*
8	261.73±20.17	467.94±36.75*	498.34±30.47*
12	208.24±27.19	421.14±35.50*	425.25±37.49*
16	154.80±20.40	349.28±32.56*	368.63±37.02*
20	128.39±17.84	275.87±31.50*	315.90±28.97*
24	98.98±11.79	205.70±23.04*	264.94±20.10*

p<0.05* Significance followed by student t test followed by Dunnet's multiple comparison test when compared with gliclazide alone group

The enhanced serum gliclazide levels in the presence mangiferin might be due to inhibition of metabolism of gliclazide by mangiferin as mangiferin is reported inhibit the CYP3A4 isozyme and the same enzyme is involved in the metabolism of gliclazide [12-13]. The mean Pharmacokinetic Parameters with Gliclazide alone and in combination with single and multiple doses of Mangiferin were shown in (Table 5).

There is significant increase in the pharmacokinetic parameters of Gliclazide like $AUC_{0-\infty}$, AUC_{0-t}, Kel, $T_{1/2}$, C_{max} and MRT and there is decrease in clearance (Cl) with single and multiple dose treatment of mangiferin. The enhancement in the serum gliclazide levels and pharmacokinetic parameters like AUC, $T_{1/2}$ and decrease in clearance indicate that there is a metabolic interaction between mangiferin and gliclazide. The interaction may be due to inhibition of gliclazide metabolism in the presence of mangiferin, since mangiferin reported to inhibit the CYP 3A4 isozyme [13]. The similar type of results were observed in rat study as well. Since the results and the pattern of the interacting selected drugs Mangiferin and gliclazide was found to be same in two dissimilar species, similar results might be possible in clinical situation also.

Table 5 Mean pharmacokinetic parameters of gliclazide alone and in combination with single and multiple doses of Mangiferin in normal rabbits

Pharmacokinetic parameter	Mean Serum Gliclazide levels (Mean ± SEM)		
	Gliclazide alone	Gliclazide+ Mangiferin (SD)	Gliclazide+ Mangiferin (MD)
C max (ng/ml)	350.53±27.78	513.55±19.37*	502.29±12.34*
T max (h)	4.00±0.00	4.00±0.00	4.00±0.00
AUC_{0-24} (ng/ml/hr)	4379.66±219.47	7741.31±262.64*	8317.99±186.76*
$AUC_{0-\alpha}$ (ng /ml/hr)	5638.66±328.15	11263.98±633.05*	14162.49±1154.12*
t ½ (h)	9.73±0.57	11.63±0.98*	16.46±1.86*
Clearance (ml/hr/kg)	1045.61±56.59	534.41±39.88*	422.93±30.42*
Vd SS (ml/kg)	16388.98±1004.82	10699.65±480.07*	11661.78±693.37*
MRT (h)	16.16±1.12	22.34±1.91*	28.39±2.48*

p<0.05* Significance followed by student t test followed by Dunnet's multiple comparison test when compared with gliclazide alone group

CONCLUSION

Significant interaction was observed between Mangiferin and Gliclazide in normal rabbits at the tested doses. This could be categorized as herb-drug interaction. The similar scenario may be observed in humans as well. Hence, caution must be exercised when the combination therapy is used.

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