

Research Article



Influence of *AyurSlim* Capsules on the Pharmacodynamics and Pharmacokinetics of Gliclazide in Rats and Rabbits

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ABSTRACT

Type 2 diabetes mellitus (T2DM) and obesity are clearly interconnected. Overweight and obesity have been estimated to account for around 65-80 percent of new cases of T2DM. A recent report suggested that obese people are up to 80 times more likely to develop T2DM than those with a body mass index (BMI) of less than 22. Poly pharmacy is very common practice in diabetic patients; the study of drug-drug interactions is an imperative rational approach with respect to safety and efficacy determination. *AyurSlim* capsules (a herbal formulation) have a favorable effect on weight reduction and lipid profiles. Gliclazide is a widely used drug for the treatment of type 2 diabetes. The objective of this study was to investigate the effect of oral administration of *AyurSlim* capsules (ASC) on the Pharmacodynamics and pharmacokinetics of Gliclazide in rats (normal and diabetic) and normal rabbits to evaluate the safety and effectiveness of the combinations. Blood-glucose levels were estimated by GOD/POD method using commercial glucose kits. The serum-Gliclazide levels were estimated by HPLC method. Diabetes was induced in rats by intraperitoneal administration of alloxan monohydrate (100 mg/kg). Gliclazide produced hypoglycemia in normal and diabetic rats with peak activity at 1h and 6h, and in rabbits at 3 h. ASC produced hypoglycemia when given alone and prolonged the effect of Gliclazide in combination during 1-12h in normal and diabetic rats and during 2-8h in normal rabbits without hypoglycemic convulsions. The serum levels and pharmacokinetics of Gliclazide were decreased in the presence of ASC. The study indicated that the combination can be used safely to obtain prolonged and sustained antidiabetic effect.

Keywords: *AyurSlim* capsules, Gliclazide, Herb-Drug interactions, Hypoglycemia, Pharmacokinetics, Pharmacodynamics.

INTRODUCTION

Obesity or overweight is a global health problem and a rate of obesity has risen steadily year on year. Obesity is not just a cosmetic or lifestyle illness but leads to many life threatening health complications such as cardiovascular disease, stroke, high blood pressure, abnormal blood cholesterol, Type 2 Diabetes Mellitus (T2DM), cancer, osteoarthritis, sleep apnea, depression, and chronic pain.^{1,2} T2DM is a chronic metabolic disorder characterized by sustained hyperglycemia. The influence of obesity on T2DM risk is determined by the degree of obesity and also by where fat accumulates. Increased upper body fat, including visceral adiposity as reflected in increased abdominal girth or waist-to-hip ratio, is associated with the metabolic syndrome, T2DM, and cardiovascular disease.³ The term diabetic dyslipidemia has been used to describe the pathophysiology surrounding the effects of insulin resistance on abnormal lipid levels. Dyslipidemia affects a diabetic patient to a greater extent than a non-diabetic patient, further reinforcing the severity of diabetic dyslipidemia.⁴

Sulfonyl ureas are oral hypoglycemic agents have been used successfully for treatment of T2DM and useful for islet cells function in those patients whose dietary and lifestyle modifications have proven to be insufficient. Among sulphonyl ureas, the most commonly prescribed drug is Gliclazide, since it has high potency, prolonged action and lower incidence of side effects and anti

oxidant activity.⁵ Only one anti-obesity medication orlistat is currently approved by the Food and Drug Administration (FDA) for long term use. Orlistat is a gastric and pancreatic lipase inhibitor that reduces dietary fat absorption by around 30%. The main treatment modalities for overweight and obese individuals remain dieting and physical exercise.^{6,7} Sibutramine, an anorectic agent has recently been withdrawn from the market due to excessive cardiovascular events.⁸

Traditional herbal medicines may have some potential in managing obesity. *AyurSlim* (a herbal preparation) is a weight reduction capsule that is manufactured by Himalaya Health Care, Bangalore, India. The efficacy and tolerability of *AyurSlim* Capsules (ASC) were investigated in thirty-two obese patients of either sex by Singh *et al.* (2008).⁹ Results revealed that ASC are effective in weight reduction in all the patients. Therefore there is a possibility for concomitant administration of Gliclazide and ASC in patients with diabetes associated with obesity. This may lead to herb-drug interaction problem with an altered Gliclazide activity which is unwanted. However, to the best of our knowledge, no evidence exists so far concerning the influence of ASC on the Pharmacodynamics and pharmacokinetics of Gliclazide. The objective of this study was to evaluate the influence of ASC on the Pharmacodynamics of Gliclazide in rats and Pharmacodynamics and pharmacokinetics of Gliclazide in



rabbits to understand the mechanism of interaction if it exists.

MATERIALS AND METHODS

Drugs and chemicals

Alloxan was purchased from Sigma-Aldrich (St. Louis, USA). AyurSlim capsules were obtained from Himalaya Health Care (Bangalore, India). Gliclazide and Isosorbide dinitrate (ISDN) are the gift samples from Wockhardt (Aurangabad, India) and Sun Pharmaceuticals (Mumbai, India), respectively. Acetonitrile (HPLC grade) was obtained from Qualigens chemicals, Mumbai, India. Orthophosphoric acid (AR grade) and dichloromethane (AR grade) were purchased from SD fine chemicals (Mumbai, India) and Loba chemie pvt. Ltd. (Mumbai, India), respectively. Glucose kits (Span diagnostics, Surat, India) were purchased from local pharmacy. All other reagents or chemicals used were of analytical grade.

Experimental animals

Rats

Male Albino rats (250-330 g) obtained from M/S. Mahaveer Enterprises, Hyderabad, India, were used in the study. They were maintained on standard pellet diet and water *ad libitum*. Animals were fasted for 18 h before the experiment and both water and food were withdrawn during the experiment.

Rabbits

Normal albino rabbits of either sex of 3 months of age, weighing between 1.5-3 Kg were procured from National Institute of Nutrition, Hyderabad, India. Animals were fed with a commercial pellet diet (Rayan's Biotechnologies Pvt Ltd., Hyderabad, India) and water *ad libitum*. The animal experiments were performed after prior approval of the study protocol by the Institutional Animal Ethics Committee. The animals were maintained under standard laboratory conditions and the study was conducted in accordance with the guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Experimental protocol

Selection of doses and preparation of oral test solution/suspension

In clinical practice, Gliclazide in a therapeutic dose will be administered orally as an antidiabetic agent. Human oral therapeutic doses of Gliclazide and ASC were extrapolated to rat/rabbit based on body surface area.¹⁰ AyurSlim capsule suspension (ASS) was prepared by dissolving in water. Gliclazide test solution was prepared by dissolving it in a few drops of 0.1 N NaOH then made up to the volume with distilled water. All the drugs were administered to the respective groups by oral gavage.

Influence of ASS on the hypoglycemic activity of Gliclazide in normal rats

Rats were divided into five groups each consisting of six animals. The dose of 0.5, 1, and 2 mg/kg of Gliclazide was administered to Group I, II, and III respectively. The Gliclazide induced hypoglycaemia was studied by administering 0.5 mg/kg, 1mg/kg, and 2 mg/kg to different groups of normal albino rats to find out the dose response relationship. After establishing the dose response effect, the sub-therapeutic dose of Gliclazide (1 mg/kg) was selected and used for studying the interaction in all sets of experiment. Group IV and V were treated with ASS (81 mg/kg) and combination of ASS and Gliclazide (1 mg/kg), respectively. Blood samples were withdrawn at 0, 1, 2, 3, 4, 6, 8, 10, and 12 h by retro-orbital puncture and analyzed for blood glucose by GOD/POD method using semi autoanalyser (Screen Master 3000, Mumbai, India).¹¹

Influence of ASS on the anti hyperglycemic activity of Gliclazide in diabetic rats

Induction of diabetes:

Diabetes was induced by intraperitoneal injection of alloxan (100 mg/kg) to rats.¹² Albino rats were fasted for 18h before alloxan injection. Since alloxan is capable of producing fatal hypoglycemia as a result of massive insulin release from the pancreas, animals were treated with 10% dextrose orally to combat the immediate hypoglycemia. Blood sugar was measured after 24-48 h of alloxan treatment to evaluate induction of diabetes. Rats with plasma glucose levels above 250 mg/dL were used for this experiment. Diabetic rats were divided into three groups each consisting of 6 animals.

Group I: treated with Gliclazide (1 mg/kg, p.o)

Group II: treated with ASS (81 mg/kg, p.o)

Group III: treated with Gliclazide (1 mg/kg, p.o) and ASS (81 mg/kg, p.o)

Blood samples were withdrawn from the retro-orbital plexus at 0, 1, 2, 3, 4, 6, 8, 10, and 12 h analyzed for blood glucose by GOD/POD method¹¹ using semi autoanalyser (Screen Master 3000, Mumbai, India).

Influence of ASS on Pharmacodynamics and pharmacokinetics of Gliclazide in normal rabbits

A group of total nine healthy albino rabbits of either sex weighing between 1.5-3 kg were used in the study. The rabbits were fasted for 18h prior to the experiment with water *ad libitum* and water was withdrawn during the experiment. Rabbits were divided into three groups each consisting of three animals. Gliclazide was administered orally to all the rabbits. After a wash out period of one week the same group of animals was administered with ASS (81mg/kg) orally. Again after a further washout period of one week, the same group was administered with ASS (81mg/kg) orally, 30 min prior to the administration of Gliclazide (1 mg/kg). Blood samples



were withdrawn at 0, 1, 2, 3, 4, 6, 8, 12, 16, 20 and 24 h intervals from marginal ear vein and were analyzed for blood glucose by GOD/POD method and serum Gliclazide by HPLC.¹² The pharmacokinetic parameters of Gliclazide were determined on subjecting the concentration-time data to non-compartmental analysis using WinNonlin (version 5.0.1) software.

Table 1: Effect of Gliclazide (0.5, 1, and 2 mg/kg), *AyurSlim* capsules (81 mg/kg) and their combination on the blood glucose levels (mg/dL) of normal rats (n=6)

Time (h)	GCZ (0.5mg/kg)	GCZ (1mg/kg)	GCZ (2 mg/kg)	ASS (81mg/kg)	GCZ (1mg/kg) + ASS (81mg/kg)
0	103.5 ± 1.28	90.83 ± 2.89	102 ± 1.16	88.50 ± 2.88	90.8 ± 2.89
1	71.66 ± 3.03	53.50 ± 3.80**	53.50 ± 2.43**	82.66 ± 2.49	53.5 ± 3.80*
2	76.50 ± 3.45	55.50 ± 3.71**	63.16 ± 2.42**	67.50 ± 2.24	44.2 ± 1.31**
3	85.83 ± 2.48	63.16 ± 3.63*	75.50 ± 2.89	55.00 ± 1.87*	52.6 ± 0.78*
4	79.50 ± 1.04	70.33 ± 5.29	76.16 ± 2.02	65.83 ± 2.28	62.5 ± 1.05*
6	72.67 ± 1.31	58.50 ± 4.10**	53.50 ± 2.62**	71.16 ± 2.02	46.8 ± 1.25**
8	76.33 ± 1.71	69.83 ± 2.25	67.50 ± 1.37*	74.83 ± 2.18	69.8 ± 2.25
10	81.16 ± 1.75	77.00 ± 2.81	72.33 ± 4.39	82.33 ± 2.52	77 ± 2.82
12	86.33 ± 2.22	85.50 ± 3.00	76.33 ± 3.57	89.66 ± 2.70	85.5 ± 3.00

All values are expressed as Mean ± SEM. ** $p < 0.01$, * $p < 0.05$ when compared to control group; (student's paired t-test). GCZ, Gliclazide; ASS, *AyurSlim* suspension (ASS).

Table 2: Effect of Gliclazide (1 mg/kg), *AyurSlim* capsules (81 mg/kg) capsules and their combination on the blood glucose levels (mg/dL) in alloxan induced diabetic rats (n=6)

Time (h)	GCZ (1mg/kg)	ASS (81mg/kg)	GCZ (1mg/kg) + ASS (81mg/kg)
0	293 ± 3.4	354.7 ± 3.3	351 ± 2.52
1	178 ± 2.6**	260.8 ± 3.7	142 ± 9.05
2	224 ± 3.5	192.16 ± 3.1	107 ± 8.33***
3	243 ± 2.1	144.2 ± 3.2**	89 ± 1.73***
4	264 ± 3.9	204.7 ± 3.2	152 ± 2.47**
6	186 ± 3.3**	249.2 ± 2.8	179 ± 14.9*
8	235 ± 3.5	293 ± 3.6	279 ± 3.64
10	249 ± 4.3	336.8 ± 4.5	302 ± 1.78
12	282 ± 3.9	357.5 ± 3.7	348 ± 1.66

All values are expressed as Mean ± SEM. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ when compared to control group; (student's paired t-test). GCZ, Gliclazide; ASS, *AyurSlim* suspension (ASS).

RESULTS

Influence of ASS on the hypoglycemic activity of Gliclazide in normal rats

Table 1 Shows the blood glucose reduction following oral administration of Gliclazide (0.5, 1, and 2 mg/kg) and ASS (81 mg/kg) and their combination in normal rats. The mean percent blood glucose reductions of Gliclazide were 30.80 ± 2.46 (1h) and 29.80 ± 0.74 (6h) with 0.5 mg/kg; 40.80 ± 4.55 (1h) and 35.50 ± 4.48 (6h) with 1 mg/kg and 47.40 ± 2.86 (1h) and 47.6 ± 2.39 (6h) with 2 mg/kg were shown in Fig. 1. The blood glucose levels were normal after 12 h. The dose of Gliclazide was selected for the

Data and Statistical analysis

Data was expressed as Mean ± Standard Error Mean (SEM). The significance was determined by applying students paired 't' test.

interaction study is 1mg/kg and ASS has significant effect on the blood glucose levels in normal rats. The combination of ASS (81mg/kg) and Gliclazide (1mg/kg) produced 59.81 ± 1.88 (3h) and 48.17 ± 2.43 (6h) in mean percent blood glucose reduction, while Gliclazide matching control produced 40.80 ± 4.55 (1h) and 35.50 ± 4.48 (6h) in mean percent blood glucose reduction (Figure 2).

Table 3: Effect of Gliclazide (5.6 mg/1.5 kg), *AyurSlim* capsules (81 mg/kg) capsules and their combination on the blood glucose levels (mg/dL) in normal rabbits (n=6)

Time (h)	GCZ (1mg/kg)	ASS (81mg/kg)	GCZ (1mg/kg) + ASS (81mg/kg)
0	94.7 ± 2.69	101 ± 1.62	73.14 ± 3.45
1	80.2 ± 3.0	84.8 ± 1.61	14.8 ± 1.01***
2	75.0 ± 2.42	69.5 ± 0.79	35.2 ± 2.13*
3	59.0 ± 1.72**	55.5 ± 1.12**	53.9 ± 1.75
4	65.7 ± 1.87*	64.0 ± 1.83*	41.3 ± 1.21
6	74.2 ± 2.46	72.5 ± 1.09	33 ± 1.15**
8	79.3 ± 2.77	82.0 ± 0.98	25.8 ± 1.76**
12	83.7 ± 2.71	90.8 ± 0.77	8.55 ± 1.92***
16	89.0 ± 1.96	96.5 ± 0.88	4.22 ± 1.79***
24	92.3 ± 2.31	104.0 ± 1.56	-0.77 ± 2.14

All values are expressed as Mean ± SEM. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ when compared to control group; (student's paired t-test). GCZ, Gliclazide; ASS, *AyurSlim* suspension (ASS).

Influence of ASS on the antihyperglycemic activity of Gliclazide in diabetic rats

The effects of Ayurslim capsules on anti hyperglycemic effect of Gliclazide in diabetic rats were found to be similar to that of the normal rats but with pronounced



effect. The blood glucose reductions with Gliclazide before and after treatment with ASS in diabetic rats were shown in Table 2. The percent blood glucose reduction were 39.68 ± 0.73 (1h) and 36.72 ± 0.46 (6h) with Gliclazide (1 mg/kg) and 78.00 ± 0.56 (4h) and 49.10 ± 4.42 (6hr) with combination of ASS and Gliclazide are shown in Fig. 3. As seen in normal rats, ASS reduced the blood glucose in diabetic rats and enhanced the anti hyperglycemic effect of Gliclazide. The results indicated the existence of interaction between ASC and Gliclazide in normal and diabetic condition in albino rats. It is also clear that since ASC alter blood glucose levels on its own, the increase in the effect of Gliclazide on blood glucose level may be due to improved activity of Gliclazide (or) improved blood Gliclazide levels. So the interaction between Gliclazide and ASC may be due to improved pharmacodynamic activity of Gliclazide (or) increased blood levels of Gliclazide (pharmacokinetic activity).

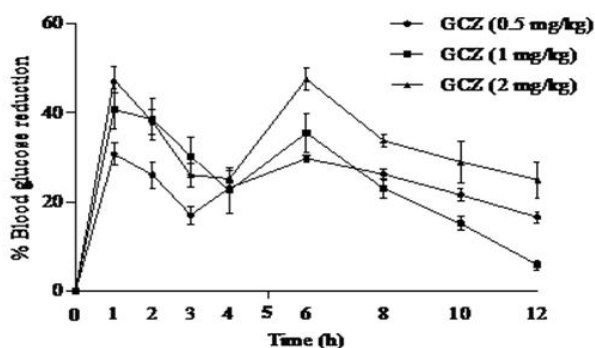


Figure 1: Percent blood glucose reduction after oral administration of Gliclazide (0.5, 1 and 2 mg/kg) in normal rats (n=6). GCZ, Gliclazide.

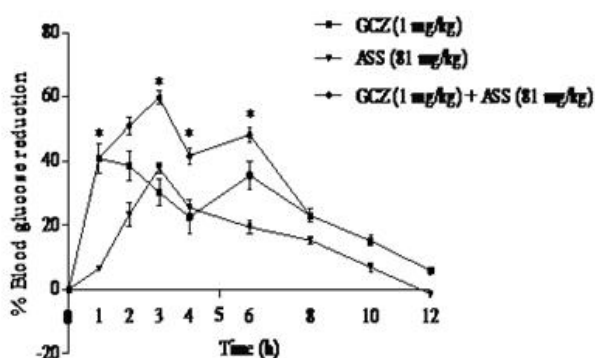


Figure 2: Percent Blood glucose reduction after oral administration of Gliclazide (1mg/kg), ASS (81mg/kg) and their combination in normal rats (n=6). GCZ, Gliclazide; ASS, AyurSlim suspension. * $P < 0.01$ when compared to GCZ control; students paired 't' test.

Influence of ASS on Pharmacodynamics and pharmacokinetics of Gliclazide in normal rabbits

Gliclazide and ASS produced hypoglycemic activity with maximum reduction of $37.2 \pm 1.2\%$ and $45.2 \pm 1.52\%$, respectively at 3 h in normal rabbits are shown in Fig. 4. ASS enhanced hypoglycemic effect of Gliclazide with

maximum reduction of $53.9 \pm 1.75\%$ in the blood glucose in normal rabbits at 3 h following oral administration are depicted in Table 3. The serum Gliclazide levels were decreased when co-administered with ASS but statistically not significant (Fig. 5). The pharmacokinetic parameters of Gliclazide like the maximum plasma concentration (C_{max}), area under the curve from zero to 24 h (AUC_{0-24}), mean residence time (MRT), Volume of distribution at steady state (V_{dss}), Clearance (CL/F), and absorption rate constant (K_a) were did not alter significant with ASS (Table 4). ASS also reduced the area under the curve from zero to infinity ($AUC_{0-\infty}$), area under the movement curve from zero to 24 h ($AUMC_{0-24}$), and area under the movement curve from zero to infinity ($AUMC_{0-\infty}$) ($P < 0.05$).

Table 4: Pharmacokinetic parameters of Gliclazide after the oral administration of Gliclazide (5.6 mg/1.5 kg) to rabbits in the presence or absence of ASS (81 mg/kg)

Parameter	GCZ (5.6 mg/1.5 kg)	GCZ (5.6 mg/1.5 kg) + ASS (81mg/kg)
C_{max} (ng/mL)	364.86 ± 8.82	344 ± 6.091^{NS}
T_{max} (h)	3 ± 0	3 ± 0
AUC_{0-24} (ng. h/mL)	3766.1 ± 43.9	3447 ± 64.54^{NS}
$AUC_{0-\infty}$ (ng. h/mL)	4593.4 ± 89.6	$4102 \pm 97.844^*$
$AUMC_{0-24}$ (ng.h ² /mL)	41542 ± 1558	$35694 \pm 1401.9^*$
$AUMC_{0-\infty}$ (ng.h ² /mL)	72190 ± 4461	$59215 \pm 3385.3^*$
K_{el} (h ⁻¹)	0.0778 ± 0	$0.103 \pm 0.004^*$
K_a	1.53 ± 0	1.5 ± 0^{NS}
$T_{1/2}$ (h)	8.9233 ± 0.31	$8.022 \pm 0.28^*$
MRT (h)	15.66 ± 0.68	14.39 ± 0.56^{NS}
V_{dss}	12.168 ± 0.35	12.26 ± 0.53^{NS}
CL/F (mL/h)	813.32 ± 15.9	911.3 ± 22.2^{NS}

All values are Mean \pm SEM. * $p < 0.05$, ^{NS} $p > 0.05$ when compared to Gliclazide control group.

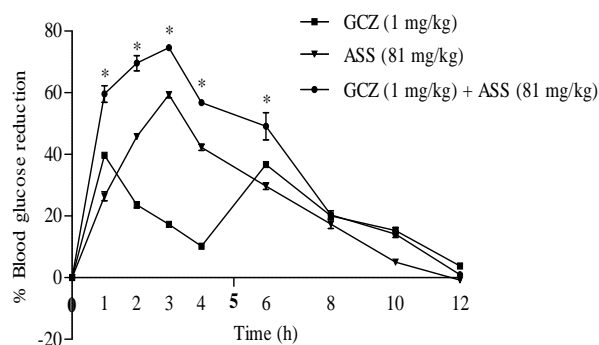


Figure 3: Percent Blood glucose reduction after oral administration of Gliclazide (1mg/kg), ASS (81mg/kg) and their combination in diabetic rats (n=6). GCZ, Gliclazide; ASS, AyurSlim suspension. * $P < 0.01$ when compared to GCZ control; students paired 't' test.

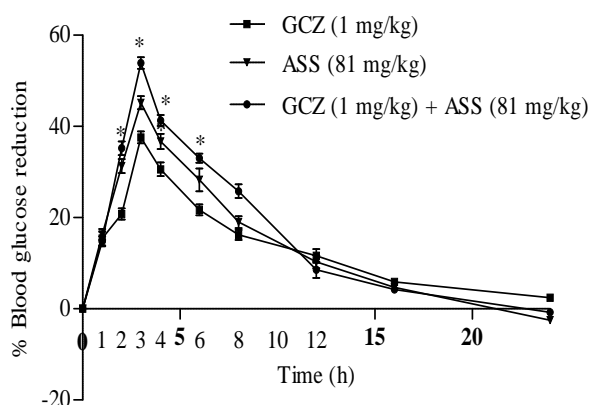


Figure 4: Percent Blood glucose reduction after oral administration of Gliclazide (5.6 mg/1.5kg), ASS (81mg/kg) and their combination in normal rabbits (n=3). GCZ, Gliclazide; ASS, AyurSlim suspension. * $P < 0.01$ when compared to GCZ control; students paired 't' test.

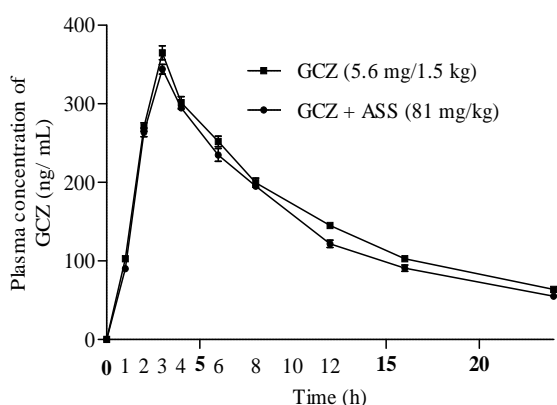


Figure 5: Mean plasma concentration–time profiles of Gliclazide (5.6 mg/1.5 kg) following an oral administration of Gliclazide to rats with or without ASS (n=3). All values are Mean \pm SEM. Bars represent the standard deviation. ASS, AyurSlim suspension; GCZ, Gliclazide.

DISCUSSION

The pharmacological treatment of hyperglycemia in T2DM is usually initiated with oral antidiabetic drug monotherapy and when the disease progresses, combination therapy with different oral antidiabetic, or parenteral therapy with insulin or glucagon-like peptide-1 (GLP-1) receptor agonists, may be needed.¹³ The ideal anti-obesity drug would produce sustained weight loss with minimal side effects. A number of agents have been evaluated for their efficacy and safety in the management of obesity. Amphetamines have a well defined abuse potential and drugs like diethylpropion and fenfluramine cause significant undesirable symptoms related to depression and pulmonary hypertension. There is need of safer and effective drug to manage obesity. Recently a herbal anti-obesity formulation (AyurSlim capsules) has been made available. Drug interactions are usually seen in clinical practice, and the mechanisms of interactions are evaluated usually in animal models (rodent and non-rodent).

Identification of drug-drug interactions (DDIs) of oral antidiabetics has often been initiated by case reports observing reduced or increased pharmacodynamic effects. Altered concentrations or effects of sulfonyl ureas due to concomitant medications have already been reported more than 30 years ago, although the exact mechanisms were largely unrecognized at that time. Today, on the basis of both in vitro data and data on the pharmacogenetics and DDIs of oral antidiabetics, the main cytochrome P450 (CYP) enzymes, including CYP2C8, CYP2C9 and CYP3A4, mediating their metabolism have been identified. Furthermore, the roles of several transporters, such as organic anion transporting polypeptide 1B1 (OATP1B1) and P-glycoprotein (P-gp), in the disposition of some of these drugs have been characterized. This forms a basis for the increased understanding of many clinically important DDIs affecting the pharmacokinetics of oral antidiabetics. Inhibition and induction of CYP enzymes and transporters are the main mechanisms of pharmacokinetic DDIs affecting oral antidiabetic agents. Both CYP2C9 and CYP2C19 can contribute to the metabolism of Gliclazide but no DDI studies in humans have been published with Gliclazide and inhibitors of these enzymes.¹⁴

Drug interactions are usually seen in clinical practice, and the mechanisms of interactions are evaluated usually in animal models (rodent and non-rodent). We studied the influence of AyurSlim capsules on the Pharmacodynamics and pharmacokinetics of Gliclazide in rats (rodents) and rabbits (non-rodent). The normal rat model served to quickly identify the interaction and the diabetic rat model served to validate the same response in the actually used condition of the drug. The rabbit model is another dissimilar species to validate the occurrence of the interaction.

Gliclazide produced hypoglycemia/antihyperglycemia in normal/diabetic rats, respectively, with peak activity at 1h and 6h, and in rabbits at 3 h. Gliclazide is metabolized to several metabolites by hepatic cytochrome P450 3A4 and 2C9 isozymes and is eliminated in urine. A part of Gliclazide is eliminated through the biliary route, which involves enterohepatic circulation in rats^{15, 16} and in humans.¹⁷ The re-absorption of Gliclazide eliminated through the biliary route might be responsible for a second peak in its hypoglycemic effect in rats. The absence of a second peak effect in rabbits indicates that it might not involve biliary excretion, and maybe excreted by the renal route only.

Since the AyurSlim capsules did not alter the pattern of double-peak effect in rats and single-peak effect in rabbit, it does not seem to interfere with the metabolic pattern of Gliclazide in both species. However, the elimination kinetics of Gliclazide in rabbits was found to be altered in the presence of the ASS, since $T_{1/2}$ and AUC were decreased. The relatively rapid elimination of Gliclazide in rabbits in the presence of the ASS might be due to the change in the distribution coupled with renal elimination.

The ASS might not have influenced biliary excretion of Gliclazide, since double-peak effects were found in the rat model in the presence, as well as absence, of the extract.

The influence of the single-dose effect of the ASS on the pharmacokinetics and hypoglycemic effect of Gliclazide was studied in normal rabbits to identify the main mechanisms involved in the interaction. When administered alone, Gliclazide produced hypoglycemia/antihyperglycemia in normal/diabetic rats with peak activity at 1 and 6h, and in normal rabbits at 3h. The ASS produced significant hypoglycemia when administered alone, and prolonged the effect of Gliclazide by 1-12h in normal and diabetic rats, and by 2-8h in normal rabbits, without hypoglycemic convulsions. ASS produced hypoglycemia/antihyperglycemia and its influence in Gliclazide activity in rats/rabbits may be caused by its insulin-secreting action. In rabbits, relatively rapid elimination of Gliclazide is expected to reduce its response. However, since Gliclazide and the extract possessed hypoglycemic activity, the combined effect appears to overcome the effect of relatively rapid elimination of Gliclazide. The observed interaction appears to be mainly pharmacodynamic, and partly pharmacokinetic, in nature. It is well established that Gliclazide (sulphonylurea derivative) produces hypoglycemia by pancreatic (insulin release) and extra pancreatic (increase in glucose uptake) mechanisms. The hypoglycemic/antihyperglycemic effect of ASS might be due to its effect on insulin release. The enhanced effect of Gliclazide in the presence of ASS might be due to the combined effect of the two drugs on insulin release. However, no convulsions were seen in rats or rabbits, even at peak hours of activity. Hence, their combination need not be contraindicated.

CONCLUSION

The interaction appears to be pharmacodynamic. Since the interaction was seen in two dissimilar species, it is likely to occur in humans also leading to increased activity of Gliclazide, which may need dosage adjustment. Hence, care should be taken when the combination is prescribed for clinical benefit in diabetic patients. Further studies are needed to establish its long-term safety in animals and humans.

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REFERENCES

- Ronald TA, Emily AF, Karen KS, Helena MH, Laura MH, David GM, Chandan S, Rationale, design, and baseline characteristics of a community-based comparative effectiveness trial to prevent type 2 diabetes in economically disadvantaged adults: The RAPID Study, *Contemporary Clinical Trials*, 37 (1), 2014, 1-9.
- Nicholas AM, Bruce JS, Peter RG, Matthew DS, From preclinical to clinical development: The example of a novel treatment for obesity, *Neurobiology of Disease*, 61, 2014, 47-54.
- Gul B, Glucose homeostasis, obesity and diabetes, *Best Practice & Research Clinical Obstetrics and Gynaecology*, 27(5), 2013, 715-726.
- David S, Paul Z, Diabetes and hyperlipidemia: A direct quantitative analysis, *World Journal of Cardiovascular Diseases*, 2, 2012, 20-25.
- Gillian MS, Drug interactions with oral hypoglycaemic drugs, *Aust. Prescr*, 24, 2001, 83-85.
- Hauptman JB, Jeunet FS, Hartmann D, Initial studies in humans with the novel gastrointestinal lipase inhibitor Ro 18-0647 (tetrahydropipstatin), *The American Journal of Clinical Nutrition*, 55, 1992, 309S-313S.
- Kyoung KK, Heuy SS, In CH, Ki DK, Influence of eating behaviors on short-term weight loss by orlistat and anorectic agent, *Eating Behaviors*, 15 (1), 2014, 87-90.
- James WP, Caterson ID, Coutinho W, Finer N, Van Gaal LF, Maggioni AP, Effect of sibutramine on cardiovascular outcomes in overweight and obese subjects, *The New England Journal of Medicine*, 363 (10), 2010, 905-917.
- Singh AK, Pralhad SP, Mitra SK, Evaluation of clinical efficacy of AyurSlim on body weight, body mass index, lipid profile and skin fold thickness: A Phase IV clinical trial, *The Antiseptic*, 105, 2008, 241-243.
- Lawrence DR, Bacharach AL, Evaluation of drug activities, *Pharmacometrics*, vol. I, New York, Academic Press, 1964.
- Trinder P, Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor, *J Clin Pathol*, 22(2), 1969, 158-161.
- Mastan SK, Eswar KK, Influence of atazanavir on the Pharmacodynamics and pharmacokinetics of Gliclazide in animal models, *International Journal of Diabetes Mellitus*, 2(1), 2010, 56-60.
- Aleksi T, Mikko N, Pertti JN, Janne TB, Drug interactions with oral antidiabetic agents: pharmacokinetic mechanisms and clinical implications, *Trends in Pharmacological Sciences*, 33 (6), 2012, 312-322.
- Elliot DJ, Identification of the human cytochromes P450 catalysing the rate-limiting pathways of Gliclazide elimination, *Br. J. Clin. Pharmacol*, 64 (4), 2007, 450-457.
- Benakis A, Glassan B, Metabolic study of 14C-labelled gliclazide in normal rats and in rats with streptozotocin-induced diabetes. In: *Gliclazide and Treatment of Diabetes*. Keen H (Ed). Academic Press and the Royal Society of Medicine, London, UK, 1980, 57-69.
- Miyazaki H, Fijii T, Yoshida K, Arakawa S, Furakawa H, Disposition and metabolism of [3H]-Gliclazide in rats, *Eur. J. Drug Metab. Pharmacokin*, 8(2), 1983, 117-131.
- Rollins DE, Klaassen CD, Biliary excretion of drugs in man, *Clin. Pharmacokin*, 4(5), 1979, 368-379.

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