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PRECLINICAL SAFETY EVALUATION OF BERBERINE IN COMBINATION WITH GLICLAZIDE

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ABSTRACT: Allopathic drugs are generally prescribed for Diabetes Mellitus (Type-II) patients. As the disease is chronic in nature, it necessitates the use of alternative medicine(s) either to supplement the existing allopathic therapy and/or to reduce the associated side effects with their chronic use. The use of herbal drugs is presumed to be safe option due to fewer side effects. However, evaluation of safety of their combination by scientific validation is very essential. Here we evaluated the safety of routinely used second generation drug Gliclazide along with Berberine (herbal drug). The study was conducted in two dissimilar species, rats, and rabbits. In rats and rabbits, the dose of 100 mg/kg of Berberine and 1 mg/Kg Gliclazide dosed separately produced~30% reduction in blood glucose. When both Berberine and Gliclazide were given in combination at similar doses, reduction in blood glucose was found to be ~60%, suggesting significant pharmacodynamic interaction between them. Also, in the rabbit study, Gliclazide pharmacokinetic parameters were studied. We observed significant changes in the pharmacokinetic parameters of Gliclazide (AUC, half-life, Cmax were increased by ~2 folds and clearance, vs. decreased by ~2-4 folds) in the combined regimen, indicating pharmacokinetic interaction. The studies in rat and rabbits (a rodent and a non-rodent), suggests that the combination (Gliclazide and Berberine) at the tested doses is not safe to be used in clinic.

INTRODUCTION: Diabetes Mellitus is a chronic metabolic disorder associated with disturbance in the carbohydrate, fat, and lipid metabolism characterized by hyperglycemia. Physical inactivity, culminating in obesity, is the primary culprit for Diabetes [1].

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Diabetes mellitus may be due to decreased synthesis of insulin in the pancreatic β-cells (type I) or may be due to decreased secretion of insulin (type II) from beta cells of islets of Langerhans of the pancreas. The treatment of DM requires lifelong administration of drugs along with diet control and exercise.

Type II DM is mainly associated with insulin resistance and can be treated with improving insulin sensitivity by reducing insulin resistance in the muscle and fat [2]. They also reduce glucose produced by the liver. As the disease is chronic in nature, managing poses challenges in terms of

treatment, management and prevention of associated complications like diabetic nephropathy, diabetic neuropathy, diabetic retinopathy, cardiomyopathy, *etc*. Sulphonylureas are still the most used drugs for Type-II diabetes [3]. Gliclazide, a second-generation sulfonylurea drug, is widely prescribed for treating type II DM, because of its selective inhibitory activity on the pancreatic K+ ATP channels, its antioxidant property [4], and Alzheimer's disease [4]. The current treatment with antidiabetic drugs is becoming very complex, and the recommended combination of drug regimens has increased the risk of pharmacokinetic interactions in diabetic patients. Although the risk of hypoglycemia with newer prolonged-release preparations of Sulphonylureas appears negligible, drug interactions may increase adverse effects and lead to safety problems [6].

There is a tremendous need for alternate treatment options which are safe and less burdensome to the such a patient population. Herbal remedies are gaining more attention as they cause less adverse effects compared with conventional allopathic medicines [7]. Many herbs and herbal isolates with hypoglycemic activity were found to enhance the hypoglycemic activity of co-administered antidiabetic drugs by synergistic effect and inhibiting the metabolic enzymes involved in the metabolism of co-administered antidiabetic drugs in the liver [8]. Berberine is one among the herbal medicines useful in managing Diabetes [9]. Berberine is a natural alkaloid obtained in barks, leaves, twigs, rhizomes, roots, and stems of several medicinal plants like *Berberis aristata, C. chinensis, C. japonica, C. rhizome, Hydratis canadensis, Phellodendron amurense, P. chinense, Tinospora cordifolia, B. aquifolium, B. heterophylla, B. beaniana, Coscinium fenestratum, Xanthorhiza simplicissima, Argemone mexicana etc* [10]. It is mainly used in traditional Indian and Chinese medicine. It is reported to have several beneficial actions like anti-diabetic, anti-atherosclerotic, anti-rheumatic, anti-angiogenic, anti-clastogenic, anti-convulsant, antioxidant *etc*. Based on the meta-analysis by Ye *et al.,* 2021. In patients with metabolic disorders as well as in healthy volunteers, Berberine has proven to reduce TG, TC, LDL *etc*. proving its potential to treat metabolic disorders like Type-II diabetes [11]. Berberine due to its lipid-lowering effect can be used as an alternative treatment in patients where statins are contraindicated [12]. Several mechanisms have been postulated for Berberine in the treatment of Diabetes Mellitus. Berberine is beneficial in combating insulin resistance.

In the current study, Berberine was selected based on its beneficial actions in diabetes and a standard drug from Sulfonyl urea class, gliclazide, was selected. The study is aimed to evaluate the safety when Berberine is administered alone and in combination with standard anti-diabetic drug Gliclazide in two dissimilar species, rats (normal and diabetic condition) and in rabbits.

MATERIALS AND METHODS: Gliclazide (Dr. Reddy's Labs, Hyderabad), Glucose Kits (Span diagnostics Ltd., Visakhapatnam), Wistar Rats (Mahaveer Enterprises, Hyderabad, Telangana), New Zealand White rabbits (Mahaveer Enterprises, Hyderabad, Telangana), Berberine (Laila Impex, Vijayawada, A.P), Acetonitrile (HPLC grade, Qualigens chemicals, Mumbai, India), Orthophosphoric acid (AR grade, SD fine chemicals, Mumbai).

Preparation of Herbal Isolate (Berberine): The herbal isolate of Berberine was dissolved in distilled water and prepared a stock of 100 mg/mL. Appropriate dilutions were made with distilled water as and when needed.

Design of Experiments: The experiments were conducted in 3 phases. Phase-I experiments were performed in healthy Wistar rats. Phase-II experiments were conducted in diabetic rats while Phase-III experiments were conducted in healthy rabbits. The experimental protocol was approved by the Institutional Animal Ethics Committee and by the regulatory body of the government (Regd No.516/01/A/CPCSEA). Both Gliclazide and Berberine or their combination was given to rats and rabbits by oral route. New Zealand White rabbits and Wistar rats of either sex was 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 and rats were fed with commercial pellet diet (Rayan's Biotechnologies Pvt. Ltd, Hyderabad, India) and water *ad libitum*. The blood samples were collected

by puncturing the retroorbital plexus in a rat model and puncturing the marginal ear vein in the rabbit at designated time intervals. The serum was separated and was analyzed for blood glucose by GOD/POD method using glucose kits in normal, diabetic rats and normal rabbits. The serum gliclazide levels were estimated by the HPLC method in normal rabbits. The pharmacokinetic parameters were calculated using Ramkin software. The significance was calculated using the student-paired t-test using graph pad prism [8].

Phase-I Experiments: In the Phase-I experiments based on prior trials doses of Berberine (100mg/Kg) and Gliclazide (1 mg/kg) which produced ~30-40% reduction of blood glucose in rats were chosen for the study. Wistar rats weighing about 200 to 250 gm were used in the study. The blood samples were collected at 0, 1,2,3,4,6,10, and 12 h intervals from all the ratsafter drug administration and were analyzed for blood glucoseby GOD/POD method given by Trinder [13].

The Dose Groups (N=6 in each group) were as follows:

Group-I: Berberine (100mg/Kg body weight, orally)

Group-II: Gliclazide (1 mg/Kg body weight, orally)

Group-III: Gliclazide (1 mg/Kg body weight, Orally) + Berberine (100mg/Kg body weight, orally)

RESULTS: In normal rats, berberine when administered alone produced approximately 30% decrease in blood glucose levels (peak level observed at 4h) at the dose of 100mg/kg compared with pre-dose samples. The hypoglycaemic effect of Berberine might be due to its insulin sensitivity effect as reported by earlier researchers [14]. Gliclazide at 1 mg/kg showed blood glucose reduction ~34-36% (peak level observed at 2 h and 8h) when compared with pre-dose sample in normal rats. In the combined treatment group (Berberine+Gliclazide), the reduction in blood glucose was up to~50%. Even though maximum reduction was observed at 4 h, significant effect was observed from 2 h till 8 h. In combination, the selected dose of berberine was found to enhance the hypoglycemic activity produced by gliclazide with single-dose treatment. This indicates there is an existence of pharmacodynamic interaction between berberine and gliclazide in normal rats. The Pharmacodynamic interaction may be due to their synergistic hypoglycemic effect or due to the inhibition of metabolism of gliclazide by berberine, as berberine was reported to have inhibitory activity on CYP 3A4, CYP 2C9 and CYP 1A2 [15]. The results are presented in **Fig. 1.**

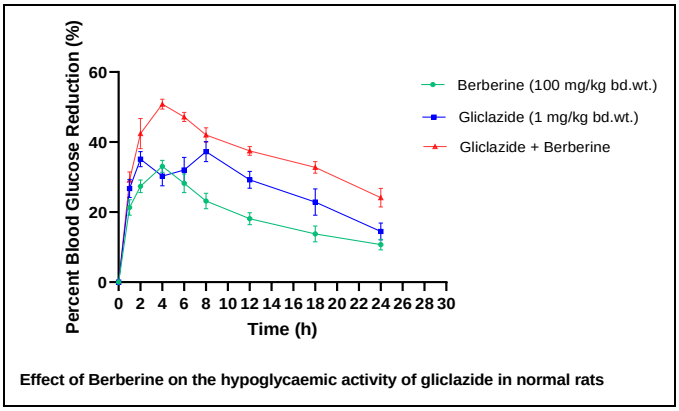

FIG. 1: MEAN PERCENTAGE REDUCTION IN BLOOD GLUCOSE LEVELS (%) IN NORMAL RATS WITH BERBERINE (ALONE), GLICLAZIDE (ALONE) AND THEIR COMBINATION

To check the validity of the observations in pathological condition, the studies were conducted in diabetic rats.

Phase-II Experiments: Phase-II experiments were performed in six diabetic Wistar rats. The rats were injected intraperitoneally with nicotinamide 100 mg/kg bd.wt. After 15 min streptozotocin (STZ 35 mg/kg b.wI.P) was administered. (STZ was dissolved in freshly prepared solution of 0.1 M citrate buffer, pH 4.5). Fasting blood glucose concentration was measured after one week of STZ injection. The rats with blood glucose level above 200 mg/dl we reconsidered diabetic and were used in the experiment. The rats were fasted for 18 h prior to the experiment with water *ad libitum*. Berberine, gliclazide and their combination were administered by oral route. The blood samples were collected at 0, 1,2,3,4,6,10, and 12 h intervals from all the rats (at different stages) after drug administration and were analyzed for blood glucose by GOD/POD method. The experiment was conducted in three stages.

Stage I: The rats were administered with standard (Gliclazide 1mg/kg bd.wt).

Stage II: The same rats (from stage-I) were administered individually with Berberine (100 mg/kg) after a washout period of one week.

Stage III: After a washout period of another one week, the same groups of rats were administered with selected doses of Berberine (100 mg/kg), 30 min prior to standard dose of gliclazide (1 mg/kg).

RESULTS: At the dose of 100mg/kg bd.wt, berberine was found to show ~40 % reduction (peak effect observed at 4h) in blood glucose levels and gliclazide 1 mg/kg bd. Wt., was found to reduce the elevated blood glucose levels by ~45% (Peak effect observed at 2 and 8 h). In the combined treatment group (Berberine+Gliclazide), the reduction in blood glucose was up to ~60%. A significant reduction was observed from 2 h till 8 h, similar to the combined treatment group in normal rats. The results are represented in **Fig. 2.**

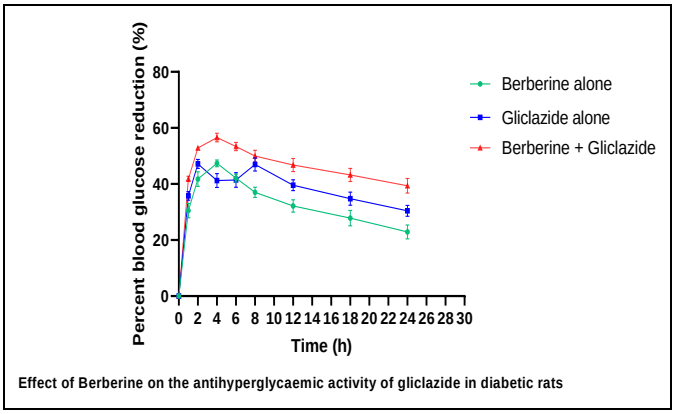

FIG. 2: MEAN PERCENTAGE REDUCTION IN BLOOD GLUCOSE LEVELS (%) IN DIABETIC RATS WITH BERBERINE (ALONE), GLICLAZIDE (ALONE) AND THEIR COMBINATION

Further, the studies were extended to normal rabbits to study the interaction of selected herbal isolates with selected dose of gliclazide to establish the safety of their combination in dissimilar species (a rodent and a non-rodent) and to study the mechanism of their interaction.

Phase III Experiments: The experiments were performed in normal healthy New Zealand White rabbits. A group of 6 normal healthy rabbits of either sex weighing between 1.35kg-1.75kg were used in the study. The blood samples were collected at 0, 1,2,3,4,6,10, and 12 h intervals after drug administration and were analyzed for blood glucose by GOD/POD method. Serum gliclazide was analyzed using the reported RP-HPLC method. The same rabbits (N=6) were used throughout the experiment, but a washout period of one week was given between each stage. In stage IV, Berberine was administered for 14 consecutive days (100 mg/1.5 kg bd.wt daily), and gliclazide was given 20 min prior to administration of Berberine on the 14th day.

The experiment was conducted in four stages.

Stage I: Vehicle control (water) and standard (Gliclazide 5.6 mg/1.5kg bd.wt).

Stage II: Berberine (100 mg/kg).

Stage III: Berberine (100 mg/1.5 kgbd.wt, single dose) + Gliclazide (5.6 mg/1.5kg bd.wt).

Stage IV: Berberine (100 mg/1.5 kgbd.wt, 14 multiple doses) + gliclazide (5.6 mg/1.5kg bd.wt).

Results: The selected dose of gliclazide (5.6 mg/1.5 Kg bd.wt) produced optimal reduction in blood glucose levels (~30%) with peak activity at 4 hr and the peak serum gliclazide was found to be at 4 h. The human therapeutic dose of gliclazide was extrapolated to rabbits based on body surface area formula as described in Ghosh MN, 2005; Paget, G. E. *et, al.* 1964 and selected herbal isolate (Berberine) for interaction study was fixed based on the hypoglycaemic study conducted in normal rats.

In normal rabbits, the selected dose of berberine produced a significant reduction in blood glucose levels, which might be due to the insulin-sensitizing activity of berberine. When administered in combination, the selected dose of berberine significantly enhanced the hypoglycemic activity of gliclazide from 1hr to 24 hr intervals with single and multiple dose treatments. The serum gliclazide levels were found to be enhanced significantly with single and multiple-dose treatments of berberine. The results of mean percent reduction in blood glucose with Berberine, Gliclazide, and their combination are presented in **Table 1, Fig. 3** and **4**. Mean serum Gliclazide levels are given in **Table 2,** and the Pharmacokinetic mean parameters of gliclazide alone and in combination with single and multiple

doses of Berberine are given in **Table 3.** This effect might be due the inhibition of metabolism of gliclazide by CYP 3A4 and CYP2C9 as both these enzymes are responsible for the metabolism of gliclazide and the same were reported to be inhibited by berberine in earlier studies [16]. There is significant increase in the pharmacokinetic parameters of gliclazide like AUC0-∞, AUMC0-t, AUMC0-∞, Kel, T1/2, Cmax and MRT and significant decrease in Clearance and Vss, with single and multiple dose treatment of berberine. The PK parameters, AUC, half-life, Cmax were increased by ~1.5 to 2.5 folds, 1.5 to 2.0 folds, and 1.2 to 1.5 folds, respectively, and Clearance, Vss was decreased by 1.3 to 1.6-foldand ~3.5-4 folds respectively. The enhancement in the serum gliclazide levels and significant changes in pharmacokinetic parameters AUC, T1/2, and clearly indicate that there is a metabolic interaction between berberine and gliclazide, which might be due to inhibition of the metabolism of gliclazide by berberine, as berberine reported to inhibit the CYP 3A4 isozyme [16].

TABLE 1: EFFECT OF SINGLE AND MULTIPLE DOSES OF BERBERINE ON THE HYPOGLYCAEMIC ACTIVITY OF GLICLAZIDE IN NORMAL RABBITS (N=6)

Time (h)	Mean Percentage reduction in blood glucose levels (%)			
	Berberine (100 mg/1.5 kg bd.wt.)	Gliclazide (5.6 mg/1.5 kg bd.wt.)	Gliclazide + Berberine (SD)	Gliclazide + Berberine (MD)
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
1	21.70±0.72	26.27±1.17	35.95±0.94*	39.41±1.07*
2	28.73±0.78	35.19±0.96	47.64±1.18*	47.75±1.27*
4	34.91±0.61	41.10±0.51	51.74±0.77*	52.87±0.61*
6	29.90±0.74	36.55±0.51	52.20±1.49*	54.66±0.24*
8	23.58±1.13	31.33±0.88	49.48±1.29*	54.62±0.69*
12	18.23±0.94	26.80±1.05	45.22±1.46*	51.18±0.95*
18	14.53±1.01	21.59±1.40	40.84±1.31*	47.57±1.23*
24	10.66±1.14	16.38±1.72	37.42±1.26*	39.84±1.80*

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

TABLE 2: MEAN SERUM GLICLAZIDE LEVELS WITH GLICLAZIDE ALONE AND IN COMBINATION WITH SINGLE AND MULTIPLE DOSES OF BERBERINE IN NORMAL RABBITS

Time (h)	Mean Serum Gliclazide levels (Mean ± SEM)		
	Gliclazide alone	Gliclazide+ Berberine (SD)	Gliclazide+ Berberine (MD)
0	0.00±0.00	0.00±0.00	0.00±0.00
1	173.63±12.75	224.21±11.95	255.74±16.79*
2	251.99±14.75	314.47±16.85*	347.25±15.20*
4	370.23±14.99	406.75±14.70*	420.96±10.13*
6	293.06±12.88	337.55±15.59*	351.22±15.13*
8	241.69±12.84	279.55±16.80*	285.89±11.27*
12	171.93±14.59	218.19±16.14*	233.39±11.89*
16	131.61±12.22	176.13±15.54*	181.83±15.46*
20	105.71±8.47	130.40±11.39*	148.08±10.32*
24	76.44±6.49	110.68±9.15*	115.18±7.96*

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

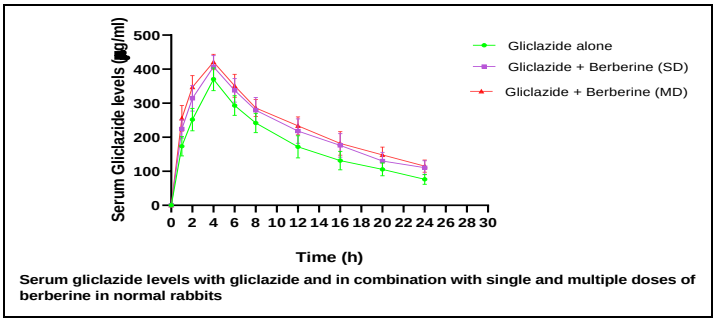

FIG. 3: MEAN SERUM GLICLAZIDE LEVELS IN NORMAL RABBITS DOSED WITH GLICLAZIDE ALONE, GLICLAZIDE WITH BERBERINE (SINGLE DOSE) AND GLICLAZIDE WITH BERBERINE (MULTIPLE DOSES)

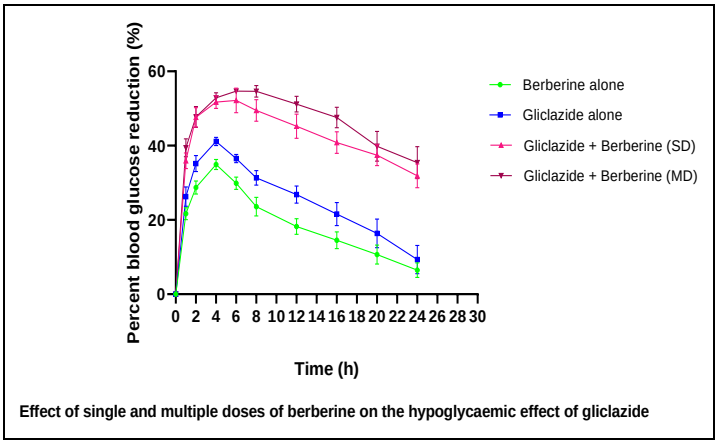

FIG. 4: MEAN PERCENT REDUCTION IN BLOOD GLUCOSE IN NORMAL RABBITS DOSED WITH BERBERINE ALONE, GLICLAZIDE ALONE, GLICLAZIDE WITH BERBERINE (SINGLE DOSE) AND GLICLAZIDE WITH BERBERINE (MULTIPLE DOSES)

TABLE 3: MEAN PHARMACOKINETIC PARAMETERS OF GLICLAZIDE ALONE AND IN COMBINATION WITH SINGLE AND MULTIPLE DOSES OF BERBERINE IN NORMAL RABBITS

Pharmacokinetic parameter	Mean Serum Pharmacokinetic parameters of Gliclazide levels (Mean ± SEM)		
	Gliclazide alone	Gliclazide+ Berberine (SD)	Gliclazide+ Berberine (MD)
AUC_{0-24} (µg/ml/hr)	4971.53±313.59	8040.24±446.52*	9861.38±206.02*
AUC_{0-24} (µg /ml/hr*h)	61809.35±4769.24	135427.26±9812.32*	148198.79±8001.38*
Kel (h^{-1})	0.07±0.00	0.04±0.00*	0.04±0.00*
$AUC_{0-\alpha}$ (µg /ml/hr)	6623.90±442.58	13956.97±1023.51*	16261.28±1098.87*
$AUMC_{0-\alpha}$ (µg /ml/hr*h)	154185.33±13413.05	376630.19±57058.51*	552519.94±58833.08*
t ½ (h)	10.06±0.48	16.19±1.41*	20.81±2.39*
Ka (h^{-1})	1.15±0.00	1.15±0.00	1.15±0.00
Clearance (ml/hr)	963.25±28.26	730.81±67.66*	597.16±67.27*
Clearance (ml/hr/kg)	940.81±53.55	509.83±42.54*	364.86±28.66*
Vd SS (ml)	67552.05±48630.47	18189.37±900.15*	*16612.96±764.44*
Vd SS (ml/kg)	16025.56±868.72	11950.76±850.82*	10203.24±358.01*
Vd area (ml)	60162.09±43009.47	15200.02±1003.79*	13435.01±816.09*
Vd area (ml/kg)	15056.03±849.57	10436.74±497.55	9015.40±371.19*
MRT (h)	18.28±1.08	27.95±1.59*	31.59±2.17*
C max (ng/ml)	406.16±17.49	518.88±13.42	611.08±20.35
T max (h)	4.00±0.00	4.00±0.00	4.00±0.00

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

DISCUSSION: Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycaemia caused by defective insulin synthesis (type I), secretion (type II) coupled with resistance to insulin action or their combination. It is a disorder prevailing worldwide irrespective of age, sex and race. It is a disorder that requires careful management of blood glucose by drugs. Too much-raised blood glucose (hyperglycaemia) leads to diabetic ketoacidosis, coma, and death. Similarly lowered blood glucose (hypoglycemia) leads to hypoglycemic coma and death. Hence patients must judiciously monitor and manage blood glucose levels. Chronic diabetes leads to precipitation of several complications including cardiac complications like angina, hypertension, cardiac dysrhythmias, and kidney failure, which are the leading causes of death in chronic diabetes if left untreated. Type II diabetes is more common than type I diabetes. Insulin is useful in type I diabetes. Sulphonylureas are the drugs of choice in type II diabetes, among which gliclazide is a second-generation, widely prescribed drug and was taken as the standard drug in the present study. The use of herbal drugs and herbal isolates is gaining importance for chronic disorders either to supplement the existing allopathic therapy and/or to reduce the associated complications and side effects with the chronic use of allopathic drugs[17]. It is a common practice for chronic diabetics worldwide. Hence, the establishment of the safety of their combination by a scientific validation is

essential. The dose of gliclazide for interaction was fixed based on the dose that produces an optimal reduction (30%-35%) in the blood glucose levels. Rats were found to be more sensitive to the gliclazide effect. Gliclazide produced biphasic response with peak effect at 2 h and 8 h intervals after oral administration in rats. The biphasic response of gliclazide in rats may be due to its enterohepatic circulation due to its biliary excretion as reported in earlier studies in rats.

The present study was planned to establish the safety of gliclazide (a most widely prescribed second-generation Sulphonylureas class drug for type II diabetes) in the presence of selected herbal drugs (Berberine) concerning blood glucose and serum insulin levels in animal models. The study was conducted in two dissimilar species of animals, a rodent (normal rats and diabetic rats) and a non-rodent (normal rabbits) with the aim that if interaction exist in two dissimilar species it is likely to occur in humans also. If the combination was found to be safe in two dissimilar species, it is likely to be safe in humans also. The studies were performed in three different phases. In the first phase the normal rats were selected as model for preliminary and quick screening of effects of selected herbal drug (Berberine) alone and in combination with gliclazide on blood glucose levels *i.e, the* pharmacodynamics of gliclazide. Rats have been selected as the experimental model, since they can be maintained easily at laboratory conditions and small volumes of blood samples can be collected at desired intervals of time.

In the second phase, based on the results obtained from normal rats, the experiments were planned and conducted in streptozotocin induced diabetic rats to determine whether the effect observed was similar in both healthy and pathological conditions. In the third phase the studies with selected drugs (Berberine), gliclazide and their combinations were extended to rabbit model to find out whether selected herbal drug (Berberine), gliclazide and their combinations produced similar effects of their own and to find out the combination was safe or not in another dissimilar species also. In the rabbit studies, pharmacodynamic and pharmacokinetic parameters were estimated to understand the mechanism of interaction between a selected herbal drug (Berberine) and gliclazide. Berberine and gliclazide were administered by oral route both in rats and rabbits. Gliclazide produced a dose-dependent reduction in blood glucose levels in both rats and rabbits. A dose of 5.6 mg/1.5 kg was found to produce optimal reduction (30-40%) in blood glucose in normal and diabetic rats. The human therapeutic dose was extrapolated to rabbits based on the body surface area selected for the interaction study.

The oral administration of gliclazide produced a dose-dependent decrease in blood glucose levels in normal rats. The doses of selected drugs for interaction study were also fixed by extrapolating the human therapeutic dose of selected drugs to rats and rabbits based on body surface area. Gliclazide is mainly metabolized in the liver by CYP2C9 and CYP3A4 isozymes [16] and is a highly protein-bound drug bound to proteins ≥95%. If the co-administered drugs inhibit/induce the above enzymes, they can modulate gliclazide levels. Similarly, drugs with a high protein binding nature may displace gliclazide from the protein binding site and enhance the levels of gliclazide in the blood when they are co-administered. In the present study berberine was selected for studying the interaction with gliclazide in preclinical models.

Berberine produced a dose-dependent reduction in blood glucose levels in normal rats. The selected dose of berberine alone significantly reduced blood glucose levels in normal rats, diabetic rats, and normal rabbits. As reported by earlier researchers, the hypoglycaemic effect of berberine might be due to its insulin sensitivity effect. In combination berberine significantly enhanced the hypoglycaemic activity of gliclazide in normal and diabetic rats and in normal rabbits with single and multiple-dose treatments. The increase in hypoglycaemic activity in rats and rabbits may be either due to their synergistic hypoglycaemic activity or to enhanced serum levels of gliclazide by their pharmacokinetic interaction. The serum gliclazide levels, as well as the pharmacokinetic parameters of gliclazide, were found to be altered with the single and multiple-dose treatment of berberine that, which indicates there is a pharmacokinetic interaction between berberine and gliclazide. This might be due to the inhibition of the metabolism of gliclazide by berberine as it was reported to inhibit the same CYP 3A4 isozyme. So,

care must be taken to adjust the dose of gliclazide or advised to maintain a dosage interval between both drugs when they are co-administered in a clinical situation. Since, the combination of Berberine with Gliclazide is not safe in two dissimilar species, care must be taken to avoid severe hypoglycemia. However, the safety of the combination needs to be confirmed with clinical trials for better assurance. The study provides preclinical scientific support for the clinical use of the combinations.

CONCLUSION: At the tested doses, there was significant interaction with Berberine when co-administered with gliclazide in two dissimilar preclinical species (rats and rabbits). The interaction is both pharmacokinetic and pharmacodynamic. This indicates severe safety concerns when such a combination is used. Hence care must be exercised when Berberine is used along with gliclazide. The hypothesis is to be tested in the clinic for better clarity.

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CONFLICTS OF INTEREST: All the authors declare that they do not have any conflict of interest.

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