CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

Available online at: http://www.iajps.com Research Article

INFLUENCE OF *GYMNENA SYLVESTRE* ON THE PHARMACOKINETICS AND PHARMACODYNAMICS OF GLIPIZIDE WITH ATORVASTATIN IN DIABETIC AND NORMAL RATS

Krishna Bheemanapally

Department of Pharmacognosy, Netaji Institute of Pharmaceutical Sciences, Toopranpet, Nalgonda, Telangana, India.

Abstract:

Diabetes mellitus is associated with oxidative stress induced micro and macro vascular complications. These complications lead to the development of dyslipidemia, hypertension and Myocardial infarction. So, these patients are treated with more than two drugs and there is a possibility of drug interactions.Many medicinal herbs and pharmaceutical drugs are therapeutic at one dose and toxic at another. Interaction between herbs and drugs may increase or decrease the pharmacological or toxicological effect of either components, and the subject is the focus of this study.Although there are many phytoconstituents that could combat diabetes and obesity, Gymnemasylvestre could be used in the treatment of both the diseases simultaneously (Kanetker et al., 2007). The sulfonylurea's Glipizide, Glimepiride, and Glibenclamide are metabolized by the CYP2C9 enzyme. Glipizide is a second-generation sulfonylurea drug and is a substrate of the P-glycoprotein drug transporter. In addition, it blocks ATP-sensitive potassium channels although there is some information on the effects of modulators of CYP enzymes and P-glycoprotein on Glipizide.Statins are recommending familiarly for maintaining of obesity patients with diabetes. Atorvastatin is a selective HMG-CoA reductase competitive inhibitor. It is metabolized by CYP 3A4 and 3A5 iso enzymes in liver. It also has moderate inhibition on metabolizing enzymes like CYP 2C9, 2D6 and 3A4. To test this hypothesis, the study was conducted to examine the effect of Gymnemasylvestre on the Pharmacokinetics and Pharmacodynamics of Glipizide with Atorvastatin in experimental diabetic and normal male Wistar Albino rats.

Key words: *Gymnemasylvestre, Glipizide, Glimepiride, and Glibenclamide etc.*

Corresponding Author:

Krishna Bheemanapally,

Department of Pharmacognosy,
Netaji Institute of Pharmaceutical Sciences,
Toopranpet, Nalgonda, Telangana, India.

Email ID: *chinna.krish19@gmail.com*

QR code

Please cite this article in press Sakthidasan et al., ***Influence of Gymnena Sylvestre on the Pharmacokinetics and Pharmacodynamics of Glipizide with Atorvastatin in Diabetic and Normal Rats,*** *Indo Am. J. P. Sci, 2017; 04(9).*

INTRODUCTION:

Drug-drug interactions occur when one therapeutic agent either alters the concentration (pharmacokinetic interactions) or the biological effect of another agent (Pharmacodynamic interactions). Pharmacokinetic drug-drug interactions can occur at the level of absorption, distribution, or clearance of the affected agent. Many drugs are eliminated by metabolism. The microsomal reactions that have been studied the most involve cytochrome P (CYP) 450 family of enzymes, of which a few are responsible for the majority of metabolic reactions involving drugs. These include the forms CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4.

Enzyme inhibition refers to the decrease in metabolic enzyme activity due to the presence of an inhibitor. Drug metabolism by CYP450 can be inhibited by any of the following three mechanisms: competitive inhibition, noncompetitive inhibition and uncompetitive inhibition. Inhibition of enzyme activity may result in higher concentrations and/or prolonged half-life of the substrate drug, which enhances the toxic side effects. The clinical significance of a specific drug-drug interaction depends on the degree of accumulation of the substrate and the therapeutic window of the substrate.

Enzyme induction is associated with an increase in enzyme activity. For drugs that are substrates of the isoenzyme induced, the effect is to lower the concentration of these substrates. The clinical consequence of the presence of an inducing agent and the resultant decrease in concentration of the substrate may mean a loss of efficacy.
Conditions when more than one drug is administered:

A] Use of drugs acting by different mechanisms of actions for the effective therapy of disease e.g., multiple drug therapy for the tuberculosis, leprosy, cancer etc.
B] Treatment of multiple disorders simultaneously with different drugs e.g., hypertension and diabetics, hypertension and other cardiac disorders, hypertension and rheumatoid arthritis, hypertension and asthma etc.
C] Use of OTC drugs e.g., Aspirin, Paracetamol, antacids etc, along with drugs for the treatment of other disorders. Antacids, H2 receptor antagonists, NSAIDS, cough and cold preparations and the anti-asthmatic drugs constitute the major part of the OTC drugs.

Gymnemasylvestre (Asclepiadaceae) a vulnerable species is a slow growing, perennial, medicinal woody climber found in central and peninsular India. Fig. 3 shows a 5-year old parent plant. It is a potent antidiabetic plant and used in folk, ayurvedic and homeopathic systems of medicine. It is also used in the treatment of asthma, eye complaints, inflammations, family planning and snakebite. In addition, it possesses antimicrobial, anti hypercholesterolemic, hepatoprotective and sweet suppressing activities. It also acts as feeding deterrents to caterpillar, Prodeniaeridania; prevent dental caries caused by Streptococcus mutans and in skin cosmetics.

Drugs and chemicals

Glipizide, Gliclazide and Atorvastatin were procured from Matrix laboratories as a gift sample. Streptozotocin (STZ) was purchased from Sigma Chemical Co. The glucose estimation (GOD- POD) kit (excel diagnostic, Hyderabad) was procured from drug store. All HPLC grade solvents (methanol and water) were procured from drug store. All other chemicals including potassium dihydrogen ortho phosphate, sodium citrate, sodium carboxy methyl cellulose, phosphoric acid, DMSO, glucose, concentrated hydrochloric acid, magnesium sulphate, potassium hydroxide, calcium chloride, diethyl ether were procured from finar chemicals Ltd., Ahmadabad. All chemicals used were analytical grade.

Animals

Experiments were performed with Male Wistar rats procured from Mahaveera Enterprises (Hyderabad, A.P., India), weighing between 180 to 230gms. The animals were housed in colony cages (four per cage) with temperature of 25±2oc with 12-h light / 12-h dark cycle. They were feed with standard pellet diet (Mahaveera Enterprises Pvt. Ltd. Hyderabad, A.P., India) and water ad libitium. Animals were fasted for 18 h before experiment and during the experiment they had withdrawn from food and water. The experiments were approved by Institutional Animal Ethical Committee (IEAC) (Approved No: 2011/10/3/3), Vaagdevi College of Pharmacy, Warangal, and A.P., India.

METHODS:

Plant meterial

The leaves of Gymnemasylvestre were collected from local forest of Namapuram, Nalgonda District, Andra Pradesh, India. Herbarium was prepared and the herbarium was identified by Dr. Vastavya S. Raju, Senior professor, former Head and Chairperson, Plant Systematic Laboratory, Department of Botany, Kakatiya University, Warangal, Andra Pradesh, India. The plant leaves were washed with distilled water, air dried under

shed at 25°C and the dried leaves were made in to a fine powder with an auto-mix blender (Venkatesan et al., 2008), passed through sieve #60 (Vivekananada et al., 2010) to obtain a homogenous leaf powder. The powder was kept in deep freezer until the time of use.

Extraction of Gymnemasylvestre leaves

5 gm of Gymnemasylvestre powder was taken in a 1000 mL round bottom flask and added with 500 mL of extraction solvent (volume ratio of methanol to water is 1:1 ratio) and 100 mL of potassium hydroxide solution (11%). The mixture was refluxed for an hour. The concentrated hydrochloric acid of 90 mL was added and refluxed again for an hour. The mixture was cooled to room temperature. The extract was filtered through 0.45 µm nylon filter (Millipore). The volume was made up to 1000 mL with extracted solvent and clear supernatant was used for HPLC analysis (Manoher et al., 2009).

Estimation of glipizide by a sensitive RP-HPLC Method

HPLC Description

A Waters HPLC system was used in the study consisting of a 515 pump and a dual wavelength UV detector (Model 2487), operating at 1ml/min, a syringe loading sample injector of 25µl capacity, a C_{18} reverse phase column of 250 X 4.6mm dimension and 5µ particle size.

Chromatographic conditions

The mobile phase consisting of methanol: water: 0.01M potassium Phosphate buffer (60:35:5,v/v/v) (Venkat*et al.,* 2011) (pH 3.0 adjusted with Ortho Phosphoric acid) were filtered through 0.45µ Ultipor N66 Nylon 6,6 membrane solvent filter, degassed and were pumped from the solvent reservoir in the ratio of 60:35:5 (v/v/v). and was pumped into the column. The flow rate of mobile phase was maintained at 1 ml/min and detection wavelength was set at 276 nm (Ashish *et al.,* 2008) with a run time of 12min. The volume of injection loop was 20 µL prior to injection of the drug solution the column was equilibrated for at least 30min with the mobile phase flowing through the system. Retention time of Glipizide and Gliclazide were 7.12 – 7.25 and 3.41 – 3.45 min respectively. The column and the HPLC system were kept in ambient temperature.

Extraction procedure

The drug was extracted from plasma with methanol. In a 2 mL micro centrifugation tube, 0.1 mL of plasma was added. The plasma was precipitated by the addition of 0.1 mL internal standard (50 µg/mL) and 0.4mL of methanol, and then the tubes were vortexes for 30 sec and centrifuged at 5000 rpm for 15 min. The supernatant was transferred to a clean, similarly labeled tube. The resulting solution was evaporated and reconstituted with 0.1 mL of methanol. From this 20 µl was injected into the HPLC (Venkatesh*et al.,* 2006).

Estimation of blood GLUCOSE

The following methods were used to estimate the blood glucose.

1. GOD-POD method
2. Folin - wu method
3. Ortho-toluidine method

In the Present study, we used the enzymatic, Glucose- Oxidase - Peroxidase (GOD-POD) method (Trinder*et al.,* 1969).

Pharmacokinetic and Pharmacodynamic studies in healthy rats

Experimental procedure

Albino Male Wistar Rats of either sex were randomly divided into eight groups of six animals each. They were housed in well ventilated aluminum cages and maintained on uniform diet and temperature with 12h light and dark cycle. Before experiment, all animals were fasted for 18 hours and water *ad libitum*, water was withdrawn during experiment. After collection of initial blood samples, drugs were administered in the following order.

Group I - Control (0.2 ml of 0.5% CMC sodium, p.o.) (Srinivas *et al.,* 2006).

Group II - Glipizide (5 mg/kg, p.o.) (Srinivas *et al.,* 2006).

Group III - *Gymnemasylvestre* extract (200 mg/kg, p.o.) (Bakrudeen*et al.,* 2010).

Group IV- Glipizide (5 mg/kg, p.o.) followed by *Gymnemasylvestre* extract (200 mg/kg, p.o.)

Group V- Atorvastatin (20 mg/kg, p.o.) (Prasad *et al.,* 2011).

Group VI- Atorvastatin (20 mg/kg, p.o.) followed by *Gymnemasylvestre* extract (200 mg/kg, p.o)

Group VII- Glipizide (5 mg/kg, p.o.) followed by Atorvastatin (20 mg/kg, p.o.)

Group VIII-Glipizide (5 mg/kg, p.o.) and Atorvastatin (20 mg/kg, p.o.) followed by *Gymnemasylvestre* extract (200 mg/kg, p.o.)

In this study, blood was collected from retro orbital using 2ml eppendroff tubes containing sodium citrate as anticoagulant. Plasma was separated by centrifugation at 5000 rpm / 10 min and stored at −20°C until further analysis. These samples are used to analyze for both blood glucose levels and Glipizide. Blood glucose levels are estimated by GOD-POD method and Glipizide was estimated by a sensitive RP-HPLC method.

Pharmacokinetic and Pharmacodynamic studies in diabetic rats:

Experimental induction of diabetes

Six adult Albino Male Wistar Rats weighing 220-300 gram (75-90 days old) were used for inducing diabetes. Diabetes was induced by single intra peritoneal injection of freshly prepared solution of Streptozotocin at the dose of 50mgkg^{-1} in 0.1M citrate buffer (pH 4.5) in volume of 1 mg kg^{-1} body weight and the rats fasted overnight. After 3days of Streptozotocin induction, blood glucose levels between 280–400 mg/dL were selected for the study (Pitchai*et al.*, 2009), (Akbarzadeh *et al.*, 2007).

Experimental procedure

Rats were randomly distributed into eight groups of six animals in each group; they were housed in well ventilated aluminum cages and maintained on uniform diet and temperature with 12h light and dark cycle. Before experiment all animals were fasted for 18hours and water ad libitum, water was withdrawn during experiment. After collection of initial blood samples, drugs were administered in the following order.

Group I - Control
Group II - Glipizide
Group III - Gymnemasylvestre extract
Group IV- Glipizide followed by Gymnemasylvestre extract
Group V- Atorvastatin
Group VI- Atorvastatin followed by Gymnemasylvestre extract
Group VII- Glipizide followed by Atorvastatin
Group VIII-Glipizide and Atorvastatin followed by Gymnemasylvestre extract

In the present study, blood was collected from orbital sinuses using 2ml eppendroff tubes containing sodium citrate as anticoagulant. Plasma was separated by centrifugation at 5000 rpm / 10min and stored at −20°C until further analysis. These samples are used to analyze for both blood glucose levels and Glipizide. Blood glucose levels are estimated by GOD-POD method and Glipizide was estimated by a sensitive RP HPLC method.

RESULTS AND DISCUSSION:

PHARMACODYNAMICS

Percentage reduction of blood glucose vs. time data of diabetic rats single drug comparison

Table 1: Percentage reduction of blood glucose vs. time data of diabetic rats single drug comparison (n = 6)

Time (Hr)	CONTROL Mean ± SD	ATV Mean ± SD	GPZ Mean ± SD	GSE Mean ± SD
0	0±0	0±0	0±0	0±0
1	0.41±0.05	3.98±0.11	15.77±1.89**	22.19±2.32***
2	0.44±0.09	6.16±0.93	24.09±2.59***	35.8±4.67***
4	0.81±0.07	5.5±0.12	50.52±3.68***	42.03±4.88**
6	0.53±0.05	4.04±0.32	34.83±3.62**	37.76±3.18**
8	0.57±0.08	4.33±0.85	23.31±2.15**	26.1±1.44***
10	0.65±0.04	3.06±0.97	9.88±1.17*	13.69±1.91**
12	0.49±0.05	4.04±0.65	9.39±1.41*	11.08±0.36**

*p<0.05, **p<0.01, *** p<0.001 Compared to control group.

Percentage reduction of blood glucose vs. time data of diabetic rats combination drugs comparison

Table 2: Percentage reduction of blood glucose vs. time data of diabetic rats combination drugs comparison (n = 6)

Time (Hr)	GPZ Mean ± SD	GPZ+ATV Mean ± SD	GPZ+GSE Mean ± SD	ATV+GSE Mean ± SD	GPZ+ATV+GSE Mean ± SD
0	0±0	0±0	0±0	0±0	0±0
1	15.77±1.89	19.47±2.12*	17.4±0.6*	9±0.9	22.41±2.17**
2	24.09±2.59	53.91±2.44***	29.13±1.53*	24.51±2.88*	46.53±6.01**
4	50.52±3.68	57.63±2.61*	63.47±1.45**	55.78±6.03*	69.01±6.56***
6	34.83±3.62	44.3±3.86***	34.25±2.65	26.75±5.96*	39.66±4.80*
8	23.31±2.15	28.797±1.52**	24.07±1.95	26.56±2.05*	18.73±1.65
10	9.88±1.17	9.75±1.05	14.12±2.1**	11.81±1.46	12.46±1.59*
12	9.39±1.41	18.1±2.63***	10.16±1.22	8.42±0.7	17.22±3.04***

***p<0.05, **p<0.01, *** p<0.001 Compared to Glipizide group.**

The mechanisms by which *Gymnemasylvestre*extract produces antidiabetic effects including recovery of β cells (Shanmugasundaram*et al.*, 1990) .Gymnemic acid formulations have also been found useful against obesity, according to the earlier reports (Yoshikawa *et al.*, 1993). This is attributed to the ability of gymnemic acids to delay the glucose absorption in the blood. The reduced glucose levels suggested that crude extract and the compound dihydroxygymnemic triacetate might exert through the insulin release by the stimulation of a regeneration process and revitalization of the remaining beta cells (Rokeya*et a*, 1999; Bolkent*et al.*, 2000).

In uncontrolled or poorly controlled diabetes, there is an increased glycosylation of a number of proteins, including Hb (Alberti*et al.*, 1982). The level of HbA1c is monitored as a reliable index of glycemic control in diabetes (Gabbay*et al.*, 1976) and useful in the management of diabetes mellitus. Significant fall in glycosylated hemoglobin indicated the efficiency of the compound in glycemic control. The glycogen content of the skeletal muscle and liver, which markedly decrease in diabetes (Prasannan*et al.*, 1965), increased significantly in the compound treated animals as compared to the diabetic control (Grover *et al.*, 2000).

The glipizide showed hypoglycemia / antihypoglycemia in normal and diabetic rats in dose dependent manner. Whereas, Atorvostatin alone did not altered blood glucose level. When administered glipizide in therapeutic dose produce the maximum effect at 4th hour and was maintained up to 6th hour in both normal and diabetic rats. In presence of Atorvastatin the onset of action of action of Glipizide was early and maintained for long duration compared to Glipizide control. Glipizide acts by stimulating insulin release. The early onset of action was noticed due to inhibition in metabolism of Glipizide, Atorvastatin inhibits hepatic CYP3A4 isoenzyme and Glipizide is partly metabolized by the same iso enzyme (Mishra *et al.*, 2010).

The blood glucose levels were calculated at various time intervals. The onset of action of action was observed at the 4th hour and the duration was observed until the 12th hour. The Percentage reduction of blood glucose in the diabetic rats (Fig. 53) at 4th hour of Glipizide, GSE, ATV, GPZ+ATV, ATV+GSE, GPZ+GSE, and GPZ+ATV+GSE were 50.52±3.68, 42.03±4.88, 5.5±0.12, 57.63±2.61, 55.78±6.03, 63.47±1.45 and 69.01±6.56 respectively. And at 6th hour of Glipizide, GSE, ATV, GPZ+ATV, ATV+GSE, GPZ+GSE, and GPZ+ATV+GSE were 34.84±3.62, 37.76±3.18, 4.04±0.32, 44.3±3.86, 26.75±5.96, 34.25±2.65 and 39.66±4.8 respectively. Whereas In presence of Atorvastatin the onset of action of action of Glipizide was early and maintained for long duration compared to Glipizide control in diabetic and normal rats.

The Percentage reduction of blood glucose in the normal rats (Fig. 54) at 4th hour of Glipizide, GSE, ATV, GPZ+ATV, ATV+GSE, GPZ+GSE, and GPZ+ATV+GSE were 36.02±7.22, 32.7±5.84, 11.11±1.14,42.04±2.28, 36.01±6.68, 50.26±4.13 and 61.09±7.19 respectively.
And at 6th hour of Glipizide, GSE, ATV, GPZ+ATV, ATV+GSE, GPZ+GSE, and GPZ+ATV+GSE were 28.5±4.9, 23.12±2.55, 8.56±1.29, 18.69±4.42, 20.68±4.63, 30.23±3.02 and 32.1±3.6 respectively. Whereas In presence of Atorvastatin the onset of action of action of Glipizide was early and maintained for long duration compared to Glipizide control in diabetic and normal rats.

So this results revel that the Glipizide in the presence of ATV and GSE showed better positive results to attain the controlling of blood glucose levels.

Atorvastatin increase the bioavailability of Digoxin, most probably by inhibition of P-gp, but does not effect pharmacokinetics of ritinovir (Lennernas*et al.*, 2003).

Both parameters was found to be statistically significant but at different time points. In the present study, we observed the synergistic effect with GSE, because it is having hypoglycaemic effect, so it produces synergistic effect with Glipizide. And with ATV inhibits metabolism of Glipizide, enhanced level of Glipizide might be responsible for the enhanced secretion of insulin in the presence of Atorvastatin (Gopalakrishna*et al.*, 2008). In the present study, we also observed that the maximum Percentage of glucose reduction at 4th hour, both in healthy and diabetic rats which correlates with peak plasma concentration of Glipizide. The above results suggested that, Atorvastatin inhibits both CYP2C9 and P-gp. It reveals the bioavailability and Pharmacokinetics of Glipizide were increased in the presence of Atorvastatin.

PHARMACOKINETICS

PlasmaConcentration Vs Time profile in Diabetic rats

Table 3: Plasma Concentration Vs Time profile in Diabetic rats (n = 6)

Time (Hr)	GPZ Mean ± SD	GPZ +ATV Mean ± SD	GPZ + GSE Mean ± SD	GPZ +ATV+GSE Mean ± SD
0	0±0	0±0	0±0	0±0
1	0.14±0.01	0.21±0.07*	0.5±0.02**	0.54±0.09***
2	0.21±0.06	0.44±0.09**	0.66±0.04***	0.34±0.02*
4	0.48±0.09	0.61±0.02**	0.87±0.06**	1.2±0.08***
6	0.82±0.07	1.01±0.04*	1.39±0.05**	1.65±0.06***
8	0.64±0.04	0.81±0.03*	1.27±0.04**	1.4±0.04**
10	0.32±0.09	0.54±0.05**	0.71±0.09**	1.04±0.01***
12	0.31±0.07	0.35±0.09	0.54±0.1*	0.58±0.09**

*p<0.05, **p<0.01, *** p<0.001 Compared to Glipizide group.

Plasma Concentration Vs Time profile in normal rats

Table 4: Plasma Concentration Vs Time profile in normal rats (n = 6)

Time (Hr)	GPZ Mean ± SD	GPZ +ATV Mean ± SD	GPZ + GSE Mean ± SD	GPZ + ATV+GSE Mean ± SD
0	0±0	0±0	0±0	0±0
1	0.02±0.001	0.19±0.01**	0.44±0.05***	0.48±0.01***
2	0.05±0.002	0.36±0.02**	0.55±0.06***	0.64±0.04***
4	0.33±0.05	0.62±0.09*	0.86±0.09**	1.2±0.04***
6	0.45±0.09	0.94±0.03*	1.23±0.06**	1.42±0.06**
8	0.25±0.08	0.52±0.05*	0.81±0.02**	1.03±0.02***
10	0.19±0.07	0.28±0.07	0.61±0.07**	0.66±0.04**
12	0.08±0.01	0.13±0.09**	0.21±0.04**	0.26±0.02***

***p<0.05, **p<0.01, *** p<0.001 Compared to Glipizide group.**

Table 5: Comparison of Pharmacokinetic parameters of Glipizide following pre treatment with ATV, GSE, ATV+GSE by oral administration in Diabetic rats (n = 6)

Parameters	GPZ Mean ± SD	GPZ +ATV Mean ± SD	GPZ + GSE Mean ± SD	GPZ + ATV+GSE Mean ± SD
C_{max}	0.87±0.08	1.04±0.09*	1.42±0.07**	1.69±0.02***
AUC (µg/mL/h)	5.39±0.02	7.4±0.15**	10.55±0.18**	11.23±0.21***
AUMC (µg/mL/h×h)	49.44±1.28	66.86±5.85**	92.91±6.2***	103.37±9.84***
Clearance (L/h/Kg)	6.19±0.12	4.63±0.45*	2.57±0.72**	1.64±0.14**
MRT (h)	8.46±0.41	9.12±0.38*	11.2±0.23**	19.25±0.16**
T_{max}	6±0	6±0	6±0	6±0

***p<0.05, **p<0.01, *** p<0.001 Compared to Glipizide group.**

Table 6: Comparison of Pharmacokinetic parameters of Glipizide following pre treatment with ATV, GSE, ATV+GSE by oral administration in normal rats (n = 6)

Parameters	GPZ Mean ± SD	GPZ +ATV Mean ± SD	GPZ + GSE Mean ± SD	GPZ + ATV+GSE Mean ± SD
C_{max}	0.44±0.06	0.95±0.09*	1.23±0.01**	1.44±0.06**
AUC (µg/mL/h)	2.53±0.08	5.76±0.07**	8.52±0.1***	10.23±0.14***
AUMC (µg/mL/h×h)	11.02±0.9	29.53±0.46**	55.4±0.39***	62.16±0.58***
Clearance (L/h/Kg)	9.22±0.02	5.43±0.52*	3.62±0.69**	3.01±0.04***
MRT (h)	6.49±0.13	7.99±0.17	9.12±0.22*	13.67±0.31**
T_{max}	6±0	6±0	6±0	6±0

***p<0.05, **p<0.01, *** p<0.001 Compared to Glipizide group.**

The % reduction of blood glucose with Glipizide + Atorvastatin, Glipizide with *Gymnemasylvestre e*xtract and *Gymnemasylvestre*extract with Glipizide and Atorvastatin in the normal rats and also diabetic rats at 4th hour were increased and showed better positive results attained the controlling of blood glucose levels. C_{max} AUC, AUMC, MRT values are also increased, this indicating that the improved systematic bioavailability of Glipizide. Butclearance was decreased, may be due to interaction and decreased metabolism but there was no change in T_{max}.In previous study shows that Atorvastatin induced the CYP2C9 in monkey hepatocytes but was an inhibitor of the CYP2C9 in human hepatocytes (Cohen *et al.*, 2000).

Finally, the systematic bioavailabilityGlipizide increased by Atorvastatin due to that may act as P-gp inhibitors (inhibits the efflux pump), and by *Gymnemasylvestre*may shows synergistic effect. Further the order of the Glipizide bioavailability was established i.e. increased by with Atorvastatin + *Gymnemasylvestre*extract > with *Gymnemasylvestre*extract > with Atorvastatin. Hence thepresent investigation warrants further studies to find out the relevance of this interaction in human beings.

CONCLUSIONS:

1. The % reduction of blood glucose with glipizide, Glipizide + Atorvastatin, Glipizide with *Gymnemasylvestre e*xtract and *Gymnemasylvestre*extract with Glipizide and Atorvastatin in the normal rats at 4th hour were 36.02±4.22, 42.04±2.28, 50.26±1.13 and 61.09±1.79 respectively.
2. In case of diabertic rats % reduction of blood glucose in the presence of glipizide, Glipizide + Atorvastatin, Glipizide with *Gymnemasylvestre*extract and *Gymnemasylvestre*extract with Glipizide and Atorvastatin at 4th hour were 50.52±3.68, 57.63±2.66, 63.47±1.45, 69.01±0.67 respectively. So this is revealed that the presence of Atorvastatin and *Gymnemasylvestre*extract showed better positive results attained the controlling of blood glucose levels.
3. The Glipizide, Glipizide + Atorvastatin, Glipizide + *Gymnemasylvestre*extract, Glipizide + Atorvastatin + *Gymnemasylvestre*extract of C_{max} AUC, AUMC, MRT values are increased, indicating improved systematic bioavailability of Glipizide. But Clearance was decreased significant result (p<0.001), interaction was may be due to decreased metabolism but there was no change in T_{max}.

4. This study also suggested that the systematic bioavailability Glipizide increased by Atorvastatin due to that may act as P-gp inhibitors (inhibits the efflux pump), and by *Gymnemasylvestre*may shows synergistic effect.
5. The order of the Glipizide bioavailability increased by with Atorvastatin + *Gymnemasylvestre*extract > with *Gymnemasylvestre*extract > with Atorvastatin.

REFFERENCES:

1. Aburuz S, Millership J, Mcelnay J. The development and validation of liquid chromatography method for the simultaneous detemination of metformin, glipizide, gliclazide, glibenclamide or glimperide in plasma. Journal of chromatography B, 2005, 817, 277-286.
2. Agarwal S, Singh S, Verma S, Lakshmi V, Sharma A. Chemistry and medicinal uses of Gymnemasylvestre Leaves: A Review. Indian drugs, 2000, 37, 354-360.
3. Akbarzadeh A, Norouzian D, Mehrabi M, Jamshidi S, Farhangi A, Verdi A, Mofidian S, Lamerad B. Induction of Diabetes by Streptozotocin in rats. Indian Journal of Clinical Biochemistry, 2007, 22(2), 60-64.
4. Alberti K, Press M, Keen H, Jarrett J. The biochemistry and the complications of diabetes. Complications of Diabetes, 1982, 43, 231-270.
5. Arimori K, Kuroki N, Hidaka M, Iwakiri T, Yamsaki K, Okumura M, Ono H, Takamura N, Kikuchi M, Nakano M. Effect of P-glycoprotein modulator, cyclosporine A on the gastrointestinal excretion of irinotecan and its metabolite SN-38 in rats. Pharma Research, 2003, 20, 910-917.
6. Ashish J, Bijaya G, Naresh R, DesaiB. Passive and inotrophoretic permeation of Glipizide. European Journal of Pharmaceutics and Biopharmaceutics, 2008, 69, 958-963.
7. Bakrudeen A, Rao S, Rao V. in-vitro callus and in-vivo leaf extract of Gymnemasylvestre stimulate β-cells regeneration and anti–diabetic activity in Wister rats. Phytomedicine, 2010, 17, 1033-1039.
8. Barnes K, Dickstein B, Cutler G, Fojo T, Bates S. Steroid transport, accumulation and antagonism of P-glycoprotein in multidrug resistant cells. Biochemistry, 1996, 35, 4820-4827.
9. Bolkent S, Yamardag R, Tabakogluoguz A, Ozsoy O. Effects of chord (Beta vulgaris L.Var.cicla) extract on pancreatic-cells in streotozotocin diabetic rats: a morphologic and biochemical study. Journal of Endocrinology, 2000, 73, 251-259.
10. Boullata J. Natural Health Product Interactions with Medication. Nutritional Clinical Practise, 2005, 20, 33-51.
11. Brahmankar D, Jaiswal B. Pharmacokinetic drug interactions, Biopharmaceutics and pharmacokinetics A Treatise. VallabhPrakashan, Pune, 2007, p 204-211.
12. Callagan R, Riordan J. Synthetic and natural opiates interact with P-glycoprotein in multidrug-resistant cells. Journal of Biological Chemistry, 1993, 268, 16059-16064.
13. Choo E, Kurnik D, Muszkat M, Ohkubo T, Shay S, Higginbotham J, Glaeser H, Kim R, Wood A, Wilkinson G. Differential in-vivo sensitivity to inhibition of P-glycoprotein located in lymphocytes, testes, and the blood-brain barrier. Journal of Pharmacology and Experimental Therapeutics, 2006, 317, 1012-1018.
14. Christopher J, Endres, Peng H, Francisco S, Jashvant D. The role of transporters in drug interactions. European journal of pharmaceutical sciences, 2006, 27, 501-517.