Original Article

Effect of *Gymnema Sylvestre* on the Pharmacokinetics and Pharmacodynamics of Gliclazide in Diabetic Rats

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ABSTRACT

Gymnema sylvestre (GS) and gliclazide both selectively promote insulin release. GS stimulate ß-cell function, increase ß-cell number, increases the enzyme activity responsible for glucose uptake and utilization. Gliclazide, a second generation sulphonylurea derivative and is preferred in therapy because of its selective inhibitory activity towards pancreatic K+ ATP channels. The effect of the combination of GS (30 mg/kg p.o.) and Gliclazide (GL) (40 mg/kg and 20 mg/kg p.o.) on the pharmacokinetic parameters of GL was studied in STZ induced diabetic rats after single dose administration and multiple dosing for 15 days. Pharmacodynamic interactions were as well studied by determing the effect of combination therapy on the serum glucose levels of STZ induced diabetic rats before and after multiple dosing for 14days. Histopathological studies were carried out by excising the pancreas after the treatment and the effect of combination on the volume of beta cells were compared to that of single (either GL alone or GS alone) administration and control. Pharmacokinetic studies revealed a decrease in the bioavailability of GL when given in combination with GS. The decrease in bioavailability was contributed by decrease in absorption rate constant and increase in clearance. Where as in the pharmacodynamic study the combination in general showed decrease in serum glucose levels, which was contributed by the hypoglycemic property of GS. But the combination did not decrease the serum glucose levels comparable to GL when given alone. Histopathological studies revealed that combination of GS with GL increased the volume of islets cells of pancreas. These observations show that *Gymnema sylvestre* is a potent hypoglycaemic but it

contributes to a decrease in hypoglycemic effect of gliclazide.

Keywords: Diabetes, glucose, *Gymnema sylvestre*, gliclazide.

INTRODUCTION

Herbal products have gained increasing popularity in the last decade. Although herbs are often perceived as "natural" and therefore safe[1]. Many Interactions between medicinal herbs and pharmaceutical drugs increase or decrease the pharmacological or toxicological effects of either component[2].

The study of mechanisms of drug interaction is of much value in selecting drug concentrations to provide rational therapy. Most of the chronic diseases (cancer, coronary artery disease, degenerative arthritis, depression, diabetes mellitus and hypertension) require multiple drug therapy[3] In the treatment of diabetes mellitus combined pharmacological therapy is required to obtain adequate blood glucose control and treatment of concurrent pathologies associated with it. While there are oral hypoglycaemic agents like sulphonylureas, biguanides and insulin to treat diabetes, none have been unequivocally successful in maintaining euglycaemia and avoiding the late complications of diabetes.

Additive glucose-lowering effects has been reported when combination of two or more hypoglycaemics are administered. The combination of sulphonylureas with thiazolidinediones or the biguanides produces more hypoglycaemia than when given alone. Although numerous herbs are reported to possess some degree of antidiabetic activity[4] Gymnema sylvestre, bitter gourd fruit (Momordica charantia), and fenugreek seeds (Trigonella foenum graecum) may be among the best in terms of efficacy and safety.

Gliclazide works in several different ways, but is known to act mainly by releasing insulin by blocking K+ channels in the pancreatic β cells[5]. Current literature shows that the drug has low incidence of hypoglycemia, inhibits platelet aggregation, increase fibrinolysis[6]. So gliclazide is used as a drug of choice in long term sulfonylurea therapy for the control of type 2 diabetes mellitus in the aged[7] Gliclazide exhibits inter-individual bioavailability variations probably due to its poor aqueous solubility.

Gymnema sylvestre (GS) is another commonly used herb in Ayurveda. The common name, gurmar, meaning "sugar-destroying," was given because of the plants antisacharogenic property (suppresses the taste of sugar).[8] This property is believed to be due to a glycoside known as gymnemic acid. Gymnemic acid is a mixture of triterpene glucuronides, which was found in the leaves of the Indian plant Gymnema sylvestre, not only inhibits glucose absorption in the small intestine[9] but also suppresses hyperglycemia and hyperinsulinaemia in an oral glucose tolerance test[10].

MATERIALS AND METHODS

Drugs and Chemicals

Albino rats of either sex weighing between 180 and 250 g obtained from National institute of Nutrition India. These animals were maintained proper conditions in animal house of Vaageswari College of pharmacy {IAEC number VCP/2012/10/6/16}. Standardized alcoholic extract of *Gymnema sylvestre* containing 25% gymnemic acid, streptozotocin (Neocare Naturals Pvt. Ltd, Hyderabad, India).

Experimental Models

Pretreatment: Albino rats of either sex (180 to 250 g) were acclimatized in an air-conditioned room at 22 ± 20 C for 2 weeks. They were housed in elevated wire cages and were provided with high fat diet (carbohydrates: proteins: fat in 42:18:40 ratios) and water *ad libitum* for a period of 12days[11].

Experimental Induction of Diabetes in Rats[12]

After 2 weeks of feeding with HFD the rats were fasted for a period of 18 hours before induction of diabetes, and were injected intra-peritonially with a single dose of Streptozocin 60 mg/kg, freshly dissolved in normal saline. After the injection, the rats had free access to food (normal pellet diet) and water *ad libitum*. Diabetes in rats was identified by moderate polydipsia and marked polyuria. After 3 days i.e. 72hrs of injection, the fasting blood glucose levels were determined by following glucose oxidase/peroxidase GOD/POD method using a commercial glucose estimation kit[13] with UV-Visible Spectrophotometer at 505nm. The rats showing fasting blood glucose more than 150 mg/dL were considered diabetic and selected for the experimentation.

Pharmacokinetic study in diabetic rats

Treatment

The Hyperglycemic rats were divided into 2 groups of 6 animals each:

Group I: gliclazide (40 mg/kg, *p.o.*) (Talari et al 2010).

Group II: combination of gliclazide (40 mg/kg, *p.o.*) (Talari et al 2010) + *Gymnema sylvestre* extract (30 mg/kg, *p.o.*)

Suspensions of gliclazide and *G. sylvestre* extract were prepared by suspending in 0.5% Na.CMC through oral gavage.

Pharmacokinetic Study

Single dose study

The overnight fasted rats were divided into two different groups (n=6); and the treatment was given as mentioned above. Posts dosing the blood samples were collected at predetermined intervals of 1, 2, 4, 8, 12 and 24 h[14] into micro-centrifugal tubes containing sodium EDTA from retro-orbital sinus under ether anesthesia. The blood was subjected to centrifugation at 10000 rpm for 10 min and the plasma was stored at -30^{0}C until analysis. The analysis for gliclazide was carried out by HPLC method, as described by Obaid and colleagues[15] and was modified in the laboratories.

Multiple dose study

The study was carried out in a similar way to single dose study. The diabetic rats were divided into two treatment groups as mentioned above and treatment was given for a period of 14 days. On the 15th day the overnight fast rats were treated as mentioned and blood samples were collected at predetermined intervals of 1, 2, 4, 8, 12, and 24 h in sodium EDTA added micro-centrifugal tubes. Plasma was separated after centrifugation and stored until analyzed.

Pharmacodynamic study in diabetic rats

Treatment

The Hyperglycemic rats were divided into 5 groups consisting of 5 animals each:

Group I: vehicle i.e. 0.5% Na.CMC suspension (1 ml/kg, *p.o.*), group II: *Gymnema sylvestre* extract (30 mg/kg, *p.o.*), group III: gliclazide (40 mg/kg, *p.o.*) 2010), group IV: combination of gliclazide (40 mg/kg, *p.o.*) (Talari et al 2010) + GS and group V: combination of gliclazide (20 mg/kg, *p.o.*) + GS .Suspensions of gliclazide and G. sylvestre extract were prepared by suspending in 0.5% Na.CMC through oral gavage.

Determination of serum glucose concentration of the diabetic rats before treatment has been given

Adult albino rats weighing 180–250g with a threshold value of fasting serum glucose >150 mg/dl were considered diabetic. Diabetes induced rats were divided into five groups and treatment was given as mentioned above. A day before the treatment was started the serum glucose concentration of the animals were determined at different time intervals of 0, 1, 2, 4, 8 and 12h[16] by GOD/POD method using UV-Visible Spectrophotometer at 505nm, and the values were considered to be zero day values.

Determination of serum glucose concentration of the diabetic rats after 14 days of treatment

After determining the glucose concentration on the zero day, the animals were given the treatment as mentioned from the 1st day until the 14th day. On the 14th day the serum glucose concentration of the overnight fasted rats were determined at predetermined time intervals of 0, 1, 2, 4, 8 and 12h[17] by GOD/POD method using UV-Visible Spectrophotometer at 505nm.

Histopathological studies

After the last blood glucose estimation, the rats were sacrificed and pancreas were excised and subjected to histopathological studies to determine the inflammatory and necrotic changes. The tissues were stained using H&E stain and observed under 100 × magnifications. The volume of islet cells in pancreas was determined using eyepiece reticule following point count method using the formula[18].

$$\text{Volume of islets (mm3)/Volume of tissue (mm3)} = \frac{\text{Number of points on the islets}}{\text{Total number of points in the reticule}} \times 100$$

Statistical Analysis

Data were expressed as mean ± standard error of the mean (SEM). The significance was determined by applying Student's unpaired t-test for determination of pharmacokinetic parameters and one way analysis of variance for pharmacodynamic data. Significance was established at a p value of less than 0.05.

Advanced software's were used to determine the pharmacokinetic and pharmacodynamic parameters. Pharmacokinetic data was calculated using PK solver software and statistical analysis was done using INSTAT graph pad software.

RESULTS & DISCUSSION

We studied the influence of *Gymnema sylvestre* on the pharmacokinetics and pharmacodynamics of gliclazide in diabetic rats. Diabetic rat model served to validate the response as seen in the actually used conditions of the drug. The multiple dose effect of GS on gliclazide activity was also studied for determining the influence of long term treatment with GS, since both are hypoglycaemics and used for chronic period.

Many studies have shown that oral administration of Gymnema extract reduces serum glucose level and improves glucose tolerance in mildly diabetic rats Studies of an ethanol leaf extract, GS, in diabetic rat and rabbit models have reported regeneration of islets of Beta cells decreases in blood glucose level, and increases of serum insulin which suggests a restorative effect of the Gymnema extract on pancreatic tissue. Administration of water extract of *Gymnema sylvestre* leaves was found to increase serum insulin level suggesting its insulin releasing effect.

Gliclazide works in several different ways, but is known to act mainly by releasing insulin by blocking K+ channels in the pancreatic β cells. Current literature

shows that the drug has good tolerability, low incidence of hypoglycemia, low rate of secondary failure, inhibits platelet adhesion and aggregation, So gliclazide appears to be a drug of choice in long term sulfonylurea therapy for the control of type 2 diabetes mellitus in the aged

Gliclazide exhibits inter-individual bioavailability variations probably due to its poor aqueous solubility. Gliclazide is metabolized via three primary methods: oxidation of the tolyl group; hydroxylation of the azabicyclo-octyl ring; and glucuronidation It is exclusively metabolized by cytochrome P450 (CYP) 2C9.

GS when combined with gliclazide reduces the hypoglycaemic activity of the latter. It was found that the $AUC_{0-\infty}$ was decreased in the combination i.e. gliclazide (40 mg/kg, *p.o.*) with GS (30mg/kg, *p.o.*) when compared to gliclazide alone (40 mg/kg, *p.o.)* (Fig: 5, 6). The decrease in the bioavailability was significantly observed in multiple dose study with a percentage decrease of 43%. Cmax was decreased by about 75% after multiple dosing which was attributed by a significant decrease in the absorption rate constant Ka by about 95 %. Apart from decrease in absorption rate constant there was increase in the clearance from 0.093 to 0.147. Plasma half life remained unaltered after multiple dosing whereas time to maximam plasma concentration was significantly increased.

The pharmacodynamic results observed suggest that, the combination of high dose of gliclazide (40 mg/kg, *p.o.*) with GS showed maximum hypoglycaemic effect and the effect produced by combination of gliclazide (20 mg/kg, *p.o.*) with *GS* was greater than the hypoglycaemic effect produced by GS (30 mg/kg, *p.o.*), but was comparably less than gliclazide (40 mg/kg, *p.o.*) (Fig: 7). The histopathological studies reveal that the combination of gliclazide (40 mg/kg, *p.o.*) and GS not only increased the volume of islets but also recovered partially destroyed beta cells.

This suggests that GS has good potential to be developed into hypoglycemic new dug. In the present study herb-drug interactions exist at pharmacokinetic level. GS was found to inhibit absorption of the gliclazide and increase the renal clearance.
GS decreases the bioavailability of gliclazide by inhibiting its absorption and by increasing its clearance thereby reducing the combined pharmacological effect. Hence care must be taken when the combination is prescribed for clinical benefit in diabetic patients. As GS decreases the effect of gliclazide it may result in increase in hypoglycemia because of lower bioavailability. Hence the study of mechanisms of drug interaction is of much value in selecting drug concentrations to provide rational therapy.

CONCLUSION

The interaction appears to be pharmacokinetic interaction at absorption, elemination. *Gymnema sylvestre* inhibits the absorption of gliclazide which results in a significant decrease in the bioavailability of the latter. Since the interaction was seen in rats it is likely to occur in humans leading to decreased activity of gliclazide, which may need dose adjustments. Hence care must be taken when the combination is prescribed for clinical benefit in diabetic patients. However the present study warrants further studies to find out the relevance of the interaction in human beings.

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Effects of *Gymnema sylvestre* on pharmacokinetics of gliclazide

Single dose study

Gymnema sylvestre reduced gliclazide $AUC_{0-\infty}$ and Cmax but the reduction was not significant. All the pharmacokinetic parameters of both the treatment groups are presented here (Table 4). There was a significant increase in the Ka and Vd. Whereas Ke and Tmax decreased significantly. Furthermore, the mean t1/2 of gliclazide after treatment with *gymnema sylvestre* decreased significantly from 0.34 to 0.04 h. clearance did not alter significantly.

Table 1. Average serum gliclazide concentrations before and after treatment with alcoholic extract of *Gymnema sylvestre* in streptozocin induced diabetic rats after single dose (n = 5).

Time (hr)	Mean (GL) ± SEM	Mean (GL+GS) ± SEM
0	0	0
1	32.87 ± 0.49	22.96 ± 1.36
2	17.97 ± 1.04	11.82 ± 0.85
4	7.92 ± 0.86	6.78 ± 0.35
8	6.17 ± 0.46	4.08 ± 0.61
12	0.38 ± 0.11	1.03 ± 0.35
24	0	0

GL: Gliclazide 40mg/kg; GS: Gymnema sylvestre leaf extract with 30mg/kg gymnemic acid; SEM: Standard error of mean

Table 2. Pharmacokinetic parameters of gliclazide before and after treatment with alcoholic extract of *Gymnema sylvestre* in diabetic rats (n=5) after single dose.

Kinetic parameter	Pharmacokinetic parameters of gliclazide (mean ± SEM)		Significance at (p < 0.05)
	Before treatment (Gliclazide alone 40mg/kg)	After treatment (combination of Gliclazide and Gymnema)	
AUC0-α (µg/ml*h)	88.45 ± 3.2	77.75 ± 5.9	NS
AUMC0- α (µg/ml*h^2)	174.63 ± 9.1	195.62 ± 27.24	NS
T1/2 (h)	0.34 ± 0.13	0.04 ± 0.002	S
Ka (h-1)	4.81 ± 2.44	18.63 ± 0.82	S
Ke (h-1)	0.71 ± 0.09	0.42 ± 0.03	S
Clearance ((mg)/(µg/ml)/h)	0.093 ± 0.005	0.10 ± 0.006	NS
Vd ((mg)/(µg/ml))	0.14 ± 0.02	0.24 ± 0.01	S
Cmax (µg/ml)	36.46 ± 3.1	29.51 ± 1.7	NS
Tmax (h)	0.68 ± 0.14	0.21 ± 0.06	S

AUC: Area under the curve; AUMC: Area under the first movement curve; NS: Not significant: S: Significant; Vd: Volume of distribution. All the values are expressed as mean ± Standard error of mean.

Multiple dose study

Gymnema sylvestre reduced gliclazide $AUC_{0-\infty}$, Cmax and Ka significantly. The reduction in $AUC_{0-\infty}$ and Cmax was by 47% and 75% respectively. Whereas CL and Tmax increased significantly. Furthermore, the mean t1/2 of gliclazide after treatment with gymnema sylvestre remained unaltered. The pharmacokinetic parameters of both the treatment groups are presented here (Table 5).

Table 3. Average serum gliclazide concentrations before and after treatment with alcoholic extract of *Gymnema sylvestre* in streptozocin induced diabetic rats after multiple dosing for a period of 15days (n = 5).

Time (hr)	Mean (GL) ± SEM	Mean (GL+GS) ± SEM
0	0	0
1	36.276 ± 3.66	14.074 ± 0.67
2	12.654 ± 0.74	8.498 ± 0.62
4	6.838 ± 0.80	4.948 ± 0.42
8	1.512 ± 0.20	1.804 ± 0.35
12	0.442 ± 0.06	0.376 ± 0.02
24	0	0

GL: Gliclazide 40mg/kg; GS: Gymnema sylvestre leaf extract with 30mg/kg gymnemic acid; SEM: Standard error of mean.

Table 4. Pharmacokinetic parameters of gliclazide before and after treatment with alcoholic extract of *Gymnema sylvestre* in diabetic rats (n=5) after a period of 15 days.

Kinetic parameter	Pharmacokinetic parameters of gliclazide (mean ± SEM)		Significance at (p < 0.05)
	Before treatment (Gliclazide alone 40mg/kg)	After treatment (combination of Gliclazide 40mg/kg and Gymnema sylvestre extract 30mg/kg)	
AUC0-α (µg/ml*h)	93.46 ± 8.69	53.56 ± 4.40	S
AUMC0- α (µg/ml*h^2)	112.83 ± 6.20	156.34 ± 23.03	NS
T1/2 (h)	0.038 ± 0.0007	0.039 ± 0.0019	NS
Ka (h-1)	18.14 ± 0.36	0.86 ± 0.04	S
Ke (h-1)	0.86 ± 0.04	0.36 ± 0.02	S
Clearance ((mg)/(µg/ml)/h)	0.093 ± 0.004	0.147 ± 0.006	S
Vd ((mg)/(µg/ml))	0.110 ± 0.01	0.410 ± 0.015	S
Cmax (µg/ml)	70.26 ± 9.38	17.56 ± 0.58	S
Tmax (h)	0.177 ± 0.004	0.224 ± 0.011	S

AUC: Area under the curve; AUMC: Area under the first movement curve; NS: Not significant: S: Significant; Vd: Volume of distribution. All the values are expressed as mean ± SEM.

Effects of *Gymnema sylvestre* on pharmacodynamics of gliclazide

Effect on streptozotocin induced diabetes rats on zero day

All the treatments showed no significant decrease in serum glucose levels compared to control on the zero day at different time intervals selected (Table 5).

Table 5. Comparison of serum glucose levels at zero day in various groups under dynamic study (n = 3)

	Control (vehicle)	GL 40mg/Kg	GL 40mg/Kg + GS 30mg/Kg	GL 20mg/Kg + GS 30mg/Kg	GS 30mg/Kg
Time (h)	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
1	236.55 ± 48	344.3 ± 41.32	328.7 ± 44.4	214 ± 4.7	182 ± 4.35
2	251.5 ± 48.55	306.6 ± 34.19	322.4 ± 44.9	204 ± 9.8	177 ± 4.8
4	235.7 ± 31.39	287 ± 32.4	314 ± 31.6	155 ± 16.4	187.7 ± 1.45
8	186 ± 23.5	268.6 ± 30.31	279 ± 45.3	185 ± 17.7	162 ± 10.7
12	179 ± 35	241.3 ± 29.16	310 ± 19.3	184 ± 5.6	160 ± 8.6

*Statistical significance $p < 0.05$ (compared with the control group);
SEM: Standard error of mean

Effect on streptozotocin induced diabetes rats after 14days of treatment

The STZ induced hyperglycemia was significantly attenuated by all the treatments (Table 7). The combination of low dose of gliclazide (20 mg/kg, *p.o.*) with GS (30 mg/kg, *p.o.*) produced greater reduction in serum glucose levels compared to that observed with GS (30 mg/kg, *p.o.*) but when compared to gliclazide (40 mg/kg, *p.o.*) the combination was less effective. The maximum effect was seen with combination of high dose of gliclazide (40 mg/kg, *p.o.*) with GS (30 mg/kg, *p.o.*).

Table 6. Comparison of serum glucose levels after 14 days of treatment in various groups under dynamic study (n = 3).

	Control (vehicle)	GL 40mg/Kg	GL 40mg/Kg + GS 120mg/Kg	GL 20mg/Kg + GS 120mg/Kg	GS 120mg/Kg
Time (h)	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
1	173.3 ± 19	37.7 ± 3.38*	28.7 ± 1.76*	42 ± 1.15*	65.7 ± 1.76*
2	158.3 ± 8.98	49 ± 5.03*	47 ± 0.57*	60 ± 1.52*	77 ± 2.08*
4	195.7 ± 31.39	80.3 ± 2.84*	72 ± 3.46*	72.3 ± 2.02*	87.7 ± 1.45*
8	222 ± 33.5	96.3 ± 2.60*	85.7 ± 4.63*	91.7 ± 0.88*	112 ± 3.21*
12	178.7 ± 18.85	118.7 ± 5.92*	103.7 ± 4.37*	106.3 ± 0.88*	125.3 ± 2.18*

* Statistical significance $p < 0.05$ (compared with the control group);
SEM: Standard error of mean

Effect on histopathology of streptozotocin induced diabetes rats

Histopathological studies revealed that the volume of islet cells in pancreas was significantly more in drug treated animals compared to the control (Table 8). The islet cells were shrunken and lytic cellular changes were observed in control (Fig). Individual treatment had improved it but combination groups with a higher dose of gliclazide showed the return of islets close to normal cytoarchitecture (Fig: ,,). In combination group, islets were big; cells were clear with good vascular pattern (Fig:). The results of combination group with a lower dose of gliclazide produced increment to the volume of islets in pancreas compared to individual treatment.

Table 7. Volume of islet cells in pancreas in different groups under dynamic study.

Group	Volumne of islets (mm3/mm3) / Volume of pancreas (mm3/mm3)
control	0.081 ± 0.002
GS (30 mg/kg, p.o.)	0.196 ± 0.053
GL (40 mg/kg, p.o.)	0.138 ± 0.009
GL (40 mg/kg, p.o.) + GS (30 mg/kg, p.o.)	0.243 ± 0.049
GL (20 mg/kg, p.o.) + GS (30 mg/kg, p.o.)	0.154 ± 0.035

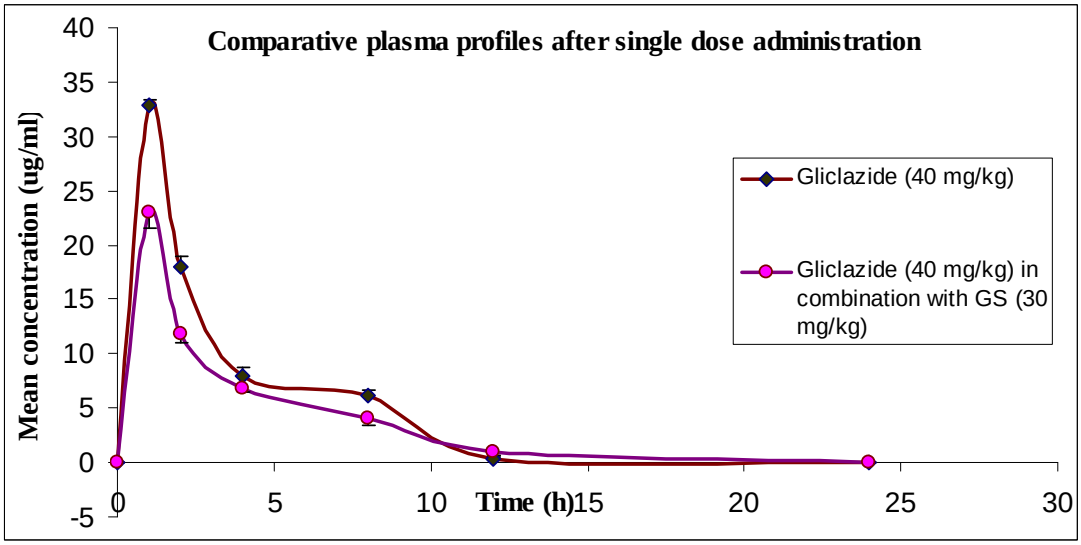

Figure.1. Mean plasma gliclazide concentrations before and after treatment with alcoholic extract of Gymnema sylvestre in streptozocin induced diabetic rats (n = 5)

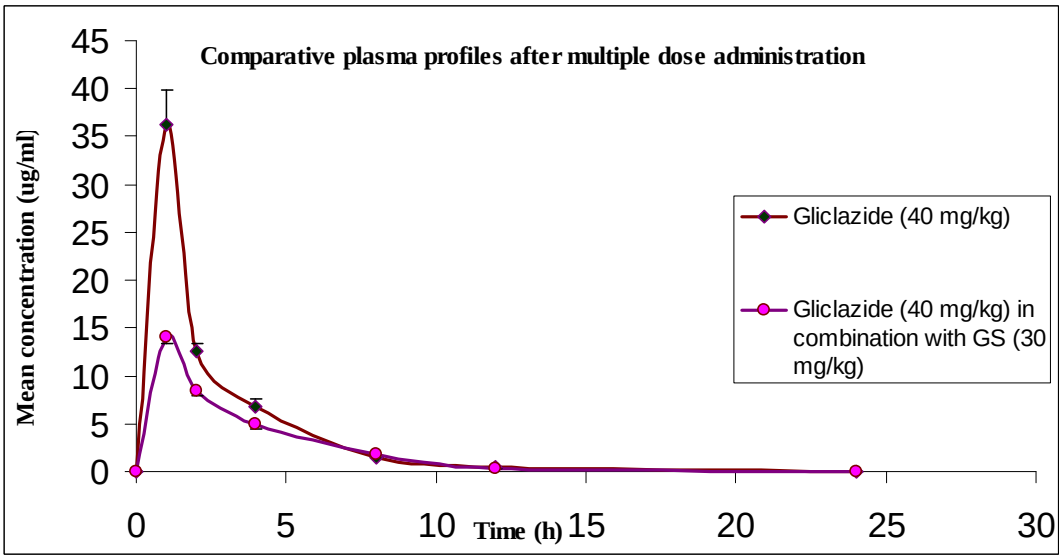

Figure.2. Mean plasma gliclazide concentrations before and after treatment with alcoholic extract of Gymnema sylvestre in streptozocin induced diabetic rats for a period of 15days(n=5)

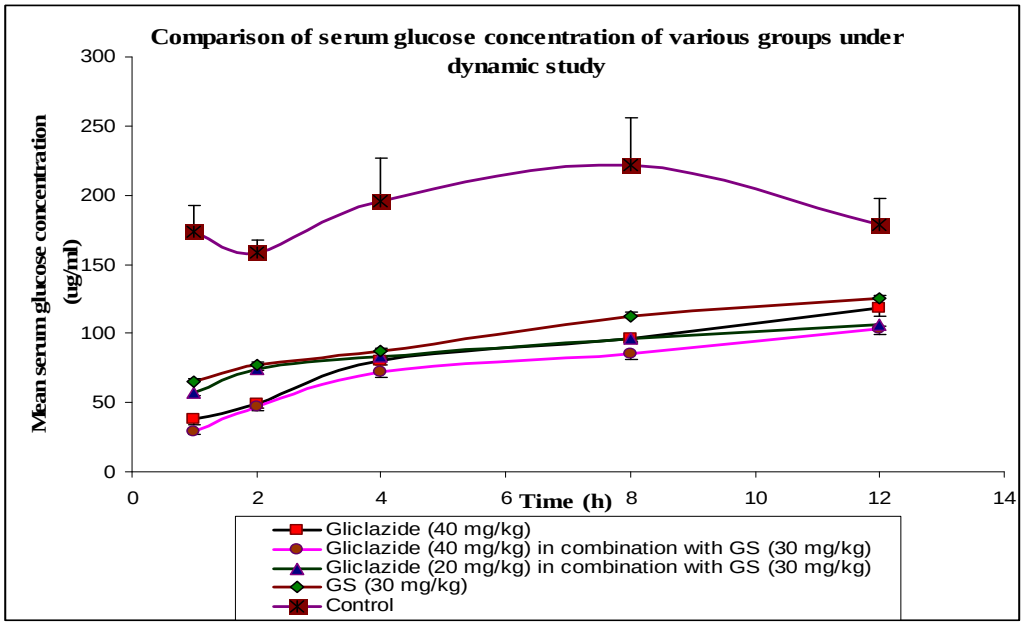

Figure.3. Comparison of mean serum glucose concentration of different groups under dynamic study after a period of 14days of treatment (n = 3)