Original Article

Safety of Gliclazide With the Aqueous Extract of Gymnema Sylvestre on Pharmacodynamic Activity in Normal and Alloxan Induced Diabetic Rats

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ABSTRACT

The present study was carried out to evaluate the hypoglycemic activity and antihyper glycemic activity of aqueous extracts of whole plant of *Gymnema sylvestre* in normal and in alloxan induced diabetic rats. The aqueous extract of *Gymnema sylvestre* (30mg/Kg, 100mg/Kg, 300mg/Kg b.w.) and the dose (100mg/Kg) that produced an optimal reduction (30-40%) of blood glucose was selected for the study. Blood samples were collected at desired intervals of time and analyzed for blood glucose by the GOD - POD method. Gliclazide a second generation sulfonyl urea was selected for the interaction study with that of the *Gymnema sylvestre*. The doses of Gliclazide (1mg/Kg, 2mg/Kg and 4mg/Kg b.w.) were given to normal and diabetic rats and the dose (2mg/Kg b.w.) that produced an optimal reduction of blood glucose was selected for the study. The prior administration of *Gymnema sylvestre* significantly increased the hypoglycemic and antihyperglycemic activity of Gliclazide.

Keywords: *Gymnema sylvestre,* hypoglycemic, antihyperglycemic activity, Gliclazide.

INTRODUCTION

Diabetes (DM) is a chronic metabolic disorder characterized by hyperglycemia caused by defective insulin secretion, resistance to insulin action or a combination of both. It is a disease prevailing throughout the world, irrespective of age, sex and race. Among diabetics, approximately 95% of patients have type 2 diabetes mellitus (NIDDM), whereas about 5% of patients have type 1 diabetes mellitus (IDDM). Patients with DM are at risk for microvascular complications like retinopathy, nephropathy and neuropathy and macrovascular complications like

myocardial infarction that increase morbidity and mortality[1].

Gymnema sylvestre (Asclepiadaceae) is an herb native to the tropical forests of southern and central India and Srilanka. Various phytochemical constituents identified for hypoglycemic / antihyperglycemic activities are ascorbic acid, chromium, manganese, niacin, gymnemic acid and gymnemosides[2-4].

MATERIALS & METHODS

Plant extracts

Whole plant of *Gymnema sylvestre* was collected and authenticated from Laila Impex, Vijayawada, Andhra Pradesh, India. Plant material was dried under shade and coarsely powdered for extraction. The coarsely powdered crude material (500g) was separately subjected to extraction using water for 10 days by cold maceration. The aqueous extract was concentrated by rotary vacuum evaporator under reduced pressure and then dried in the open air.

The present study was carried out in the institute with CPCSEA approval reference number: 1175/ac/08/CPCSEA.

Animals

Wistar Albino rats of either sex procured from Mahaveer Enterprises, Hyderabad, India were used in the study. They were maintained under standard laboratory conditions at ambient temperature of 25±2°C and 50±15% relative humidity with 12 hours light/12 hours dark cycle. Rats were fed with commercial pellet diet (Rayan's Biotechnologies Pvt. Ltd, Hyderabad, India) and water *ad libitum*. Rats were fasted for 18 h prior to the experiment, allowing access to water and during the experiment food and water were withdrawn.

In the present study doses of the extract was decided based on 'IRWINS' method, i.e. minimum dose selected was 30 mg/kg b.w. And further doses were selected on the logarithmic basis method mentioned in Turners book as 100 mg/kg b.w. and 300 mg/kg b.w.

Experimental Design[5]

For the evaluation of antidiabetic activity rats (200-240 g) were divided into twelve groups containing six animals each group.

Normal Rats

Group-I : Normal control (water).
Group-II : *Gymnema sylvestre* (30mg/Kg b.w.orally)
Group-III : *Gymnema sylvestre* (100mg/Kg b.w., orally)
Group-IV : *Gymnema sylvestre* (300mg/Kg b.w., orally)
Group-V : Gliclazide (1mg/Kg b.w.,orally)
Group-VI : Gliclazide (2mg/Kg b.w., orally)
Group-VII : Gliclazide (4mg/Kg b.w., orally)
Group-VIII : *Gymnema sylvestre* +Gliclazide (100mg +2mg/Kg b.w., orally)

Diabetic Rats

Group-IX : Normal control (water).
Group-X : *Gymnema sylvestre* (100mg/Kg b.w., orally)
Group-XI : Gliclazide (2mg/Kg b.w., orally)
Group-XII : *Gymnema sylvestre* +Gliclazide (100mg +2mg/Kg b.w., orally)

Induction of Diabetes in Experimental Animals[5]

Animals were injected with freshly prepared aqueous solution of alloxan monohydrate in two doses of 100 mg/kg and 50 mg/kg body weight intraperitonial route

for two consecutive days. Then 10% dextrose was administered to combat the immediate hypoglycemia. Blood sugar was measured and rats showing fasting blood sugar levels above 250 mg/dL were selected for the study. Blood was collected from the retro orbital plexus of rats. The blood samples were collected at 0, 1, 2, 3, 4, 6, 8, 10, and 12 h intervals from all the groups of rats after drug administration and were analyzed for blood glucose by GOD/POD method[16].

STATISTICAL ANALYSIS

All the values of percentage reduction of blood glucose were expressed as mean ± standard error of mean (S.E.M.) and analyzed for ANOVA and post hoc Dunnet's t-test.

RESULTS AND DISCUSSION

Normal Rats

Gymnema sylvestre induced hypoglycemia was studied by administering it in different doses namely 30,100,300 mg/kg body weight for the dose response effect. *Gymnema sylvestre* produced 24.00±0.71%, at 4 h, 35.41±0.91% and 43.81±0.73% at 3h reduction in blood glucose levels by 30,100 and 300mg/kg b.w. respectively (Table-1).

Gliclazide was used as standard drug, tested at 1, 2, and 4 mg/kg b.w doses and 2 mg/kg b.w was found to produce optimal reduction in blood glucose. Gliclazide induced hypoglycemia was studied by administering a dose of 2 mg/kg b.w. Gliclazide produced 34.07±0.54% and 31.28±0.64% reduction in blood glucose levels at 3 h and 8 h respectively (Table-2).

The influence of 100 mg/kg b.w. of aqueous extract of *Gymnema sylvestre* was studied on the hypoglycemic effect of Gliclazide (2 mg/kg b.w.) in normal rats. This combination produced a peak effect of 38.24±0.54 % (reduction in blood glucose) at 4 h and percentage reduction was maintained above 30% from 3 h to 8 h intervals.

Diabetic rats

The aqueous extract of *Gymnema sylvestre* (100 mg/kg body weight) produced peak effect of 33.78 ± 0.11% reductions in blood glucose at 3 h. Gliclazide 2 mg/kg body weight produced a biphasic response in peak effect in 38.62±0.42% and 35.81±0.19% reduction in blood glucose at 3 h and 8 h respectively (Table-3).

The selected dose of aqueous extract of *Gymnema sylvestre* enhanced the antihyperglycaemic effect of Gliclazide with a peak reduction in blood glucose level of 46.18±0.36 at 3 h and 46.18±0.37% at 8 h (Table-4). The enhanced activity was shown to be sustained for 3 h to 10 h.

The sulphonylureas act by stimulating the release of insulin from pancreatic beta cells of islets of Langerhans and by increasing the sensitivity of peripheral tissues to insulin. The predominant effect of sulfonylureas is on the insulin secretion[6]. Sulphonylureas also stimulate the release of somatostatin, and they may suppress the secretion of glucagon slightly[7]. The reduction in blood glucose levels with *Gymnema sylvestre* might be due to the presence of active constituents like gymnemic acid and gymnemosides[2-4].

The possible mechanisms of hypoglycemic action of *Gymnema sylvestre* were increased secretion of insulin, promotion of regeneration of islets cells, reduction in glucose absorption, increase in utilization of glucose by insulin dependent pathways [8,9]. Hence increase in Gliclazide action in the presence of *Gymnema sylvestre* could be due to their combined action in pancreatic and extra pancreatic mechanisms[13-15]. To check the validity of the observations seen earlier in normal rats, the studies were repeated in diabetic rats. The observations were considered to be significant if they exist in diabetic condition also.

The aqueous extract of selected dose of *Gymnema sylvestre* (100 mg/kg b.w.) produced a significant antihyperglycaemic

activity with peak activity at 3 h when administered alone and was found to enhance the antihyperglycaemic activity of Gliclazide during 2 to 10 h in combination. The antihyperglyceamic activity of *Gymnema sylvestre* might be due to the presence of active constituents (gymnemic acid and gymnemosides)[10-12] which were reported to produce antihyperglycaemic activity. The hypoglycemic effect was qualitatively similarly, but quantitatively 2-8 % more in diabetic condition compared to matched control in rats.

CONCLUSION

The drug interaction studies of *Gymnema sylvestre* with Gliclazide was conducted in normal and diabetic rats based on pharmacodynamic (blood glucose) response for 12h. The normal rat model served to quickly identify the interaction and diabetic rat model served to validate the same response in actual use condition of drugs.

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Table 1. Dose response relationship of *Gymnema sylvestre* on % blood glucose reduction in normal rats

Time (h)	Percentage blood glucose reduction in rats (Mean ±SEM)		
	GS (30mg/kg)	GS (100mg/kg)	GS (300mg/kg)
0	0.00±0.00	0.00±0.00	0.00±0.00
1	6.90±0.48	11.22±0.59	12.90±0.95
2	13.06±0.38	21.28±0.77	26.24±1.09
3	19.00±0.57	35.41±0.91	43.81±0.73
4	24.00±0.71	29.21±0.55	38.86±1.33
6	16.15±0.69	21.84±1.60	30.16±2.08
8	8.77±1.08	14.63±0.81	18.72±1.64
10	3.32±0.59	9.60±0.78	10.29±1.14
12	0.02±0.99	5.27±0.49	4.91±0.30

Results are expressed as ± SEM (n=6). Data processed by one way ANOVA followed by Dunnett's test,*

Table 2. Dose response relationship of Gliclazide on % blood glucose reduction in normal rats

Time (h)	Percentage blood glucose reduction in rats (Mean ±SEM)		
	Gliclazide (1mg/kg)	Gliclazide (2mg/kg)	Gliclazide (4mg/kg)
0	0.00±0.00	0.00±0.00	0.00±0.00
1	8.78±1.19	7.36±0.68	18.60±0.58
2	18.82±1.09	19.69±1.14	26.15±0.89
3	29.99±0.80	34.07±0.54	35.26±0.31
4	19.27±0.98	23.51±2.39	31.60±2.48
6	14.75±1.17	22.34±1.10	24.94±1.69
8	24.98±1.35	31.28±0.64	34.57±0.80
10	12.72±1.06	13.34±0.51	17.70±2.17
12	10.47±0.85	7.36±0.61	7.04±1.73

Results are expressed as ± SEM (n=6). Data processed by one way ANOVA followed by Dunnett's test,*

Table 3. The percent blood glucose reduction Vs time with Gymnema sylvestre, Gliclazide and their combination in normal rats

Time (h)	Gliclazide (Mean ±SEM)	Gymnema sylvestre (Mean ±SEM)	GS+GL (Mean ±SEM)
0	0.00±0.00	0.00±0.00	0.00±0.00
1	7.36±0.68	11.22±0.59	13.33±0.46
2	19.69±1.14	21.28±0.77	26.43±0.51
3	34.07±0.54	35.41±0.91	38.24±0.54
4	23.51±2.39	29.21±0.55	35.33±0.90
6	22.34±1.10	21.84±1.60	34.58±0.62
8	31.28±0.64	14.63±0.81	33.36±0.35
10	13.34±0.51	9.60±0.78	17.63±0.55
12	7.36±0.61	5.27±0.49	12.37±0.37

Results are expressed as ± SEM (n=6). Data processed by one way ANOVA followed by Dunnett's test,

Table 4. The percent blood glucose reduction Vs time with Gymnema sylvestre, Gliclazide and their combination in diabetic rats

Time (h)	Gliclazide (Mean ±SEM)	Gymnema sylvestre (Mean ±SEM)	GS+GL (Mean ±SEM)
0	0.00±0.00	0.00±0.00	0.00±0.00
1	12.46±0.28	5.50±0.36	18.92±0.21
2	21.65±0.51	22.08±1.04	28.14±0.10
3	38.62±0.42	33.78±0.11	46.18±0.36
4	31.87±0.64	25.94±1.23	47.37±0.39
6	27.03±0.50	18.87±1.28	42.28±0.38
8	35.81±0.19	12.09±1.10	46.18±0.37
10	25.10±0.47	7.00±1.18	32.38±0.44
12	14.05±0.66	3.18±0.47	21.11±0.66

Results are expressed as ± SEM (n=6). Data processed by one way ANOVA followed by Dunnett's test,*

Figure 1. *Gymnema sylvestre*

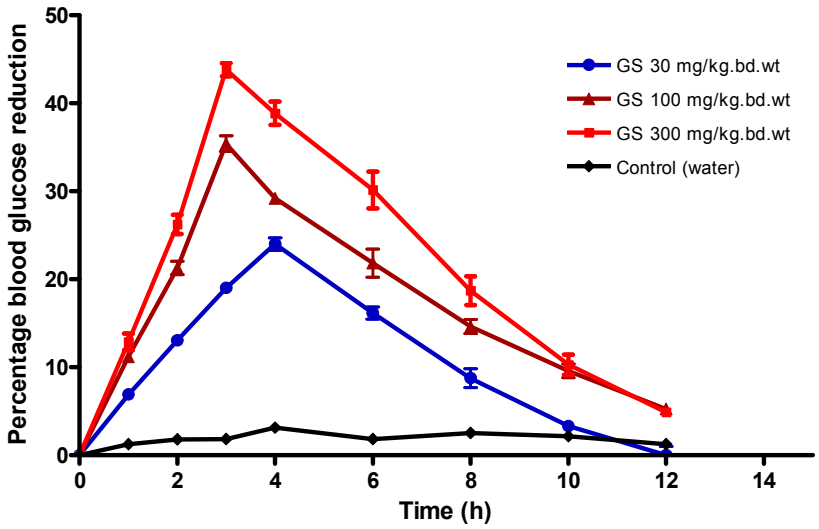

Figure 2. Dose response relationship of *Gymnema sylvestre* on % blood glucose reduction in normal rats

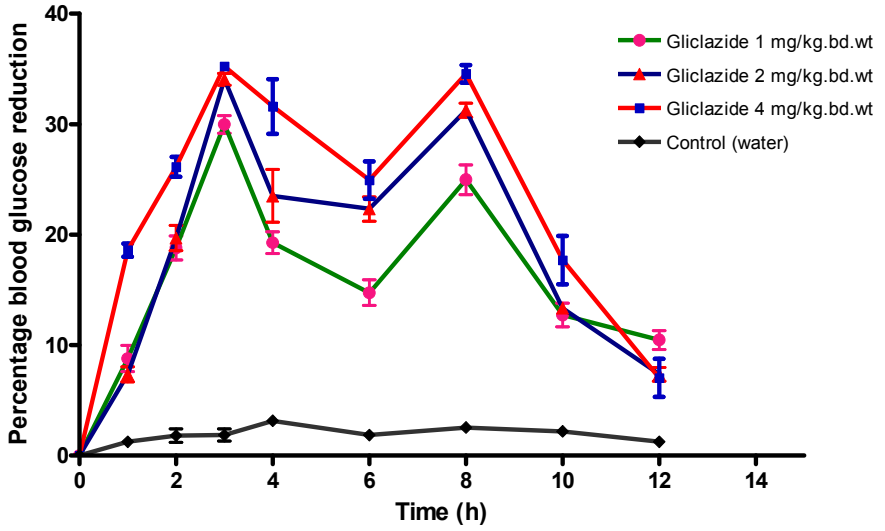

Figure 3. Dose response relationship of Gliclazide on % blood glucose reduction in normal rats

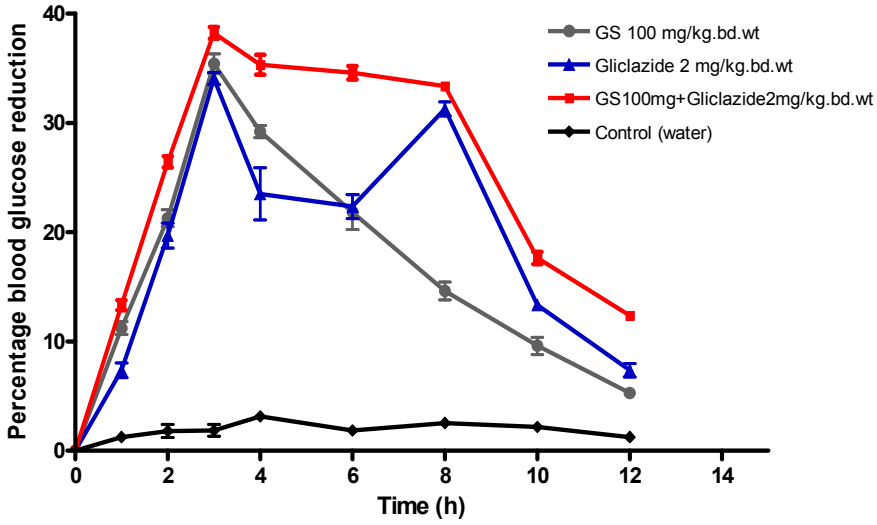

Figure 4. The percent blood glucose reduction Vs time with *Gymnema sylvestre,* Gliclazide and thier combination in normal rats

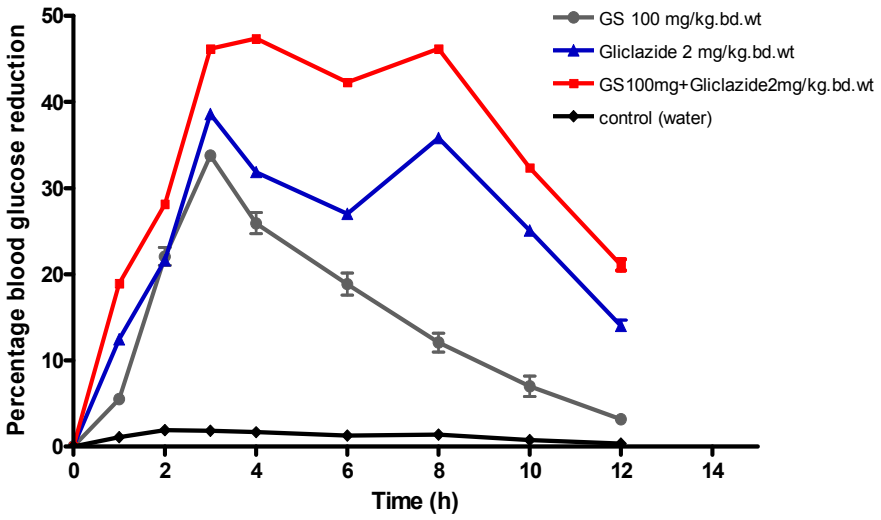

Figure 5. The percent blood glucose reduction Vs time with *Gymnema sylvestre,* Gliclazide and their combination in diabetic rats