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Research Article

Evaluation of *In-vivo* Antidiabetic Activity of *Gymnema sylvstre* in Streptozotocin Induced Diabetic Rats

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ABSTRACT

As per WHO recommendations the search for newer antidiabetic drugs with no or minimal side effects from herbal medicinal plants is a challenge because the synthetic drugs like Oral hypoglycemic agents have one or more side effects. *Gymnema sylvestre* is a slow growing vulnerable species belonging to family Asclepiadaceae and has been shown to exhibit various pharmacological activities. The antidiabetic effect of leaves has been demonstrated. The present study was carried out to assess the antidiabetic effect in STZ induced diabetic rats. Body weight and blood glucose level were observed on 0, 7, 14 and 21st day of post treatment. After 21st days of the treatment with *Gymnema sylvestre* extract, the biochemical parameters were evaluated. The efficacy of the test sample was compared to the standard drug Glibenclamide. The test sample HF01 and HF02 showed extremely significant ($p<0.001$) reduction in blood glucose level. It provided significant effect ($p<0.05$) on body weight of STZ induced diabetic rats. It showed significant effect ($p<0.05$) on TC and LDL whereas it showed extremely significant effect ($p<0.001$) on HDL level. It exerted marked significant ($p<0.001$) reduction in SGOT, SGPT and ALP levels. The histopathological results of the HF01 and HF02 sample exhibited the regenerative effect on pancreatic β-cells in diabetic rats. Diabetic rats responded favorably to treatment with *Gymnema sylvestre* extract which exhibit antidiabetic, antihyperlipidemic and hepatoprotective effects.

Keywords: *Gymnema sylvestre*, Animal model, Diabetes mellitus, Anti-diabetic activity, Streptozotocin, Biochemical parameters.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder caused by an absolute or relative lack of insulin or reduced insulin activity, which results in hyperglycemia and abnormalities in carbohydrate, protein and fat metabolism[1-3]. It is associated with reduced quality of life and increased risk factors for mortality and morbidity. The long-term hyperglycemia is an important factor in the development and progression of micro and macro vascular complications, which include nephropathy, retinopathy, neuropathy, and cardiomyopathy[4-5].

Despite considerable progress in the treatment of diabetes by oral hypoglycemic agents such as biguanides, sulphonylurea, thiozolidinedions and α-glycosidase inhibitors, search for newer drugs continues because the existing synthetic antidiabetic drugs have several limitations and harmful side effects[6-7]. Therefore there is a growing interest in herbal remedies, due to less or no side effects associated with these therapeutic agents, Because of their perceive effectiveness, minimal side effects in clinical experience and relatively low costs, herbal drugs are prescribed widely[4,8]. A variety of ingredients present in medicinal plants are thought to act on a variety of targets by various mechanisms. They have the potential to impart therapeutic effect in complicated disorders like diabetes and its complications. Hence the present study was carried out to evaluate the antidiabetic effect of *Gymnema sylvestre* in a rat model of diabetes.

MATERIALS AND METHODS

Collection of plant material and preparation of extract

The plant material *Gymnema sylvestre* leaves were procured from a farm in Udupi district, Karnataka state. The leaves were cleaned, shade-dried, powdered coarsely and passed through 40 meshes and stored in labeled, closed vessels for further use.

The dried powder material was subjected separately to continuous Soxhlet extraction successively with petroleum ether, chloroform and methanol. The final methanolic extract (known henceforth as GSE for *Gymnema sylvestre* methannolic extract) was concentrated in vacuo and dried under reduced pressure. Plant Authentication was done based on organoleptic and Macroscopic examination of fresh sample. Reg No. of certificate PC/2014/GS01

Qualitative phytochemical screening[9]

The extract of *Gymnema sylvestre* leaves were subjected to various qualitative tests for the presence or absence of different phytochemical constituents.

Experimental Animals

Healthy adult albino Wistar rats (200±25gm) were used for all experiments in the study. Animals were housed in a group of three in separate cages under standard

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Table 1: Effect of *Gymnema sylvestre* extract on Body weight of the rats.

S.N.	Treatment	Body weight (gm)			
		0 Day	7 Day	14 Day	21 Day
1	Normal Control	193.03±11.03	195.47±10.54	196.56±10.65	196.95±10.66
2	Diabetic Control	196.62±11.70	191.55±11.70	183.68±11.20	178.34±9.64
3	STZ + GBC	193.23±11.36	202.28±9.28	208.17±9.02	212.30±11.87
4	STZ + HF01	192.67±10.93	199.17±8.99	204.00±7.79	207.83±7.38
5	STZ + HF02	189.75±12.53	193.62±12.36	196.17±13.01	198.90±14.55

Table 2: Effect of *Gymnema sylvestre* extract on Blood glucose level in diabetic rats.

S.N.	Treatment	Blood glucose level (mg/dl)			
		0 Day	7 Day	14 Day	21 Day
1	Normal Control	86.56±6.19	85.64±5.89	86.83±5.67	87.66±5.73
2	Diabetic Control	263.38±8.34	268.75±7.25	273.13±6.04	273.45±5.34
3	STZ + GBC	284.65±9.86	203.38±9.36	146.97±8.28	137.78±7.36
4	STZ + HF01	282.00±8.19	226.22±6.09	186.90±7.69	170.85±8.29
5	STZ + HF02	281.93±11.89	241.18±7.41	201.10±10.07	181.12±12.44

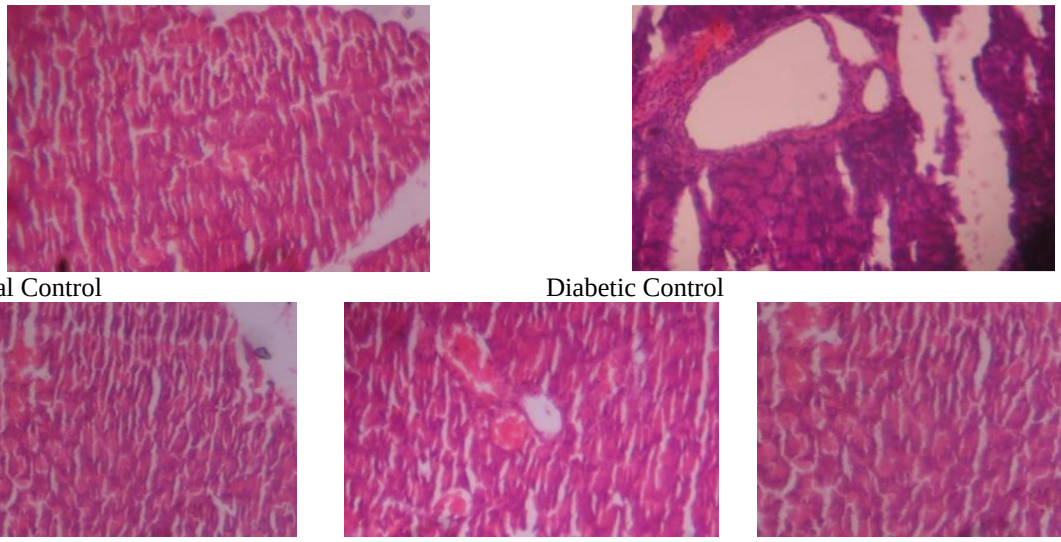
Table 3: Effect of *Gymnema sylvestre* extract on Lipid profile in STZ induced diabetic rats.

S.N.	Treatment	Lipid Profile (mg/dl)		
		Total Cholesterol (TC)	LDL	HDL
1	Normal Control	54.46±0.89	22.27±0.90	18.44±0.93
2	Diabetic Control	95.96±0.76	53.92±0.65	16.00±0.90
3	STZ + GBC	91.88±7.15	39.15±6.30	42.28±4.55
4	STZ + HF01	102.33±6.84	50.43±8.29	37.47±5.49
5	STZ + HF02	112.17±6.84	56.48±6.46	29.15±5.06

Table 4: Effect of *Gymnema sylvestre* extract on Liver Function in diabetic rats.

S.n.	Treatment	Liver Function Test			
		SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Total Bilirubin (m/dl)
1	Normal Control	54±6.31	45±4.77	183.6±5.84	0.82±0.02
2	Diabetic Control	160±5.49	149±5.42	213.47±6.10	1.6±0.01
3	STZ + GBC	50.98±5.14	54.27±4.82	92.78±4.65	0.52±0.05
4	STZ + HF01	57.73±5.91	61.30±5.85	114.02±6.48	0.58±0.06
5	STZ + HF02	67.92±8.97	70.73±5.81	132.05±8.80	0.77±0.05

All results are expressed as mean ± SD. Statistical significance (p) analyzed by Repeated measures one way ANOVA followed by Dunnett's Multiple test for comparison.



Normal Control Diabetic Control

Standard (Glibenclamide) Test sample HF01 Test Sample HF02

Figure 1: Histopathology of pancreas in experimental rats after 21 days of treatment.

environmental conditions of temperature (22±2ºC), relative humididty (60±5%) and 12:12 light:dark cycle. The rats were fed on a standard diet and water *ad libitum*. The experimental work was conducted in Pinnacle Biomedical Research Institute (PBRI) Bhopal (Reg No. 1283/PO/c/09/CPCSEA) in accordance with the current guidelines. Institutional Animal Ethics Committee (IACE) approved the study.

Experimental Groups

In the experiment, total 30 wistar rats were used. Animals were divided in five groups of six each. Group 1: Normal Control (administered 0.1 M citrate buffer); Group 2: Diabetic Control (administered Streptozotocin (60mg/kg) in 0.1 M citrate buffer, pH 4.5 intra-peritoneally); Group 3: STZ induced diabetic rats were administered standard drug Glibenclamide (0.6mg/kg) orally; Group 4: STZ induced diabetic rats were administered test sample HF01 (GSE-300mg/kg) orally; Group 5: STZ induced diabetic rats were administered test sample HF02 (GSE-500mg/kg) orally.

Induction of Diabetes[10-11]

Diabetes was induced with single dose of STZ (60mg/kg) intra peritoneally. It was prepared by dissolving STZ in ice cold 0.1M citrate buffer. To overcome STZ induced hyperglycemia the animals were allowed to drink 5% glucose solution overnight. Control rats were administered with 0.1 M citrate buffer alone as placebo. If the blood glucose value was above 240mg/dl on 3rd day of STZ injection, the animals were considered as diabetic. The treatment was started on 4th day after STZ injection, considering it as 1st day of treatment, the treatment being continued till 21 days body weight and blood glucose level were observed on 0, 7, 14 and 21 day of post treatment.

Estimation of Biochemical Parameters[10-11]

The blood samples from the retro-orbital plexus of the rats were collected after induction of diabetes in animals and after 21st day of the treatment with GSE, serum was separated, and the biochemical estimations of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP), total bilirubin. Decreased blood glucose level was measured. Low density lipoprotein

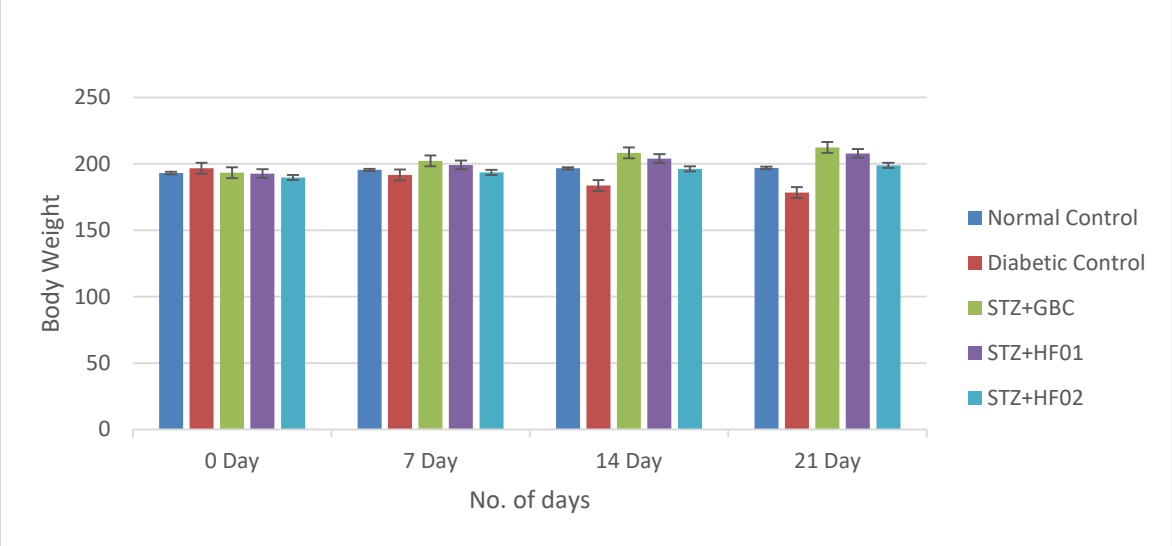


Figure 2: Effect of *GS* extract on Body weight of experimental rats

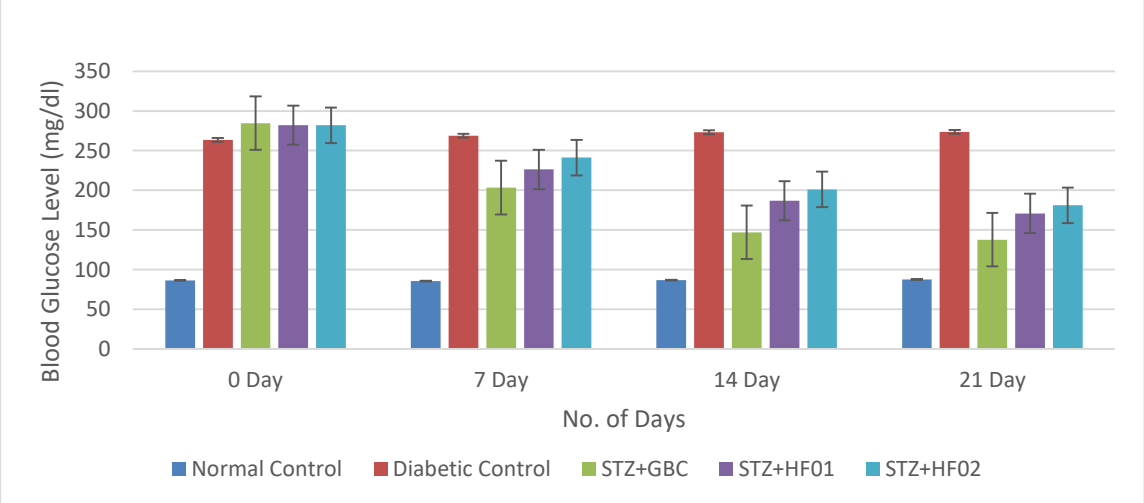


Figure 3: Effect of *GS* extract on blood glucose level in experimental rats.

(LDL), Total cholesterol (TC), and High density lipoprotein (HDL) in all groups were measured.

Histopathology study[12]

On the day of 21st, all animals were sacrificed by under mild ether anesthesia. After sacrificing the animal, the whole pancreas from animal was removed and was kept in 10% formalin solution, and immediately processed by the paraffin technique. For histological examination, sections of 5 micron thickness were cut and stained by haematoxylin and eosin.

Statistical analysis

Results were statistically evaluated using one-way Analysis of Variance (ANOVA), followed by GraphPad Prism software. The values were considered significant when $p<0.05$ and extremely significant when $p<0.001$.

RESULTS

Antidiabetic activity

Effect of Gymnema sylvestre extract on body weight in STZ induced diabetic rat

Reduction in food and water consumption is an important sign of deterioration of health or an indicator of poor health, and generally results in loss of bodyweight. Changes in the body-weight have also been used as an indicator of adverse effects of drugs and chemicals. At the end of 21 days treatment, the body weight of normal rats, *G. sylvestre* extracts (HF01 and HF02) and standard drug treated groups, increased significantly, whereas bodyweight of diabetic control group rats decreased. The study showed that the extract is safe as no significant changes were observed in the behavior and body weight of the animals in the treated groups compared to that of the control group. The results are shown in Table 1.

Effect of Gymnema sylvestre extract on blood glucose level in STZ induced diabetic rat

On repeated administration of *G. sylvestre* extracts (i.e. HF01 and HF02) for 21 days showed significant antidiabetic activity in diabetic rats. The results are shown in Table 2.

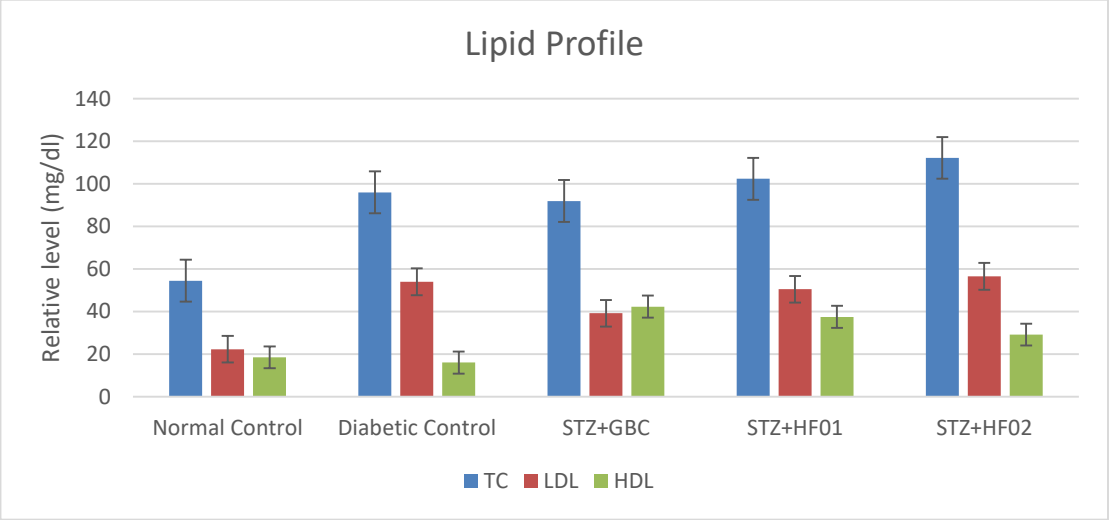


Figure 4: Effect of *GS* extract on TC, LDL, HDL in experimental rats.

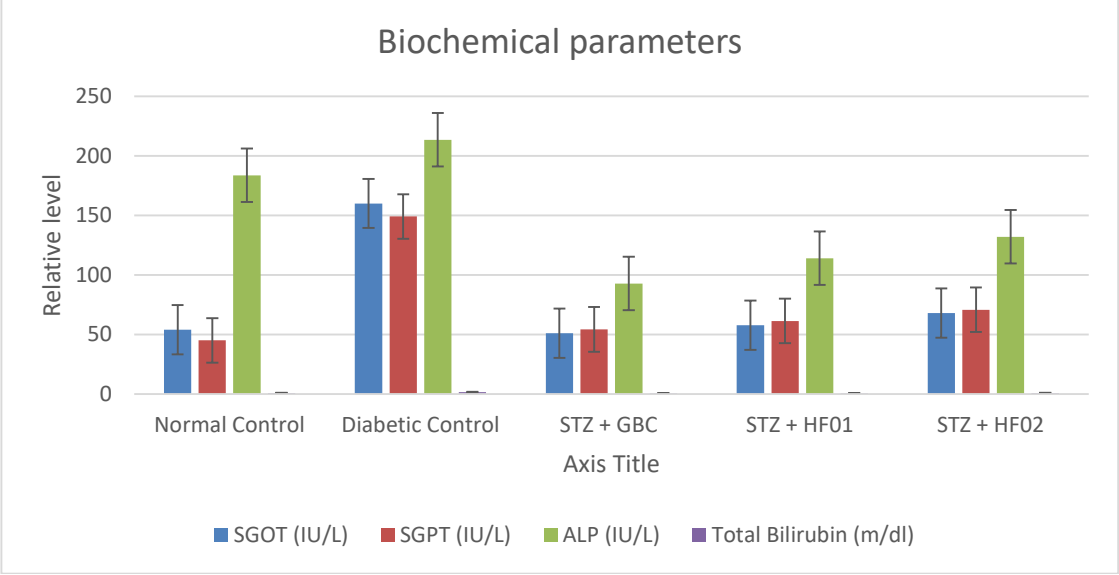


Figure 5: Effect of extract on Biochemical parameters in STZ induced diabetic rats.

Effect of Gymnema sylvestre extract on lipid profile in STZ induced diabetic rats

On repeated administration of *G. sylvestre* extracts (HF01 and HF02) for 21 days showed significant reduction in lipid profile in diabetic rats. The results are shown in Table 3.

Effect of G. sylvestre extract on SGPT, SGOP, ALP and Total bilirubin

The significant elevation in activities of SGPT, SGOP, ALP and Total bilirubin was observed in diabetic rats. After treatment with *Gymnema sylvstre* extracts (HF01 and HF02) and standard drug, the SGPT, SGOP, ALP and Total bilirubin were significantly reduced as compared to diabetic control rats. The results are shown in Table 4.

Histopathology study

Histology of the fine sections of pancreas of rats revealed that all the sections of pancreas were normal in vehicle control group. The blood vessels, connective tissues, islets of Langerhans, acinar cells, inter and intralobular ducts were clearly seen. There was no inflammation. The structure and arrangement of islets of Langerhans was normal and they were tightly arranged. They were distributed in the lobule unevenly. In STZ diabetic control group the cells of pancreas were inflamed with a decrease in number of islet, increased gaps between islets and their size. The interlobular and intralobular duct had clear widening.

In standard glibenclamide and test sample treated groups normal hepatocellular architecture with normal nucleus, cytoplasm and distinct hepatic layer. The test sample both HF01 and HF02 had protected and generated the cells which looked similar to normal and standard groups. Thus the histological examination confirms very good protective and regenerative property of extract.

DISCUSSION

Streptozotocin, β-cytotoxin, induces "chemical diabetes" in various animal species by selectively damaging the insulin secreting pancreatic β-cell. Thus it was used as diabetic inducing agent to produce DM irreversibly with a single dose of i.p. administration by relative necrotic action on the β-cell of pancreas leading to insulin deficiency. The study revealed that the body weight of normoglycemic rats were not altered after 21 days. However in diabetic control group the body weight was selectively decreased and there was found slight increase in body weight with HF01 and HF02 treated rats when compared with diabetic rats. The present investigation showed antidiabetic effect of the test sample by significantly reducing the blood glucose level in diabetic rats treated with HF01 and HF02 compared to that of untreated diabetic rats by increasing the release of insulin from pancreatic β-cells or may be due to potentiating the effect of insulin. In this study antihyperlipidamic effect of HF01 and HF02 were shown by significantly increasing in HDL-cholesterol as compared to untreated diabetic rats. STZ induced rats exhibited the elevated levels of SGOT, SGPT and ALP due to destruction of hepatocytes. Then it was found to have significantly decreased levels of SGOT, SGPT and ALP. Thus the study revealed the hepatoprotective effect of drug in diabetic rats as it decreased SGOT, SGPT and ALP level significantly. The histopathological results of the HF01 and HF02 sample showed the regenerative effect on pancreatic β-cells in diabetic rats.

CONCLUSION

It was concluded that *Gymnema sylvestre* exhibit the antidiabetic activity and showed marked antihyperlipidemic, and hepatoprotective effect in diabetes. Therefore it can be used as an adjuvant along with allopathic medicine for the treatment of diabetes mellitus as well as to delay the late complications of diabetes.

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