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ANTI-DIABETIC EFFECTS OF *GYMNEMA SYLVESTER* EXTRACT ON STREPTOZOTOCIN INDUCED DIABETIC RATS AND POSSIBLE β-CELL PROTECTIVE AND REGENERATIVE EVALUATIONS

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Gymnema sylvestre (Asclepiadaceae) is emerging as a potential treatment for the management of diabetes mellitus. The leaves are used in herbal medicine preparations. The aim of the present study was to identify the potential ability of *Gymnema sylvestre* to regenerate pancreatic β-cells. In the current investigation, an active extract of *Gymnema sylvestre* with the dose of 200 and 400mg/kg was administered orally to streptozotocin induced diabetic rats for 40 days for the assessment of plasma glucose, insulin, glycosylated hemoglobin (HbA1c), tissue and liver glycogen, lipid parameters such as triglycerides, total cholesterol, LDL-cholesterol, and HDL-cholesterol in normal as well as streptozotocin diabetic rats. These results indicate that *Gymnema sylvestre* extract shows significant change in the all above said biochemical parameters when compared to control group. The histopathological study shows the significant recovery of damaged β-cells in diabetic *Gymnema sylvestre* treated rats, when compared to diabetic control ones. In conclusion these results indicate that *Gymnema sylvestre* extract, possessed hypoglycemic and hypolipidemic activity in long-term treatment and is also capable of regenerating β-cells and hence it could be used as a drug for treating diabetes mellitus. Because it has regenerating ability of β-cells, at least the people in the earliest stages of the disease could be treated to delay or prevent full-blown clinical diabetes.

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Keywords: Gymnema sylvestre extract, β-cells, diabetes mellitus

1. Introduction

DM is a group of metabolic diseases characterized by hyperglycemia, dyslipidemia, and protein metabolism that results from defects in both insulin secretion and/or insulin action. The disease 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 neuropathy, nephropathy, cardiovascular and cerebro-vascular diseases [1, 2].

The underlying goal of all diabetes treatment and management is to maintain an adequate blood glucose concentration. The treatment of DM is based on oral hypoglycemic agents and insulin. Four major classes of oral hypoglycemic agents have been used extensively *viz.* insulin secretagogues, biguanides, thiazolidinediones and alpha-glycosidase inhibitors [3]. Each class of drugs works on different mechanism of actions including stimulation of insulin secretion, reduction of hepatic gluconeogenesis, increase in insulin receptor sensitivity and delay of digestion and absorption of carbohydrate, respectively. The use of orally administered drug is limited by the adverse side effects including hematological, cutaneous and gastrointestinal reactions,

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hypoglycemic coma and disturbance of liver and kidney functions [4]. In addition these are contraindicated for use during pregnancy [5]. Presently, there is growing interest in herbal remedies due to the side effects associated with the oral hypoglycemic agents (therapeutic agent) for the treatment of DM [6].

Several investigators have shown that cumarins, flavonoids, terpenoids and a host of other secondary plant metabolites, including arginine and glutamic acid exhibit anti-diabetic properties [7, 8]. *Gymnema sylvestre* is a plant used in India and parts of Asia as a natural treatment for diabetes or "sweet urine." The hypoglycemic action of Gymnema leaves was first documented in the late 1920s [9]. Gymnema is reported to increase glucose uptake and utilization. It also improve the function of pancreatic β-cells and may also decrease glucose absorption in the gastrointestinal tract [10-12].

Phytochemically the plant has been reported to contain gymnemagenin- the sapogenin. Gymnemic acid (Fig.1) -III, -IV, -V, -VIII, and -IX, were isolated in pure states from the hot water extract of leaves of *G. sylvestre* [13]. The present study was aimed at assessing the regenerating ability of the extract of *Gymnema sylvestre* R.Br. leaves.

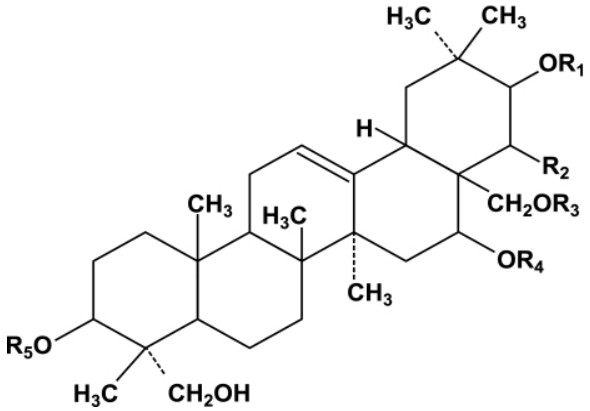

Fig. 1: Structure of Gymnemic acid

2. Materials and Methods

2.1 Materials

Animals

Adult male Wistar rats (180 ± 10 g) were obtained from Central Animal House of Govt College of Pharmacy Karad, Maharashtra India. The animals were housed in groups of six in polypropylene cages at an ambient temperature of 25 ± 1ºC and 45-55% relative humidity, with a 12:12 hr light/dark cycle. They were provided with commercial food pellets and water *ad libitum*. The animals were used for this experiment with the permission of Institutional animal ethical committee, Govt. College of Pharmacy Karad, Maharashtra, India (R.No.209/CPCSEA). The Animals were acclimatized to laboratory conditions for at least one week before using them for experiments and were subjected only once to the experimental conditions. Principles of laboratory animal care (NIH publication number 85-23, revised 1985) guidelines were followed.

***Gymnema Sylvester* extract**

The standardized dry extract of *GS* 75% was obtained from Shivpuri Botanicals Pvt Ltd, Shivpuri Madhya Pradesh (India).Ref no-SBPL/47,dt 09/09/2010.

Streptozotocin

Streptozotocin was purchased from Sigma-Aldrich, St. Louis USA.

2.2 Induction of Diabetes

A freshly prepared solution of Streptozotocin dissolved in citrate buffer saline (pH-4.5) was administered intra-peritonialy in male Wistar rats were made diabetic with a dose of 50 mg/kg body weight. Diabetes was established in STZ treated rats over a period of 7 days. After 7 days the animals with blood sugar level of 300-350mg/dl were considered as diabetic and included in the study.

2.3 Drug administration

Standardized extract of *Gymnema sylvestre* was suspended in 0.3% carboxy methyl cellulose (CMC) and administered orally through oral gavage at the doses of 200 and 400 mg/kg of body weight per day. Doses were selected on the basis of available literature on the aqueous extract of *GS*.

2.4 Experimental Design

Six groups of animals were taken for the study each containing 6 rats. The grouping of the animals was done as tabulated in Table 1.

Table 1: Experimental design.

Group	Animal
I	Normal rats+ saline
II	Normal rats+(400mg/kg) GS
III	Diabetic rats (STZ (50mg/Kg)
IV	Diabetic rat+(200mg/kg) GS
V	Diabetic rat+(400mg/kg) GS
VI	Diabetic rat+ insulin (3IU/kg)

At the end of the 40 days study, the rats were anesthetized with diethyl ether following a fast of 12 hrs. Blood was drawn by milking the tail into fresh EDTA added tubes. It was then centrifuged at 3000 rpm for 20 minutes using refrigerated centrifuge at 4°C. The plasma was used testing for the blood glucose level, lipid profile and serum insulin. The liver and muscle pieces were removed dried on tissue paper, weighed and stored at -80^0C for determining glycogen content.

2.5 Biochemical Analysis

Biochemical analysis was performed to estimate blood glucose, Glycosylated hemoglobin, Liver glycogen, Serum total cholesterol and Serum insulin. Blood glucose was determined using glucometer (Accu-Check). Glycosylated hemoglobin was determined by using the diagnostic kit from Biosystems, Spain. Liver glycogen was also estimated using the Grover method. Tissue glycogen was determined in fed rats by the method by Morales et al [15]. Serum total cholesterol was assessed by using the Cholesterol oxidase/peroxidase method and the phosphate oxidase/peroxidase method was used to estimate the triglycerides. Serum HDL- cholesterol and LDL-cholesterol were determined using Diagnostic kit, Beacon Diagnostics, Kbilpore, Navsari, India. Serum insulin was assayed using the radioimmunoassay kit purchased from (Disasorin, Italy).

2.6 Statistical Method

Student's t-test and probability level of $p<0.05$ were chosen as the criteria of statistical significance. Values reported are mean ± SD. All statistical analysis was performed using One Way ANOVA and expressed as ± SD using SPSS 14.0 Version

3. Results and discussion

3.1 Determination of Fasting blood glucose level

Before treatment schedule, fasting blood glucose level in all animals was within normal range (Table-2). After 24hr treatment with STZ, the fasting blood glucose level was significantly changed in the range of 300-350mg/dl and it was significantly ($P<0.005$) reduced by 40 days treatment with extract of *Gymnema Sylvester*. On the progression of treatment with *Gymnema Sylvester,* fasting blood glucose reduced from 10^{th} day. At the end of experiment (40^{th} day) fasting blood glucose (FBG) level was 185.52 ±20.06 and 112.85±13.68 mg/dl in the doses of *Gymnema Sylvester* 200 and 400 mg/kg respectively. So the GS extract in 400mg/kg dose in diabetic rats proved to have significant hypoglycemic properties.

Table 2: Effect of G. sylvestre extract on blood glucose levels in normal and STZ-induced diabetic rats.

Groups	Blood Glucose level in mg/dl			
	0 day	10 day	20 day	40 day
I	82.1 ± 6.2	83.3 ± 5.1	79.2 ± 6.3	82.5 ± 6.6
II	83.3 ± 6.5	85.32 ± 4.5	88.6 ± 5.50	89.9 ± 6.36
III	341.42 ± 25.32	369.06 ± 15.25[a]	390.07 ± 20.36[a]	410.05 ± 18.56[a]
IV	339.25 ± 22.10	310.26 ± 18.23[a]	225.32 ± 12.45[a,b]	185.52 ± 20.06[a,b]
V	345.25 ± 20.55	292.12 ± 12.54[a]	212.16 ± 15.55[a,b]	112.85 ±13.68[b]
VI	325.16 ± 15	95.42 ± 12.54[b]	85.56 ± 9.56[b]	88.95 ± 12.5[b]

a. $p < 0.05$ by comparison with normal rats.
b. $p < 0.05$ by comparison with streptozotocin diabetic rats.

3.2 Determination of Glycosylated hemoglobin & Body weight

In diabetic conditions the erythrocytes are more prone to oxidative stress and hence exhibit high glycosylated hemoglobin levels. In the present study, treatment with *Gymnema sylvestre* extract decreased the HbA1c level when compared to diabetic control rats. The glyosylated Hemoglobin was recovered in GS treated rats, when compared to diabetic rats, aslo there is a good recovery in body weight. The results of effects of GS extract on body weight and HbA1c are shown in Table 3.

Table 3: Effect of G. sylvestre extract on body weight and Glycosylated hemoglobin in normal and diabetic rats after 40 days of treatment

Groups	Body weight (g)			
	Before Induction	After induction	After treatment	Glycosylated hemoglobin
Normal rats+ saline	202.5 ± 8.4	203.3 ± 6.6	209 ± 5.7	0.53 ± 0.04
Normal rats+(400mg/kg)GS	198.7 ± 4.6	198.2 ± 4.5	208 ± 4.5[b]	0.49 ± 0.05[b]
Diabetic rats(STZ 50mg/Kg	195.4 ± 5.3	187.6 ± 5.25	139.7 ± 6.3[b]	0.93 ± 0.06[a]
Diabetic rat+(200mg/kg)GS	193.5 ± 5.1	188.6 ± 6.23	180.3 ± 5.4[b]	0.65 ± 0.06[b]
Diabetic rat+(400mg/kg)GS	188.2 ± 7.55	184.2 ± 5.54	202.6 ± 5.5[b]	0.55 ± 0.08[b]
Diabetic rat+ insulin (3IU/kg)	199.6 ± 5.8	195.4 ± 6.54	208.5 ± 9.56	0.53 ± 0.05[b]

a $p < 0.05$ by comparison with normal rats.
b $p < 0.05$ by comparison with streptozotocin diabetic rats

3.3 Determination of Glycogen, and insulin level in tissue

The serum insulin decreases in diabetic animals. Insulin plays a crucial role in lowering blood glucose level by enhancing glycogenesis in liver and muscles. Glycogen content of skeletal muscles and liver markedly decreases in diabetes [16].

After 40 days treatment with *Gymnema sylvester* extract (200 and 400 mg/kg), liver glycogen level (20.79±0.43 and 25.84±1.52) was significantly increased with respect to diabetic control group (12.31±0.63) but this was not restored to the normal group glycogen level (35.20±0.89) (Table3). In the present investigation, we confirm that daily administration of Gymnema extract (400mg/kg) resulted to be effective, in satisfactory restore back to near normal capacity of liver to synthesize glycogen. [17,18.]

The insulin level was restored to (16.85±2.18[b]).When Compaired to diabetic rats(5.52±1.56[a]) the value is almost in comparison with control rats (17.85 ±1.26). This indicated an overall blood glucose control due to the improvement in insulin secretion to a considerable level in GS extract treated rats.

Table 4: Effect of G. sylvestre extract on Muscle glycogen liver glycogen and plasma insulin in normal and diabetic rats after 40 days of treatment

Groups	Muscle glycogen (mg/g tissue)	Liver glycogen (mg/g tissue)	Plasma insulin (µU/ml)
I	9.11 ± 0.8	45.12 ± 4.8	17.85 ± 1.26
II	9.3 ± 0.78[b]	47.45 ± 4.6[b]	15.69 ± 1.45[b]
III	1.96 ± 0.52[a]	18.39 ± 4.3[a]	5.52 ± 1.56[a]
IV	8.25 ± 0.71[b]	42.32 ± 4.45[b]	12.52 ± 1.60[b]
V	9.25 ± 0.65[b]	46.52 ± 4.85[b]	16.85 ± 2.18[b]
VI	9.42 ± 0.54[b]	48.56 ± 4.56[b]	4.95 ± 2.5[a]

a $p < 0.05$ by comparison with normal rats.
b $p < 0.05$ by comparison with streptozotocin diabetic rats.

3.4 Determination of lipid profile level

Abnormalities in lipid profile are one of the most common complications in diabetes mellitus; this is found in about 40% of diabetics[19]. Insulin deficiency is associated with hypercholesterolemia and hypertriglyceridemia[20,21]. Acute insulin deficiency initially causes an increase in free fatty acid mobilization from adipose tissue. This results in increased production of cholesterol-LDL particles[22]. Hypertriglyceridemia is also associated with metabolic consequences of hypoinsulinemia, insulin resistance and glucose intolerance[23]. Significant lowering of total cholesterol and rise in HDLcholesterol is a very desirable biochemical state for prevention of atherosclerosis and ischemic conditions[24,25].

The *Gymnema sylvestre* extract was more effective in decreasing the serum lipids. This hypolipidemic effect may be due to an increase in insulin secretion that ultimately led to a decrease in the synthesis of cholesterol and fatty acid.These extracts improved the biochemical parameters. After 40 days experiment, cholesterol, triglycerides in the *G. sylvester* Extract treated groups were reduced to as compare to diabetic control groups. There was significant improvement by treatment of *Gymnema sylvester* Extract. In standard GS treated group, HDL was effectively increased than normal & LDL is restored as normal when compared to STZ treated group (Table 5).

Table 5: Effect of G. sylvestre extract on Total cholesterol, Triglyceride Serum HDL and Serum LDL in normal and diabetic rats after 40 days of treatment

Groups	Total cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
I	11 ± 4.18	16.17 ± 1.81	54.98 ± 4.1	80.12 ± 5.25
II	95.13 ± 3.78[b]	14.45 ± 1.26[b]	52.72 ± 4.36[b]	79.26 ± 3.14[b]
III	241.96 ± 4.52[a]	38.39 ± 2.31[a]	33.67 ± 3.85[a]	146.4 ± 3.8[a]
IV	161.25 ± 6.71[b]	26.32 ± 1.45[b]	46.32 ± 3.8[b]	82.66 ± 4.26[b]
V	112.25 ± 7.65[b]	16.52 ± 1.15[b]	64.78 ± 3.96[b]	87.88 ± 4.32[b]
VI	98.42 ± 5.54[b]	15.56 ± 0.86[b]	55.61 ± 6.15[b]	88.42 ± 1.2[b]

a $p < 0.05$ by comparison with normal rats.
b $p < 0.05$ by comparison with streptozotocin diabetic rats.

3.5 Histopathological Results

Histology of normal pancreas and treated pancreas are shown in Figures 2, 3 and 4. There is a high adaptability of endocrine pancreas to damage and the potential capacity that remained in exocrine pancreas to reestablish homeostasis. We found that regeneration of β-cells is possible

with the treatment of *Gymnema sylvestre* extract. Regeneration of β-cells and neogenesis is mainly from acinar cells. Neogenesis of β-cells through differentiation of acinar cells was amply documented [26, 27]. The acinar cells were even proposed as precursors of ductal cells in focal regions, which can differentiate into β-cells. The marked increase in acinar cells we found could as the ultimate providers of cells that will become β-cells, The Histopathological results showed that severe congestion with sever decrease in number of islets of langerhans and β-cells in streptozotocin treated diabetic rats (Fig-3). But in GS treated diabetic rats it shows decrease in congestion with mild decrease in islets and increase in normal β-cells (Fig- 4a and 4b). This clearly indicates that there is a reversal of the endocrine damage in GS treated rats.

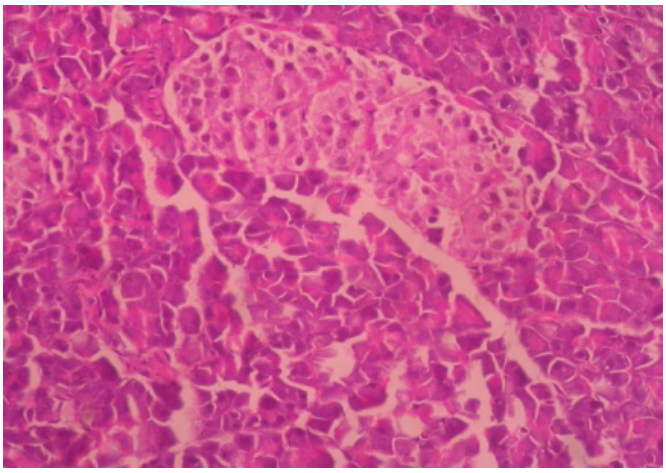

Fig. 2: Normal pancreatic acini and islets of langherhans with normal cellularity

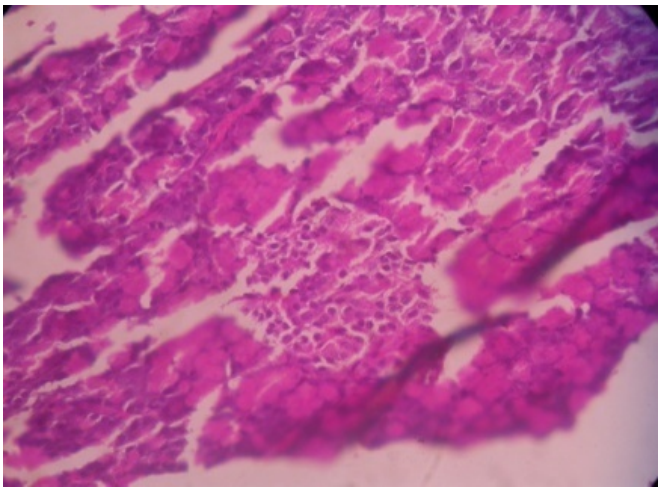

Fig. 3: STZ induced damaged pancreas

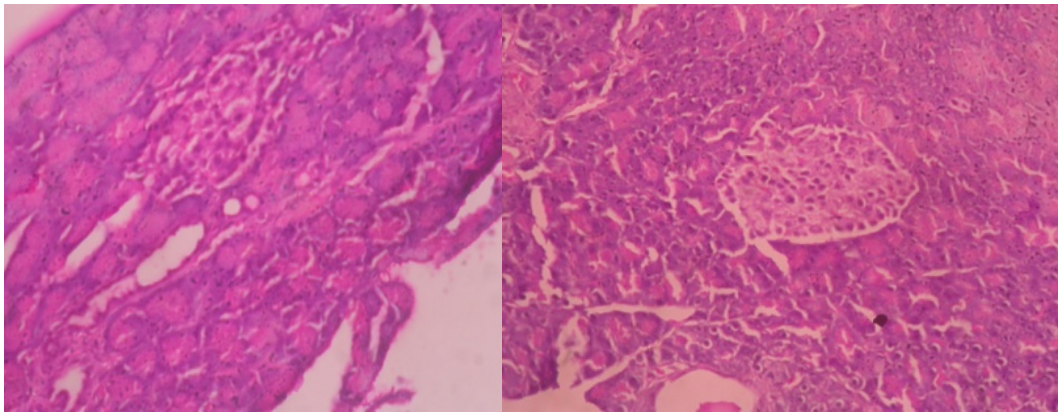

Fig. 4: Pancreas treated by GS at a dose 400 mg/kg

4. Conclusion

In conclusion these results indicate that *Gymnema sylvestre* extract, possessed hypoglycemic and hypolipidemic activity in long-term treatment and is also capable of regenerating β-cells and hence it could be used as a drug for treating diabetes mellitus. Because it has regenerating ability of β-cells, at least the people in the earliest stages of the disease could be treated to delay or prevent full-blown clinical diabetes.

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