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Antidiabetic effect of *Gymnema sylvestre* in Streptozotocin induced diabetes in rats

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Abstract

The purpose of the study was to study the hypoglycemic effect of *Gymnema sylvestre* in streptozotocin induced diabetic rat model. *G. sylvestre* was administered at the dose rate of 50 and 100 mg/kg b w and was compared with standard hypoglycemic drug, glibenclamide for its anti hyperglycemic effect. There was improvement in various parameters such as body weight, haemoglobin, serum glucose, cholesterol, triglycerides, AST, ALT and antioxidant enzymes in all the treatment groups. *G. sylvestre* elicited dose dependent effect with 100 mg/kg b w being more effective in alleviating most of the diabetic clinical signs. The findings were clearly substantiated by histopathology and immunohistochemistry. In conclusion, *G. sylvestre* has significant antidiabetic effect at 100 mg/kg b w when administered daily for 45 days.

Key words: hypoglycemic, streptozotocin, *Gymnema sylvestre*, glibenclamide, antidiabetic.

Introduction

Diabetes mellitus (Type 1 and 2) is a metabolic disorder of multiple etiologies characterised by hyperglycemia due to defects in insulin secretion, insulin action, or both. Diabetes has a tendency to become an epidemic in India (25). Most of the deaths among diabetic patients are due to chronicity of the disorder with long term complications (1). The prevalence of diabetes is predicted to increase from 2.8% in 2000 to 4.4% in 2030. Every fifth diabetic in the world is an Indian because of which India is considered as diabetes capital of the world (14).

Streptozotocin (STZ) induces type I diabetes upon single intraperitoneal injection (21). STZ is a naturally occurring broad-spectrum antibacterial compound produced by soil bacterium *Streptomyces achromogenes* (11). It has been widely used for the induction of diabetes in experimental animals. STZ is a DNA synthesis inhibitor in bacterial and mammalian cells (7).

Sulphonylureas and biguanides are oral hypoglycaemic drugs used for diabetes treatment. Diabetes and related complications continue to be a major medical problem although there are plenty of hypoglycemic agents available for treatment of diabetes. Folk medicine with a variety of plant extracts has been used orally to treat patients with diabetes since many years. A number of plants have been mentioned for the treatment of diabetes in ancient literature of India (4). The World Health Organization (WHO) has listed 21,000 plants, which are used for medicinal purpose around the world. Among these 2,500 species are in India, out of which 150 species are used commercially on a fairly large scale. India is the largest producer of medicinal herbs and is called as botanical garden of the world (26). India is the largest producer of medicinal herbs and most commonly used antidiabetic herbs include *Allium sativum*, *Eugenia jambolona, Momardica charantia, Tinospora cardifolia, Phyllanthus amarus, Trigonella foenum graceum, Pterocarpus marsupium* and *Withnia somnifera*. Medicinal

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plants are gaining popularity because of their lesser side-effects and natural origin (20)

Gymnema sylvestre is a perennial woody climber plant belonging to Asclepiadeceae family (22). Its leaf extract is emerging as a potential remedy for the management of diabetes. *Gymnema sylvestre* is a bitter, astringent, acrid, thermogenic, anti-inflammatory, anodyne, digestive, liver tonic, emetic, diuretic, stomachic, stimulant, anthelmintic, alexipharmic, laxative, cardiotonic, expectorant, antipyretic and uterine tonic in nature (10).

The objective of the study was to evaluate the efficacy of an herb, *Gymnema sylvestre* in alleviation of STZ induced type I diabetes in rats.

Materials and methods

Experimental animals

Female albino Wistar rats weighing 180-200 g obtained from Central Animal Facility, NIMHANS, Bangalore, India were used for the present study. They were maintained under standard laboratory conditions and offered *ad libitum* quantity of standard commercial rat feed and clean drinking water. The animals were kept for acclimatization for 2 weeks. The study was carried out with a prior approval by the Institutional Animal Ethical committee. The experiment was carried out for a period of 45 days.

Streptozotocin

STZ from Sigma Aldrich, St. Louis, USA was used to induce diabetes in rats.

Plant extract

Alcoholic leaf extract of *Gymnema sylvestre* (75% purity) was procured from Himalaya Drug Company (Bangalore, India).

Glibenclamide solution

Glibenclamide (Daonil, 5 mg), an oral hypoglycaemic drug was dissolved in distilled water (82.33 mL) which resulted in a concentration of 60 µg/mL which was used as a stock solution and administered orally at a dose of 600 µg/kg b w (6).

Experimental design

Diabetes was induced in rats by a single intraperitoneal injection of a freshly prepared solution of STZ (45 mg/kg b w) in 0.1 M cold citrate buffer of pH 4.5 (6). After the induction of diabetes, the animals were divided randomly into five different groups with ten rats each. Group I control rats were administered with normal saline; Group II diabetic rats without any treatment (diabetic control); Group III diabetic rats treated with glibenclamide at the dose rate of 600 µg/kg body weight; Group IV diabetic rats treated with alcoholic leaf extract of *Gymnema sylvestre* at the dose rate of 50 mg/kg b w; Group V diabetic rats treated with alcoholic leaf extract of *Gymnema sylvestre* at the dose rate of 100 mg/kg b w. All the groups received their respective treatments orally through oral gavaging daily for a period of 45 days.

Confirmation of diabetes

The blood glucose levels were estimated 72 hours post STZ injection using Span diagnostic kit with semi-automatic biochemical analyser in order to confirm the diabetic state in animals. The animals with blood glucose levels above 200 mg/dL were considered as diabetic.

Collection of serum samples

Blood was drawn from the retro-orbital plexus of the rats under light ether anaesthesia on 3rd, 15th, 30th and 45th day post STZ injection. Serum samples were stored at -20^{o}C for evaluation of biochemical parameters.

Body weight

The rats were weighed on the day of commencement of experiment and on 15th, 30th and 45th day of the experiment to evaluate the effects of various treatments on body weight.

Haemoglobin (Hb)

Blood was collected in EDTA on 15th, 30th and 45th day of experiment for Hb estimation.

Biochemical analysis

The serum samples collected at various intervals were subjected for biochemical estimation of serum concentration of glucose, cholesterol, triglycerides, ALT and AST using a Semi-Automatic Biochemical Analyser with commercial biochemical kits (27).

Estimation of antioxidant enzymes

The liver was processed for the estimation of activity of antioxidant enzymes. Sample of liver was rapidly excised in ice cold normal saline and then blotted dry and stored at -20^{o}C for further analysis. Liver tissue was homogenized with ice cold 0.1 mol/L Tris–HCl buffer of pH 7.4 to make 10% homogenate w/v (1 g of liver crushed in 10 ml of ice cold 0.1 mol/L Tris–HCl buffer of pH 7.4). This homogenate was centrifuged at 1,500 g for 10 min. The supernatant was collected and used for

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estimation of total proteins, superoxide dismutase, catalase and glutathione peroxidase levels.

Protein content of the liver was estimated. The phenolic groups of tyrosine and tryptophan residues in a protein will produce a blue purple complex with maximum absorption in the region of 660 nm wavelength with Folin-ciocalteau reagent which consists of sodium tungstate molybdate and phosphate, thus intensity of color depends on the amount of these aromatic amino acids present and will thus vary for different proteins (17).

Superoxide dismutase (SOD) was estimated (19). Superoxide, an ion is an intermediate in the auto-oxidation of pyrogallol which occurs at pH 8.2. The ability of SOD to inhibit the auto-oxidation of pyrogallol at pH 8.2 provides the basis for enzyme activity. The enzyme activity was expressed in terms of units per minute per mg of protein. One unit of enzyme corresponds to the amount of enzyme that inhibits pyrogallol auto-oxidation reaction by 50 per cent.

Catalase was estimated (8). The decrease in absorbance due to decomposition of hydrogen peroxide was monitored spectrophotometrically at 240 nm. The difference in extinction coefficient per unit time was a measure of catalase activity. Enzyme activity was expressed as µmol of H_2O_2 decomposed per minute per mg of protein.

Glutathione peroxidase (GPx) was estimated (24). GPx reacts with H_2O_2 and reduced glutathione resulting in oxidoreductase which forms a coloured complex with dithio bis-nitrobenzoic acid (DTNB). The intensity of color development is directly proportional to the amount of GPx present in the tissue. The enzyme activity was expressed as µg of GSH utilized/min/mg protein.

Histopathology

Representative tissue samples (pancreas, liver, kidney, spleen, heart, intestine and lungs) of 3-5mm thickness were collected in 10% buffered formalin for histopathological examination. The tissues were processed by routine paraffin embedding technique and 5 µm sections were stained with Hematoxylin and Eosin staining (18).

Immunohistochemical demonstration of insulin

Pancreatic sections from all the treatment groups (Group I to V) were subjected to immunohistochemistry to demonstrate insulin in pancreatic islets at different intervals. Ready-to-Use FLEX Polyclonal Guinea Pig anti-insulin (Code No. IS002) from Dako (Denmark) was used as primary antibody and polyclonal rabbit anti guinea pig immunoglobulin conjugated with HRPO (Catalog. No. P0141) (Dako, Denmark) at a dilution of 1:75 as secondary antibody. Diamine benzedrine tetra hydrochloride and Harris hematoxylin were used as substrate and nuclear counter stain respectively. To determine the percentage of positive cells for insulin production, the number of cells positive for insulin by immunohistochemistry in 1000 β-cells (approximately 10-12 islets) was counted under high magnification and was expressed in percentage.

Special staining for beta cells

Pancreatic sections were stained by Gomori's chrome alum hematoxylin and phloxine stain for demonstration of β-cells (13).

Statistical analysis

Statistical analysis was performed using the statistical software Graphpad Prism, version 5 for Windows. Mean values and standard error were calculated and all values were expressed as mean (± SE). The data were analysed by two way analysis of variance (ANOVA) for all biochemical parameters and by one way analysis of variance (ANOVA) for antioxidant enzymes and percentage positivity of insulin secreting cells.

Results

In the present study all the rats from Group II to V became diabetic and showed hyperglycaemia with a significant ($P \leq 0.001$) increase in mean serum glucose levels (Table 1) 72 hours post-STZ injection.

The diabetic control (Group II) rats revealed a significant reduction ($P \leq 0.001$) in the body weight and hemoglobin concentration (Table 2 and 3) from Day 3 to Day 45. Clinical signs included progressive polyuria, polydipsia, polyphagia, poor body condition and restlessness. The diabetic control rats also revealed a significant ($P \leq 0.001$) increase in the mean serum glucose, cholesterol, triglyceride, AST and ALT levels in comparison with normal rats (Table 1, 4, 5, 6 and 7).

Grossly, pancreas of diabetic control rats (Group II) showed slight congestion and progressive decrease in the size which was appreciable from 15th day and appeared as a thin gelatinous strip on 45th day. Liver was pale red. The other organs such as kidney, heart, lungs and intestine did not reveal any appreciable gross lesions throughout the study period.

Microscopically, pancreas of diabetic control rats (Group II) revealed lesions in both exocrine and endocrine components. The exocrine portion revealed loss of normal lobular architecture with reduction in the lobular size which were widely separated out. The acini were lined by highly vacuolated degenerating and necrotic cells with loss of zymogen granules. Exocrine portion also revealed presence of bluish tinged amorphous material consisting of scattered zymogen granules extruded from degenerating and necrotic cells both intra and interlobularly. Those lobules which were intact revealed large number of apoptotic cells. The endocrine component of pancreas revealed reduction in number of islets which varied in their

shape and size. There was a loss of clear demarcation between islets and adjacent exocrine portion. Islets revealed loss of normal architecture and appeared irregular in shape and showed loss of normal distribution of α and β cells in comparison with islets of normal control rats (Fig. 1A). The β-cells were either highly swollen with vacuolated cytoplasm or elongated and fusiform with condensed nucleus. The cytoplasmic granularity of β-cells was reduced. There was a progressive increase in the severity of lesions with the islets assuming star fish appearance (Fig. 1C) on 45th day of the experiment. Liver revealed swelling of hepatocytes with highly vacuolated cytoplasm. Heart muscle fibres appeared atrophied, and a few diabetic animals during 45th day of experiment revealed renal tubular vacuolar degeneration. Spleen revealed drastic depletion of lymphocytes from the periarteriolar sheath as well as from the follicles (Fig. 3).

Immunohistochemistry revealed complete absence of insulin positive cells (Fig. 1D) which was also supported by special staining where in there was absence of β-cells in diabetic control (Group II) rats.

In the present study, the activity of antioxidant enzymes- superoxide dismutase, catalase and glutathione peroxidase was found to be significantly ($P \leq 0.001$) reduced in the liver of diabetic control rats (Group II) when compared to normal control rats (Table 8).

Diabetic rats treated with glibenclamide showed significant ($P < 0.05$) improvement in various parameters such as glucose, body weight, haemoglobin, cholesterol, triglycerides, AST, ALT and antioxidant enzymes compared to diabetic control rats (Tables 1-8). There was also improvement in clinical signs, gross and microscopic architecture of pancreas in diabetic rats treated with glibenclamide.

The diabetic rats treated with *G. sylvestre* leaf extract at the dose rate of 50 mg/kg b w (Group IV) and 100 mg/kg b w (Group V) revealed alleviation of STZ induced effects. In both treatments there was a significant improvement in the mean ± SE values of glucose, cholesterol, triglycerides, AST, ALT and antioxidant levels from Day 15 to Day 45 (Table 1, 4, 5, 6, 7 and 8). *G. sylvestre* extract at 100 mg/kg b w was more effective in alleviating the diabetic effects than 50 mg/kg b w.

Table 1. Mean (±SE) animal glucose (mg/dL) values of normal control, diabetic and diabetic treatment groups at different intervals of time.

Groups	3rd day	15th day	30th day	45th day
Group I	110.83 ± 4.15	109.33 ± 3.52	105.33 ± 3.45	108.00 ± 5.58
Group II	403.66 ± 8.99^{a}	437.66 ± 9.30^{a}	462.33 ± 7.86^{a}	485.16 ± 6.89^{a}
Group III	400.50 ± 6.23^{a}	331.83 ± 4.20^{ab}	248.00 ± 3.17^{ab}	193.50 ± 2.74^{ab}
Group IV	401.83 ± 6.14^{a}	324.83 ± 1.97^{ab}	268.33 ± 2.30^{abc}	216.50 ± 2.06^{abc}
Group V	399.50 ± 6.09^{a}	319.33 ± 2.85^{ab}	262.66 ± 2.12^{ab}	194.50 ± 2.83^{ab}

Group I: Normal control, Group II: Diabetic control, Group III: Diabetic rats treated with glibenclamide, Group IV: Diabetic rats treated with *G. sylvestre* at 50 mg/kg b w, Group V: Diabetic rats treated with *G. sylvestre* at 100 mg/kg b w
^{a}Comparison with Group I, ^{b}Comparison with Group II, ^{c}Comparison with Group III
Values are statistically significant at $P < 0.05$

Table 2. Mean (±SE) haemoglobin (g/dL) values of normal control, diabetic and diabetic treatment groups at different intervals of time.

Groups	15th day	30th day	45th day
Group I	13.80 ± 0.20	13.75 ± 13.75	14.08 ± 0.21
Group II	11.73 ± 0.16^{a}	09.78 ± 0.13^{a}	08.03 ± 0.20^{a}
Group III	11.81 ± 0.20^{a}	12.26 ± 0.15^{ab}	13.10 ± 0.17^{ab}
Group IV	11.65 ± 0.24^{a}	12.35 ± 0.15^{ab}	13.33 ± 0.15^{ab}
Group V	11.91 ± 0.19^{a}	12.65 ± 0.14^{ab}	13.90 ± 0.17^{bc}

Group I: Normal control, Group II: Diabetic control, Group III: Diabetic rats treated with glibenclamide, Group IV: Diabetic rats treated with *G. sylvestre* at 50 mg/kg b w, Group V: Diabetic rats treated with *G. sylvestre* at 100 mg/kg b w
^{a}Comparison with Group I, ^{b}Comparison with Group II, ^{c}Comparison with Group III
Values are statistically significant at $P < 0.05$

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Table 3. Mean (±SE) body weight (g) values of normal control, diabetic and diabetic treatment groups at different intervals of time.

Groups	3rd day	15th day	30th day	45th day
Group I	165.66 ± 2.76	176.66 ± 3.37	183.33 ± 3.62	192.83 ± 2.82
Group II	170.00 ± 1.52	157.00 ± 1.26[a]	138.50 ±1.94[a]	121.50 ± 2.09[a]
Group III	165.50 ± 1.60	166.00 ± 1.91	173.00 ± 3.02[b]	182.50 ± 3.54[b]
Group IV	183.83 ± 4.41[a]	172.33 ± 4.57	177.16 ± 4.63[b]	186.16 ± 4.48[b]
Group V	188.16 ± 10.03[abc]	176.50 ± 9.42[b]	182.83 ± 9.85[b]	195.33 ± 9.84[b]

Group I: Normal control, Group II: Diabetic control, Group III: Diabetic rats treated with glibenclamide, Group IV: Diabetic rats treated with *G. sylvestre* at 50 mg/kg b w, Group V: Diabetic rats treated with *G. sylvestre* at 100 mg/kg b w
[a]Comparison with Group I, [b]Comparison with Group II, [c]Comparison with Group III
Values are statistically significant at $P < 0.05$

Table 4. Mean (±SE) cholesterol (mg/dL) values of normal control, diabetic and diabetic treatment groups at different intervals of time.

Groups	3rd day	15th day	30th day	45th day
Group I	80.68 ± 0.61	75.84 ± 0.88	77.82 ± 0.61	78.16 ± 0.58
Group II	146.17 ± 1.05[a]	166.20 ± 0.79[a]	177.21 ± 0.88[a]	186.46 ± 0.74[a]
Group III	147.25 ± 0.80[a]	128.87 ± 1.71[ab]	116.63 ± 1.34[ab]	106.80 ± 1.13[ab]
Group IV	149.62 ± 1.10[a]	126.38 ± 1.00[ab]	116.40 ± 1.47[ab]	107.02 ± 1.28[ab]
Group V	148.08 ± 1.03[a]	116.07 ± 0.84[abc]	105.86 ± 1.53[abc]	96.08 ± 0.70[abc]

Group I: Normal control, Group II: Diabetic control, Group III: Diabetic rats treated with glibenclamide, Group IV: Diabetic rats treated with *G. sylvestre* at 50 mg/kg b w, Group V: Diabetic rats treated with *G. sylvestre* at 100 mg/kg b w
[a]Comparison with Group I, [b]Comparison with Group II, [c]Comparison with Group III
Values are statistically significant at $P < 0.05$

Table 5. Mean (±SE) triglyceride (mg/dL) values of normal control, diabetic and diabetic treatment groups at different intervals of time.

Groups	3rd day	15th day	30th day	45th day
Group I	94.41 ± 1.19	95.33 ± 0.79	94.71 ± 0.96	93.82 ± 0.93
Group II	186.49 ± 1.027[a]	197.70 ± 0.89[a]	216.72 ± 1.38[a]	238.23 ± 0.81[a]
Group III	185.98 ± 0.69[a]	165.92 ± 0.74[ab]	157.05 ± 0.86[ab]	146.46 ± 0.88[ab]
Group IV	186.06 ± 0.69[a]	165.30 ± 1.11[ab]	156.14 ± 0.76[ab]	145.88 ± 0.79[ab]
Group V	186.78 ± 0.66[a]	154.48 ± 0.51[abc]	145.95 ±0.69[abc]	135.45 ± 0.76[abc]

Group I: Normal control, Group II: Diabetic control, Group III: Diabetic rats treated with glibenclamide, Group IV: Diabetic rats treated with *G. sylvestre* at 50 mg/kg b w, Group V: Diabetic rats treated with *G. sylvestre* at 100 mg/kg b w
[a]Comparison with Group I, [b]Comparison with Group II, [c]Comparison with Group III
Values are statistically significant at $P < 0.05$

Table 6. Mean (±SE) ALT (IU/L) values of normal control, diabetic and diabetic treatment groups at different intervals of time.

Groups	3rd day	15th day	30th day	45th day
Group I	62.81 ± 0.73	63.95 ± 0.75	63.80 ± 0.94	63.73 ± 0.90
Group II	132.13 ± 0.71[a]	147.16 ± 0.83[a]	167.44 ± 0.85[a]	182.32 ± 0.64[a]
Group III	131.14 ± 0.64[a]	119.65 ± 0.60[ab]	114.14 ± 0.83[ab]	107.32 ± 0.83[ab]
Group IV	133.63 ± 0.64[a]	124.66 ± 0.62[abc]	119.56 ± 0.53[abc]	110.04 ± 0.55[abc]
Group V	132.80 ± 0.76[a]	117.26 ± 0.63[abc]	112.60 ± 0.47[ab]	105.01 ± 0.42[ab]

Group I: Normal control, Group II: Diabetic control, Group III: Diabetic rats treated with glibenclamide, Group IV: Diabetic rats treated with *G. sylvestre* at 50 mg/kg b w, Group V: Diabetic rats treated with *G. sylvestre* at 100 mg/kg b w
[a]Comparison with Group I, [b]Comparison with Group II, [c]Comparison with Group III
Values are statistically significant at $P < 0.05$

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Table 7. Mean (±SE) AST (IU/L) values of normal control, diabetic and diabetic treatment groups at different intervals of time.

Groups	3rd day	15th day	30th day	45th day
Group I	83.67 ± 0.68	83.09 ± 0.85	84.62 ± 0.97	84.63 ± 0.53
Group II	161.44 ±0.50[a]	186.12 ± 0.80[a]	205.57 ± 0.96[a]	225.75 ± 1.26[a]
Group III	161.65 ± 0.65[a]	150.07 ±0.54[ab]	139.99 ± 0.83[ab]	131.44 ±0.65[ab]
Group IV	162.64 ± 0.54[a]	153.43 ± 0.81[abc]	143.44 ± 0.60[abc]	135.10 ± 0.67[abc]
Group V	162.47 ± 0.53[a]	146.68 ±0.57[abc]	135.80 ± 1.14[abc]	125.44 ± 1.22[abc]

Group I: Normal control, Group II: Diabetic control, Group III: Diabetic rats treated with glibenclamide, Group IV: Diabetic rats treated with *G. sylvestre* at 50 mg/kg b w, Group V: Diabetic rats treated with *G. sylvestre* at 100 mg/kg b w
[a]Comparison with Group I, [b]Comparison with Group II, [c]Comparison with Group III
Values are statistically significant at $P < 0.05$

Table 8. Mean (±SE) activities of SOD, CAT and GPx of normal control, diabetic and diabetic treatment groups on 45th day of the experiment.

Groups	SOD	CAT	GPx
Group I	19.08 ± 0.35	75.78 ± 0.90	44.25 ± 0.68
Group II	4.67 ± 0.24[a]	17.27 ± 0.49[a]	12.88 ± 0.15[a]
Group III	7.04 ± 0.27[ab]	29.17 ± 0.44[ab]	19.85 ± 0.39[ab]
Group IV	8.08 ± 0.19[ab]	25.80 ± 0.43[abc]	22.64 ± 0.50[abc]
Group V	9.90 ± 0.082[abc]	36.86 ± 0.69[abc]	28.03 ± 0.47[abc]

Group I: Normal control, Group II: Diabetic control, Group III: Diabetic rats treated with glibenclamide, Group IV: Diabetic rats treated with *G. sylvestre* at 50 mg/kg b w, Group V: Diabetic rats treated with *G. sylvestre* at 100 mg/kg b w
[a]Comparison with Group I, [b]Comparison with Group II, [c]Comparison with Group III
Values are statistically significant at $P < 0.05$

Table 9. Mean (±SE) percentage positivity of insulin secreting cells of normal control, diabetic and diabetic treatment groups**.**

Groups	15th day	30th day	45th day
Group I	79.62 ± 0.39	79.05 ± 0.49	78.73 ± 0.92
Group II	2.36 ± 0.60	2.88 ± 0.23	3.50 ± 0.33[a]
Group III	11.17 ± 0.85	13.21 ± 0.76	18.54 ± 0.28[ab]
Group IV	11.43 ± 1.00	15.3 ± 0.35	19.62 ± 0.27[ab]
Group V	14.59 ± 1.05	20.36 ± 0.5	49.51 ± 0.34[abc]

Group I: Normal control, Group II: Diabetic control, Group III: Diabetic rats treated with glibenclamide, Group IV: Diabetic rats treated with *G. sylvestre* at 50 mg/kg b w, Group V: Diabetic rats treated with *G. sylvestre* at 100 mg/kg b w
[a]Comparison with Group I, [b]Comparison with Group II, [c]Comparison with Group III
Values are statistically significant at $P < 0.05$

Microscopically, there was an appreciable improvement in the architecture of pancreas with appearance of multiple islets of varying size consisting of compact arrangement of cells with more number of β-cells having abundant granular eosinophilic cytoplasm on 45th day of the study in diabetic rats treated with *G. sylvestre* at 100 mg/kg b w (group V) (Fig. 2A). Immunohistochemical staining revealed increase in the number of β-cells consisting of abundant brownish granules consisting of insulin in the islets of diabetic rats treated with *G. sylvestre* at 100 mg/kg b w (group V) (Fig. 2B).

The special staining (Gomori's) for β and α cells also revealed an increase in β-cells with deep blue granular cytoplasm at the centre and α cells with reddish pink cytoplasm at periphery of islets of diabetic rats treated with *G. sylvestre* at 100 mg/kg b w (group V) (Fig. 2C) when compared to diabetic control rats (Fig. 2D). The results of both IHC and special staining technique indicated the regeneration of β-cells in *G. sylvestre* treated diabetic rats.

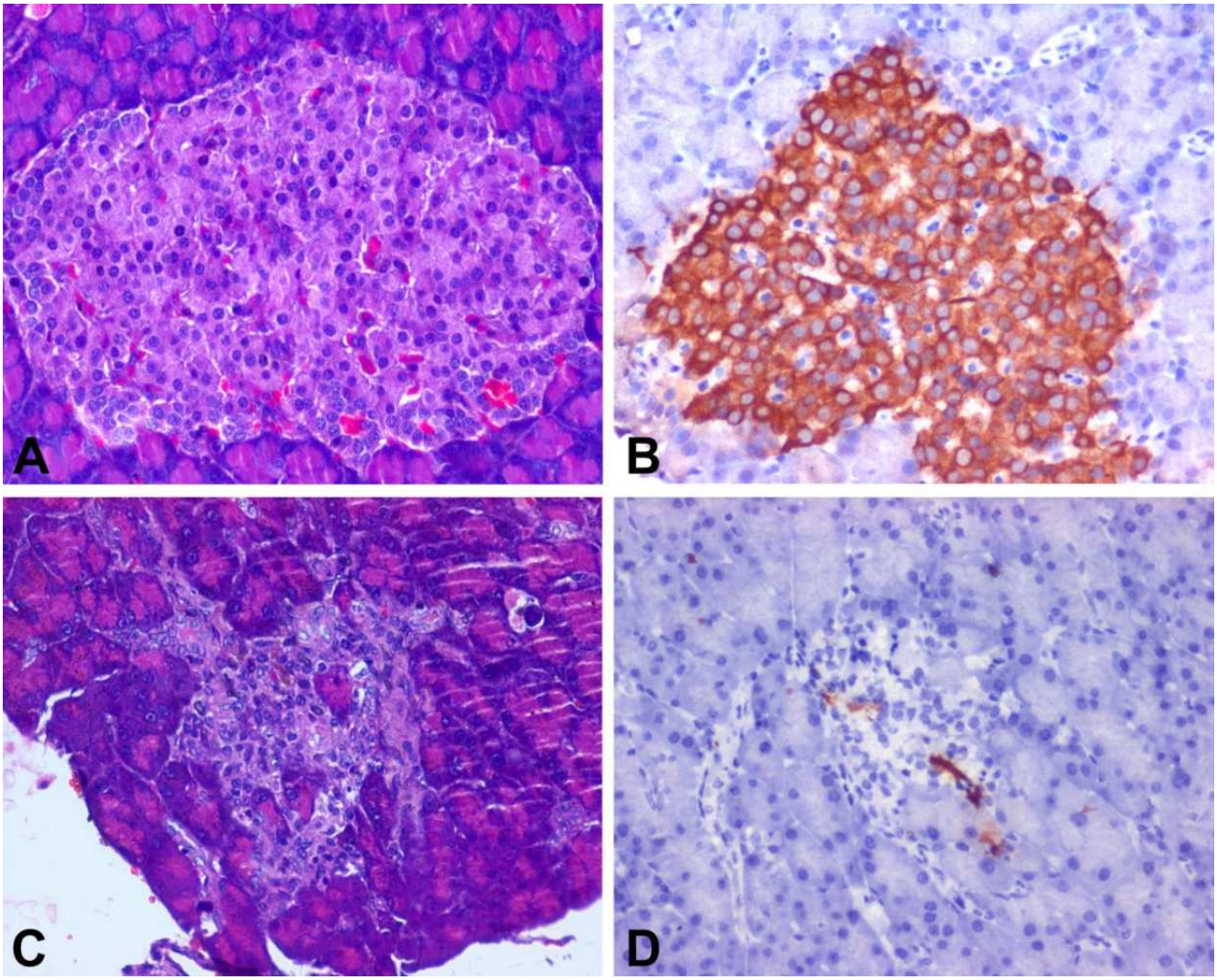


Figure 1. Islet of Langerhans. (**A**) Normal control animal showing large, round beta cells with abundant eosinophilic cytoplasm occupying the core and alpha cells at the periphery (Hematotoxylin and eosin, 200X). (**B**) Normal control animal showing compactly arranged dark brown insulin positive granules in the cytoplasm with unmarked nucleus by immunochemistry (Immunohistochemistry, 200X). (**C**) Diabetic control animal showing loss of normal appearance of β-cells with extension of endocrine portion into the surrounding exocrine portion giving "star fish" appearance on 45th day of the study (Hematotoxylin and eosin, 200X). (**D**) Diabetic control rat showing few insulin positive cells on 30th day of the study by immunohistochemistry (Immunohistochemistry, 200X).

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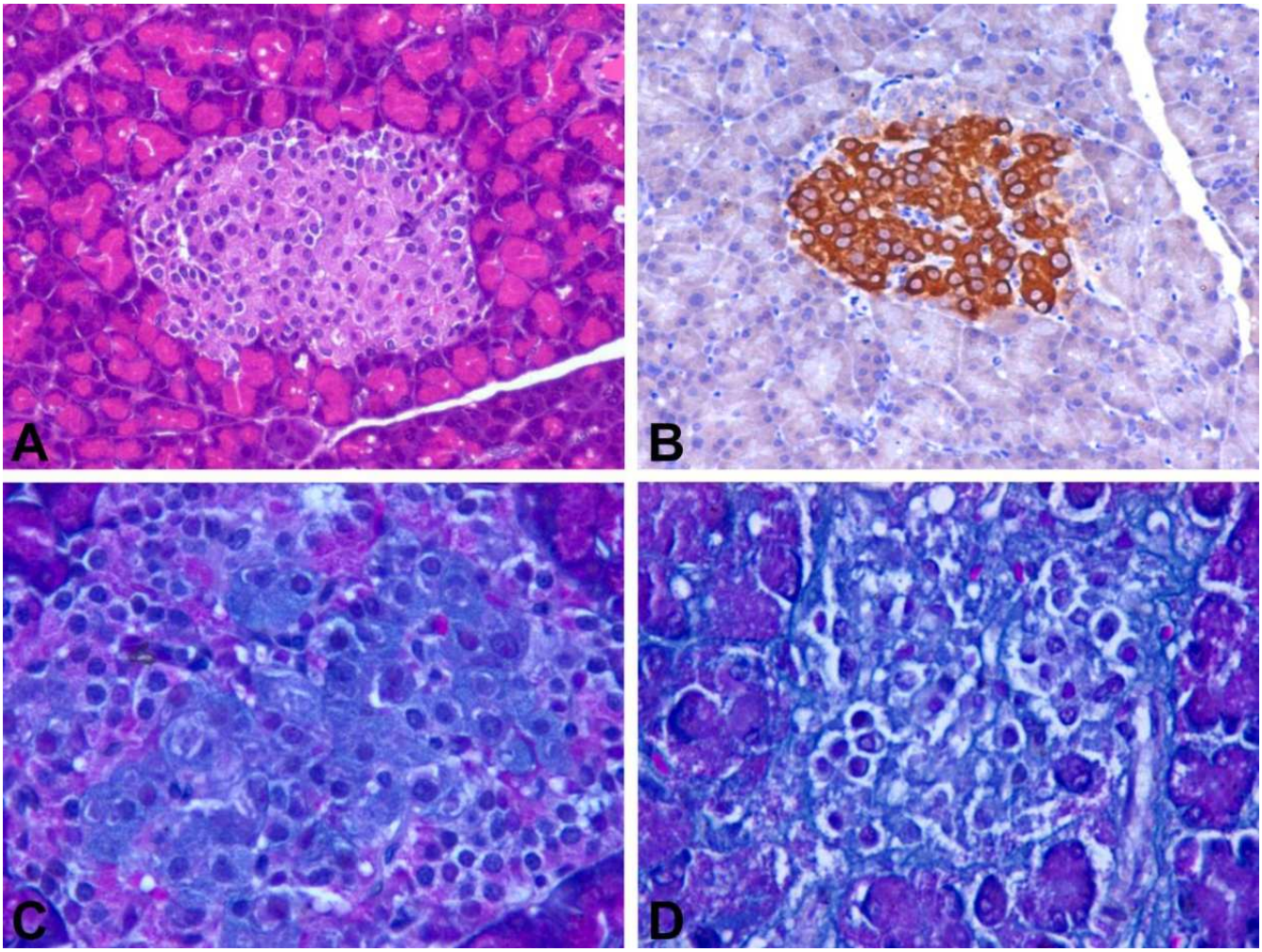


Figure 2. Islet of Langerhans. (**A**) Diabetic rat treated with *G.sylvestre* 100 mg/kg b w in distilled water showing large sized islet with almost normal morphology on 45th day of the study (Hematotoxylin and eosin, 200X). (**B**) Diabetic rat treated with *G.sylvestre* 100 mg/kg b w showing increase in number of brownish insulin positive cells on 45th day of the study by immunohistochemistry (Immunohistochemistry, 200X). (**C**) Diabetic rat treated with *G.sylvestre* at 100 mg/kg b w showing increased number of β-cells with deep blue granular cytoplasm at the center and α cells with reddish pink cytoplasm at periphery on 45th day of the study (Gomori's staining, 400X). (**D**) Diabetic control rat showing decreased cellularity with loss of normal appearing β-cells on 45th day of the study (Gomori's staining, 400X).

Discussion

The induction of diabetes by STZ, a nitrosourea derivative has been attributed to the selective destruction of insulin producing beta cells by induction of necrosis (6). The selective beta-cell toxicity of STZ is related to the glucose moiety in its chemical structure, which enables STZ to enter the cell via the low affinity glucose transporter GLUT2 in the plasma membrane (12).

The significant ($P \leq 0.001$) decrease in body weight, hemoglobin and antioxidant enzymes and increase in serum glucose, cholesterol, triglycerides, AST and ALT levels in diabetic rats could be attributed to the selective destruction of β-cells by the STZ leading to insulin deficiency.

G. sylvestre is emerging as a potential treatment for the management of diabetes. It is a valuable herb belonging to the family Asclepiadaceae, and widely distributed in India, Malaysia, Srilanka, Australia, Indonesia, Japan, Vietnam, tropical Africa and the south-western region of the People's Republic of China.

Both the dried leaf and dried root of the plant have been reported to be used for the treatment of various diseases (28). The leaves of the plant in particular have been reported to have antidiabetic property (5). Gymnemic acids present in callus and leaf extracts of *G. sylvestre* have been reported to increase the regeneration of β-cells in Alloxan induced diabetic rats (2).

Gymnemic acid is the active principle of *G. sylvestre* responsible for the antihyperglycaemic effect of the plant. The possible mechanisms by which gymnemic acid exerts its antidiabetic effect have been reported to be through promotion of regeneration of islet cells, secretion of insulin, inhibition of glucose absorption from intestine,

increased utilization of glucose through activation of enzymes responsible for utilization of glucose by insulin-dependent pathways, increase in phosphorylase activity and decrease in gluconeogenic enzymes and sorbitol dehydrogenase (15). The gymnema extract has also shown antioxidant activity *in vitro* by inhibiting DPPH (2,2-diphenyl-1-picrylhydrazyl), scavenging super oxide, hydrogen peroxide and their reduction by the presence of flavonoids, phenols, tannins (Phenolic compounds) and triterpenoids (23). *G. sylvestre* has also been reported to have neuroprotective effect due to its inhibition of activation of inflammatory molecules and oxidative stress mediators (3).

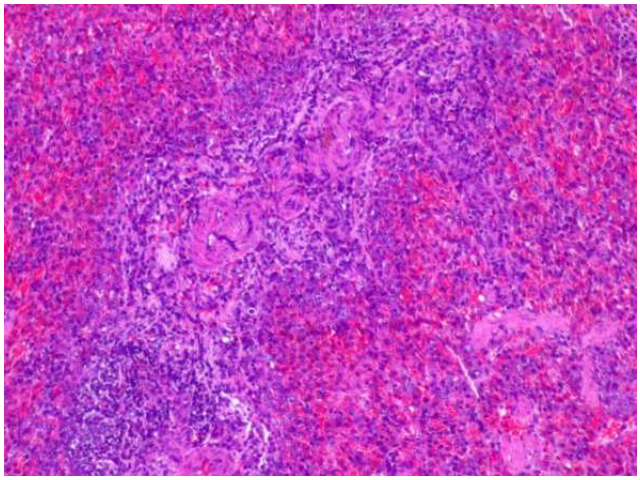


Figure 3. Section of spleen from diabetic control animal showing severe depletion of lymphoid cells on 15th day of the study from periarteriolar sheath (Hematotoxylin and eosin, 200X).

The immunohistochemical and special staining technique which showed an increase in the β-cells upon *G. sylvestre* supplementation point out the possible regeneration of β-cells from precursor cells or transdifferentiation of α-cells to β-cells (9).

The improvement seen in glibenclamide treated rats could be attributed to stimulation of insulin release that rapidly follows the drug's binding to a surface membrane receptor and a subsequent rise in cytoplasmic free calcium concentration. It has been reported that prolonged exposure to glibenclamide maintains high intracellular calcium levels and several calcium dependent signalling pathways, which is responsible for activation of translation and subsequent protein synthesis in beta cells under sustained glibenclamide influence (29).

In the present study, *G. sylvestre* was found to be more effective than glibenclamide in alleviation of STZ induced diabetes in rats.

In conclusion, *G. sylvestre* represents a novel candidate for alternative medicine in the management of diabetes mellitus in view of its effects on the blood glucose level and associated biochemical parameters and also improvement in gross and microscopic architecture of pancreas with increase in β-cells in pancreatic islets of STZ induced diabetic rats.

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