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Antidiabetic and Hypolipidemic Effects of Extracts of *Gymnema Sylvestre* in Streptozotocin Induced Type I Diabetes in Rats

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Abstract

Gymnema sylvestre of the family Asclepiadaceae is one of the most important medicinal plants of the central eco-region. It is popularly known as *Gurmar,* which means "sugar killer". Extract of leaves is reported to have tannins, gum, flavonoids, proteins and saponins. It has displayed a wide array of pharmacological activities. This study was aimed to investigate the antidiabetic and hypolipidemic effects of *Gymnema sylvestre* extract in experimentally induced diabetes in rats. Diabetes was produced in adult Wistar rats with single dose of streptozotocin (STZ) @ 60 mg/kg b.wt. intraperitoneally. After the confirmation of diabetes on 7th day (sugar >200 mg/dl), alcoholic and aqueous extracts of *G. sylvestre* (400 mg/kg) were administered orally to the experimental rats from 8th day and continued for 42 days thereafter. The antidiabetic and hypolipidemic activity was estimated by measuring blood glucose, lipid profile and histopathological examination of various tissues from all the groups. Administration of STZ resulted in a significant ($p<0.01$) increase in blood glucose and lipid profile and histopathological alterations in Diabetic control group as compared to healthy control group. *Gymnema* treatment demonstrated significant ($p<0.01$) antidiabetic effect indicated by restoration of blood glucose compared to STZ control group. The study concluded that extracts of *Gymnema sylvestre* improved the altered glucose and lipid profile in diabetic rats, suggesting that the *Gymnema Sylvestre* extracts exhibit the antidiabetic and hypolipidemic activity.

Keywords: Diabetes mellitus, *Gymnema sylvestre,* Streptozotocin, Blood glucose, Lipid profile.

Introduction

Diabetes is known to exist since ancient times in man and animals, particularly in dogs and cats, but has also been reported occasionally in cattle, horse, sheep and pigs (Kaneko and Rhode, 1964). There is relative or absolute deficiency of insulin resulting in hyperglycemia. Diabetes mellitus (DM) remains incurable and can only be controlled with antidiabetic drug along with restricted diet and exercise. The treatment of DM is based on oral hypoglycaemic agents and insulin. However, DM is also treated in Indian traditional medicine using anti-diabetic medicinal plants. The *Gymnema sylvestre* belongs to *Apocynaceae* family and *Asclepiadoideae* subfamily. It is also known as Cowplant, Australian Cowplant, Gurmari, Gurmarbooti, Gurmar, Meshasringa, Madhunashini, Chakkarakolli, Podapatri, etc. in various Indian languages. It is a climbing woody plant which is native

to the tropical forests of central and peninsular India (Gaurav *et al.,* 2007). Extract of leaves is reported to have tannins, gum, flavonoids, proteins, saponins and also a minute amount of fixed oil. The leaves of the plant are particularly used as antiviral, diuretic, anti-allergic, hypoglycemic, hypolipidemic, antibiotic and in stomach pains and rheumatism (Saneja *et al.,* 2010).

Gymnema sylvetsre is an effective natural treatment for diabetes, which have been used in traditional medicine since the 6th century BC (Gholap and Kar, 2003). The leaves contain gymnemic acids, which have been shown to slow the transport of glucose from the intestine to the bloodstream having antidiabetic, anti-sweetener and anti-inflammatory activities (Liu *et al.,* 1992; Murugami *et al*., 1996). When the leaf extract is administered to a diabetic patient, there is stimulation of the pancreas by virtue of which there is an increase in insulin release (Laddha and Kamat, 2004). Hence, the present study was undertaken to investigate antidiabetic and hypolipidemic effects of alcoholic and aqueous extracts of *Gymnema sylvestre* in STZ induced type I diabetes in rats through evaluation of haematobiochemical parameters and histopathological alterations in liver, kidneys, pancreas and spleen tissues.

Materials and Methods

The study was conducted on 36 adult male albino Wistar rats (Age: 6-8 weeks, weight: 150-200 g). Prior to study, the approval of institutional animal ethics committee was taken vide letter No AAU/GVC/CPCSEA-IAEC/129/2015. Rats procured from Animal Research Facility, Zydus Research Center, Moraiya, Ahemdabad, Gujarat, were kept under standard management conditions at temperature 22 ± 3^{o}C, humidity 70 ± 5 %, and 12:12 hrs light-dark cycles. Animals were maintained in polypropylene cages on rat pellets (Keval Sales Corporation, Laboratory animal feed suppliers, Baroda) and water was given *ad-libitum*. Streptozotocin (STZ) injection was obtained from Himedia chemical Ltd Mumbai. Leaves of *G. sylvestre* were purchased from the Medicinal and Aromatic Plants Unit, Anand Agricultural University, Anand.

Preparation of Extracts of Gymnema sylvestre and Phytochemical Analysis

Leaves of *G. sylvestre* were taken and dried under shade, then powdered by mechanical grinder, sieved (sieve no: 10/44) and stored in air tight containers. Exactly 100 g of coarse powdered material of *G. sylvestre* was successfully extracted in soxhlet extractor with water and methanol for aqueous and alcoholic extract, respectively**.** Extracts so obtained were decanted in beaker and then concentrated to 1/6th of total volume in water bath. The extracts were preserved in the refrigerator. The per cent extractability of plant was calculated by the formula:

$$\text{Percent extractability} = \frac{\text{Total amount of extract obtained}}{\text{Total weight of powder taken for extraction}} \times 100$$

The aqueous extract of *Gymnema sylvestre* was reconstituted in Milli Q water for chromatography separation, which was done by Gas Chromatography/Mass Spectrometry (GC-MS). Analysis of extract was carried out at "Sophisticated Instrumentation Centre for Applied Research and Testing" (SICART), Vallabh Vidyanagar, Anand with GC-MS (Perkin Elmer, Autosystem XL GC, USA) equipped with HP-5 MS capillary column (30m x 0.25mm x 0.25µm).

Experimental Groups and Induction of Insulin-Dependent Diabetes Mellitus (IDDM)

The study was conducted for a period of 42 days. The rats were divided into 6 groups having six rats in each (n=6). Group I was kept as normal control. Group II was kept as induced diabetic control. Groups III and IV received 400 mg/ kg b.wt. of aqueous and alcoholic extracts of *Gymnema sylvestre* orally, while groups V and VI were kept as aqueous extract control and alcoholic extract control respectively. At the commencement of experiment, all rats except normal control and diabetic control were injected with streptozotocin (@ 60 mg/kg b.wt.) intraperitoneally for induction of diabetes (Pellegrino *et al.,* 1998). Hyperglycemia was confirmed by the elevated glucose levels in blood, which

was determined by glucometer (Easytouch G, code No. 7128) at 0 and 72 hrs and then weekly after injection till 7th week. The threshold value of fasting glucose to diagnose diabetes was taken as >200 mg/dl (Pari and Suman, 2010). Rats found with permanent IDDM (Insulin Dependent Diabetes Mellitus) were used for the anti-diabetic study. At the end of the study period, blood was collected by retro-orbital sinus puncture for estimation of biochemical parameters. The rats were then sacrificed and organs were collected in 10% formalin for histopathological examination.

Serum Biochemical Estimation

Blood samples were centrifuged at 2000 RPM and serum was separated. Serum was used for various biochemical determinations. Cholesterol level was measured by Oxidase-Peroxidase (CHOD-PAP) method. Triglycerides were estimated by Glycerol Phosphate oxidase peroxidase (GPO-POD) method while creatinine was estimated by modified Jaffe kinetic method using Coral Kits (Coral Clinical Systems, Goa, India) on Chemistry Analyzer (BS 120, Mindray) (Pragathi *et al.,* 2015).

Histopathology

The tissues like pancreas, kidneys, liver, spleen and intestine were collected from sacrificed animals and subsequently preserved in 10 per cent formal saline for at least 24-48 hrs. All the organs were processed in automatic tissue embedding lab. The 5 micron thickness sections of all organs were cut with automatic section cutting machine (Leica RM 2255, Germany) and stained with Haematoxylin and Eosin (H & E) stain (Luna, 1968).The stained slides were observed under light microscope and the lesions observed were recorded.

Statistical analysis

The data generated on various parameters was subjected to statistical analysis using completely randomized design (CRD) on IBM SPSS 20.00 Statistics (Snedecor and Cochran, 1986). One-way-analysis of variance was used and statistical significance was assumed, if $p<0.01$.

Results and Discussion

Phytochemical analysis

A study of physical characteristic of plant *Gymenma sylvestre* revealed semi solid brownish green aqueous extract and dark greenish alcoholic extract. The per cent extractability of the plant *Gymnema sylvestre* ranged from 15.9 % (alcoholic extract) to 16.3 % (aqueous extract). The preliminary phytochemical analysis of *Gymnema sylvestre* aqueous extract showed presence of alkaloids, phenols, saponins, tannins, and terpenoids.

Biochemical parameters

Induction of DM with streptozotocin (60 mg per kg, i.p.) significantly increased the blood glucose levels. Treatment of rats in group III, IV with aqueous and alcoholic extract of *Gymnema sylvestre* significantly reduced the elevated blood glucose levels (Table 1). While the values of total cholesterol, creatinine and triglyceride levels were increased in comparison to control groups and after treatment they were reduced in comparison to Diabetic control group (Table 2).

Histopathological Observations:

Pancreas: In groups I, V and VI (healthy/control), the pancreas appeared normal. The microscopic lesions in pancreas of STZ induced diabetic rats (group II) were characterized by distortion and destruction of beta cells and congestion (Fig. 1) of pancreatic islets as compared to normal control rats. They also showed reduction in the number of islet cells at places. There was vacuolation, increased vascularity and deterioration of shape of pancreatic islet at places (Fig. 2). The histopathological lesions in rats of groups treated with extracts of *Gymnema sylvestre* were comparatively less severe and the structural architectural details were restored. They showed

Table 1: Comparison of weekly blood glucose of different groups of Wistar rats

No	Description of group	Blood glucose, Mean ± SE (mg/dl)							
		First week	Second week	Third week	Forth week	Fifth week	Sixth week	Seventh week	Overall
I	Normal control	143.67 ±4.21	139.67 ±10.55	151.00 ±5.29	139.67 ±5.62	136.17 ±8.36	149.33 ±4.73	150.67 ±5.68	147.34 ±3.13^{p}
II	Diabetic control	535.67 ±18.92	533.33 ±19.64	517.67 ±22.98	512.83 ±19.17	530.83 ±18.13	525.50 ±19.62	531.00 ±18.44	527.52 ±8.41^{r}
III	GS (aq. Ex) 400 mg/kg	525.83 ±38.41	494.33 ±37.68	449.00 ±37.40	413.67 ±36.30	365.17 ±32.82	319.50 ±36.51	248.83 ±37.02	352.95 ±16.08^{q}
IV	GS(alc. Ex) 400 mg/kg	548.17 ±20.86^{d}	458.50 ±19.99^{cd}	404.17 ±15.61^{c}	351.00 ±15.30^{bc}	312.50 ±12.14^{b}	266.33 10.46^{ab}	194.50 ±8.92^{a}	324.28 ±8.75^{qr}
V	Ext control-I (aqu.)	133.00 ±10.24	144.17 ±6.62	167.33 ±7.62	143.50 ±5.44	143.00 ±7.65	153.50 ±6.02	139.67 ±5.35	113.12 ±3.82^{p}
VI	Ext control II (alc.)	160.67 ±7.69	157.33 ±5.36	167.00 ±6.42	146.00 ±5.28	159.33 ±9.51	155.50 ±6.61	146.00 ±7.29	153.19 ±3.18^{p}

Overall means with superscript, p, q, r vary highly significantly between groups (P<0.01).

Means with superscript, a, b, c, d vary highly significantly between weeks within the group (P<0.01).

Table 2: Effect of different treatment on various biochemical parameters (mg/dl) of different groups of Wistar rats on 42nd day

Group No.	Description of group	Mean ± SE		
		Cholesterol	Creatinine	Triglycerides
I	Normal control	65.70±3.09^{q}	0.39±0.01^{q}	180.93±15.57^{pq}
II	Diabetic control	214.54±11.71^{p}	1.50±0.12^{p}	391.08±23.49^{r}
III	GSE-I(aq.) 400 mg/kg	76.96±4.90^{q}	0.26±0.03^{q}	169.29±8.25^{p}
IV	GSE-II(alc.) 400 mg/kg	76.49±8.97^{q}	0.31±0.04^{q}	155.37±7.14^{q}
V	Ext. control-I (aqu.)	73.99±6.34^{q}	0.39±0.13^{q}	148.54±8.49^{pq}
VI	Ext. control-II (alc.)	74.69±6.36^{q}	0.25±0.03^{q}	199.89±21.25^{pq}

Mean values with different superscript vary highly significantly between groups (p<0.01).

restoration in the number as well as the size of the islets almost near to normal.

Kidneys: The histopathological changes in kidneys revealed damage in both cortex and medulla in group II rats. The microscopic lesions in this group were characterized by congestion (Fig. 3) and scattered haemorrhages. In addition to vascular changes, tubular degeneration and necrosis (Fig. 3) were also observed. The diabetic rats treated with extracts of *Gymnema sylvestre* showed almost normal architectural details in kidneys, except tubular degeneration.

Liver: The microscopic lesions in liver in streptozotocin induced diabetic rats (group II) showed congestion of central vein (centrilobular congestion)(Fig. 4) with mild sinusoidal dilatation. At some places there was focal hepatic necrosis around central vein. At parenchyma fatty changes (Fig. 4) were observed. Rats of groups treated with extracts of *Gymnema sylvestre* showed very mild degree congestion only.

Intestine: The microscopic lesions were observed especially in the group II (STZ control) rats only in both-small and large intestine. The lesions comprised of severe necrosis (Fig. 5) at mucosa and brush borders. Except these no marked changes were evident.

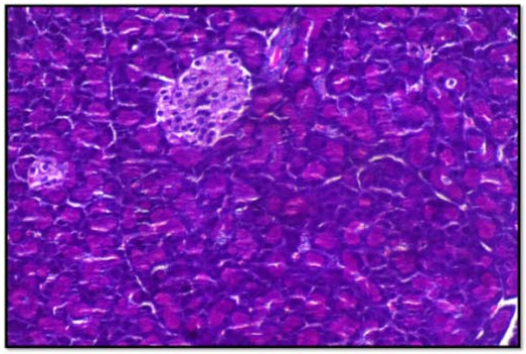

Fig. 1: Congestion of β-cells of pancreatic islets (200 x, H & E stain)

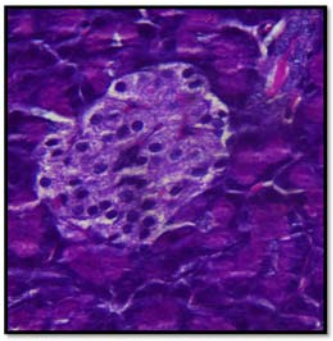

Fig. 2: Vacuolation of pancreatic islets (400 x, H & E stain)

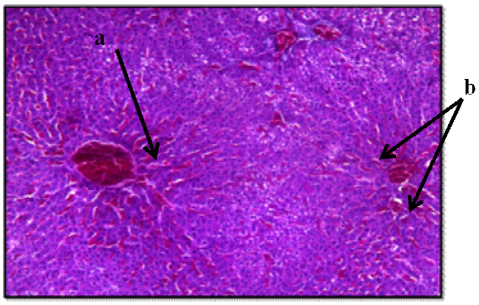

Fig. 3: Centrilobular congestion (a) and parenchymal fatty changes (b) in kidney (100 x, H & E)

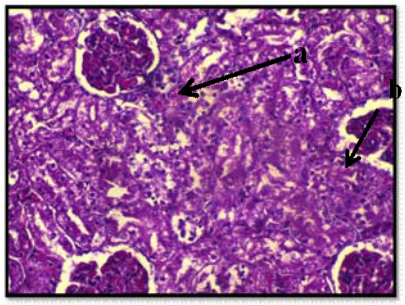

Fig. 4: Congestion (a) and necrosis (b) in liver section (200 x, H & E stain)

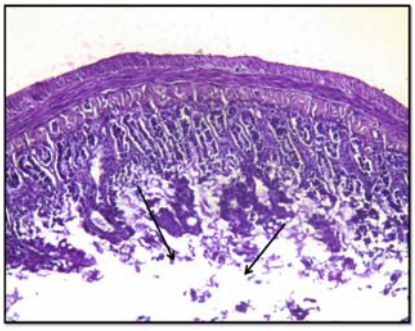

Fig. 5: Severe necrosis at intestinal mucosa (100 x, H & E stain)

Gymnema sylvestre is an important medicinal plant used in different systems of medicine. It has antidiabetic effect as given in literature. Diabetes mellitus is one of the common metabolic disorders with micro and macro vascular complications that results in significant morbidity and mortality. Streptozotocin is a β-cytotoxin, induces "irreversible chemical diabetes" in a wide variety of animal species including rat by selectively damaging the insulin-secreting β-cells of the pancreas with a single dose intraperitoneal leading to insulin deficiency (Kokate *et al.,* 2002).

Gymnema sylvestre induces hypoglycemia (Shenoy *et al.,* 2012). 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 (Parijit *et al.,* 2007; Shenoy *et al.,* 2012; Pragathi *et al.,* 2015). Present study supports the earlier findings that the extract of *Gymnema sylvestre* leaves significantly ($p<0.01$) decreased elevated blood glucose levels.

In the current study, treatment with aqueous and alcoholic extracts of *Gymnema sylvestre* significantly ($p<0.01$) improved the altered lipid profile. It significantly decreased circulating cholesterol and triglycerides levels also. It promotes the fecal excretion of cholesterol and cholic acid derived bile acids. It also decreases serum triglycerides and cholesterol and improves hypertriglyceridemia and hypercholesterolemia. These actions might be due to phytochemical constituent saponins (Yumiko *et al.,* 1999). *Gymnema sylvestre* leaves have been reported to be rich in saponins, a well known antioxidant which account for the scavenging of free radicals and further might have lead to its ameliorative effect.

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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Conflict of Interest: All authors declare no conflict of interest.

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