

## Research Article



## In Vivo Antidiabetic Evaluation of *Gymnema sylvestre* Extract in Streptozotocin Induced Diabetic Rats

\*Shravan Kumar Dholi<sup>1</sup>, Ramakrishna Raparla<sup>2</sup>, Kannappan<sup>3</sup>

<sup>1</sup>Research Scholar (Ph.D), Department of Pharmacology, JNTUK, Kakinada, A.P. India.

<sup>2</sup>Department of Pharmaceutics, Vaageswari Institute of Pharmaceutical Sciences, Ramakrishna Colony, Karimnagar, A.P. India.

<sup>3</sup>Department of Pharmacy, Annamali University, Annamali Nagar, Tamil Nadu, India.

\*Corresponding author's E-mail: [shravanjntuk@rediffmail.com](mailto:shravanjntuk@rediffmail.com)

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### ABSTRACT

*Gymnema sylvestre* has been used medicinally throughout history by many different cultures. Many compounds have been found in the exudates of the *Gymnema sylvestre* plant that have been used medically by humans. We have examined the pharmacological hypoglycemic action of *Gymnema sylvestre* in diabetic rats. *Gymnema sylvestre* 100mg/kg (single dose study) reduced glucose, cholesterol, triglycerides, urea, creatinine, and lipids after treatment for 24 hrs. In chronic study (multiple dose study) also *Gymnema sylvestre* reduced creatinine, urea, lipids, triglycerides and glucose after 15days and significantly reduced glucose levels at 15th day in diabetic rats. In glucose tolerance test in diabetic rats with *Gymnema sylvestre* 100 mg/kg demonstrated glucose levels were found significantly less compared to the control group. *Gymnema sylvestre* serves as an important alternative source in the management of diabetes mellitus involved in reducing increased blood glucose during diabetes which should be examined further by oral hypoglycemic therapy.

**Keywords:** Cholesterol, Creatinine, Diabetes, Glucose, *Gymnema sylvestre*, Triglycerides, Urea.

### INTRODUCTION

Diabetes is a metabolic disease in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced. This high blood sugar produces the classical symptoms of Polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger). Based on the WHO recommendations hypoglycemic agents of plant origin used in traditional medicines are important.<sup>1</sup> Plant drugs and formulations are frequently considered to be less toxic and more free side effects than synthetic one.<sup>2</sup>

*Gymnema sylvestre* is one of the important anti-diabetic medicinal plant, there is a growing demand for *Gymnema sylvestre* leaves in the pharmaceutical trade. Gymnemic acid, the active ingredient of this plant, is extracted from leaves and used for different diseases.<sup>3</sup>

The "destroyer of sugar" is a traditionally used term for *Gymnema sylvestre* because chewing the leaves shall abolish the taste of sweetness. *Gymnema sylvestre* belongs to "Asclepiadaceae" family, *Laticiferous* genus. *Gymnema sylvestre* is found in central and peninsular India. It is a large or more pubescent shrub with young stems and branches, Leaves are opposite, 2.5-6 cm long, usually ovate or elliptical. Flowers are small, yellow, in umbellate cymes and follicles are terete, lanceolate, up to 3 inches in length.<sup>4-7</sup>

### MATERIALS AND METHODS

#### Animals

Wistar rats of weight between 150 to 200 g obtained from NIN, Hyderabad, India, were used in the study. The

animals were maintained under standard conditions in animal house of Vaageswari college of pharmacy [IAEC number VCP/2012/10/6/16]. The rats were males 8-10 weeks old with average weight of 150-200g. Animals were housed 3-4 per cage in a temperature-controlled (22±1) AC room, with a light/dark cycle of 12hr for a week following their arrival; the animals were allowed free access to the standard rat chow diet and tap water they were acclimating to the environment. Rats were also monitored daily and cages cleaned thrice weekly. At the start of the experiment animals were randomly distributed so that body weights, initial triglycerides (TG), total cholesterol (TC), other parameters in all the experimental groups were similar.

#### Preparation of plant extract

#### Extraction procedure of *Gymnema sylvestre*



500gm of *Gymnema sylvestre* leaves were collected from Botanical garden of vaageswari institute of pharmaceutical sciences and plant is authenticated by Pharmacognosy Department of Vaageswari institute of pharmaceutical sciences. A small amount of dust present



as dust was removed by shifting through a sieve of mesh number 30. Initial identification was done by chewing few leaves for a minute. The mouth rinsed clean with water few grains of sugar were placed in mouth and disappearance of sugars sweetness was felt. 1gm of powdered material was shaken vigorously with water and examine more than 30 minutes for froth test confirmed presence of saponin glycoside that is gymnemic acid. 500gm of powdered dry leaf powder was packed soxhlet thimble and extracted continuously with 80% of ethanol until the material was completely exhausted. The final product was dark green amorphous powder after evaporation of solvent.<sup>8</sup>

### Experimental protocol

The test samples were suspended in distilled water. Glibenclamide (2.5 mg/kg) was used as reference control during the study. All the test samples were administered through oral route.<sup>9</sup>

### Single dose study (Acute study)

#### In normoglycemic rats

The rats were fasted for 18 h, but were allowed free access to water before and throughout the duration of experiment. At the end of the fasting period, taken as zero time (0 h), blood was withdrawn (0.1 ml) from the retro orbital route of each rat under mild ether anesthesia. Plasma was separated following centrifugation the glucose was estimated by using Glucose estimation kit from 'One touch ultra', Horizon. U.S.A. The normal rats were then divided into five groups of five rats each. Group I and II were noted as normal control and diabetic control. Groups III and IV received the test extract at a dose of 100 and 250 mg/kg, respectively, through oral route. Group V (standard) received glibenclamide (2.5 mg/kg) and served as reference control. All the test samples were administered in a similar manner. Blood glucose levels were examined after 1, 2, 4, 6, 8, 12 and 24 hrs of administration of single dose of test samples.

#### Induction of Diabetes in Rats by using 60mg/kg of streptozocin<sup>10</sup>

After 2 weeks of feeding with high fat food the rats were fasted for a period of 18 hours before induction of diabetes, and were injected intra-peritoneally with a single dose of Streptozocin 60 mg/kg (Sigma-Aldrich, St. Louis, MO, USA), freshly dissolved in normal saline solution. After the administration, the rats had free access to food (normal pellet diet) and water *ad libitum*. Diabetes in rats was identified by moderate polydipsia and marked polyuria. After 3 days i.e. 72hrs of injection, the fasting blood glucose levels were determined by following glucose oxidase/peroxidase GOD/POD method using a commercial glucose estimation kit with UV-Visible Spectrophotometer at 505nm. The rats showing fasting blood glucose more than 150 mg/dL were considered diabetic rats.

### Experimental Design

The rats were then divided into five groups of five rats each.

**Group I** – Normal Control and rats received only vehicle that is distilled water.

**Group II** – Diabetic control and rats received only vehicle that is distilled water.

**Group III** – Rats received Ethanol Extract of *Gymnema sylvestre* (100 mg/kg/day p.o) suspended in distilled water.

**Group IV** - Rats received Ethanol Extract of *Gymnema sylvestre* (500 mg/kg/day p.o) suspended in distilled water.

**Group V** – Rats received Glibenclamide (2.5 mg/kg p.o) suspended in 2% v/v Tween 80 solution.

### Multidose study (Chronic Study)

The selected rats were treated with similar test samples as above, but the blood glucose level was measured on 1, 3, 5, 7, 9 and 14 days of treatment. Glucose testing kit utilized for the measuring of plasma glucose levels was manufactured by Excel Diagnostic Pvt. Ltd.

### Estimation of Lipid Profile

Estimation of Lipid profile such as Total Cholesterol, Triglycerides, HDL, LDL, VLDL and serum glucose level was conducted appropriately as per specifications. Cholesterol- EGD test kit manufactured by Excel Diagnostics Pvt. Ltd. was used for this purpose. The test kit utilizes CHOD/ POD method for cholesterol analysis. Triglycerides testing kit utilized for measuring the triglycerides in the plasma was also manufacture by Excel Diagnostics Pvt. Ltd.

### Estimation of Urea and Creatinine

Urea and Creatinine levels were also checked using the respective kits that were both manufactured by Excel Diagnostics Pvt. Ltd.

### Statistical Analysis

The data were expressed as mean  $\pm$  standard error mean (SEM). The Significance of differences among the group was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnet's test p values less than 0.05 were considered as significance.

### Data Analysis

All data are expressed as the standard error of the mean. Comparisons among the control and treatment groups were made using analysis of variance followed by a Student- Newman-Keuls t-test using the Graph pad instat statistical program. With all analyses, an associated probability (p value) of less than 5% (P<0.05) was considered significant.

**RESULTS**

Upon administration of ethanolic extract of *Gymnema sylvestri*, significant changes were recorded in blood glucose levels, triglycerides, total cholesterol levels, urea and creatinine levels both in acute as well as in chronic study groups. It was observed that the higher dosage of *Gymnema sylvestri* exhibited increased reduction in the values of parameters compared to low dosage

administration. The values of the blood glucose levels observed by treating diabetes induced rats with ethanolic *Gymnema sylvestri* extract was comparable to the values obtained by treating with glibenclamide. Recorded values showed a dose dependant reduction of blood glucose levels, total cholesterol, triglycerides and urea levels in the alloxan induced diabetic rats treated with ethanolic extract of *Gymnema sylvestri*.

**Table 1:** Effect of *Gymnema sylvestri* on serum glucose levels in STZ induced diabetic rats after single dose administration

Groups	Drug	Dose	0 Hr	1 Hr	2 Hr	4 Hr	6 Hr	8 Hr	12 Hr	24 Hr
I	Normal control	5% w/v Tween 80	116.0 ±3.54	115.4 ±7.46	114.0 ±8.85	111.9 ±4.97	111.2 ±7.73	111 ±8.30	110.1 ±2.42	110 ±4.72
II	Diabetic Control	5% w/v Tween 80	284.4 ±31.1	281.5 ±95.21	282.4 ±88.76	278 ±40.9	285.4 ±37.2	283.4 ±42.4	285.1 ±38.1	282.3 ±35.50
III	<i>Gymnema sylvestri</i>	100 mg/Kg	281.2 ±24.2	280 ±18.1	261.6 ±20.8	231.8 ±24.5	191.8 ±29.4	131.2 ±14.8	102.6 ±8.73	86.6 ±10.5
IV	<i>Gymnema sylvestri</i>	500 mg/Kg	288 ±66.2	278 ±68.2	259 ±78.7	232.6 ±86.1	186.8 ±75.1	128.2 ±75.0	99.2 ±48.8	84.4 ±68.45
V	Diabetic Control+ Glibenclamide	2.5 mg/kg	289.6 ±3.8	282.8 ±4.9	272 ±6.51	240.2 ±.96	191.8 ±5.4	132 ±4.89	101.9 ±.59	85.66 ±7.24

**Table 2:** Effect of *Gymnema sylvestri* on triglyceride levels in serum in STZ induced

Groups	Drug	Dose	0 Hr	1 Hr	2 Hr	4 Hr	6 Hr	8 Hr	12 Hr	24 Hr
I	Normal control	5%w/v Tween 80	91.3 ±2.3	91.33± 2.4	91.22 ±8.6	92.7± 3.0	91.3± 3.1	91.16± 2.2	93.62± 2.0	92.91± 3.4
II	Diabetic Control	5%w/v Tween 80	180.3± 4.3	180.2± 4.5	179.4 ±4.1	179.5± 4.1	177.01 ±4.1	178.7± 4.2	182.91± 6.1	172.2± 4.6
III	<i>Gymnema sylvestri</i>	100 mg/Kg	169.23± 4.4	166.18± 4.3	163.8 ±4.2	159.6± 4.1	156.48± 4.1	152.8± 4.0	148.02± 3.2	140.1± 2.6
IV	<i>Gymnema sylvestri</i>	500 mg/Kg	170.4± 1.9	168.2± 1.2	164.51± 3.3	161.2± 2.2	152.63± 3.4	151.72± 3.2	146.62± 2.4	143.43± 2.4
V	Diabetic Control+ Glibenclamide	2.5 mg/kg	170± 3.8	168.6± 3.4	165.2 ±3.6	162.3± 3.2	153.86 ±5.8	149.4± 2.8	147.42± 3.4	137.43± 3.22

**Table 3:** Effect of *Gymnema sylvestri* on total cholesterol in STZ induced diabetic rats after single dose administration

Groups	drug	Dose	0 Hr	1 Hr	2 Hr	4 Hr	6 Hr	8 Hr	12 Hr	24 Hr
I	Normal control	5%w/v Tween 80	71.10 ±1.39	70.88 ±1.0	76 ±1.4	70.02 ±0.8	78 ±1.2	69.04 ±1.3	70.84 ±0.8	70 ±0.9
II	Diabetic Control	5%w/v Tween 80	298.2 ±3.9	298.0 ±3.2	297.9 ±3.2	297.4 ±2.7	293.6 ±2.7	296.5 ±0.24	294.8 ±1.8	298.3 ±1.76
III	<i>Gymnema sylvestri</i>	100 mg/Kg	301 ±3.5	292 ±4.3	276.2 ±4.8	263 ±4.0	249 ±4.91	197 ±5.2	160.8 ±3.7	132 ±4.3
IV	<i>Gymnema sylvestri</i>	500 mg/Kg	302.2 ±5.7	287.2 ±7.7	268 ±8.7	259 ±9.52	247.6 ±8.87	194 ±9.9	158.2 ±9.3	127.6 ±8.2
V	Diabetic Control+ Glibenclamide	2.5 mg/kg	299.7 ±3.2	278.0 ±3	237.1 ±3	217.1 ±3.4	196.8 ±2.5	172.5 ±2.7	143.2 ±1.8	121.1 ±1.7

**Table 4:** Effect of *Gymnema sylvestre* on serum creatinine levels in STZ induced diabetic rats after single dose administration

Groups	Drug	Dose	0 Hr	1 Hr	2 Hr	4 Hr	6 Hr	8 Hr	12 Hr	24 Hr
I	Normal control	5% w/v Tween 80	0.386 ±0.02	0.384 ±0.02	0.374 ±0.02	0.368 ±0.01	0.378 ±0.02	0.386 ±0.02	0.388 ±0.02	0.372 ±0.02
II	Diabetic Control	5% w/v Tween 80	6.22 ±0.31	6.38 ±0.32	6.26 ±0.30	6.32 ±0.33	6.28 ±0.3	6.31 ±0.3	6.36 ±0.23	6.25 ±0.19
III	<i>Gymnema sylvestre</i>	100 mg/Kg	6.24 ±0.35	6.18 ±0.17	5.94 ±0.19	5.64 ±0.27	5.24 ±0.24	4.76 ±0.11	3.56 ±0.20	2.62 ±0.19
IV	<i>Gymnema sylvestre</i>	500 mg/Kg	6.36 ±0.24	6.29 ±0.22	6.12 ±0.25	5.82 ±0.33	5.48 ±0.31	4.62 ±0.41	3.34 ±0.28	2.49 ±0.19
V	Diabetic Control+ Glibenclamide	2.5 mg/kg g	6.32 ±0.15	6.24 ±0.17	6.02 ±0.02	5.68 ±0.2	5.32 ±0.21	4.54 ±0.19	3.12 ±0.29	2.14 ±0.31

**Table 5:** Effect of *Gymnema sylvestre* on urea levels in serum in STZ induced diabetic rats after single dose administration

Groups	Drug	Dose	0 Hr	1 Hr	2 Hr	4 Hr	6 Hr	8 Hr	12 Hr	24 Hr
I	Normal control	5% w/v Tween 80	29.1 ± 1.0	28.4 ±1.6	28.6 ±6.89	28.9 ±1.4	29.4 ±1.4	30.2 ±1.3	29.2 ±1.3	29.6 ±1.5
II	Diabetic Control	5% w/v Tween 80	147.1 ±1.5	144.6 ±3.22	145.0 ±1.9	144.8 ±2.6	143.6 ±2.2	144.56 ±2.6	144.3 ±2.7	145.3 ±2.8
III	<i>Gymnema sylvestre</i>	100 mg/Kg	146.1 ±4.9	144.9 ±5.2	134.5 ±4.9	125.1 ±7.4	117.8 ±6.8	108.76 ±5.06	95.6 ±6.6	85.9 ±7.8
IV	<i>Gymnema sylvestre</i>	500 mg/Kg	147.3 ±13.4	143.0 ±14.1	130.8 ±40.8	120.9 ±11.6	115.2 ±7.2	106.9 ±7.06	93.1 ±7.6	82.4 ±7.2
V	Diabetic Control+Glibenclamide	2.5 mg/kg	148.1 ±8.6	142.4 ±9.8	123.5 ±12.9	117.3 ±12.6	111.1 ±12.44	102.4 ±9.4	92.4 ±9.9	78.5 ±7.3

**Table 6:** Effect of *Gymnema sylvestre* on serum glucose levels in STZ induced diabetic rats after prolonged treatment

Groups	Drug	Dose	1 day	3 day	5 day	7 day	14 day
I	Normal control	5% w/v Tween 80	111.6 ±6.34	113.2 ±7.43	114.4 ±8.84	111±9.97	164 ± 7.73
II	Diabetic Control	5% w/v Tween 80	284.8 ±93.55	283.8 ±91.25	279.6 ±88.71	282 ±43.92	281.6 ±37.44
III	<i>Gymnema sylvestre</i>	100 mg/Kg	287.2 ±13.2	263.2 ±18.5	198.6 ±20.8	152.8 ±24.54	131.8 ±29.4
IV	<i>Gymnema sylvestre</i>	500 mg/Kg	288 ±66.2	261.3 ± 68.2	187.4 ±78.72	104.6 ±86.1	128.8 ±75.1
V	Diabetic Control+ Glibenclamide	2.5 mg/kg	289.6 ±3.8	258.8 ±4.9	163.2 ±6.51	137.2 ± 3.96	125.8 ±5.4

**Table 7:** Effect of *Gymnema sylvestre* on serum triglycerides levels in STZ induced diabetic rats after prolonged treatment

Groups	Drug	Dose	1 day	3 day	5 day	7 day	14 day
I	Normal control	5% w/v Tween 80	91.8±2.8	91.52±2.8	91.68±3.1	92.74 ±3.04	91.36±3.4
II	Diabetic Control	5% w/v Tween 80	180.2 ±4.4	181.2±4.4	179.4±4.2	179.5 ±4.8	177.02±4.7
III	<i>Gymnema sylvestre</i>	100 mg/Kg	178.9 ±4.5	157.18±4.2	149.4±4.4	145.6 ±4.2	133.48±4.3
IV	<i>Gymnema sylvestre</i>	500 mg/Kg	177.4 ±1.5	151.22±1.9	147.5±3.7	141.8 ±2.8	131.66±3.0
V	Diabetic Control+ Glib-enclamide	2.5 mg/kg	175.22 ±3.4	154.66 ±3.3	141.26 ±3.2	138.5 ±3.1	129.82 ±5.3

**Table 8:** Effect of *Gymnema sylvestre* on total cholesterol in STZ induced diabetic rats after prolonged treatment

Groups	Drug	Dose	1 day	3 day	5 day	7 day	14 day
I	Normal control	5% w/v Tween 80	71.18±1.39	70.88 ±1.0	70.76 ±1.4	70.92 ±0.8	70.78±1.2
II	Diabetic Control	5% w/v Tween 80	298.2±3.9	298.0 ±3.2	297.9 ±3.2	297.48 ±2.7	293.6±2.7
III	<i>Gymnema sylvestre</i>	100 mg/Kg	301±3.5	245±4.3	192.2 ±4.8	143±4.0	125.4±4.91
IV	<i>Gymnema sylvestre</i>	500 mg/Kg	302.2±5.7	238.2 ±7.7	187±8.7	139±9.52	121.6±8.87
V	Diabetic Control+ Glibenclamide	2.5 mg/kg	299.76±3.2	228.0±3	174.1±3	125.1 ±3.4	116.89±2.5

### Single Dose Study

Administration of single dose of *Gymnema sylvestre* 100 mg/Kg and 500 mg/Kg, oral, each to two study groups which are diabetes induced by streptozotocin, significant reduction ( $P>0.05$ ) in blood glucose levels was observed. The study period encompassed 24hrs. The results were significantly comparable to the standard drug glibenclamide. *Gymnema sylvestre* at 250 mg/Kg exhibited better blood glucose level reduction compared to *Gymnema sylvestre* administered at 100 mg/Kg.

### Chronic study

During chronic study which encompassed a period of 15 days, the *Gymnema sylvestre* (100 and 500 mg/kg, oral) produced a significant ( $P>0.05$ ) in BGL of the diabetic rats compared to control. *Gymnema sylvestre* at the dose of 500 mg/kg body weight exhibited better BGL reduction than 100 mg/kg body weight.

### DISCUSSION

*Gymnema sylvestre* is one of the oldest herbs known for their beneficial uses. *Gymnema sylvestre* have been shown to have multiple benefits in patients with diabetes such as reduction of blood sugar and its complications. Many earlier studies whether using the whole extracts showed that *Gymnema sylvestre* decreased fasting blood sugar levels in animals.

At present, the treatment of diabetes mainly involves a sustained reduction in hyperglycemia by the use of Biguanides, thiazolidinediones, sulphonylureas in addition to insulin. However, due to unwanted side effects there is a demand for new compounds for the treatment of diabetes. Hence; plants have been suggested as a rich source of potentially useful antidiabetic drugs.

Our results showed that oral administration of *Gymnema sylvestre* for 24 hrs effectively controlled hyperglycemia. Maintenance of normoglycemia, normalization of serum lipid profile was maintained through seeds extract of *Gymnema sylvestre*. The *Gymnema sylvestre* maintains the blood glucose to normoglycemia during diabetes, which acts as an essential trigger for both liver and kidney to revert to their normal metabolic homeostasis.

### CONCLUSION

The mode of action of *Gymnema sylvestre* is reducing the increased blood glucose level, thereby preventing hyperglycemia during diabetes and also reducing the lipid profile (cholesterol, triglycerides) which protects from the risk factor of coronary heart disease. The further studies are carrying out in isolation of the active constituents involved in *Gymnema sylvestre* for obtaining the more valuable results in treatment of the disorders facing by the mankind. Therefore, *Gymnema sylvestre* serves as an important alternative source in the medicinal study.

In conclusion, the leaves of *Gymnema sylvestre* act as for alternative or complementary medicine in the management of diabetes mellitus.

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