**In vivo anti diabetic evaluation of gymnemic acid in streptozotocin induced rats**

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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 gymnemic acid in diabetic rats and influence of gymnemic acid in streptozotocin induced diabetic rats. In single dose study 100 mg and 500 mg/kg of Gymnemic acid reduced glucose, cholesterol, triglycerides, urea, creatinine, and lipids after treatment for 24 hrs. In chronic study (multiple dose study) also, Gymnemic acid reduced creatinine, urea, lipids, triglycerides and glucose after 15 days and significantly reduced glucose levels at 15th day in diabetic rats. In glucose tolerance test in diabetic rats with Gymnemic acid 100 mg/kg and 500 mg/kg, demonstrated glucose levels were found significantly less compared to the control group. Gymnemic acid has influence on the regenerative influence on pancreatic tissue in histological studies. Gymnemic acid 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**: Diabetes, glucose, Gymnemic acid, Cholesterol, Urea, Creatinine, Triglycerides.

1. **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 \(^1\). Plant drugs and formulations are frequently considered to be less toxic and more free side effects than synthetic one \(^2\). Gymnema sylvestre is used in different systems of medicine as a remedy for the treatment of diabetes, rheumatism, and cough \(^3\). The major phytoconstituents of Gymnema sylvestre are gymnemic acids, gudmarin and saponins. Gymnemic acid (C\(_{43}\)H\(_{68}\)O\(_{14}\)) is a pentacyclic triterpenoid and is the main active phytoconstituents of Gymnema sylvestre, exhibiting potent anti-diabetic activity \(^4\).

2. **Materials and Methods**

2.1 **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 150-200 g. Animals were housed 3-4 per cage in a temperature-controlled (22±1) AC room, with a light/dark cycle of 12 hr 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.

2.2 **plant material collection, Extraction, Isolation and identification of Gymnemic acid from Gymnemic sylvestre**

2.2.1 **Plant material**

Gymnema leaves are collected from botanical garden of Vaageswari Institute of
pharmaceutical sciences, Karimnagar. Identification and authentication of samples was done by using standard botanical monographs. They were further confirmed with the Department of Pharmacognosy, Vaageswari Institute of pharmaceutical sciences, Karimnagar, Andhra Pradesh.

2.2.2 Extraction Procedure
The plant material is then extracted with 90% methanol. 90% methanol was added and the extraction was carried out for 24-36 hours till the total methanol soluble extract was obtained. The methanol soluble extract was distilled and finally 175 gm of the thick paste were obtained.

2.2.3 Isolation of pure gymnemic acid from methanol extract
175 gm thick paste of methanol soluble extract was dissolved in 1% aqueous KOH solution on continuously stirring for 45 min to 1 hour. The solution is then filtered through filter paper to separate the un-dissolved particles. Diluted HCl was added slowly under constant stirring, during that the gymnemic acids were precipitated. Precipitated solution was filtered under suction and precipitate was dried. The pure gymnemic acid was obtained.

Gymnemic acid gave positive test for phenolics, steroids and glycoside

Phenolic test: A pinch of gymnemic acid was taken into a clean test tube and dissolved 2 ml of methanol. Then a few drops of 1% alcoholic ferric chloride were added.
Steroid test: A pinch of gymnemic acid was added to a solution of 2 ml CHCl3 and 1ml of acetic anhydride. A few drops of Conc. H2SO4 were added from the sides of the tubes.

Glycoside test: A pinch of gymnemci acid was taken in a dried test tube and dissolved in 2 ml of methanol. 1 ml of alpha naphthol alcoholic solution was added from the sides of the test tube.

2.2.4 HPTLC Screening of Gymnemic acid
High Performance Thin Layer Chromatography is a planar Chromatography where the separation of the sample components is achieved on high performance layers with detection and acquisition using an advanced workstation. CAMAG HPTLC System, equipped with a Linomat IV sample applicator, a twin chamber tank, a model III Thin Layer Chromatography (TLC) Scanner and wincats software (1.21 version) was used in the study. TLC Aluminium sheets (20 x 10 cms) of silica gel GF254 were used. A 11g/ll stock solution of gymnemic acid, reference standard of 92% purity was prepared in methanol. Three identical plates were prepared for concurrent results. The plates were developed up to 80 nm under chamber saturation conditions. After air drying the solvent, the plates were scanned using scanner III at 290 nm wavelength in absorbance mode (D2 and W lamp).

2.3 Experimental protocol
The test samples (Gymnemic acid) 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 [5].

2.3.1 Induction of Diabetes in Rats by using 60 mg/kg of streptozocin [6]
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 intraperitoneally 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. 72 hrs 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 505 nm. The rats showing fasting blood glucose more than 150 mg/dL were considered diabetic rats and selected for the grouping in experimentation.
2.3.2 Experimental Design
The rats were divided into 5 groups, 6 animals in 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 Gymnemic acid (100 mg/kg/day p.o) suspended in distilled water.
- **Group IV** - Rats received Gymnemic acid (500 mg/kg/day p.o) suspended in distilled water.
- **Group V** – Rats received Glibenclamide (2.5 mg/kg p.o) suspended in 2% w/v Tween 80 solution.

2.3.3 Single dose study (Acute study)
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 500 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.

2.3.4 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 Diagnostics Pvt. Ltd.

2.3.5 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 manufactured by Excel Diagnostics Pvt. Ltd.

2.3.6 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.

2.3.7 Statistical Analysis
The data were expressed as mean ± standard error of the 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 significant.

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 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.

3. Results
Upon administration of Gymnemic acid, 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 Gymnemic acid 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 methanolic Gymnemic acid 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 Gymnemic acid.

3.1 Single dose study
Administration of single dose of Gymnemic acid 100 mg/Kg and 500 mg/Kg, oral, each to two study groups which are diabetes induced rats with streptozotocin, significant reduction (P<0.05) in blood glucose levels was observed. The study period encompassed 24 hrs. The results were significantly comparable to the standard drug glibenclamide. Gymnemic acid at 500 mg/Kg exhibited better blood glucose level reduction compared to Gymnemic acid administered at 100 mg/Kg.

<table>
<thead>
<tr>
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<th>Drug</th>
<th>Dose</th>
<th>0 Hr</th>
<th>1 Hr</th>
<th>2 Hr</th>
<th>4 Hr</th>
<th>6 Hr</th>
<th>8 Hr</th>
<th>12 Hr</th>
<th>24 Hr</th>
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<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>5% w/v</td>
<td>116.0±</td>
<td>115.4±</td>
<td>114.0±</td>
<td>111.9±</td>
<td>111.2±</td>
<td>111±</td>
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<td>110±</td>
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<tr>
<td>II</td>
<td>Diabetic Control</td>
<td>5% w/v</td>
<td>284.4±</td>
<td>281.5±</td>
<td>282.4±</td>
<td>278 ±</td>
<td>285.4±</td>
<td>283.4±</td>
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<td>282.3±</td>
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<tr>
<td>III</td>
<td>Gymnemic acid</td>
<td>100 mg/Kg</td>
<td>281.2±</td>
<td>280 ±</td>
<td>261.6±</td>
<td>231.8±</td>
<td>191.8±</td>
<td>131.2±</td>
<td>102.6±</td>
<td>86.6±</td>
</tr>
<tr>
<td>IV</td>
<td>Gymnemic acid</td>
<td>500 mg/Kg</td>
<td>288 ± 66.2</td>
<td>278 ± 68.2</td>
<td>259 ± 78.7</td>
<td>232.6 ± 86.1</td>
<td>186.8± 75.1</td>
<td>128.2± 75.0</td>
<td>99.2± 48.8</td>
<td>84.4± 68.45</td>
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<tr>
<td>V</td>
<td>Glibenclamide</td>
<td>2.5 mg/kg</td>
<td>289.6± 3.8</td>
<td>282.8± 4.9</td>
<td>272 ± 6.51</td>
<td>240.2± .96</td>
<td>191.8± 5.4</td>
<td>132± 4.89</td>
<td>101.9± .59</td>
<td>85.66± 7.24</td>
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Table 1: Effect of Gymnemic acid on serum glucose levels in streptozotocin induced diabetic rats after single dose administration.
Table 2: Effect of Gymnemic acid on serum glucose levels in streptozotocin induced diabetic rats after prolonged treatment

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>DRUGS</th>
<th>DOSE</th>
<th>1 day</th>
<th>3 day</th>
<th>5 day</th>
<th>7 day</th>
<th>14 day</th>
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<tr>
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<td>Normal</td>
<td>5% w/v Tween 80</td>
<td>71.16</td>
<td>70.98</td>
<td>70.83</td>
<td>70.87</td>
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<tr>
<td>II</td>
<td>Diabetic Control</td>
<td>5% w/v Tween 80</td>
<td>201.2</td>
<td>201.0</td>
<td>200.7</td>
<td>200.5</td>
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<td>III</td>
<td>Gymnemic acid</td>
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<td>255</td>
<td>224</td>
<td>218</td>
<td>213</td>
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<tr>
<td>IV</td>
<td>Gymnemic acid</td>
<td>500 mg/kg</td>
<td>302</td>
<td>256</td>
<td>224</td>
<td>218</td>
<td>213</td>
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<tr>
<td>V</td>
<td>Glyburide</td>
<td>2.5 mg/kg</td>
<td>202.8</td>
<td>202.8</td>
<td>202.8</td>
<td>202.8</td>
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</tr>
</tbody>
</table>

Table 3: Effect of Gymnemic acid on triglyceride levels in serum in streptozotocin induced diabetic rats after single dose administration

Table 4: Effect of Gymnemic acid on triglycerides levels in streptozotocin induced diabetic rats after prolonged treatment

Table 5: Effect of Gymnemic acid on total cholesterol in streptozotocin induced diabetic rats after single dose administration

Table 6: Effect of Gymnemic acid on total cholesterol in streptozotocin induced diabetic rats after prolonged treatment

Table 7: Effect of Gymnemic acid on serum creatinine levels in streptozotocin induced diabetic rats after single dose administration

Table 8: Effect of Gymnemic acid on serum creatinine levels in streptozotocin induced diabetic rats after prolonged treatment

Table 9: Effect of Gymnemic acid on urea levels in serum in streptozotocin induced diabetic rats after single dose administration

Table 10: Effect of Gymnemic acid on urea levels in serum in streptozotocin induced diabetic rats after prolonged treatment

3.2 Multiple dose study
During chronic study which encompassed a period of 15 days, the Gymnemic acid (100 and 500 mg/kg, oral)
produced a significant (P>0.05) in BGL of the diabetic rats compared to control. Gymnemic acid at the dose of 500 mg/kg body weight exhibited better blood glucose levels reduction than 100 mg/kg body weight and results shown in Table. No. 2, 5, 6, 8, 10.

4. Discussion
Gymnemic acid 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 seeds or extracts showed that Gymnemic acid decreased fasting blood sugar levels in animals [7]. 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 [8, 9]. Hence; plants have been suggested as a rich source of potentially useful antidiabetic drugs. Our results showed that oral administration of Gymnemic acid for 24 hrs effectively controlled hyperglycemia. Maintenance of normoglycemia, normalization of serum lipid profile was maintained through Gymnemic acid. The Gymnemic acid 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 [10-12]. Histopathological studies revealed that the volume of islet cells in pancreas was significantly more in drug treated animals compare to the Diabetic control. The islet cells were shrunken and lytic cellular changes were observed in Diabetic control, drug treatment had improved and showed the return of islets close to original cytoarchitecture. In 100 mg and 500 mg/kg of Gymnemic acid group, islets were big and cells were clear with good vascular pattern.

5. Conclusion
The mode of action of Gymnemic acid 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. Therefore, Gymnemic acid serves as an important alternative source in the medicinal study. In conclusion, Gymnemic acid acts as for alternative or complementary medicine in the management of diabetes mellitus.

6. Conflict of interest statement
We declare that we have no conflict of interest.

7. Acknowledgement
I take this privilege and pleasure to acknowledge the contributions of many individuals who have been inspirational and supportive throughout my work undertaken and endowed with the precious knowledge to see success in my endeavour.

8. References