Effect of *Gymnema sylvestre* leaf extract on Streptozotocin induced diabetic rats

A Mishal, R Saravanan, S Sakthi Atchitha, K Santhiya, M Rithika, S Sanju Menaka and T Thiruvalluvan


Abstract

Use of traditional plant as remedies to treat Diabetes mellitus plays a major role in the inexpensive alternative medicine with fewer or no side effects. *Gymnema sylvestre* is an herbal plant used by Indian natives for several years to treat Diabetes mellitus. The aim of the present study was to evaluate the anti-diabetic activity of *Gymnema sylvestre* leaf extract. The anti-diabetic activity was studied using Streptozotocin induced diabetic wistar albino rats. The preliminary phytochemical analysis of the extract of *Gymnema sylvestre* shows the presence of phytoconstituents such as alkaloids, saponins, phenolic compound, tannins, flavonoids, terpenoids, cardiac glycosides and absence of carbohydrates, steroids. Methanolic leaf extract did not produce mortality or morbidity while acute oral toxicity studies in wistar albino rats at 500mg/Kg body weight. The anti-diabetic activity of *Gymnema sylvestre* leaf extract was confirmed by oral administration of the extract to a Streptozotocin induced diabetic rat.

Keywords: Anti-diabetic, *Gymnema sylvestre*, herbal plant, streptozotocin

Introduction

Diabetes – polyuria, ‘mellitus’ – honey. The name ‘diabetes mellitus’ was coined by Thomas Willis. Diabetes mellitus is a metabolic disorder characterized by high blood glucose level, associated with other manifestations [Sembulingam, K. Essentials of medical physiology, 6th edition]. According to WHO about 422 million people worldwide have diabetes, the majority living in low and middle income countries. Diabetes mellitus is caused by destruction of β-cells by viral infections or during autoimmune diseases. It is due the development of antibodies against β-cells, availability of insulin receptors may be less, absent or abnormal result in insulin resistance, genetic disorders. If untreated, possible complications such as kidney failure, leg amputation, vision loss and nerve damage can occur. These complications are seen in millions of cases around the world. Oral medications such as -α-glucosidase inhibitor, biguanides, meglitinides, thiazolidinediones and sulfonylureas are indicated to the patients who are unable to achieve glycemic control despite diet and exercise. These drugs act by various mechanisms to control blood glucose level, but many side effects have been reported such as low blood sugar, unusual weakness, sleepiness, bloating, B-12 deficiency, upset stomach. Serious conditions include lactic acidosis, trouble breathing and muscle pain. Medications require monitoring, have serious side effects and less effective than lifestyle changes. Despite this, many clinicians are giving high-risk patients medication for the delay or prevention of diabetes, in combination with lifestyle changes [Dipiro, Joseph T., Talbert, Robert L., Yee, Gary, Matzke, 6th edition]. Keeping in mind of all above facts, researches are involved in discovering new herbal products due to their natural origin and less side effects. According to WHO, traditional herbal medicines are naturally occurring, plant derived substances with minimal or no industrial processing that have been used to treat illness within local or regional healing practices. *Gymnema sylvestre* also known as ‘sugar destroyer’ is used to treat Diabetes mellitus from ancient days. Chewing of these leaves have significant property of paralyzing the taste glands for few hours against the sweet and bitter flavor. It is used in treatment of diabetic mellitus, stomachic, stimulant, laxative and diuretic. In India, the leaf has been used in early medicinal practice of Ayurveda for snakebites.

Taxonomical classification of *Gymnema sylvestre*: [Kokate, C.K., Purohit, A.P., Gokhale, S.B. Pharmacognosy, 55th edition]

- Kingdom: Plantae
- Subkingdom: Tracheobionta
Methods and Materials
Collection of the drug
The leaf of Gymnema sylvestre has been collected from the wild of Thiruvallur district.

Authentication of the drug
The plant for the present study has been authenticated.

Preparation of plant extract
The fresh leaves of Gymnema sylvestre have been cleaned with clean water, desiccated for 7 days under silhouette and crushed into abrasive powder. The extraction was processed by cold maceration method; stoppered glass container was used to macerate abrasive powder of Gymnema sylvestre with the solvent 85% methanol. The extract was transferred to a rotary vacuum after 3 days with frequent interval (4hours) of agitation. The filtrate yields brown semisolid extract of methanol and the extract were preserves in refrigerator for further use.

Phytochemical studies
Preliminary analysis was done to test the presence or absence of alkaloids, saponins, phenolic compound, tannins, flavonoids, terpenoids, cardiac glycosides, carbohydrates and steroids of methanolic extract of Gymnema sylvestre leaves were performed by standard test.

Experimental animals
Inbred wistar albino rats of both sexes, weighting between 150gm – 200gm were used. They have been maintained in polypropylene cage under standard laboratory conditions at ambient temperature around 23±1°C with 50-60% humidity. Standard food and tap water were provided. Fasting animals have been fasted overnight before commencing the experiment, but had free access to water. The project was approved by IAEC (Institutional Animal Ethical Committee) of CPCSEA (Committee for the Purpose of Control and Supervision of Experimentation of Animals). IAEC Reference No: IAEC/XIII/03/CLBMCP/2008-2009 dated 16/06/2008.

Acute toxicity studies
OECD guidelines (Organization of Economic Cooperation and Development) 423 (acute toxic class) was followed in the procedure. Definite doses (5, 50, 500 mg/ kg body weight) have been used for the acute toxic studies with 3 male animals in step by step procedure. The results have been classified according to the global harmonized system (GHS).

Diabetogenic agent
Streptozotocin (STZ) was used as diabetogenic agent, which was given as a single intraperitoneal dose (45mg/kg body weight) dissolved in citrate buffer (pH 4.5).

Standard drug
Gliclazide was used as a standard drug, administered as intragastrically at a dose of 25mg/kg body weight.

Induction of diabetes mellitus
Diabetes mellitus was experimentally induced in 32 adult inbred Wistar albino rats of either sex were fasted for 12 hours with freshly prepared Streptozotocin (STZ) (45mg/kg body weight) dissolved in citrate buffer (pH 4.5) intraperitoneally. After this, the animal had free access to food and instead of drinking water 5% glucose solution was given for 48 hours until sustained hyperglycemia was established. 6 normal rats received 1ml citrate buffer as vehicle. The development of diabetes mellitus was confirmed after 48hours of Streptozotocin injection by the collected blood samples from lateral tail vein of rats which was fasted overnight (10-12Hours). glucose level was measured using single one touch glucometer based on glucose oxidase method. Rats having glucose ranging from 180-200 mg/dl was considered as mild diabetic were included in the experiment, where else 4 rats were omitted due to the sub diabetic condition. 4 groups of 24 animals (6 in a group) were formed and 4 rats died before grouping.

Experiment design
The animals were divided into 5 groups (6 rats on each group).

Group I: Normal rats received 0.5% CMC 5ml/kg body weight for 14 days.

Group II: STZ induced diabetic rats received 0.5% CMC 5ml/kg body weight for 14 days

Group III: STZ induced diabetic rats received standard drug Gliclazide 25mg/kg body weight for 14 days.

Group IV: STZ induced diabetic rats treated with methanolic extract of Gymnema sylvestre 50mg/kg body weight for 14 days.

Group V: STZ induced diabetic rats treated with methanolic extract of Gymnema sylvestre 100mg/kg body weight for 14 days.

The blood samples were collected over night fasted animals on 0th, 7th, 15th day to estimate blood glucose levels using glucometer.
Results
Preliminary phytochemical analysis of methanolic extract of Gymnema sylvestre
Table 1 shows the result of preliminary phytochemical analysis of leaf extract of Gymnema sylvestre. Methanolic extract shows the presence of alkaloids, saponins, phenolic compound, tannins, flavonoids, terpenoids and cardiac glycosides. However, the extract shows negative test for carbohydrates and steroids.

Acute oral toxicity
OECD 423 guideline (Acute toxic class method) was followed in acute oral toxicity. The starting dose of 500mg/kg body weight was administrated as single dose of methanolic extract in 3 male rats and observed for 14 days. After and before the treatment of extract, there was no considerable change in body weight and no signs of toxicity were observed. The results are shown in table 2.

Effect of sub-acute treatment of methanolic extract of the leaves of Gymnema sylvestre on blood glucose level in STZ induced diabetic rats
STZ induced diabetic rats were treated with methanolic extract 50mg and 100mg/kg body weight for the duration of 14 days. Decrease in blood glucose level after 7th day onwards was observed in 50mg treated rats – p<0.01. Drop in blood glucose level after 7th day onwards was observed in 100mg treated rats – p<0.01. STZ induced diabetic rats which were treated with Gliclazide shows significant drop in blood glucose level after 7th day – p<0.05. Results are shown in table 3.

Table 1: Preliminary Phytochemical analysis of methanolic leaf extract of Gymnema sylvestre

<table>
<thead>
<tr>
<th>S. No</th>
<th>Constituents</th>
<th>Test</th>
<th>Methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloids</td>
<td>Dragendoff’s test</td>
<td>Present</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hagner’s test</td>
<td>Present</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mayer’s test</td>
<td>Present</td>
</tr>
<tr>
<td>2.</td>
<td>Carbohydrates</td>
<td>Benediet’s test</td>
<td>Absent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fehligh’s test</td>
<td>Absent</td>
</tr>
<tr>
<td>3.</td>
<td>Saponins</td>
<td>Froth test</td>
<td>Present</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Foam test</td>
<td>Present</td>
</tr>
<tr>
<td>4.</td>
<td>Phenolic compound</td>
<td>Ferric chloride test</td>
<td>Present</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lead acetate test</td>
<td>Present</td>
</tr>
<tr>
<td>5.</td>
<td>Tannins</td>
<td>Gelatin test</td>
<td>Present</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ferric chloride test</td>
<td>Present</td>
</tr>
<tr>
<td>6.</td>
<td>Flavonoids</td>
<td>Alkaline reagent test</td>
<td>Present</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lead acetate test</td>
<td>Present</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ferric chloride test</td>
<td>Present</td>
</tr>
<tr>
<td>7.</td>
<td>Steroids</td>
<td>Liebermann – Burchard’s test</td>
<td>Absent</td>
</tr>
<tr>
<td>8.</td>
<td>Terpenoids</td>
<td>Salkowski test</td>
<td>Present</td>
</tr>
<tr>
<td>9.</td>
<td>Cardiac glycosides</td>
<td>killer killani test</td>
<td>Present</td>
</tr>
</tbody>
</table>

Table 2: Results of Acute Oral Toxicity studies (OECD 423 guidelines)

<table>
<thead>
<tr>
<th>S. No</th>
<th>Treatment</th>
<th>Dose</th>
<th>Weight of animal (in gms)</th>
<th>Signs of toxicity</th>
<th>Onset of toxicity</th>
<th>Reversible or Irreversible</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Before test</td>
<td>After test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>GSM</td>
<td>0.5g/kg</td>
<td>100</td>
<td>100</td>
<td>No signs of toxicity</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>2.</td>
<td>GSM</td>
<td>0.5g/kg</td>
<td>125</td>
<td>130</td>
<td>No signs of toxicity</td>
<td>Nil</td>
<td>Nil</td>
</tr>
</tbody>
</table>

Table 3: Results of sub-acute administration of GSM extract on STZ induced diabetic rats

<table>
<thead>
<tr>
<th>S. No</th>
<th>Day</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0th day</td>
<td>69.2 ± 2.3</td>
<td>254.41 ± 6.3**</td>
<td>242.2 ± 6A**</td>
<td>248.5 ± 1.23C**</td>
<td>243.3 ± 6.13**</td>
</tr>
<tr>
<td>2.</td>
<td>7th day</td>
<td>94.51 ± 6.0</td>
<td>275.3 ± 3.31**</td>
<td>178.3 ± 7.1**</td>
<td>200.8 ± 3.11**</td>
<td>181.0 ± 7.41**</td>
</tr>
<tr>
<td>3.</td>
<td>14th day</td>
<td>108.7 ± 7.9</td>
<td>293.21 ± 4.11**</td>
<td>118.2 ± 7.1**</td>
<td>156.6 ± 5.12**</td>
<td>122.2 ± 4.41**</td>
</tr>
</tbody>
</table>

The values are expressed as mean ± SME (standard mean error) of 4 animals; a – group II compared with group I; b – group III, IV, V as compared with group II. Statistical significant test for comparing was done by one way ANOVA followed by Dunnett’s multiple comparison test using graph pad prism software, Inc. version 4.03, 1992 – 2005.

Discussion
Antidiabetic activities are present in many species and have been reported. Working on the same line we have undertaken a study on Gymnema sylvestre for its antidiabetic property. Alkaloids, saponins, phenolic compound, tannins, flavonoids, terpenoids and cardiac glycosides are present in Gymnema sylvestre methanolic leaf extract and absent of carbohydrates, steroids on phytochemical analysis.

In experimental rats no mortality or sign of toxicity were produced by the methanolic leaf extract (500mg/kg body weight) on acute oral toxicity studies. In overnight fasted normal rats have no significant suppression of blood glucose level was observed at doses 50 and 100mg/kg body weight of GSM but showed significant improvement in glucose tolerance in glucose fed hyperglycemic normal drugs. In sub-acute studies the sugar level of standard group which have been treated with Gliclazide were decreased from the first day of the treatment. GSM 50mg and 100mg treatment produces significant decrease in blood glucose level from the 7th day of the treatment thereafter a steady decrease in blood glucose level was observed. The number of pancreatic islet cells, as well as insulin levels, was elevated in diabetic rats treated orally with Gymnema sylvestre extract, suggesting a positive repair or regeneration of endocrine pancreas [Bashar Saad, Hilal Zaid, Siba Shanak, Sleman Kadan. Anti – diabetes and Anti – obesity Medicinal Plants and Phytochemicals, 1st edition, 2017]. Hypoglycemic activities of methanolic extract have been revealed from the study of blood glucose level in Streptozotocin induced diabetic rat.

Conclusion
Based on the obtained result the extract of Gymnema sylvestre possesses significant anti-diabetic effect at 100mg/Kg body weight. Acute oral toxicity studies reveal no sign of toxicity or mortality in the experimental rats at a dose of 500mg/Kg body weight was observed. Hence it can be used as a treatment or supportive therapy for Diabetic mellitus.

References


