ANTI-DIABETIC ACTIVITY OF GYMNEMA SYLVESTRE LEAF EXTRACT ON ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT
The concerned study reveals the anti-diabetic effect of Gymnema sylvestre in control of blood glucose level in Alloxan induced Diabetic rats. The recent studies shows the active constituent is Gymnemic acid a triterpenoid saponin has significant effects on the intestinal absorption of glucose in to the blood and stimulation of b-cells of pancreas in the production of Insulin. Being a traditional anti diabetic plant in many alternative systems of medicine, the dried aqueous leaf extract of Gymnema sylvestre at the dose of 100, 200, 300 mg/kg was given orally to the alloxan induced diabetic rats for a period of 21 days and the blood glucose levels was sampled every week. The study showed a significant reduction in the blood glucose levels showing the potential of anti-diabetic activity of Gymnema sylvestre.

Key Words: Alloxan, Gymnema sylvestre, Triterpenoids

INTRODUCTION
Diabetes mellitus is a metabolic disorder of multiple aetiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular disease [1]. Two major classes of diabetes mellitus and named them, IDDM or Type 1 or Juvenile onset Diabetes which is caused by destruction of b-cells of pancreatic Islets, and NIDDM or Type 2 or maturity onset diabetes caused by abnormality in gluco-receptor, reduced sensitivity of peripheral tissues to insulin, excess of hyperglycemic hormones like glucagons [2]. Many synthetic Allopathic medicines are currently available in the market for the treatment. But due to their side effects these medicines are comparatively replaced by many alternative systems of medicines out of which the ancient herbal remedies are gaining popularity due to their less side effects on continuous use compared with that of synthetic medicines.
**Fig. 1: Gymnema sylvestre herb**

*Gymnema sylvestre* is a medicinal plant belonging to the family Asclepiadaceae. *G. sylvestre* is regarded as one of the plants with potent antidiabetic properties. The “destroyer of sugar” is a traditionally used term for *Gymnema sylvestre* because chewing the leaves will abolish the taste of sweetness. It is a woody plant found in tropical forests of India and Africa has been proven as antidiabetic drug [3]. Recent studies showed that *Gymnema* has anti-microbial activity [4]. The major chemical constituents of *Gymnema* are gymnemic acid and gurmarin. Gymnemic acids have antidiabetic, anti-sweetener and anti-inflammatory activities. The Gymnemic acid is made up of a group of oleanane type triterpenoid saponins which intern contain several acylated (tigloyl, methylbutyroyl etc,) derivatives of deacylgymnemic acid (DAGA). The individual gymnemic acids (saponins) include gymnemic acids I-VII, gymnemosides A-F, gymnema saponins [5]. The atomic arrangement of gymnemic acid molecules is similar to that of glucose molecules. These molecules fill the receptor locations on the taste buds thereby preventing its activation by sugar molecules present in the food, thereby curbing the sugar craving. Besides impairing the ability to discriminate sweet taste, increase enzyme activity responsible for the glucose uptake and utilization. It may stimulate pancreatic b–cell function, increase b-cell number and increase insulin release by increasing cell permeability to insulin [6], Gymnemic acid molecules fill the receptor location in the absorptive external layers of the intestine thereby preventing the sugar molecules absorption by the intestine, which results in low blood sugar level. It is extensively used to treat the Type-II Diabetes.

**Mechanism of Action of Gymnemic Acids:**

The main constituent of gymnema is believed to be gymnemic acid, a mixture of at least 17 different saponins. Gymnemic acid formulations have been found useful against obesity, according to recent reports. This is attributed to the ability of gymnemic acids to delay the glucose absorption in the blood. The atomic arrangement of gymnemic acid molecules is similar to that of glucose molecules. These molecules fill the receptor locations on the taste buds thereby preventing its activation by sugar molecules present in the food, thereby curbing the sugar craving. Similarly, Gymnemic acid molecules fill the receptor location in the absorptive external layers of the intestine thereby preventing the sugar molecules absorption by the intestine, which results in low blood sugar level. There are some possible mechanisms by which the leaves and especially Gymnemic acids from *G. sylvestre* exert its hypoglycemic effects are:
• It increases secretion of insulin
• It promotes regeneration of islet cells
• It increases utilization of glucose:
• It is shown to increase the activities of enzymes responsible for utilization of glucose by insulin-dependent pathways, an increase in phosphorylase activity, decrease in gluconeogenic enzymes and sorbitol dehydrogenase and
• It causes inhibition of glucose absorption from intestine, the exact action being unknown. It could be involve one or more mechanisms [7].

Fig. 2: Structure of Gymnemic acid [8]

MATERIALS AND METHODS
PROCESS OF EXTRACTION: The dried leaf powder was collected from the locally marketed preparation called Podapathri of about 500g. The process of extraction is based up on the nature of compound present in the plant material and the selection of solvents is based on the affinity towards it. Hooper’s method is followed for the extraction of gymnemic acids [9].

• STEP-1: DEFATTING OF LEAF POWDER: 100g of dried leaf powder was packed in to a clean Soxhlet extraction apparatus and 200ml of petroleum ether was added and extracted at 50-60 C for a period of 24-36 hrs till all the components were soluble in petroleum ether. This process for defatting of leaf powder in which most of the fats and fatty acids are solubilised leaving the compound free and makes the extraction efficacious. Care should be taken that the defatted leaf powder should be used for the next step of extraction immediately, on storage, the active ingredient may exhaust and concentration of the product reduces.

• STEP-2: EXTRACTION OF GYMNEMIC ACID: Distilled water is used as a solvent in the next step of extraction of plant material and the extraction process is continued for a period of 24-36 hrs till all the components are soluble. The thick paste obtained was collected and dissolved in 1% NaOH on continuous stirring. The solution is then filtered and dilute Hcl was added slowly under constant
stirring during which the gymnemic acids were precipitated. It was dried and used as Gymnema leaf extract. The absorbance was determined by using UV-Visible spectrophotometer and it was found to be 220nm.

![Gymnema sylvestre Leaf extract](image)

**Fig. 3: Gymnema sylvestre Leaf extract**

**PHYTOCHEMICAL SCREENING:** [10, 11]

- **Test for Alkaloids:** Alkaloids are basic nitrogenous compounds with definite physiological and pharmacological activity. Alkaloid solution produces white yellowish precipitate when a few drops of Mayer’s reagents are added. Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer’s reagent.

- **Test for Cardiac glycosides (Keller-Killiani test):** Five ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was under layered with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

- **Test for Anthraquinones:** Bomtragers test was used for the detection of anthraquinones. 5 gm of plant extract was shaked with 10 ml of benzene. This was filtered and 5.0 ml of 10% ammonia solution was added to the filtrate. The mixture was shaken and the presence of violet color in the lower phase indicated the presence of free hydroxyl anthraquinones.

- **Test for Tannins:** About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

- **Test for Phenols:** The solvent plant extract was treated with few drops of neutral ferric chloride solution 5% intense color developed which indicated the presence of phenols.

- **Test for Terpenoids (Salkowski test):** Five ml of each extract was mixed in 2 ml of chloroform, and concentrated Sulfuric acid (3 ml) was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids.
• **Test for Steroids:** Two ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml Sulfuric acid. The color changed from violet to blue or green in some samples indicating the presence of steroids.

• **Test for Saponins:** About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

• **Test for Flavonoids:** 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated sulfuric acid. A yellow coloration observed in the extract indicated the presence of flavonoids.

**EXPERIMENTAL DESIGN:**

Albino rats of both sex of weight 150-180g were kept in separate cages under standard temperature and fed with standard diet, water and libitum. These animals were divided in to 6 groups of 6 animals each of either sex. These groups are as follows.

- **GROUP-1:** Normal
- **GROUP-2:** Diabetic control
- **GROUP-3:** Standard drug (Metformin) 100mg/kg
- **GROUP-4:** Gymnema leaves extract 100mg/kg
- **GROUP-5:** Gymnema leaves extract 200mg/kg
- **GROUP-6:** Gymnema leaves extract 300mg/kg

**ACUTE TOXICITY STUDIES:** The acute toxicity study was carried out in albino rats of either sex by the "fix dose" method of OECD (Organization for Economic Co-operation and Development) Guideline. The aqueous solution of leaf extract was administered orally at a dose level of 3000 mg/kg bwt. Then the animals were observed continuously for 3 hours for general behavioral, neurological, and autonomic profiles and then every 30 minutes for next 3 hours and finally for mortality after 24 hours till 7 days. No mortality was observed at the end of 7th day.

**INDUCTION OF DIABETES:** Before the induction of diabetes the weights and glucose levels of each rat were pre-determined by using a standard glucometer based on glucose oxidase method. Blood samples were collected from the tip of the tail at defined time patterns. Then Alloxan monohydrate of 150mg/kg body weight was given intraperitoneally to each rat. After two days of alloxan administration blood samples were collected and diabetic state was confirmed when blood sugar level was above 200mg/dl. The rats with moderate diabetes were used in the experiment.

**DRUG ADMINISTRATION:** The group-1 control rats received normal saline and fed on normal diet. The group-2 was diabetic control. The group-3 rats received a standard marketed anti-diabetic drug Metformin of 100mg/kg orally dissolved in normal saline solution. The *Gymnema* leaf extract at the dose of 100, 200 and...
300mg/kg body weight was given orally to the groups - 4, 5 and 6 respectively. Then blood glucose levels are estimated on every 1st, 7th, 14th and 21st days.

RESULTS AND DISCUSSION

17gm of dried powder was collected from the thick paste of about 22.5gm obtained during the soxhlet extraction of dried leaves powder of Gymnema sylvestre. Phytochemical screening was done to detect various chemical compounds showed the presence of Alkaloids, Cardiac glycosides, Tannins, Phenols, Terpenoids, Saponins and Flavanoids. The anti-Diabetic activity of Gymnema sylvestre was may be due to the presence of Gymnemic acid a Triterpenoid Saponin. (Table: 1)

The aqueous extract was tested for anti-diabetic activity in rats shows the protocol for anti-diabetic activity. The aqueous extract showed a significant reduction in blood glucose level in diabetic rats. However, the possible mechanism by which the drug brings about its hypoglycemic actions may be by competitively inhibiting the glucose receptors at intestinal level, either by increasing the pancreatic secretions of Insulin from the cells of Islets of Langerhans or its release from bound insulin. The anti-diabetic activity was found to be significant and dose dependent. All the doses showed maximum reduction of blood glucose level on the 7th day. Out of three doses, 100 mg/kg and 200mg/kg showed a significant reduction but the dose 300mg/kg showed more significant reduction of blood glucose levels when compared with that of standard. (Table: 2)

Table 1: Qualitative Phytochemical screening of leaf extract of Gymnema sylvestre

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Gymnema leaf extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
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<td>Terpenoids</td>
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<td>+</td>
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<tr>
<td>Fats and Lipids</td>
<td>-</td>
</tr>
<tr>
<td>Flavanoids</td>
<td>+</td>
</tr>
</tbody>
</table>

(+) Denotes Presence, (-) Denotes Absence
Table-2: Effect of Gymnema sylvestre leaf extract on Alloxan induced Diabetic rats

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>Blood glucose levels mg/dl</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0day</td>
</tr>
<tr>
<td>I</td>
<td>114.5±0.7638</td>
</tr>
<tr>
<td>II</td>
<td>252.3±1.563</td>
</tr>
<tr>
<td>III</td>
<td>248.7±1.054*</td>
</tr>
<tr>
<td>IV</td>
<td>251.5±0.763*</td>
</tr>
<tr>
<td>V</td>
<td>239.5±0.763**</td>
</tr>
<tr>
<td>VI</td>
<td>251.5±0.763*</td>
</tr>
</tbody>
</table>

Note: Values are given as mean ± SEM for groups of six animals each *P < 0.01 & **P< 0.05 (Dunnet -test). Diabetic control was compared with the vehicle control and extract treated groups were compared with the diabetic control.

CONCLUSION

The present study reveals the antidiabetic effect of Gymnema sylvestre leaf extracts by a significant reduction in the blood glucose levels of alloxan induced diabetic rats. As Diabetes a clinical entity with several complications, this study proves the activity against it. Not only Gymnema but also many herbals have been identified with anti-diabetic properties and research is going on based on their activity in India been regarded as Capital for Diabetic medicine of world. This show traditional anti diabetic plants will be the future in the treatment of Diabetes and also an alternative to the present system of therapy.
REFERENCES


2) Killeddar SG, Rathod AM, Salunkhe RM, Bhore N V, Mahmuni SS. Parameters studied for the development of Gymnema sylvestre leaf extract: As injectable antidiabetic. IJRAP.2012: 3(2); 1


