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Challenges in Biochemical Engineering and Biotechnology for Sustainable Environment

## Hypoglycemic Effect of *Gymnema Sylvestre* Leaf Extract on Normal and Streptozotocin Induced Diabetic Rats

<sup>1</sup>K.Yogalakshmi\*, <sup>1</sup>J.Vaidehi, <sup>1</sup>P.Ramakotti

<sup>1</sup>Department of Zoology Faculty of Science, Annamalai University,  
Annamalai Nagar- 608002

\*Corres.author: yogalakshmi4537@gmail.com

**Abstract :** The aim of the present study was to evaluate the hypoglycemic activity of *Gymnema sylvestre* leaf extract in streptozotocin induced diabetic rats. Diabetes mellitus was induced by a single intraperitoneal injection of STZ (60 mg/kg body weight). The experimental set up was farmed as follows: Group I – Control, non-diabetic, Group II – Control, diabetic, Group III – Diabetic, treated with standard drug (Glibenclamide 10mg/kg bodyweight/30days, Group IV- Diabetic, treated with methanolic extract of *Gylmnema syvestre* leaves (100mg/kg body weight/30days) orally, Group V- Diabetic, treated with methanolic extract of *Gymnema sylvestre* leaves (200mg/kg body weight/30 day) orally, Group-VI- Diabetic, treated methanolic extract of *Gymnema sylvestre* leaves (300 mg/kg body weight /30 days) orally. The results of the study indicates that *Gymnema syvestre* extract significantly ( $P<0.01$ ) reduced the blood sugar level. The oral administration of methanolic extracts at doses of 300 mg / kg lead to a significant blood glucose reduction. This may lay the foundation to study the active compounds of such anti-diabetic plants that are responsible for the hypoglycemic activities.

**Key words:** *Gymnema sylvestre*, hypoglycemic, methanolic extract, diabetic rat.

### Introduction:

Diabetes mellitus (DM) is a major global health problem and is now recognized as one of the leading causes of death worldwide, where the high prevalence of the disease could be attributed to improved nutritional status<sup>1,2</sup>. More than one-fifth of them are Indians. According to the International Diabetes Federation, India has been declared as “Diabetic Capital of the World” at the recently concluded conference in Paris . Plants have been used as sources of drugs for the treatment of diabetes in developing countries where the cost of conventional medicines is a burden to the population<sup>3</sup>. DM is defined as a metabolic disorder characterized by an elevation of the blood glucose concentration due to absolute or relative lack of insulin leading to hyperglycemia. DM is associated with abnormal metabolism of carbohydrates, fat and protein<sup>4,5,6</sup>. The anti-diabetes bioactive compounds in Chinese medicinal plants include polysaccharides, terpenoids, flavonoids, sterols, and alkaloids<sup>7</sup>. In many countries, much attention has been paid to discover natural antidiabetic drugs from various medicinal plants<sup>8</sup>. Though different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes mellitus, there is increasing demand by patients to use natural products with antidiabetic activity. Insulin cannot be used orally and continuous use of the synthetic antidiabetic drugs causes side effects and toxicity<sup>9,10</sup>. The variances in weights of experimental rat organs are also monitored for indirect diabetes diagnosis<sup>11</sup>. Reported that the weights of the liver and kidney were increased in diabetic rats. Based on

the results, it had been found that *P. baumii* EPS administration led to the diabetogenic effect of STZ and significantly reduced the degree of diabetes. This result encouraged the researchers to believe that oral administration of *P. baumii* EPS might have a potential benefit in preventing diabetes, since pancreatic damage induced by environmental chemicals and other factors is a cause of diabetes<sup>12</sup>. In Morocco, the phytotherapy is an integral part of national culture. A lot of plants are currently used to treat diabetes mellitus<sup>13</sup>. The main constituents of streptozotocin were flavonoids, saponins and tannins<sup>14</sup>. Flavonoids are considered as active principles in many medicinal plants<sup>15</sup> and natural products with positive effect for human health<sup>16</sup>. Substantial evidence in the literature indicates that hyperglycemia can cause oxidative stress by various mechanisms. Many indigenous Indian medicinal plants have found to be useful to successfully manage diabetes. One of the greatest advantages of traditional medicinal plants is that these are readily available and have no side effects. Even WHO<sup>17</sup> has suggested the evaluation of the potential of plants as effective therapeutic agents, especially in areas in which safe modern drugs are lacking. Although, numerous traditional medicinal plants are reported to have antidiabetic and hypoglycemic properties. A member of the milkweed (family: Asclepiadaceae) *Gymnema sylvestre* sculpt is a woody plant found in tropical forests of India and Africa that has been proven as antidiabetic drug. The medicinally active parts of the extracts of the plant leaves and the roots although is unknown. Besides impairing the ability to discriminate sweet taste, it increases the enzyme activity responsible for the glucose uptake and utilization. The objective of the present study thus focuses on the hypoglycemic activity of methanolic extract of *Gymnema sylvestre* leaves in streptozotocin induced diabetic rats.

## Materials and Methods:

### 1. Preparation of extract:

The leaves of *Gymnema sylvestre* were procured from the Botanical garden, Department of Botany, Annamalai University, Tamil Nadu. The dried leaves were pulverized and passed through 40 mesh sieve. The coarse powder was extracted with 95% v/v methanol at 600 – 750<sup>0</sup> C for 48 hours. The extract was filtered, concentrated and dried under reduced pressure by rotating evaporator (yield 6.3%) and the residue was kept in desiccators. The suspension of methanolic extract was prepared by using 0.5% Tween-80 in saline.

### 2. Animal:

Healthy adult male Wistar albino rats between 2-3 months of age and weighing 150-200 gm were used for the present study. The animals were housed individually in polypropylene cages, maintained under standard conditions (12-hr light and 12-hr dark cycle, 25±5°C and 40-60% humidity). They were fed with standard rat pellet diet (Hindustan Lever Limited, Mumbai) and provided water ad libitum. The experimental protocols were conducted in accordance with internationally accepted standard guidelines for care and use of laboratory animals. The animals treatment and protocol employed were approved by the TAEC, Annamalai university (Registration Number -1084/2014/CPCSEA)

### 3. Toxicity study:

All these rats were fasted overnight before the administration of Streptozotocin. Diabetes was induced in rats by intra peritoneal injection of streptozotocin dissolved in 0.1 M sodium citrate buffer of pH 4.5 at the dose of 50mg/kg body weight. After the injection they had free access to food and water. The animals were allowed to drink 5% glucose solution overnight to overcome hypoglycemic shock. The development of diabetes was confirmed after 48hrs of streptozotocin injection. The animals having fasting blood level of more than 200g/dl were considered as diabetic rats and used for further the experimentation. Diabetic animals were grouped five days after the induction of diabetes.

### 4. Experimental Design:

The experimental rats were divided into the following groups with six animals each: Group I – Control, non-diabetic, Group II– Control, diabetic, Group III– Diabetic, treated with standard drug (Glibenclamide (10mg/kg bodyweight/30days). Group IV- Diabetic, treated with methanolic extract of *Gymnema sylvestre* leaves (100mg/kg body weight/30days) orally. Group V- Diabetic, treated with methanolic extract of *Gymnema sylvestre* leaves (200mg/kg body weight/30 day) orally, Group-VI- Diabetic, treated with methanolic extract of *Gymnema sylvestre* leaves (300 mg/kg body weight /30 days) orally.

Rats were fasted overnight and the blood was withdrawn from the orbital sinus of the eye on the 10<sup>th</sup>, 20<sup>th</sup> and 30<sup>th</sup> days post induction to determine the blood glucose by GOD-POD kit method. The change of body weight was observed throughout the treatment period in experimental animals.

#### 4. Statistical Analysis:

All values were expressed as Mean  $\pm$  S.D. The differences between control and treatment groups were tested for significance using Anova followed by Dunnet's t test. P<0.05 were considered as significant.

#### Results and Discussion:

In the present study, STZ-induced diabetic rats models were indulged as to investigate the hypoglycemic effect of *Gymnema sylvestre* leaf extract. Photochemical analyses of the crude extract revealed the presence of flavonoids, steroids, alkaloids and tannins and saponins. However, treatment of the alloxan induced diabetic rats with the aqueous suspension of *Lupinustermis*, Halfabarror Kammunquaramany reduced their plasma glucose levels by 59, 57 and 39%, respectively, when compared with the diabetic group in agreement with the findings<sup>(18)</sup> Previous studies have shown that these hypoglycemic herbs contained saponins, alkaloids, tannin and quinovic acid<sup>19,20,21</sup>. It is well established that some saponins have hypoglycemic activity, which may be due to the inhibition of liver gluconeogenesis or glycogenolysis<sup>22</sup>. A hypoglycemic substance related to the biguanide class of oral antidiabetic drugs, has been detected in edible mushroom<sup>23</sup>. The ability of lectins isolated from mushroom (*A. campestris* and *A. bisporus*) to enhance insulin release by isolated rat islets of Langerhans from rats has been documented<sup>24,25</sup>. However obtained an adverse result regarding the hypoglycemic activity from the Sam<sup>26</sup>. This study elucidated the extent of mortality up to the dose of 200mg/kg body weight. Hence these extracts were considered safe for long term administration. The effect of the treatment with the extract for 30 days led to a dose dependent fall in blood glucose levels. Maximum effect seems to reach constant thereafter. Normal healthy control was found to be stable in their body weight but diabetic rat showed reduction in body weight. In this study, the decrease of body weights were significantly diminished (P<0.05) by the extract after 30 days of treatment (Table-1). Furthermore, the effects of the treatment of extract and Glibenclamide on blood glucose concentration in normal fasted and diabetic rats after treatment are shown (Table 2). At the end of the experiment after 30 days, blood glucose level was 209.80 $\pm$ 1.65 and 178.56 $\pm$ 1.53 mg/dl in the diabetic rats treated with 200 and 300 mg/kg b.w of the leaves extract respectively. The present study revealed that the methanolic extract of the leaves of *Gymnema sylvestre* exhibited good ant hypoglycemic activity in streptozotocin induced diabetic rats. The present findings from the methanolic extract of leaves of the *Gymnema sylvestre*, could be incorporated into diabetes biology and pharmacology that may lead to significant development in diabetes treatment.

**Table-1: Effect of *Gymnema sylvestre* leaf extract on the body weight in streptozotocin induced diabetic rats**

Groups	Body weight in gms(Mean $\pm$ SD)		
	Post induction days		
	10 <sup>th</sup> day	20 <sup>th</sup> day	30 <sup>th</sup> day
I	167.41 $\pm$ 1.19	164.55 $\pm$ 1.10	159.20 $\pm$ 1.18
II	198.25 $\pm$ 1.23	194.56 $\pm$ 1.26	192.16 $\pm$ 1.28
III	192.45 $\pm$ 1.23	185.69 $\pm$ 1.38	178.55 $\pm$ 1.43
IV	221.41 $\pm$ 1.41	218.55 $\pm$ 1.39	215.36 $\pm$ 1.48
V	219.55 $\pm$ 1.35	205.48 $\pm$ 1.45	195.31 $\pm$ 1.46
VI	331.46 $\pm$ 1.39	301.46 $\pm$ 1.55	229.30 $\pm$ 1.62

**Table-2: Effect of *Gymnema sylvestre* leaf extract on the blood sugar level in streptozotocin induced diabetic rats.**

Groups	Blood glucose level in mg/dl(Mean±SD)		
	Post induction days		
	10 <sup>th</sup> day	20 <sup>th</sup> day	30 <sup>th</sup> day
I	106.10±1.50	104.69±1.65	108.20±1.52
II	229.23±1.54	230.59±1.62	174.29±1.68
III	201.82±1.60	180.40±1.58	172.43±2.16
IV	219.78±1.27	206.21±1.97	166.65±1.54
V	208.61±1.52	190.67±1.62	178.56±1.53
VI	209.52±1.52	187.70±1.54	209.80±1.65

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