

## Antidiabetic Activity of Glycoside Isolated from *Gymnema sylvestre* in Streptozotocin Induced Diabetic Rats

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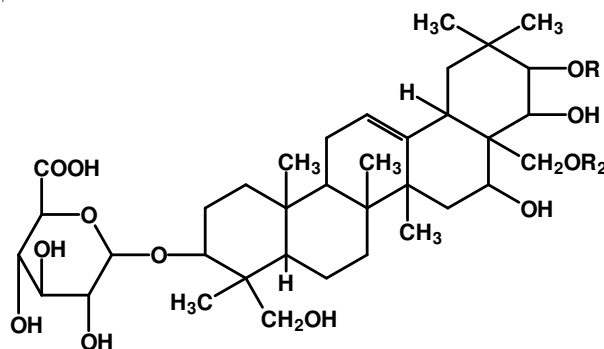
A study was undertaken to evaluate the antidiabetic activity of alcoholic extract of leaf of *Gymnema sylvestre* in streptozotocin induced diabetic rats. Glycoside is isolated from *G. sylvestre* investigated hypoglycemic activity of normal and streptozotocin induced diabetic rats and compared with glibenclamide reference drug. *Gymnema sylvestre* (250 mg/kg/body weight) decrease blood glucose levels significantly ( $p < 0.05$ ) at 2 and 4 h after the glucose load in glucose tolerance test. The results suggested that the alcoholic extract of *Gymnema sylvestre* possess significant hypoglycemic activity in normal and streptozotocin induced diabetic rats.

**Key Words:** Antidiabetic, Glibenclamide, *Gymnema sylvestre*, Hypoglycemic, Streptozotocin.

### INTRODUCTION

Diabetes mellitus generally termed as Madhumeha in ayurvedic terminology is frequent clinical problem. The incidence of diabetes has been on a constant rise during few decades particularly in the developing countries like India. Insulin is a hormone produced by Islet of Langerhans in pancreas. This hormone implicates vital role in the metabolism of carbohydrates and utilization of glucose<sup>1,2</sup>. Diabetes is characterized by the defective synthesis of insulin<sup>3,4</sup>.

The leaves of *Gymnema sylvestre* are reported to possess many medicinal properties in the ayurvedic literature<sup>5</sup>. Locally it is called Gurmar or destroyer of sugar. Leaves contain glycoside designated as Gymnemic acid (I) a bitter principle; tartaric acid and calcium oxalate and hentriacontane (C<sub>31</sub>H<sub>64</sub>) have been isolated from leaves and demonstrated antidiabetic activity. The leaves of plant find used as antidiabetic remedy in ancient medicine since 2000 years<sup>6</sup>. The alcoholic extract of leaves yields water soluble acidic fraction, which shows good hypoglycemic activity. The present study is aimed to evaluate the antidiabetic activity in streptozotocin induced diabetic rats after administration of extract of *Gymnema sylvestre*.



Structure of gymnemic acid (I)

## EXPERIMENTAL

*Gymnema sylvestre* commonly known as Gurmar belonging to the family Asclepidaceae are collected from Sanchi in Raisen district of Madhya Pradesh.

**Preparation of plant extract:** *Gymnema sylvestre* leaves collected and air-dried in shade at room temperature. The dried leaves were powdered and sieved using the fine muslin cloth. The fine powdered leaves were kept with 90 % alcohol in Soxhlet apparatus to get the crude.

**Analysis of crude drug:** The separation and purification of plant constituents is mainly carried out using thin layer chromatography and column chromatography. TLC was carried out using *n*-butanol:acetic acid:distilled water (16:4:20), which gave three fractions. The fractions were tested chemically for glycosides showing the presence of mono and disaccharides in the compound. Molish and Fehling tests confirmed the presence of glycosides.

**Acid hydrolysis of purified fraction:** 5 mL each fraction from column chromatography was mixed with 2 mL conc. HCl (10 %) and refluxed for 4 h in evaporator. After 4 h, reaction was diluted with 3 mL of water and again evaporated to remove ethanol. The aqueous solution was extracted in chloroform. The chloroform extract after evaporation was neutralized with 10 % NaOH and concentrated under reduced pressure<sup>7</sup>.

**Methylation:** The extract of acid hydrolysis used for methylation. The methanol was added to residue and left it for evaporation till it converted to crystal form.

**Animals:** Wister albino rats of in breed colony weighed 150-250 g procured from germ free animal house of Bharat Traders, Bhopal. These rats were kept under gridded cages in air-controlled room where the congenial temperature of  $25 \pm 5$  °C and 12 h light and dark cycle were maintained. The animals fed on a pallet diet and water *ad libitum*. The animals were kept under standard condition for 7 d with access and food before the

experiment commenced. The experiments were conducted according to the Animal Ethics Committee Guidelines.

#### **Antidiabetic evaluation**

**Using normoglycemic rats:** The normal rats were divided into three groups having 6 in each group. The animals were put to fast for 12 h and allowed access to the water before and throughout the duration of experiment. At the end of the fasting period, zero time (0 h) blood was withdrawn from the Jugular vein<sup>8</sup> and blood sugar level was determined by 0-toluidine method<sup>9</sup>. The animals having blood sugar concentration 110-220 mg % were used.

**Diabetes induced:** Normal rats having sugar level 110-220 mg % after 12 h fast were used. Diabetes was induced by a single intraperitoneal injection of a freshly prepared streptozotocin 55 mg/kg body weight of rats in 0.1 M citrate buffer (pH 4.5)<sup>10,11</sup>. Animal showing glycosuria (indicated by brick red test of urine) after 36 h and hyperglycemic after 48 h after streptozotocin injection considered as diabetic.

**Experimental bioassay:** The animals were divided into group I (normal), group II (diabetic) and group III (diabetic). Group I (normal) received normal saline, group II (diabetic) received *Gymnema sylvestre* extract 250 mg/kg body weight and group III (diabetic) received glibenclamide 5 mg/kg body weight by the oral dose. Blood samples were collected from the jugular vein prior to glucose administration and at 1 and 2 h after glucose loading serum was separated and blood glucose level were measured by glucose oxidase method.

### **RESULTS AND DISCUSSION**

Based on preliminary study, the alcoholic extract of *Gymnema sylvestre* is found to be safe for biological study as no lethal effect was observed at 600 mg/kg orally in mice. Mitra *et al.*<sup>12</sup> found that extract of leaves and stem is non-toxic to human being. Oral administration of alcoholic extract of leaves of *G. sylvestre* showed significant hypoglycemic effect on blood glucose level in normal fasted rats. Effects of alcoholic extract of *Gymnema sylvestre* on glucose tolerance has been shown in Table-1. At 1 h after glucose administration, the blood glucose levels were found to be increased. The blood glucose was measured prior to, 2, 4 and 6 h of administration of extracts.

The test for alcoholic extract of *Gymnema sylvestre* have shown significant hypoglycemic activity in streptozotocin induced rats as compared to reference antidiabetic drug glibenclamide (dose 5 mg/kg)<sup>13</sup>. The results were statistically significant as against glycemic effect in streptozotocin induced rats ( $p < 0.05$ ).

TABLE-1  
EFFECT OF ALCOHOLIC EXTRACT OF *Gymnema sylvestre* (250 mg/kg)  
ON ORAL GLUCOSE TOLERANCE IN RATS

Group	Fasting	2 h	4 h	6 h
I A (Normal) (Glucose loaded)	82.86±3.46	102.40±6.46	94.21±2.48	84.26±1.22
II B (Diabetic) <i>Gymnema sylvestre</i>	186.16±2.23	168.70±1.23	141.32±1.16	122.23±2.89
III C (Diabetic) Glibenclamide	181.10±2.10	153.36±3.60	136.36±1.65	124.34±0.21

Value are given as mean ± SD group of 6 animal each; p < 0.05.

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