

## Antidiabetic and Hypolipidemic Activity of *Gymnema sylvestre* in Alloxan Induced Diabetic Rats

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**Abstract:** The concerned study reveals the experimental investigation of the biological activity of *Gymnema sylvestre* r.br. Ex schult (Family: Asclepiadaceae) used as a traditional antidiabetic and hypolipidemic agent in past and present culture. To study the effect of *Gymnema sylvestre* in both normal and alloxan induced diabetic rats. The aqueous leaf extract of *Gymnema sylvestre* at the dose of 400, 600 and 800 mg kg<sup>-1</sup> body weight was administered orally once a day to the groups for 30 days. The fasting blood glucose, cholesterol, HDL-cholesterol and serum triglyceride content were estimated in both normal and alloxan induced diabetic rats. The fasting blood glucose, cholesterol and serum triglyceride content were found to be significantly reduced ( $p < 0.05$ ) in treated rats whereas the extract also showed the potent elevation in the level of serum HDL-cholesterol. The study reveals that *Gymnema sylvestre* has significant antidiabetic activity and a hypolipidemic activity in alloxan induced and normal fasting rats. The extract seems promising for the development of a phytomedicine for diabetes mellitus.

**Key words:** Antidiabetic • Diabetes mellitus • *Gymnema sylvestre* • Hypolipidemic activity

### INTRODUCTION

Diabetes mellitus is syndrome, initially characterized by a loss of glucose homeostasis resulting from defects in insulin secretion, insulin action both resulting impaired metabolism of glucose and other energy-yielding fuels such as lipids and protein [1]. Diabetes mellitus is a common disorder among the Indian population. It is estimated that diabetes would affect approximately 57 million people by the year 2025 [2]. Therapeutic options for diabetes are diet, exercise, oral hypoglycemic drugs and insulin therapy. Plant drugs are frequently considered to be less toxic and free from side effects than synthetic one [3].

Although, there are numerous traditional medicinal plants reported to have antidiabetic and hypoglycemic properties [4]. A member of the milkweed family (Family: Asclepiadaceae) *Gymnema sylvestre* r.br. Ex schult is a woody plant found in tropical forests of India and Africa has been proven as antidiabetic drug [5]. The medicinally active parts of the plant are the leaves and the roots although the exact mechanism is unknown. Besides impairing the ability to discriminate sweet taste,

increase enzyme activity responsible for the glucose uptake and utilization. It may stimulate pancreatic  $\beta$ -cell function, increase  $\beta$ -cell number and increase insulin release by increasing cell permeability to insulin [6]. Drug interaction occurs from the additive effect when used concomitantly with hypolipidemic agent. Hence in the present study an attempt is made to elucidate the possible antidiabetic and hypolipidemic activity of *Gymnema sylvestre* aqueous leaf extract on both normal and alloxan induced diabetic rats.

### MATERIALS AND METHODS

**Plant Material:** The leaves of *G. sylvestre* were collected from Auraon Research Centre, Botanical garden of National Botanical Research Institute, Lucknow. The plant leaves were air dried under shed at 25°C and the dried leaves were made in to a fine powder with an auto-mix blender. The powder was kept in deep freezer until the time of use.

**Preparation of Aqueous Extract:** One hundred grams of dry fine powder was suspended in 250 ml of water for two

hours and then boiled at 60°C to 65°C for 30 minutes (since boiled decoction of the leaf of this plant has been used as remedy for diabetes). The collected extract was pooled and passed through a fine cotton cloth. The filtrate upon evaporation at 40°C yielded 20 percent semi-solid extract [7].

**Drugs and Chemicals:** Alloxan monohydrate was purchased from BDH Chemicals, Poole, England. All other chemicals used were of analytical grade.

**Experimental Animals:** Male Wistar albino rats of body weight 140 g to 180 g were obtained from Central Animal House, Moti Lal Nehru Medical College, Allahabad University. The animals were maintained at Animal House and fed a standard diet (Hindustan Lever, Mumbai, India) and water ad libitum. The rats used in the present study was maintained in accordance with guidelines of the National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India and the study approved by the ethical committee (Vide No. 88, 2002).

**Drug Administration:** After seven days of alloxan induction, the aqueous leaf extract was administered orally through intragastric tube at the following doses of 400, 600 and 800 mg kg<sup>-1</sup> body weight.

**Experimental Induction of Diabetes:** The rats were injected intraperitoneally with alloxan monohydrate dissolved in sterile normal saline at a dose of 150 mg kg<sup>-1</sup> body weight. Blood samples were collected before the administration of alloxan and after 5 days of alloxan administration. Diabetic state was confirmed when the blood sugar level was above 200 mg/dl. The rats with moderate diabetes and hypolipidemia were used for the experiment.

**Animal Allotment:** After the induction of diabetes the rats were divided in to a five different groups of six rats each.

- Group I: Control rats received normal saline and fed on normal diet.
- Group II: Diabetic control.
- Group III: Diabetic rats received *G. sylvestre* leaf extract (400 mg kg<sup>-1</sup> body weight) daily using an intragastric tube for 30 days.
- Group IV: Diabetic rats given *G. sylvestre* leaf extract (600 mg kg<sup>-1</sup> body weight) daily using an intragastric tube for 30 days.

- Group V: Diabetic rats received *G. sylvestre* leaf extract (800 mg kg<sup>-1</sup> body weight) daily using an intragastric tube for 30 days.

At the end of 0<sup>th</sup>, 10<sup>th</sup>, 20<sup>th</sup> and 30<sup>th</sup> day blood was collected in tubes containing potassium oxalate and sodium fluoride solution for the estimation of glucose and lipid profile.

## BIOCHEMICAL ANALYSIS

**Estimation of Blood Glucose:** Blood glucose was determined by the O-toluidine method [8].

**Estimation of Total Cholesterol (TC):** Total cholesterol level was determined by the commercially available reagent kit (Erba Mannheim, Transansia biomed, Daman, India). It is based on (CHD\_PAP) enzymatic methods

**Estimation of HDL-cholesterol:** HDL-cholesterol level was determined by commercially available reagent kit (Erba Mannheim, Transansia biomed, Daman, India) based on phosphotungstate method.

**Estimation of Triglyceride (TG):** Triglyceride level was estimated by commercially available kit (Erba Mannheim, Transansia biomed, Daman, India). Its working is based on enzymatic colorimetric method. This reagent kit was made for *in vitro* quantitative determination of triglycerides in serum or plasma. Our study was carried out by serum.

**Statistical Analysis:** Data were expressed as mean±SE. Statistical analysis was done using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison. Values were considered statistically significant when at p<0.05.

## RESULTS

The various doses of aqueous extract of *G. sylvestre* leaves were given to the diabetic rats once a day and changes in fasting blood glucose, total cholesterol, HDL-cholesterol and triglyceride were measured on day 10<sup>th</sup>, 20<sup>th</sup> and 30<sup>th</sup> from the day of first dose of experiment. An effective reduction in fasting blood glucose level was observed on above mentioned time. Reduction was examined at all doses of given plant extracts but highest concentration (800 mg kg<sup>-1</sup> body weight) was resulted maximum on all observed days. Although, a drastic

Table 1: Changes in fasting blood glucose levels of control and experimental animals

S. No.	Group	Fasting blood glucose (mg/dl)			
		0 <sup>th</sup> day	10 <sup>th</sup> day	20 <sup>th</sup> day	30 <sup>th</sup> day
1	Normal	85±1.6	87.6±2.4	87.3±2.6	89.3±2.4
2	Diabetic (control)	299±3.4	311±2.3	310.2±2.4	301±2.6
3	A. extract (400 mg kg <sup>-1</sup> )	284±2.3	123.6±2.8	119.2±3.1	110.5±2.3
4	A. extract (600 mg kg <sup>-1</sup> )	291.6±2.1	112.6±2.0	106.2±2.7	100±2.8
5	A. extract (800 mg kg <sup>-1</sup> )	289.6±2.6	98±2.9	95.7±2.8	90±2.5

Results are the means of triplicate determinations on±standard deviation

Table 2: Changes in total cholesterol (TC) levels of control and experimental animals

S. No.	Group	Total cholesterol (mg/dl)			
		0 <sup>th</sup> day	10 <sup>th</sup> day	20 <sup>th</sup> day	30 <sup>th</sup> day
1	Normal	138.6±1.6	138.6±3.2	136±2.5	134±2.9
2	Diabetic (control)	265.3±2.1	265.7±2.8	279.5±2.9	275±3.2
3	A. extract (400 mg kg <sup>-1</sup> )	261.6±3.3	213.5±2.6	166±3.2	151±2.5
4	A. extract (600 mg kg <sup>-1</sup> )	256.3±3.3	210±2.5	157.7±2.8	145.2±3.0
5	A. extract (800 mg kg <sup>-1</sup> )	259±2.9	210±3.5	145.5±4.7	139±2.7

Results are the means of triplicate determinations on±standard deviation

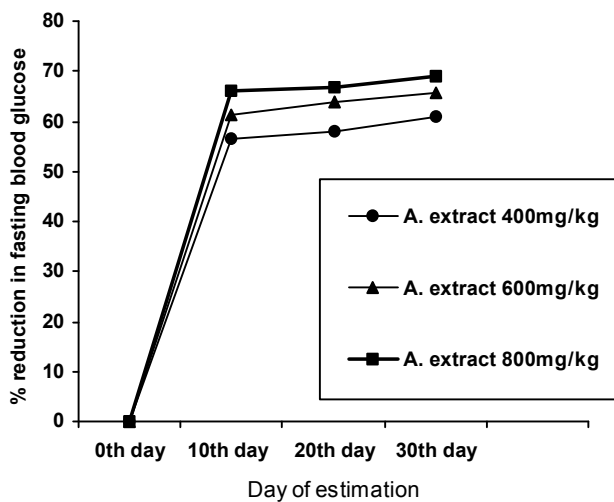


Fig. 1: Percentage reduction in fasting blood glucose level on 0<sup>th</sup>, 10<sup>th</sup>, 20<sup>th</sup> and 30<sup>th</sup> day

reduction of fasting blood glucose was found to be at 400 mg kg<sup>-1</sup> body weight compared with other doses (Table 1). Diabetic control showed negligible change whereas maximum percentage reduction of 61, 66 and 69% were recorded on 30<sup>th</sup> day in group fed aqueous leaf extract at 400, 600 and 800 mg kg<sup>-1</sup> body weight respectively (Fig. 1).

The change in the cholesterol, HDL-cholesterol and triglyceride level was measured and observed on potent reduction in serum cholesterol, triglycerides and effective elevation in HDL-cholesterol level over diabetic control

when the rats fed aqueous leaf extract. The level of serum cholesterol was lower in normal rats that were not treated with alloxan and elevation were found in diabetic control (Table 2) whereas maximum percentage reduction 19, 44 and 46 % were seen on 10<sup>th</sup>, 20<sup>th</sup> and 30<sup>th</sup> day during the course of 800 mg kg<sup>-1</sup> body weight treatment (Fig. 2). In respect to HDL-cholesterol, it showed decrement in normal rats (Table 3) but maximum elevation of 20, 29 and 30% were recorded on 10<sup>th</sup>, 20<sup>th</sup> and 30<sup>th</sup> day due to 800 mg kg<sup>-1</sup> body weight concentration of aqueous leaf extract (Fig. 3). However, similar trends of HDL-cholesterol



Table 3: Changes in HDL-cholesterol content of control and experimental animals

S. No.	Group	Serum HDL (mg/dl)			
		0 <sup>th</sup> day	10 <sup>th</sup> day	20 <sup>th</sup> day	30 <sup>th</sup> day
1	Normal	44.3±1.2	42±2.5	40±2.4	40±2.3
2	Diabetic (control)	49.6±1.8	50.2±3.1	51.7±2.7	54.5±2.2
3	A. extract (400 mg kg <sup>-1</sup> )	51±2.9	44.5±2.8	59.2±2.6	61.5±1.6
4	A. extract (600 mg kg <sup>-1</sup> )	48±2.9	55.2±2.9	61.7±2.7	61.7±2.2
5	A. extract (800 mg kg <sup>-1</sup> )	49.3±2.3	59±2.2	63±2.9	64.2±1.9

Results are the means of triplicate determinations on±standard deviation

Table 4: Changes in serum triglyceride (TG) content of control and experimental animals

S. No.	Group	Serum triglyceride (mg/dl)			
		0 <sup>th</sup> day	10 <sup>th</sup> day	20 <sup>th</sup> day	30 <sup>th</sup> day
1	Normal	80.6±1.6	81.5±2.6	78±2.7	79.7±2.5
2	Diabetic (control)	187±2.6	192.5±2.5	186.7±1.4	179.5±2.5
3	A. extract (400 mg kg <sup>-1</sup> )	191.3±2.3	111.7±2.6	107±3.0	106.5±2.2
4	A. extract (600 mg kg <sup>-1</sup> )	189.6±2.6	115±2.2	101±2.5	101.5±2.3
5	A. extract (800 mg kg <sup>-1</sup> )	186±2.9	110.5±2.6	97.2±2.9	92.5±2.3

Results are the means of triplicate determinations on±standard deviation

elevation were observed at all doses of treatments with given time periods. Rats fed aqueous leaf extract were showed inhibition in serum triglyceride content (Table 4) which was recorded in percentage 44, 47 and 50% on 10<sup>th</sup>, 20<sup>th</sup> and 30<sup>th</sup> day but remarkable reduction of serum triglyceride was noted on feeding at 400 mg kg<sup>-1</sup> body weight extract at all days of observations (Fig. 4).

## DISCUSSION

Alloxan, a  $\beta$ -cytotoxin, induces “chemical diabetes” in a wide variety of animal species by damaging the insulin secreting pancreatic  $\beta$ -cells, resulting in a decrease in endogenous insulin release, which paves the ways for the decreased utilization of glucose by the tissue [9]. In our study, we have observed that *G. sylvestre* decreases fasting blood glucose in alloxan diabetic rats that may be due to increase the activity of enzymes responsible for utilization of glucose by insulin-dependent pathway [10] or regenerate  $\beta$ -cells in pancreatic islets of [5]. Like the plant extract, glibenclamide also produced significant reduction in blood glucose levels of alloxan diabetic rats, the present findings appear to be in consonance with the earlier suggestion of [11].

In this study, the feeding of *G. sylvestre* leaf extract resulted in significantly decreased total cholesterol and serum triglycerides and significantly increased HDL-cholesterol level; these findings are correlated with the [12] experiment. Ingestion of *G. sylvestre* produced a

significant lowering of cholesterol in a hypertension model [13]. Insulin is potent inhibitor of lipolysis, since it inhibits the activity of the hormones sensitive lipases in adipose tissue and suppresses the release of triglycerides [14]. The increase in HDL-cholesterol levels may be beneficial owing to the negative correlation between HDL-cholesterol levels and cardiovascular diseases. This could be due to the presence of other hypolipidemic agents such as  $\beta$ -sitosterol in the aqueous leaf extract [15].

## CONCLUSION

Diabetes mellitus is a well known clinical entity with various late complications like retinopathy, neuropathy, nephropathy etc. *G. sylvestre* has significant antidiabetic as well as hypolipidemic activity so that it can be used as an adjuvant along with allopathic treatment of medicine to treat diabetes as well as to delay the late complications of diabetes. Further study is underway in our laboratory to isolate the active principle and to study the mechanism of its action.

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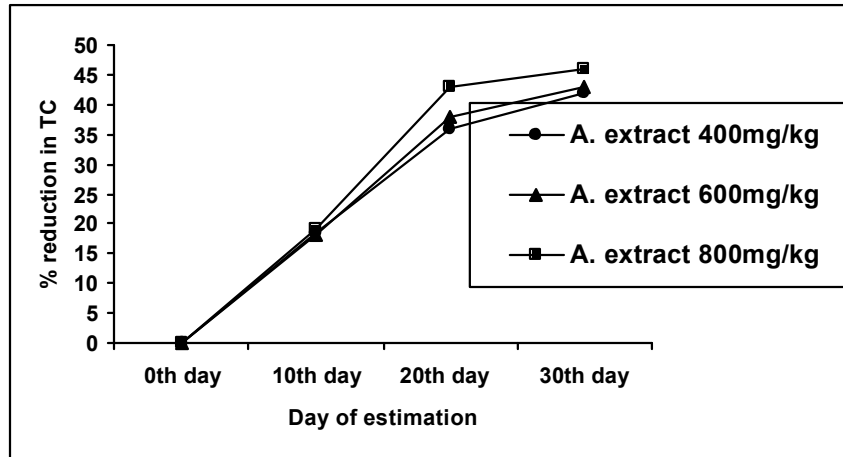


Fig. 2: Percentage reduction in total cholesterol (TC) content on 0<sup>th</sup>, 10<sup>th</sup>, 20<sup>th</sup> and 30<sup>th</sup> day

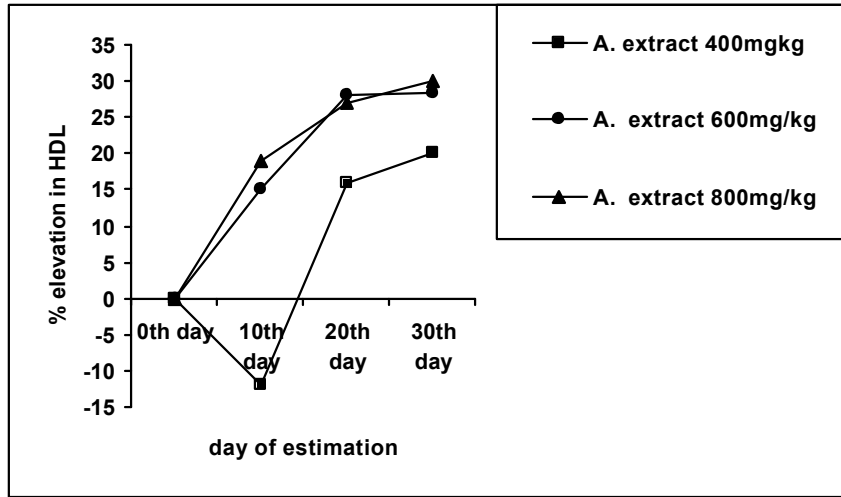


Fig. 3: Percentage elevation in serum HDL-cholesterol on 0<sup>th</sup>, 10<sup>th</sup>, 20<sup>th</sup> and 30<sup>th</sup> day

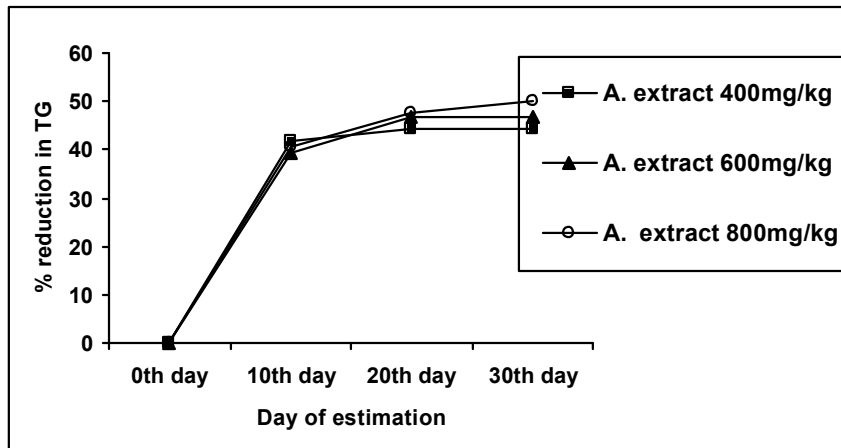


Fig. 4: Percentage reduction in serum triglyceride (TG) on 0<sup>th</sup>, 10<sup>th</sup>, 20<sup>th</sup> and 30<sup>th</sup> day