



Short Communication

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## Effects of *Gymnema lactiferum* leaf on serum glucose and cholesterol levels of streptozotocin induced diabetic rats

Bandara THUSHARI<sup>1</sup>, Rokeya BEGUM<sup>2\*</sup>, Ekanayake SAGARIKA<sup>1</sup> Ali LIAQUA T<sup>2</sup>,  
Jansz R. ERROL<sup>1</sup> and Balasubramaniam KANDIAH<sup>3</sup>

<sup>1</sup>Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka.

<sup>2</sup>Biomedical Research Group, BIRDEM, 122 KN Islam Avenue, Dhaka, Bangladesh.

<sup>3</sup>Biotech (Pvt) Ltd, Jaffna, Sri Lanka.

\* Corresponding author, E-mail: [b\\_rokeya@yahoo.com](mailto:b_rokeya@yahoo.com); Tel.: +880-2-8617130; Fax: 880-2-8611138

### ABSTRACT

*Gymnema lactiferum* var. *lactiferum* is distributed in Assam, Malay Peninsula, Malaysia and Sri Lanka. The leave of this plant is popular as a treatment for diabetes. However, scientific data is not available on this. The present study was designed to determine the effects of *G. lactiferum* leaf on glycaemic and lipidemic status in type 2 diabetic rats. Fasting blood sugar (FBS) levels of the diabetic rats reduced significantly ( $p=0.001$ ) after 28 days of the treatment. A significant decline ( $p=0.015$ ) was also seen in the serum total cholesterol levels. Results indicate that *G. lactiferum* leaf powder possesses hypoglycaemic and hypocholesterolaemic effects on type 2 diabetic rats and therefore it is postulated that it would make a good functional food for type 2 diabetic subjects.

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**Keywords:** *Gymnema lactiferum* var. *lactiferum*, Type 2 diabetic rats, hypoglycaemic, hypocholesterolaemic

### INTRODUCTION

*Gymnema lactiferum* var. *lactiferum* is a green leafy vegetable which is used in salads, curries and herbal gruels. This plant closely resembles *Gymnema sylvestri* and both plants belong to the same family, *Asclapiadaceae*. *G. lactiferum* is distributed in Assam, Malay Peninsula, Malaysia and in Sri Lanka (Dasanayake and Fosberg, 1983). In Sinhala it is popularly known as 'Kuringnan' (The Sri Lankan Forester, 1974; Dasanayake and Fosberg, 1983). However, *G. sylvestri* (Sinhala - Masbedda, Muva kiri- vel or binnuga) is also known as 'Kuringnan'. The plant *G. sylvestri* has been proven of

hypoglycaemic property (Baskaran et al., 1990). Extensive work has been carried out with *G. sylvestri* both on animals and humans. A study on the effects of a water extract of *G. sylvestri* (GS4) on insulin-dependent diabetic patients has shown a significant reduction in blood glucose, glycosylated hemoglobin and glycosylated plasma proteins (Shanmugasundaram et al., 1990). This study has further indicated that the GS4 therapy has been effective in returning the serum lipid profiles to near normal levels in insulin-dependent diabetic patients (Shanmugasundaram et al., 1990). There is a folkloric history among the people in Jaffna, Sri Lanka,

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of using the leaves of the plant *G. lactiferum* as a treatment for diabetes, over a long period of time. The present study was designed to analyze the effects of *G. lactiferum* leaf powder on the serum glucose and cholesterol levels of streptozotocin (STZ)-induced diabetic rats. This is the first scientific animal study which has been carried out on the leaf of *G. lactiferum* to evaluate its therapeutic potential on diabetes.

## MATERIALS AND METHODS

### Preparation of the leaf powder

Plants were identified at the National Herbarium, Sri Lanka. They were grown in Jaffna, Sri Lanka without using insecticides and fungicides and were supplied with organic composts. Mature leaves were dried hygienically in sunlight for 4-5 days. The dried leaves were ground and sieved to prepare the leaf powder.

### Experimental animals and their standard diet

Study was carried out on male Long Evans rats (weight 150-210 g), inbred in the Research division, Bangladesh Institute for the Research and Rehabilitation of Diabetes Endocrine and Metabolic disorders (BIRDEM), Dhaka, Bangladesh. Ethical clearance was obtained from the ethical committee, BIRDEM. Around 8-10 rats were included in each experimental group.

Type 2 diabetes was induced by a single intraperitoneal injection of STZ at a dose of 90 mg/kg B.W. /10 mL 0.1 M citrate buffer (pH 4.5), to 48 h old rat pups (Bonner et al., 1981). Before the experiments (after 3 months of STZ injection), rats were tested to confirm whether they are diabetic. For this purpose their fasting blood glucose levels after 30 min of an oral glucose load (2.5 g/kg B.W.) were determined. Rats with fasting blood sugar value ranging from 7.5-10 mmol/L and value of 15 mmol/L or above after 30 min were used in the study.

Standard rat pellet contained wheat (40%), wheat bran (20%), rice polishings (5%), fish meal (10%), oil cake (10%), gram (3.9%), pulses (3.9%), milk (3.8%), soya bean oil (1.5%), molasses (0.95%) and salt

(0.95%). Embavit GS (vitamin mixture) 250 g was added per 100 kg of rat food.

### Acute effect of *G. lactiferum* leaf on blood glucose levels

Rats were fasted for 12 h with free access to water. Fasting blood samples were taken from the tip of tail. *G. lactiferum* leaf powder (1.25 g/kg B.W.) was administered to diabetic rats orally, 30 min before a glucose load (2.5 g/kg B.W.), using a syringe with a metallic tube that was smooth and curved at the end which led the feed direct to the stomach. The control rats were fed with an equal volume of deionized water. A Glibenclamide group (positive control) was fed with a dose of 5 mg/kg B.W./day. Blood samples drawn after 60 min and 105 min were analyzed for glucose.

### Effects on the chronic consumption of *G. lactiferum* leaf

Diabetic rats were divided into three groups (n=8 in each group) and they were treated as follows.

1. Vehicle group - given water
2. Standard drug treated group - Glibenclamide was administered at a dose of 5 mg/kg B.W./day for four weeks.
3. Test group - treated with *G. lactiferum* leaf powder at a dose of 1.25 g/kg B.W. for four weeks.

Body weights of the rats were measured each week. Blood was drawn on day zero, 7, 14 and 21 by amputation of the tail tip. On the 28<sup>th</sup> day the animals were sacrificed and blood samples were collected, centrifuged at 4000 rpm for 10 min and re-centrifuged at 3000 rpm for 5 min. The level of serum glucose was determined (by GOD-PAP kit) using micro plate reader (Kunst et al., 1984), from the 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> day samples. Total cholesterol (Allain et al., 1974), triglycerides (Tietz, 1990), HDL-cholesterol (Hainline et al., 1980), LDL-cholesterol (Friedewald et al., 1972), glucose (Kunst et al., 1984), insulin (Kratzsch et al., 1990), alanine transaminase (International Federation of Clinical Chemistry, 1980) and creatinine levels (Toro and Ackermann, 1975) were measured from

serum samples of the day zero and last day. Liver glycogen contents of the rats were determined by Anthrone-sulfuric acid method (Vries, 1954) at the end of the study period.

#### Statistical analysis

Results are presented as mean  $\pm$  standard deviation (SD). Groups of data were compared using paired student's t-test and One-way ANOVA test as indicated with the results. Differences were considered significant at  $p < 0.05$ .

### RESULTS AND DISCUSSION

#### Acute experiment

*G. lactiferum* leaf powder did not produce any significant acute effect on blood glucose levels of type 2 diabetic model rats when leaf powder was given 30 min before an oral glucose load ( $p = 0.430$  at 60 min,  $p = 0.117$  at 105 min and  $p = 0.229$  for the increment over basal value as compared by one-way ANOVA test). However, the chronic consumption of the leaf powder brought about many beneficial effects for the type 2 diabetic model rats.

#### Chronic experiments

The changes in the body weights of rats were not remarkably different between different treated groups during the treatment period ( $p = 0.865$ , as compared by one-way ANOVA test). Oral administration of the *G. lactiferum* leaf powder for 28 consecutive days resulted in a gradual reduction in serum fasting glucose levels (15.5% and 37.3% on the day 14 and 28 respectively) and on the day 28 the levels were significantly low ( $p = 0.001$ ) as shown in Table 1. Glycogen metabolism in the liver regulates the blood glucose level. The control of the synthesis and breakdown of glycogen in the liver is central to the regulation of glucose homeostasis. Liver glycogen level was estimated to determine if *G. lactiferum* leaf powder could increase liver glycogen content thereby exerting a hypoglycemic action. In the present study there was no significant effect ( $p = 1.000$ ) on glycogen deposition in the liver.

Unlike *G. sylvestre*, *G. lactiferum* did not affect the serum insulin levels as well.

Fasting serum insulin content did not change in *Gymnema* treated group ( $p = 0.893$ ) whereas a 26.3% increase was observed in glibenclamide treated group ( $p = 0.612$ ).

Therefore, it is evident that the hypoglycaemic effect of *G. lactiferum* is brought about by some extra pancreatic factors, which are not related to the level of serum insulin. However, any factor which can either trigger insulin activity or which can overcome insulin resistance or which can inhibit the intestinal glucose absorption may be contributory to the hypoglycaemic effect.

Dyslipidaemia is an important risk factor for atherosclerotic complications of diabetes (Laakso and Lehto, 1997). Hypercholesterolaemia and hypertriglyceridaemia have been reported to occur in STZ diabetic rats (Sharma et al., 1997). Therefore, it was considered that estimating serum cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol levels was worthwhile. Serum total cholesterol levels of the *Gymnema* treated group reduced significantly ( $p = 0.015$ ) with 9.01% reduction as shown in Table 2. On the other hand HDL-cholesterol level increased by 39.42% ( $p = 0.165$ ). Serum TG level and LDL-cholesterol level reduced by 23.5% and 12.3% respectively in the *Gymnema* treated group. However, those reductions were not significant ( $p = 0.465$  and  $p = 0.189$  respectively) as shown in Table 3. The observed hypocholesterolaemic effect may probably be due to the indirect effect of blood glucose lowering or due to any other active principle which can inhibit the cholesterol synthesis or increase cholesterol or bile salt excretion which is yet to be confirmed.

Chronic consumption of the leaf powder did not indicate any adverse effects on liver and kidneys in the type 2 diabetic rats as determined by their serum ALT and creatinine levels. Serum creatinine levels remained unchanged throughout the study period among the different groups whereas serum ALT levels reduced significantly in glibenclamide group ( $p = 0.014$ ) while the values of other groups remained steady (Table 4).

**Table 1:** Effect of *G. lactiferum* leaf powder on glycaemic status of type 2 diabetic rats.

Group	Fasting serum glucose levels (mmol/L), Mean $\pm$ SD				
	Day 0	Day 7	Day 14	Day 21	Day 28
Water (n=6)	9.03 $\pm$ 0.68	9.13 $\pm$ 0.90	8.54 $\pm$ 1.63	7.81 $\pm$ 1.99	7.79 $\pm$ 1.82
Glibenclamide(n=6)	9.50 $\pm$ 1.15	9.82 $\pm$ 1.52	10.30 $\pm$ 1.15	10.33 $\pm$ 4.07	7.19 $\pm$ 1.50
<i>G. lactiferum</i> (n=6)	8.95 $\pm$ 1.36	10.18 $\pm$ 4.23	7.76 $\pm$ 3.54	8.75 $\pm$ 0.86	5.56* $\pm$ 0.72

\*p = 0.001 in comparison to day 0 (as determined by the paired student's t-test)

**Table 2:** Effect of *G. lactiferum* leaf powder on serum total cholesterol and HDL levels.

Group	Serum levels (mg/dL), Mean $\pm$ SD			
	Total Cholesterol		HDL	
	Day 0	Day 28	Day 0	Day 28
Water (n=6)	67.00 $\pm$ 3.58	69.67 $\pm$ 13.77	29.33 $\pm$ 3.72	34.83 $\pm$ 8.70
Glibenclamide (n=6)	70.83 $\pm$ 10.96	66.00 $\pm$ 9.90	31.50 $\pm$ 6.95	32.33 $\pm$ 7.15
<i>G. lactiferum</i> (n=6)	76.00 $\pm$ 7.18	69.00* $\pm$ 6.07	27.67 $\pm$ 4.67	36.67 $\pm$ 10.13

\*p = 0.015 in comparison to day 0 (as determined by the paired student's t-test)

**Table 3:** Effect of *G. lactiferum* leaf powder on serum LDL and TG levels.

Group	Serum levels (mg/dL), Mean $\pm$ SD			
	LDL		TG	
	Day 0	Day 28	Day 0	Day 28
Water (n=6)	19.37 $\pm$ 7.76	19.93 $\pm$ 7.95	91.50 $\pm$ 20.03	74.67 $\pm$ 22.32
Glibenclamide (n=6)	20.23 $\pm$ 4.77	20.80 $\pm$ 6.90	95.50 $\pm$ 26.22	64.33* $\pm$ 14.94
<i>G. lactiferum</i> (n=6)	28.17 $\pm$ 12.91	14.90 $\pm$ 11.06	100.83 $\pm$ 21.78	87.00 $\pm$ 43.29

\*p = 0.018 in comparison to day 0 (as determined by the paired student's t-test)

**Table 4:** Effect of *G. lactiferum* leaf powder on liver and kidney function.

Group	ALT (U/I), Mean $\pm$ SD		Creatinine (mg/dL), Mean $\pm$ SD	
	Day 0	Day 28	Day 0	Day 28
Water (n=6)	90.17 $\pm$ 19.29	95.17 $\pm$ 13.72	0.64 $\pm$ 0.06	0.65 $\pm$ 0.07
Glibenclamide (n=6)	94.33 $\pm$ 11.13	80.50* $\pm$ 13.64	0.64 $\pm$ 0.06	0.67 $\pm$ 0.06
<i>G. lactiferum</i> (n=6)	97.83 $\pm$ 15.82	101.00 $\pm$ 21.93	0.62 $\pm$ 0.07	0.67 $\pm$ 0.08

\*p = 0.014 in comparison to day 0 (as determined by the paired student's t-test)

From the data obtained it could be concluded that *G. lactiferum* leaf powder possesses hypoglycaemic and hypocholesterolaemic effects on type 2 diabetic rats and it is postulated that it would make a good functional food for type 2 diabetic subjects. These findings have been confirmed by a

subsequent study carried out on type 2 diabetic patients (Bandara et al., 2009).

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