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Original Article

Antihyperglycemic and Glucose Homeostasis Effect of Ethanol and DCM Extract of Gymnema Sylvestre on Type 1 Diabetic Model Rat

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Abstract

Bacground: *Plants have formed the basis for the treatment of diseases in traditional medicine systems for thousands of years and continue to play a major role in the primary health care of about 80% of the world inhabitants. Gymnema sylvestre is one of the most studied herbs which has been claimed to be active against both type of diabetes mellitus.* ***Objective:*** *The dried powder leaves of Gymnema extracted with ethanol and dichloromethane (DCM) and then its effect were studied in streptozotocin induced type 1 diabetic model rat at different prandial state.* ***Materials and Methods:*** *In the present study both of the extracts (GS EthOH and GS DCM) of Gymnema sylvestre produced a significant antihyperglycemic effect in type 1 diabetic model rats when the extract was given 30 minutes before glucose load. The effectiveness of the extracts in type 1 diabetic rats with residual insulin secretion indicates that the hypoglycemic effect of active plant compound(s) is probably mediated either by improving insulin secretion from the existing β cells or by increasing its sensitivity.* ***Results:*** *The baseline value of fasting serum glucose reflects that the degree of damage of β cells by the toxic effect of streptozotocin was gradual. The findings at one week show some spontaneous recovery in water control group and better recovery in all the treated groups. After two weeks, fasting serum glucose level was improved in all the extract treated groups.* ***Conclusion:*** *The data suggest that Gymnema sylvestre leaf extracts improve the glycemic status in type 1 diabetic model rat.*

Key words*: Gymnema sylvestre; Type 1 diabetes; Streptozotocin; Antihyperglycemic*

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Introduction

Plants have formed the basis for the treatment of diseases in traditional medicinal systems for thousands of years, and continue to play a major role in the primary health care of about 80% of the world's inhabitants.[1] Traditional antidiabetic plants might provide a useful source of new oral hypoglycemic compounds for development as pharmaceutical entities or as simple dietary adjuncts to existing therapies.[2] Many of the currently available drugs have been derived directly or indirectly from plants. However, only a few have, till now, been subjected to scientific and systematic medicinal studies to assess their efficiency. Various

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plant extracts have been found to be active both in type 1 and type 2 which may potentially be the pharmacological target of modern antidiabetic drug research.

Gymnema sylvestre a plant used in the Ayurvedic medicine of India for the treatment of diabetes mellitus for over 2000 years by Ayurvedic and Unani practitioners.[3] It is one of the most studied herbs which has been claimed to be active against both type of diabetes mellitus.[4] The plant belongs to the family Asclepiadaceae and grows in open woods in India, China, Indonesia, Japan, Malaysia, Sri Lanka, Vietnam and South Africa.

Gymnema sylvestre possesses tremendous importance as a medicinal plant. The hot water extract of *Gymnema sylvestre* leaves is usually taken orally for the treatment of diabetes.[5,6] Hypoglycemic effects of water and methanol extracts of *Gymnema sylvestre* leaves have been reported in diabetic rats and humans.[7,8] A pure compound conduritol also isolated from methanol extract.[9] It has been claimed that, oral administration of water soluble acidic fraction of ethanol extract normalized blood sugar level in Streptozotocin treated rat by secretion of endogenous insulin, possibly due to regeneration of beta cell.[10] The present work was undertaken to study the glucose-lowering effects of the ethanol extract and DCM extracts in streptozotocin-induced types 1 diabetic rat at different prandial states.

Materials and Methods

Collection & identification of Gymnema sylvestre leaves

Dried *Gymnema sylvestre* leaves (5 kg) were purchased from a local herbal medicinal shop of old Dhaka town. Botanical identification was performed and the accession no is 30381 by National Herbarium, Bangladesh. The leaves were cleaned, separated from petiole and stems and dried in an oven at 40 ^{0}C. Finally they were grounded to powder and stored in a well-stopper plastic container.

Extraction and fractionation procedure

Gymnema sylvestre leaves powders were extracted with 80% ethanol in the extraction tank for about 4 days at room temperature by changing ethanol every alternate day. The extracts were filtered and evaporated by rotary evaporator and finally freeze-dried to obtain a 210 g extract. The dry samples were stored in reagent bottles at 4^{o}C in a freezer.

The ethanol extract (100 g) was partitioned between Dichloromethane and water. DCM part was separated and evaporated to dryness to get DCM soluble material, which was kept, separately for chemical analysis. The residual aqueous part was condensed by rotary evaporator and finally freeze-dried (60 g).

Animal preparation

Adult male Long-Evans rats weighing 180−220 gm were used throughout the study. The animals were bred at BIRDEM (Bangladesh) and maintained on 12 hour light-dark cycle, fed on a standard laboratory pellet diet and with water supplied *ad libitum*. Animal described as fasted were deprived of food for at least 12 hr but allowed free access to drinking water.

Induction of Type 1 diabetes in rats

Type 1 diabetes was induced by intraperitoneal injection of streptozotocin (stz, 65 mg/kg body weight) dissolved in citrate buffer (pH 4.5) immediately before use to 3 month old, healthy adult rats fasted for 18 hours. The blood glucose level was checked on the 5th day after injection of STZ. Animals having high blood glucose levels (>20 mM) were considered as type 1 diabetic.

Administration of extracts and glucose loading

Rats were kept fasted for 12 h before use. Solutions or suspensions of plant extracts (1.25 g/kg body weight in 10 ml of water) were administered orally by gastric intubations. For postprandial condition the extracts were fed 30 minutes before glucose (2.5 g/kg body weight in 10 ml of water) load following the standard procedure developed in BIRDEM laboratory. The corresponding negative control rats were fed orally with deionized water (10 mL) and positive control rats were given injection Insulin (Actrapid HM-40 U: 1mL).

Experiment on glucose homeostasis

For chronic study, type 1 diabetic rats were fed plant

extracts (1.25 g/kg bw) by a metallic tube once daily for 14 days. Control rats were administered water (10 ml/kg bw). The extract treated rats were divided into 3 subgroups depending on the administration of the extracts. GS-0 group received extracts immediately after injection of streptozotocin. The GS-3 group and GS-5 group were fed with the extracts after 3 days and 5 days of giving streptozotocin injection. All the groups remained under similar environmental conditions, and were provided with enough food and water throughout the experiment. Body weight of each rat was recorded every 7th day. Blood samples were collected at the beginning of the experiment from the tail tips under mild ether anaesthesia and at the end directly from heart/abdominal aorta under pentobarbital anaesthesia. Serum was separated by centrifugation for the analysis of glucose and insulin. Serum was preserved immediately at –70 ^{0}C and stored until analyzed.

Blood collection from the experimental rats and biochemical analysis

Blood samples were collected by cutting tail tip under mild ether anesthesia at 0 min, 60 min and 105 min. After collecting blood at 0 min, extracts, water and insulin were given to rats; the experiment was followed by glucose load 30 min after feeding extracts. The glucose level was measured immediately by the glucose-oxidase method using ACCUTREND GC blood glucose analyzer from Boehringer Mannheim GmbH (Germany).

Statistical analysis

Results are expressed as mean ± SD or median (range) as appropriate. Between groups, comparison of data was done by using One-way ANOVA with Post hoc Bonferroni test. The level of significance was set at 0.05. Data were managed using the computer software *Statistical Package for Social Sciences (SPSS*) *for Windows,* Version 10.0.

Results

Acute effect of G sylvestre on serum glucose level

Ethanol extract of *G sylvestrae* (GS-EthOH) was fed 30 min before oral glucose load. Statistical analysis indicated that oral administration of the ethanol extract of *G sylvestrae* (GS-EthOH) significantly opposed the rise of serum glucose at 60 min (p=0.033) compared to water control (WC) but it did not bring significant effect on the group, which was fed DCM extract (GS-DCM) in compared to WC at 60 min (p=0.06). At 105 min, serum glucose level of the WC and GS-EthOH groups was comparable. Compared to WC, GS-DCM extract significantly opposed the rise of serum glucose at 105 min (p=0.01) (Table I).

Table I: Effect of 80% ethanol extract and aqueous part of DCM extract of *on* serum glucose level of Type 1 diabetic model rats when the extract was fed 30 minutes before glucose load

Groups	Min 0 (mmol/L)	Min 60 (mmol/L)	Min 105 (mmol/L)
WC (n=10)	22.2 ± 4.2	31.3 ± 3.0	28.4 ± 4.3
IC (n = 12)	16.2 ± 3.9	3.9 ± 0.37	7.5 ± 1.7
GS_{eth} (n=11)	18.8 ± 5.1	23.1 ± 3.2	24.4 ± 3.3
GS_{dcm} (n=9)	20.3 ± 2.7	26.0 ± 3.4	21.7 ± 4.8
		Bonferroni p values	
WC vs IC	0.198	**<0.001**	**<0.001**
WC vs GS_{eth}	0.375	**0.033**	0.684
WC vs GS_{dcm}	2.871	0.06	**0.010**
IC vs GS_{eth}	1.809	**<0.001**	**<0.001**
IC vs GS_{dcm}	**0.045**	**<0.001**	**<0.001**
GS_{eth} vs GS_{dcm}	0.168	0.726	1.206

Results are expressed as Mean ± SD; WC, Water Control; IC, Insulin Control; GS, *Gymnema sylvestre*; eth, Ethanol; dcm Dichloromethane. One way ANOVA with post hoc Bonferroni test was performed as the test of significance.

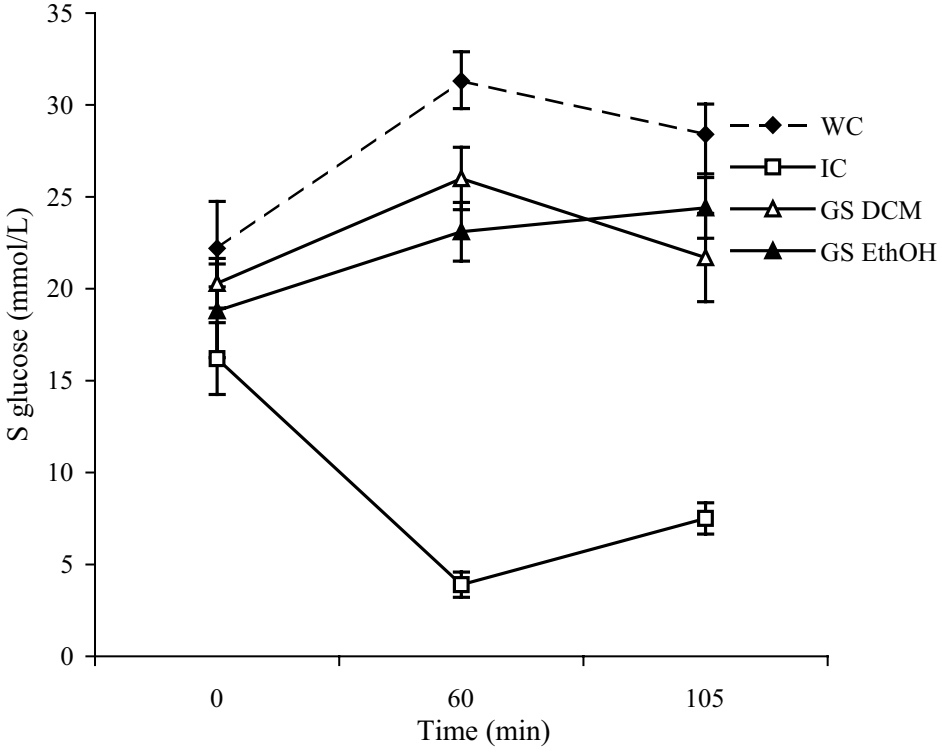

Fig 1. Effect of Gymnema sylvestre (GS) ethanol (EthOH) and DCM extracts on serum glucose level when fed 30 min after glucose load. DCM, Dichlormethane; WC, water control; IC, insulin control

Table II: Effect on fasting serum glucose of the study rats after 14 days

Groups	**S Glucose** Baseline (mmol/L)	**S Glucose** 1 wk (mmol/L)	**S Glucose** 2 wk (mmol/L)	t/p value		
				Baseline vs 1 wk	Baseline vs 2 wks	1 wk vs 2 wks
WC (n = 17)	22.2 ± 4.1	15.5 ± 4.9	17.6 ± 5.1	3.36/**0.02**	0.33/0.75	1.58/0.13
GS-0 (n=9)	8.5 ± 3.1	14.1 ± 4.7	11.0 ± 4.3	1.35/0.35	1.02/0.25	0.25/0.80
GS-3 (n=7)	16.7 ± 6.4	6.4 ± 1.4	8.4 ± 4.1	2.10/0.17	2.81/**0.04**	1.95/0.10
GS-5 (n=10)	20.9 ± 3.5	11.4 ± 6.7	10.4 ± 5.6	1.77/0.15	1.33/0.27	0.04/0.96

	Bonferroni p values		
WC vs GS-0	**0.003**	1.506	**0.012**
WC vs GS-3	0.055	**0.003**	**0.006**
WC vs GS-5	0.483	0.348	**0.033**
GS-0 vs GS-3	**0.032**	**0.003**	0.567
GS-0 vs GS-5	**0.012**	0.972	2.364
GS-3 vs GS-5	0.124	0.144	1.212

Results are expressed as Mean + SD; WC, Water control; GS, Gymnema sylvestre; GS-0, Extract feeding started on 0 day of STZ injection; GS-3, Extract feeding started on 3 day of STZ injection; GS-5, Extract feeding started on 5 day of STZ injection; Between group comparison was done using One way ANOVA with post hoc Bonferroni test. Within group comparison was done using paired t test.

Chronic effect of G sylvestre on glucose homeostasis

Results of fasting serum glucose (FSG) level of the study rats at baseline (before onset of feeding), at 1 week and at 2 week after feeding is shown in Table II.

At baseline, the mean fasting serum glucose (FSG) (mmol/L) of the GS-O group was significantly lower than the WC group (p=0.003). The level of FSG (mmol/L) at baseline of the GS-3 & GS-5 group was lower than the WC group but was not statistically significant.

At 1 week, the value of FSG (mmol/L) level of the GS-3 group was significantly lower than the WC group (p=0.003). The mean FSG (mmol/L) at 1 week of the group GS-3 was significantly lower than the GS-0 group (p=0.003). Compared to WC, the level of FSG at 1 week of the GS-0 and GS-5 groups were not significantly different.

At 2 week, the level of fasting serum glucose level of the group GS-0, GS-3 and GS-5 were significantly lower (p=0.012, p=0.006 and p=0.033 respectively) compared to WC.

Discussion

Diabetes is a serious disorder that has a significant impact on health and life expectancy of the patient. The cost of treating this disease already put a heavy burden on the health care system. Among the non-communicable diseases diabetes now considered as an emerging epidemic in Bangladesh.[11] People living with diabetes are especially facing an increasing demand for long term continuous care of health service and burden of health care cost. Currently available drug regimens for management of diabetes have certain drawback. There is need for safer and more effective antidiabetic drugs. The present study was undertaken to assess hypoglycemic properties and to investigate the underlying mechanism of action of *G sylvetre* leaf extracts in type 1 diabetic model rats.

In the present study both of the extracts (GS EthOH and GS DCM) of *G sylvetre* produced a significant antihyperglycemic effect in type 1 diabetic model rats when the extract was given 30 minutes before glucose load. The effectiveness of the extracts in type 1 diabetic rats with residual insulin secretion indicates that the hypoglycemic effect of active plant compound(s) is probably mediated either by improving insulin *secretion* from the existing β cells or by increasing its *sensitivity*. The antihyperglycemic properties of *G sylvetre* was supported by the results of others.[7,8,12,13]

In the chronic study, the baseline values of fasting serum glucose (at 0, 3 and 5 days of giving streptozotocin) reflects that the degree of damage of β cells by the toxic effect of streptozotocin was gradual. The findings at one week show some spontaneous recovery in water control group and better recovery in all the treated groups. After 2 weeks, fasting serum glucose in the water control group deteriorated whereas it was improved in all the *extract treated* groups.

The data suggest that *Gymnema sylvestre* leaf extracts improve the glycemic status in type 1 diabetic model rat.

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