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Gymnema sylvestre R. Br. suspension cell extract show antidiabetic potential in Alloxan induced diabetic albino male rats

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ABSTRACT

Objective: To study the antidiabetic effects of suspension cell extract of *Gymnema sylvestre* (*G. sylvestre*) along with field grown and wild plants. **Methods:** The effect of ethanolic extracts of the *in vitro* grown suspension cells of *G. sylvestre* along with field grown and wild plant leaves of *G. sylvestre* was tested on alloxan induced diabetic rats. **Results:** While oral administration of the extracts reduced the glucose content in blood and urine, sugar and lipids in serum significantly ($P \leqslant 0.05$), it also increased the body weight, total haemoglobin and plasma protein content. **Conclusions:** It can be concluded that *G. sylvestre* suspension cell extract show excellent antidiabetic potential against alloxan induced diabetic albino male rats therefore be considered as potent antidiabetic drug.

1. Introduction

Indiscriminate collection of *Gymnema sylvestre* (*G. sylvestre*) (Asclepiadaceae), a potent antidiabetic plant used in folk, ayurvedic, homeopathic systems and even in modern medicine, from wild has been classified as endangered [1-5]. The pharmacological properties of *G. sylvestre* are attributed to a group of triterpene saponins, known as gymnemic acids (I - XVIII and gymnemosaponins I - V). Reports indicate that oral administration of *G. sylvestre* to exert diverse range of effects[6] targeting several of the etiological factors connected to diabetes, including chronic inflammation [7], obesity [8, 9], enzymatic defects, and pancreatic β-cell function [10]. The capacity for plant cell cultures to produce and accumulate many of the same valuable chemical compounds as the parent plant in nature has been recognized almost since the inception of in vitro technology and plant cell cultures have been used as an attractive source for production of bioactive compounds[11]. *G. sylvestre* cell suspension culture established in the laboratory was administered to alloxan induced diabetic albino rats to study the antidiabetic effects of suspension cell extract of *G. sylvestre* along with field grown and wild plants.

2. Materials and methods

2.1. Plant and cell materials

G. sylvestre plants were collected from Muniyankudisai, Tamilnadu, India, authenticated using standard manuals [12, 13] and established in the field garden at Shri AMM Murugappa Chettiar Research Center (MCRC). An extensive, spherical, yellow aggregate cell suspension with chlorophyll accumulation (GSCIK2) obtained in MS medium supplemented 56.8 μM/L Indole acetic acid 4.6 μM/L Kinetin was established and maintained in 250 mL glass Erlenmeyer flasks and incubated in orbital shaker (95 rpm) and 16/8 photoperiod at (22±1) ℃.

2.2. Extract preparation

Shade dried wild and field grown *G. sylvestre* leaf samples and oven dried (37 ℃ for three days) suspension cells were extracted with 70% EtOH and the aliquots were concentrated to dryness in a rotary evaporator at a temperature of 40 ℃ under reduced pressure. The dark green colour extract from field grown and wild plant leaves (yield = 14.74% w/w), and pale yellow colour extract from suspension cells (yield =

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1.24 % w/w) were dissolved in normal saline and stored in refrigerator.

2.3. Experimental animals

Male albino Wistar rats with body weights of 200 to 240 g were obtained from Tamil Nadu Veterinary and Animal Sciences University, Chennai, India. Animals were housed in polypropylene cages in controlled room temperature (24±2℃), with (50±10)% humidity and an automatically controlled cycle of 12/12 hours light and dark. The animals were fed with standard pellet diet (Hindustan lever limited, Bangalore, India) and water *ad libitum*. The animals used for the study were approved by the Institutional Animal Ethical Committee (IAEC No. 03/017/09).

2.4. Experimental induction of diabetes

Rats were injected intraperitoneally with a freshly prepared solution of *Alloxan monohydrate* in normal saline at a dose of 40 mg/kg of body weight [14]. Because Alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 20% glucose solution (5 to 10 mL) orally after 6 h. The rats were then kept for the next 24 h on 5% glucose solution bottles in their cages to prevent hypoglycemia. After 2 weeks, rats with moderate diabetes having glycosuria and hyperglycemia (i.e., with blood glucose levels of above 200 mg/dL) were chosen for the experiment.

2.5. Experimental procedure

A total of 42 rats (36 diabetic surviving rats and 6 normal rats) were divided into seven groups, with each group comprising six rats respectively; Group 1: Normal rats + saline (Control- NC), Group 2: Diabetic control rats (Alloxan induced - DC), Group 3: Diabetic rats + *G. sylvestre* cell extract (50 mg/kg body weight - GCE1), Group 4: Diabetic rats + *G. sylvestre* cell extract (250 mg/kg of body weight - GCE2), Group 5: Diabetic rats + *G. sylvestre* cell extract (500 mg/kg of body weight - GCE3), Group 6: Diabetic rats + Field *G. sylvestre* extract (500 mg/kg of body weight - FGE), Group 7: Diabetic rats + Wild *G. sylvestre* extract (500 mg/kg of body weight - WGE). During the study period, the animals were deprived of food overnight. Blood was collected from tail vein in two separate tubes. One tube containing potassium oxalate and sodium fluoride was used for the estimation of glucose. The other tube containing the blood was allowed to clot at room temperature and the serum obtained after centrifugation was used for estimation of lipids. Apart from this fasting blood glucose [15], plasma protein [16] total haemoglobin[17], serum cholesterol[18], serum phospholipids[19], free fatty acids in serum[20] were also estimated.

2.6. Statistical analysis

All the data were statistically evaluated and the significance of various treatments was calculated using DMRT ($P \leqslant 0.05$). All the results were expressed as mean± S.D. of six replications.

3. Results

Much of the research on *G. sylvestre* looked at the hypoglycemic effects of the plant extracts have demonstrated that *G. sylvestre* may exert its antidiabetic effect through a number of pathways [6]. Present study describes the effect of suspension cells of *G. sylvestre*, field grown and wild grown plant extract in alloxan induced diabetes albino rats. An increase in the fasting blood glucose and urine sugar levels of alloxan treated rats observed was attributed to insulin deficiency that destructs pancreatic beta cells leading to hyperglycaemia [14]. A significant decrease in body weight, total haemoglobin content and plasma protein were also observed. Reduction in weight was attributed to increased catabolic reactions leading to muscle wasting [21], dehydration and catabolism of fats and proteins [22], and excessive break down of tissue proteins [23].

Oral feeding of alcoholic extracts of suspension cells (GCE1, GCE2 and GCE3) as well as field and wild grown plant leaves (FGE, WGE) for 21 days was found to reduce hyperglycemia, urine sugar, serum cholesterol, phospholipids and free fatty acids and increase body weight, plasma protein, total haemoglobin in alloxan induced rats as compared to untreated diabetic rats. While the results of the field and wild grown plant leaf extracts were comparable to the suspension cell extract with highest concentration (500 mg/kg), the impact of suspension cell extracts was found to be dose dependent (Table 1, Figure 1 and 2). Administration of the extracts was found to reverse the blood glucose considerably. The same trend was observed in urine sugar levels also (Table 1). The ability of alcoholic extracts to

Table 1
Effect of ethanolic extracts of *G. sylvestre* suspension cells and leaves on blood glucose, body weight and urine sugar in Alloxan induced diabetic rats observed for 21 days.

Groups	Blood glucose (mg/dL)				Change in the body weight (g)	Urinesugar (%)
	Initial	Day 7	Day 14	Day 21		
NC	97.0±2.5	$95.3\pm3.9^{\#a}$	$96.5\pm2.6^{\#a}$	$96.0\pm2.0^{\#a}$	$13.3\pm1.6^{\#a}$	Nil
DC	221.1±3.2	$228.0\pm3.0^{d\#b}$	$245.0\pm6.5^{d\#b}$	$265.0\pm4.0^{d\#b}$	$-53.5\pm0.5^{f\#b}$	++
GCE1	213.6±5.9	199.0 ± 7.4^{c}	142.5 ± 10.1^{bc}	132.0 ± 3.2^{c}	-30.8 ± 0.6^{e}	+
GCE2	221.8±5.6	199.6 ± 6.8^{c}	146.6 ± 4.5^{c}	119.0 ± 4.2^{b}	-23.4 ± 1.3^{d}	+
GCE3	224.0±4.6	191.5 ± 8.8^{b}	144.8 ± 9.6^{c}	118.8 ± 6.7^{b}	-18.2 ± 0.6^{c}	Nil
FGE	212.6±5.3	180.0 ± 4.7^{a}	133.5 ± 12.2^{ab}	100.0 ± 9.6^{a}	-15.3 ± 0.4^{b}	Nil
WGE	214.8±6.4	175.0 ± 5.3^{a}	129.0 ± 9.5^{a}	100.6 ± 10.0^{a}	-14.0 ± 0.5^{a}	Nil

Samples were collected after 12 hours of fasting. Values are given as mean ± S.D. for six rats in each group. Values not sharing a common superscript letter differ significantly at $P \leqslant 0.05$ (DMRT). # indicates statistical comparison of diabetic control with normal, $ - 150 mg/kg body weight; ++ : ⩽ 2%; + : ⩾2%.

protect body weight loss also seems to be as a result of its ability to reduce hyperglycaemia[9]. The possible mechanism by which the extracts brings about the hypoglycaemic action may be by potentiating of the insulin effect of plasma by increasing either the pancreatic secretion of insulin from β −cells or its release from the bound form [24–26]. The same insulin metabolism could be related to the reduction and increase in the plasma protein content and haemoglobin in the alloxan induced diabetic rats and diabetic rats administered with the ethanolic extracts (Figure 1).

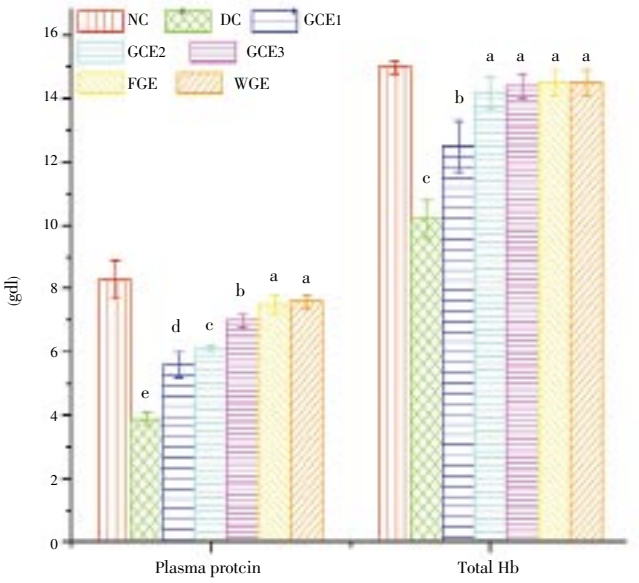

Figure 2. Effect ethanolic extracts of *G. sylvestre* suspension cells and plant leaves on plasma protein and total haemoglobin in alloxan induced diabetes in rats.
Results were observed after 21 days of treatment.Values are given as mean ± S.D. for six rats in each group. Values not sharing a common superscript letter differ significantly at $P \leq 0.05$ (DMRT). Experimental groups were compared with diabetic control.

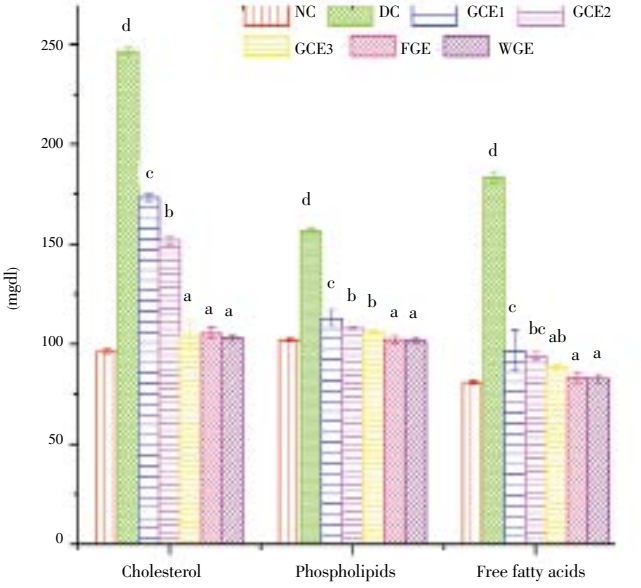

Figure 2. Effect ethanolic extracts of *G. sylvestre* suspension cells and plant leaves on serum lipids in alloxan induced diabetes in rats.
Results were observed after 21 days of treatment.Values are given as mean ± S.D. for six rats in each group. Values not sharing a common superscript letter differ significantly at $P \leq 0.05$ (DMRT). Experimental groups were compared with diabetic control.

The level of serum cholesterol, phospholipids and free fatty acids was high in diabetic rats as compared to normal rats (Figure 2). Oral administration of alcoholic extracts lowered the values significantly as compared to untreated diabetic rats. It was reported that there is a significant alteration in the fatty acid composition of serum in experimental diabetic rats [27, 28] and good glycemic control and elevated levels of HDL cholesterol and decreased levels of triglycerides are reported to correlat with the phospholipid levels [29]. The restoration of phospholipids by plant extracts was attributed to controlled mobilization of triglycerides and improved insulin secretion and action [19,25,30]. Regression of the diabetic state with an increase in the utilization of glucose, thereby depressing the mobilization of fat, on administration of ethanolic extracts observed in this study was in consonance with the above findings.

4. Discussion

Evaluation of antidiabetic activity of *G. sylvestre* looked at the hypoglycemic effects of the plant extracts have demonstrated that *G. sylvestre* may exert its antidiabetic effect through a number of pathways [6]. Present study describes the effect of suspension cell, field grown and wild grown plant extract in alloxan induced diabetes albino rats. An increase in the fasting blood glucose and urine sugar levels of alloxan treated rats observed was attributed to insulin deficiency that destructs pancreatic beta cells leading to hyperglycaemia[14]. A significant decrease in body weight, total haemoglobin content and plasma protein were also observed. Reduction in weight was attributed to increased catabolic reactions leading to muscle wasting [21], dehydration and catabolism of fats and proteins[22], and excessive break down of tissue proteins[23]. Oral feeding of alcoholic extracts of suspension cells (GCE1, GCE2 and GCE3) as well as field and wild grown plant leaves (FGE, WGE) for 21 days was found to reduce hyperglycemia, urine sugar, serum cholesterol, phospholipids and free fatty acids and increase body weight, plasma protein, total haemoglobin in alloxan induced rats as compared to untreated diabetic rats. While the results of the field and wild grown plant leaf extracts were comparable to the suspension cell extract with highest concentration (500 mg/kg), the impact of suspension cell extracts was found to be dose dependent The possible mechanism by which the extracts brings about the hypoglycaemic action may be by potentiating of the insulin effect of plasma by increasing either the pancreatic secretion of insulin from β −cells or its release from the bound form[24–26]. The same insulin metabolism could be related to the reduction and increase in the plasma protein content and haemoglobin in the alloxan induced diabetic rats and diabetic rats administered with the ethanolic extracts Oral administration of alcoholic extracts lowered the values significantly as compared to untreated diabetic rats. It was reported that there is a significant alteration in the fatty acid composition of serum in experimental diabetic rats [27, 28] and good glycemic control and elevated levels of HDL cholesterol and decreased levels of triglycerides are reported to correlat with the phospholipid levels [29]. The restoration of phospholipids by plant extracts was attributed to controlled mobilization of triglycerides and improved insulin

secretion and action [19,25,30]. In conclusion, the results of this study show that regression of the diabetic state with an increase in the utilization of glucose, thereby depressing the mobilization of fat, on administration of ethanolic extracts observed in this study was in consonance with the above findings.

Conflict of interest statement

We declare that we have no conflict of interest.

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