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RESEARCH ARTICLE

Anti-Hyperglycemic and Renal Protective Activities of *Andrographis paniculata* Roots Chloroform Extract

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ABSTRACT

The chloroform extract of *Andrographis paniculata* roots was tested for its antihyperglycemic activity in alloxan induced diabetic rats using chronic and acute studies. The blood glucose lowering activity was determined after oral administration at doses of 50, 100 and 150 mg/kg body weight in acute study. Where as in case of chronic study blood glucose, protein, albumin and creatinine levels were estimated after 4 weeks of treatment at the dose of 300 mg/kg. Significant reductions in blood glucose levels were observed in both acute and chronic studies. The extract significanly inhibited the induction of albuminuria, proteinemia and uremia. The present study clearly indicated a significant antidiabetic activity with the chloroform extract of A. paniculata roots and supports the traditional usage of the plant by Ayurvedic physicians for the control of diabetes. Also the extract is useful in preventing the incidence of long-term complication, diabetic nephropathy.

Keywords: *Alloxan, Blood glucose, Rats, Albuminuria, Proteinemia, Uremia*

Diabetes mellitus is a major endocrine disorder affecting nearly 10% of population all over the world [1]. Inspite of the introduction of hypoglycemic agents, diabetes and related complications continue to be a major medical problem. Since time immemorial, patients with non-insulin dependant diabetes mellitus have been treated orally with a variety of plant extracts. In the indigenous Indian system of medicine (Ayurveda), a mention was made on good number of plants for the cure of diabetes or 'madhumeha' and some of them have been experimentally evaluated and the active principles have been isolated [2-8]. How ever search for new Antidiabetic drugs continues.

Andrographis paniculata has a long history of use in traditional forms of oriental medicine and it has enjoyed popular use in Scandinavia for a century. In addition to its well-known effect in supporting normal immune function, and in contrast to other popular immune enhancing nutritionals, such as mushroom extracts or nutrient combinations, it has a wider range of biological activities. It has been shown to be help to support liver function (ie, it is "hepatoprotective") [9] and in malaria treatment [10]. It also helps to support normal body temperature and circulatory and cardiovascular function [11]. It is also useful in the treatment of hypertension [12] and myocardial infarction [13-15]. In previous studies aqueous extract [16], ethanolic extract [17] of *A. paniculata* whole plant, ethanolic extract of the aerial parts of *A. paniculata* [18] had shown significant antihyperglycemic activities against streptozotocin induced diabetic rats. Andrographolide, an active principle in the leaves of *A. paniculata* [19] has shown significant antihyperglycemic action in streptozotocin-induced diabetic rats in another study. In the present study effect of *Andrographis paniculata* roots chloroform extract in alloxan induced diabetic rats was evaluated.

MATERIALS AND METHODS

Collection of Plant Material and Extraction

Fresh roots of *A. paniculata* were purchased from local traders in Visakhapatnam and shade dried until a completely dried product is obtained. A voucher specimen (No. SP-26) of the plant has been preserved in our Department. The authenticity of the sample was identified by botanist Dr. M. Venkaiah, Department of Botany, Andhra University, Visakhapatnam. The dried roots were then milled to a fine powder. This powder (2 Kg) was extracted with chloroform in a Soxhlet apparatus for 24 h and the extract was evaporated to dryness under vacuum and dried in a vacuum desiccator (184.5 g).

Chemicals Used

Glipizide was a generous gift from M/s Micro Labs Ltd., Pondicherry, Glibenclamide was a gift from M/s Sun Pharmaceuticals Ltd., Baroda and alloxan was purchased from Sigma-Aldrich, St. Louis, USA. Glucose assay kit was obtained from diagnostic division of Dr. Reddy's Laboratories, Hyderabad. All other chemicals used were of analytical grade.

Animal Experiments

Sprague-Dawley strain rats procured from Mahaveer Enterprises, Hyderabad were used for the study. All the animal experiments were conducted according to protocols that were approved by the Institutional Animal Ethics Committee (Reg. No. 516/01/a/CPCSEA). They were divided into eight groups of five each and were fed with standard diet (Ratan Brothers, Hyderabad) and water *ad libitum*. They were kept in clean and dry cages and maintained in a well-ventilated animal house with 12 h light–12 h dark cycle. Rats were rendered diabetic by injecting a freshly prepared aqueous solution of alloxan monohydrate (50 mg/kg, i.v.) [20] after base-line blood glucose estimation. After 48 h when the condition of diabetes was stabilized, animals with blood glucose levels above 300 mg/dl were selected for the study.

Acute Study. Groups I, II and III were given orally the *A. paniculata* roots chloroform extract (suspended in 1% sodium CMC) in the form of mucilage at doses of 50, 100 and 150 mg/kg body weight, respectively. Group IV served as diabetic control and received appropriate volumes of vehicle orally. Group V received glibenclamide at a dose of 0.040 mg/kg body weight and served as standard. Animals were fasted for 16 h prior to drug administration allowing access only to water. Blood samples were collected from the eye orbital plexus of the rats at 0.5, 1, 2, 4, 6, 8, 12 and 24 h after extract administration. Samples were analyzed for blood glucose content by using glucose-oxidase method [21] with optical density measured by visible spectrophotometer at 505 nm.

Chronic study. Group VI was given orally, the *A. paniculata* roots chloroform extract (suspended in 1% sodium CMC) in the form of mucilage at the dose of 150 mg/kg body weight. Group VII served as diabetic control while group VIII received glibenclamide at a dose of 0.040 mg/kg body weight and served as standard. All the administrations were given orally after analyzing zero hour blood samples. The administrations were continued for 28 days. Blood glucose levels were estimated at weekly intervals.

Study of Renal Activity. At the end of the experiment (4 weeks) 24 h urine samples of group VI, VII and VIII were collected and analyzed for protein, albumin and urea levels using standard diagnostic kits.

STATISTICAL ANALYSIS

Data are expressed as mean ± standard error of mean. Statistical analysis was made using one-way analysis of variance (ANOVA) and post-hoc comparisons were done using Dunnet's t-test. p-values < 0.05 were considered as significant.

RESULTS

The chloroform extract of *A. paniculata* roots produced a dose-dependent hypoglycemia in alloxan-induced diabetic rats in acute study. It produced significant reduction in blood glucose with doses of 50, 100 and 150 mg/kg body weight respectively (Table 1) compared to control group. Glibenclamide (40 μg/kg) produced a significant reduction in blood glucose compared to control group. Maximum reduction of blood glucose levels was 22.23% (4 h, $p<0.05$), 41.01% (2 h, $p<0.01$) and 51.69% (4 h, $p<0.01$) with doses of 50, 100 and 150 mg/kg body weight respectively. At the dose of 150 mg/kg the hypoglycemic effect was observed up to 24 h. Where as glibenclamide produced maximum reduction of 50.44% (4 h, $p<0.01$) compared to control group.

Chronic administration of *A. paniculata* to alloxan-induced diabetic rats for four weeks produced significant blood glucose reduction. Significant reduction was observed from the first week by both extract and glibenclamide at the doses of 150 mg/kg and 0.040 mg/kg. At the end of 4th week extract produced significant blood glucose reduction of 59.15% ($p<0.001$). On the other hand, glibenclamide produced significant blood glucose reduction of 62.02% ($p<0.001$). The activity of the extract (150 mg/kg) is not significantly different ($p<0.05$) from the standard drug glibenclamide (0.040 mg/kg). The results are given in Table 2.

Significant increase in urinary secretion of proteins, albumin and urea is observed in alloxan-induced diabetic rats at the end of 4 weeks. Where as no significant increase is observed in *A. paniculata* extract (150 mg/kg) and glibenclamide (0.040 mg/kg) treated group of rats. Results are shown in Fig 1.

DISCUSSION

Diabetes mellitus is possibly the world's largest

Table 1. Percent blood glucose reduction produced by *A. paniculata* root chloroform extract after oral administration in alloxan induced diabetic rats.

Group (n=5)	Dose (mg/kg)	Percent Blood Glucose Reduction at Various Time Intervals							
		0.5 h	1 h	2 h	4 h	6 h	8 h	12 h	24 h
Diabetic Control	----	-1.32±3.95	-1.29±2.95	-8.48±4.79	-7.09±9.85	-4.79±3.25	-3.19±1.94	-7.03±8.15	-5.13±7.58
A. paniculata	50	1.00±5.19	12.33±10.98	20.44±4.72*	22.23±6.95*	20.76±5.45*	13.51±9.60	13.36±6.35	10.31±7.28
A. paniculata	100	2.19±6.18	22.95±7.55*	41.01±10.56**	37.88±5.45**	32.14±3.90**	28.92±8.72*	22.86±8.20*	20.40±3.95*
A. paniculata	150	0.51±6.84	38.65±14.90**	49.16±9.64**	51.69±8.32**	44.40±9.28**	40.04±7.15**	37.12±9.48**	34.62±8.24**
Glibenclamide	0.040	25.64±9.80*	46.37±14.60**	48.72±10.22**	50.44±8.40**	46.56±4.60**	23.11±6.75*	10.26±11.03	6.66±8.95

Values are represented as mean ± S.E.M.; n = number of animals

Significant difference from diabetic control at identical times, $^{*}p<0.05$; $^{**}p<0.01$

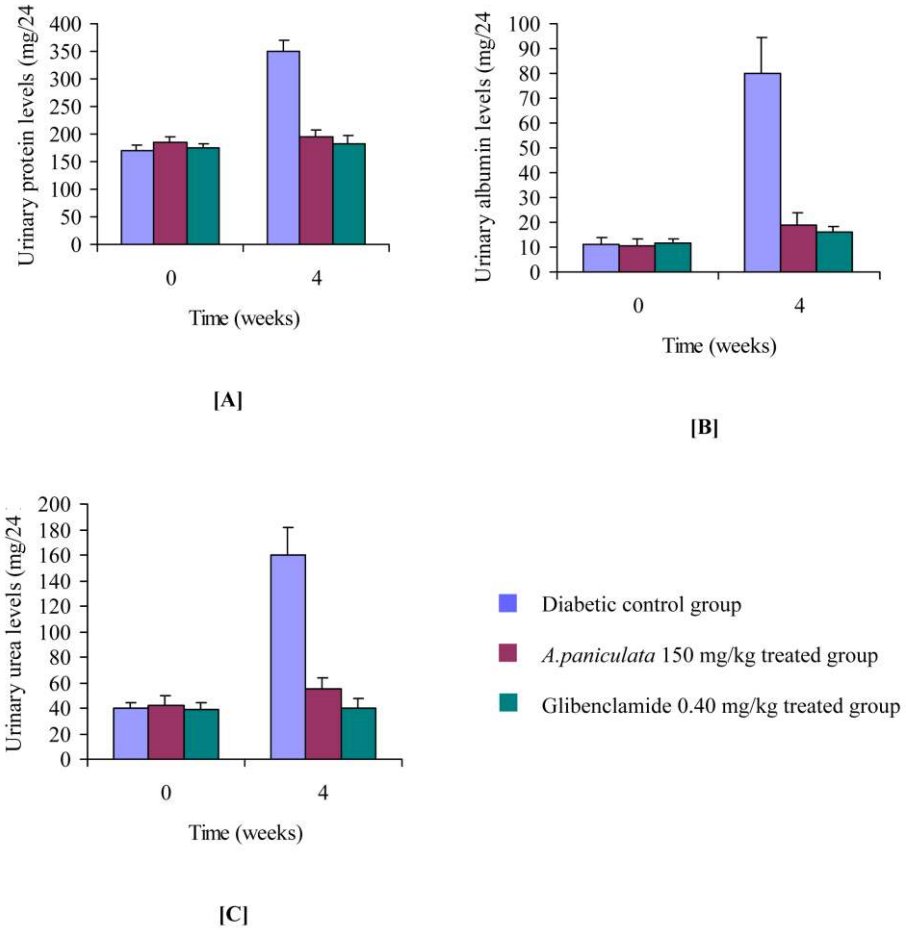

Fig 1. Effect of *A. Paniculata* on urinary excretion of protein, albumin and urea in alloxan induced diabetic rats.

growing metabolic disorder, and as the knowledge on the heterogeneity of this disorder is advanced, the need for more appropriate therapy increases [22]. Traditional plant medicines are used throughout the world for a range of diabetic complications. The study of such medicines might offer a natural key to unlock a diabetologist's pharmacy for the future.

A. paniculata is used traditionally by diabetic patients in India. Due to this reason the root chloroform extract of the plant was evaluated and the data also confirmed the traditional indications. Earlier investigations on the antidiabetic activity of the organic extracts of *A. paniculata* whole plant [16, 17] and aerial parts [18] by various authors also substantiate the results of our studies in rats, Moreover, our results on root extract also indicate potent action in acute study and a prolonged duration of antidiabetic action in chronic study could be due to multiple sites of action possessed by the active principles of *A. paniculata*. The study also revealed that *A. paniculata* had more effectively inhibited the incidence of nephropathy.

In the previous study significant antidiabetic activity was observed with the ethanolic extract (400 mg/kg) of whole plant aqueous extract (50 mg/kg) in streptozotocin induced diabetic rats. In our present study, root chloroform extract (100 mg/kg) showed significant antidiabetic activity against alloxan induced diabetic rats. This indicates that *A. paniculata* has significant effect on both streptozotocin and alloxan induced diabetic rats. Andrographolide (1.5 mg/kg), an active principle in the leaves of *A. paniculata* has been shown to have significant antidiabetic activity [19] and suggested that andrographolide can increase glucose utilization to lower plasma glucose in diabetic rats lacking insulin. Earlier investigations also revealed absence of insulin releasing mechanism by the *A. paniculata*. However, possibility of extra pancreatic action such as increased glucose uptake by *A. paniculata* cannot be ruled out. The study also revealed that the extract was useful in preventing the long-term complication of diabetic nephropathy. Further work on fractionation, purification, identification of active principle(s) and detailed mechanistic

Table 2. Percent blood glucose reduction produced by *A. Paniculata* after chronic oral administration in alloxan induced diabetic rats.

Group (n=5)	Dose (mg/kg)	Percent Blood Glucose Reduction			
		1	2	3	4 (Weeks)
Diabetic Control	----	-17.80±9.85	-11.95±5.90	-8.50±6.15	-14.65±8.90
A. paniculata	150	40.98±9.60**	48.75±12.85**	52.81±8.75**	59.15±5.85**
Glibenclamide	0.040	51.48±12.75**	59.40±9.72**	60.15±4.59**	62. 02±3.98**

Values are represented as mean ± S.E.M.; n = number of animals
Significant difference from diabetic control at identical times, *p<0.05; **p<0.01

evaluation is obviously required on the roots of *A. paniculata*.

Our study clearly indicated a significant antidiabetic activity and nephroprotective activity with the chloroform extract of *A. paniculata* roots and supported the traditional usage of the plant by the Ayurvedic physicians for the control of diabetes. Hence it helps in preventing diabetic complications and serves as a good adjuvant in the present armamentarium of antidiabetic drugs.

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