

Hypoglycemic and Antihyperglycemic Effect of *Aristolochia Indica* Normal and Alloxan Induced Diabetic Rats

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Summary

The antihyperglycemic effect of methanolic extract of root of *Aristolochia indica*. Was investigated in alloxan induced diabetic rats. The *A. indica* shows a dose dependent hypoglycemic effect and prevented rise in blood glucose levels in normal rats. The blood glucose levels were measured at 0, 1,2,4,6 and 8 h after the treatment. The alcoholic extract of *A. indica* at a doses of 200 and 400 mg/kg reduced the blood glucose levels of the normal rat from 94.55 ± 19.14 to 74.71 ± 13.64 mg/dl, 113.51 ± 14.78 to 82.65 ± 12.09 mg/dl, at 6 h after oral administration of the extract ($P < 0.05$) and also significantly lowered blood glucose levels in alloxan induced diabetic rats from 429.90 ± 10.4 to 305.34 ± 10.94 mg/dl respectively at 6 h after oral administration of the 400 mg/kg body weight extract ($P < 0.05$).The antihyperglycemic activity of *A. indica* was compared with Glibenclamide (10 mg/kg), an Oral hypoglycemic agent.

Key words: Antihyperglycemic, *Aristolochia indica*, Alloxan, Glibenclamide

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Introduction

Diabetes mellitus is an endocrine disorder, which characterized with hyperglycemia and effecting nearly 10% of the population all over the world (Burke et al., 2003). In modern medicine no satisfactory effective therapy is still available to cure diabetes mellitus (Suman and Suryawashi, 2001).there is increasing demand by patients to use natural products with antidiabetic activity due to side effects associated with the use of insulin and oral hypoglycemic agents (Holman and Turner, 1991;Prout, 1974; Kameswararao et al., 1997). The world health organization (WHO) (1980) has also recommended the evaluation of the effectiveness of plants in condition where we lack safe mode drugs (Upadhyaya and pandt,1984).

Aristolochia indica is plant belonging to the family of Aristolochiaceae is grows as weed in most tropical countries, but some areas is semi cultivated a leafy vegetable and is sometimes inter cropped with other annual field crops (Waithaka et al., 1991). It is widely distributed throughout the India is commonly called “dulagooda, eswaramul, Thella usirika” in Telugu and “Arkamula, ishwari, Jafa” in Sanskrit (Parrotta, J.A *et al.*, 1986). The root of the plant is used in indigenous system of medicines as a antidote for the snake bites, gastric stimulant, bitter tonic. Among the tribal inhabitants, the roots are ground with black pepper seeds and made into pills administered to treat rheumatism, diabetes (Irfan Ali Khan et al., 2005). A decoction of the roots is considered stimulant febrifuge. Ground root and leaves – Asthma, Leaf juice – diarrhoea, cholera. Seeds – inflammations, dry cough. The Preliminary Phytochemical studies reveal the presence of glucosinolates, glucoiberine, glucocapparine etc., and flavones, flavanoids, terpenes, fatty acids. But no scientific work have been carried out on the roots. The present study focused to evaluate alcoholic extract of *A. indica* roots at various doses in normal and alloxan induced diabetic rats.

Materials and methods

Plant material

Aristolochia indica roots were collected freshly in and around Kakatiya university campus, Warangal, South India. The plant was identified by the botanist Dr.V.S. Raju, department of botany, Kakatiya University, Warangal.

Alcoholic extraction

Alcoholic extract was prepared from a powder of the root of *A. indica* prepared in electric grinder. The 500 g powder was extracted with alcohol (95%v/v) in soxhlet apparatus. The extract was evaporated to dryness under vacuum and dried in vacuum desiccator (15.5%w/w).

Animals

Laboratory bred Sprague Dawley rats of either sex weighing 150-200 g were selected. The rats were maintained under standard laboratory conditions at $25 \pm 2^\circ\text{C}$, relative humidity $50 \pm 15\%$ and normal photo period (12 h dark / 12 h light) were used for the experiment. Commercial pellet diet (Ratan Brothers, India) and water were provided *ad libitum*. The experimental protocol has been approved by the Institutional Animal Ethics committee and by the Regulatory body of the government.

Induction of diabetes

Animals were allowed to fast 24 h and were injected with alloxan monohydrate dissolved in sterile normal saline at a dose of 150 mg/kg body weight intraperitoneally (Kameswararao et al., 1999). After 2 weeks, rats with moderate diabetes and hyperglycemic (250-350 mg/dl) were used for the experiment.

Experimental design

In the experiment a total of 42 rats (12 diabetic surviving rats, 30 normal rats) were used. The rats were divided into eight groups after the induction of alloxan diabetes. In the experiment six rats were used in each group. Group 1 treated with vehicle (5%gum acacia) and served as normal untreated group, and Group 2, and 3 treated with alcoholic extract of *A. indica* at a doses of 200 and 400 mg/kg respectively, Group 4 Diabetic rats treated with vehicle

(5% gum acacia) served as diabetic control, Group 5 diabetic rats treated with alcoholic extract of *A. indica* at a dose of 400 mg/kg respectively, Group 6 diabetic rats treated with 10 mg/kg dose of Glibenclamide.

After an overnight fasting, the plant extract suspended in 5% gum acacia was fed by gastric intubations with a syringe. Blood samples were collected for the measurement of blood glucose by puncture of retro-orbital plexus at 0, 1, 2, 4, 6 and 8 h after feeding the plant extracts. The samples were collected into glass vials containing of a small quantity of a mixture of potassium oxalate and sodium fluoride as anticoagulant. The blood glucose levels were determined by using GOD—POD method (Trinder, 1964).

Oral glucose tolerance test

After an overnight fasting, a 0-min blood sample (0.2ml) was taken from the rats of normal, diabetic control, normal rats treated with plant extract and diabetic rats also treated with plant extract groups by orbital sinus puncture (Waynforth, 1980). Glucose solution (2 g/kg) was administered orally immediately. Four more samples were taken at 30, 60, 90 and 120 min after glucose administration (Whittington *et al.*, 1991).

Statistical analysis

Data were expressed as means±S.E.M. Statistical comparison between different groups were done using one-way analysis of variance (ANOVA) followed by the Tukey–Kramer multiple comparison test, to judge the difference between various groups. Significance was accepted at $P < 0.05$.

Results

Effect on normal rats

The effect of different doses of alcoholic extract of *A. indica* on fasting blood sugar level was assessed in normal rats at various time intervals (Table 1). It produce significant ($P<0.05$) maximum reduction in blood glucose level $15.79\pm\%$, $20.62 \pm 4.72 \%$, $27.19 \pm 3.76 \%$ and 30.2% of normal rats treated with alcoholic extract of *A. indica* at a doses of 100, 200 and 400 mg/kg respectively, after 6 h of the treatment.

Effect on alloxan induced diabetic rats

The antihyperglycemic effect of the extract on the fasting blood glucose levels on diabetic rats is shown in Table 2. the alcoholic extract of *A. indica* at a dose of 400 mg/kg produced the significant ($P<0.001$) maximum fall of 28.94 ± 2.8 on the blood glucose levels of diabetic rats after 6 h of the treatment.

Table 3 shows the changes in the levels of blood glucose in normal, diabetic control and experimental groups after oral administration of glucose (2 g/kg). The diabetic rats showed that significant increase in the blood glucose at 60 min and 90 min. In *A. indica* treated animals blood glucose concentration was significantly ($P<0,05$) decreased after 60 min and 90 min. *A. indica* treated animals tend to bring the values to near normal. *A. indica* (400mg/kg) were more effective then Glibenclamide.

Table 1

: Hypoglycemic effect of the extracts in normal rats (Mean \pm SD)

Group	Dose (mg/kg)	Blood Glucose levels (mg/dl)				
		Pretreatment (h)	Post treatment (h)			
			0	2	4	6
Control (5% gum acacia in water)	-	74 \pm 11.22	74.05 \pm 11.36 (0.09 \pm 2.27)	71.12 \pm 11.75 (3.99 \pm 2.10)	69.68 \pm 11.81 (5.97 \pm 2.57)	72.16 \pm 10.96 (2.44 \pm 2.57)
Glibenclamide	10	87.16 \pm 3.88	67.74 \pm 7.57* (22.37 \pm 6.96)	58.01 \pm 2.92** (33.35 \pm 4.10)	68.94 \pm 2.9** (20.86 \pm 2.59)	77.86 \pm 6.00** (10.74 \pm 4.10)
AIM	200	94.55 \pm 19.14	84.86 \pm 18.85 (10.49 \pm 2.32)	79.63 \pm 15.10 (15.59 \pm 2.61)	74.71 \pm 13.64 (20.62 \pm 4.70)	81.91 \pm 18.05 (13.40 \pm 7.10)
AIM	400	113.51 \pm 14.78	100.41 \pm 9.48 (11.13 \pm 4.67)	87.72 \pm 11.87** (22.15 \pm 10.22)	82.65 \pm 12.09** (27.19 \pm 3.76)	93.09 \pm 7.62* (17.54 \pm 4.19)

* Statistically significant $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, compared to 0 h of their respective group; values given in parenthesis are percent blood glucose reduction.

Table 2:

Antihyperglycemic effect of the extract in alloxan induced diabetic rats (Mean ±S.D)

Group	Dose (mg/kg)	Blood Glucose levels (mg/dl)				
		Pretreatment (h) 0	Post treatment (h)			
			2	4	6	8
Diabetic Control	-	266.84±11.86	260.59±13.86 (2.36±1.06)	252.03±11.22 (5.52±2.08)	239.48±10.50 (10.19±1.61)	250.08±10.91 (6.21±3.76)
Glibenclamide	10	364.36±15.86	279.94 ± 15.29*** (23.07 ± 4.89)	224.86 ± 11.27*** (38.12 ± 11.27)	273.11 ± 14.88*** (24.97 ± 4.49)	312.21 ± 4.91*** (14.15 ± 4.73)
AIM	400	429.90±10.40	339.09±12.83*** (21.08±3.40)	325.54±13.54*** (24.26±4.07)	305.34±10.94*** (28.94±2.80)	318.97±20.24*** (25.72±5.67)

***Statistically significant p<0.001, compared to 0 h of their respective group; values

Table 3
Oral glucose tolerance test in normal and alloxan induced diabetic rats (Mean \pm S.D)

Group	Dose (mg/kg)	Blood Glucose levels (mg/dl)				
		Pretreatment (min) 0	Post treatment (min)			
			30	60	90	120
Normal Control (5% gum acacia)	-	65.84 \pm 8.12	127.01 \pm 8.33	115.71 \pm 12.94	89.36 \pm 13.79	70.32 \pm 9.55
Diabetic control		247.84 \pm 4.46	322.24 \pm 5.47	377.01 \pm 10.59	349.28 \pm 3.77	316.66 \pm 3.57
Glibenclamide	10	64.47 \pm 7.73	91.22 \pm 20.09**	65.94 \pm 6.79	56.77 \pm 6.88	49.20 \pm 3.11
AIM	400	72.72 \pm 9.21	120.19 \pm 3.94***	102.96 \pm 13.43***	77.98 \pm 8.89	57.78 \pm 6.72*
Diabetic + A. indica	400	220.64 \pm 3.05	207.02 \pm 5.49**	183.66 \pm 7.66**	149.06 \pm 5.11*	112.16 \pm 3.64*
Diabetic + Glibenclamide	10	222.12 \pm 1.88	207.1 \pm 4.62**	187.34 \pm 5.93**	148.92 \pm 4.95*	104.62 \pm 1.92*

*Statistically significant $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, compared to 0 min of their respective group

Discussion

In this study the alcoholic extract of roots of *A. indica* at different doses produce a significant fall in the blood glucose level in both normal and diabetic rats in a dose dependent manner and this was evident 1 h after the administration of the extract. On the other hand Glibenclamide caused significantly more hypoglycemia in comparison with the plant extract (400 mg/kg). As emphasize is laid on glucose homeostasis as a severe hypoglycemia can result in life threatening situation. Therefore, lesser hypoglycemia with plant extract in comparison with Glibenclamide is a desirable feature. *A. indica* might enhance glucose utilization because it significantly decreased the blood glucose level in glucose loaded rats. It seems to suggest that *A. indica* may have a mechanism of action similar to that of Glibenclamide. This may be due to restoration of delayed insulin response or due to inhibition of intestinal absorption of glucose. The mechanism of this hypoglycemic effect of the extract is not elucidated in this study. Further studies will be focused on the determination of the mechanism(s) of action, as well as on the isolation of bioactive principles.

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