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Hypoglycemic and antihyperglycemic effect of *Argyreia speciosa* Sweet. in normal and in alloxan induced diabetic rats

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Abstract

Objective: To investigate the hypoglycemic and antihyperglycemic activities of methanolic extract of stem of *Argyreia speciosa* Sweet (*A. speciosa*) in normal and alloxan induced diabetic rats. Materials and method: The blood glucose levels were measured at 0 h and 1, 2, 4, 6, 8, 12, 16 and 24 h after the treatment. Oral glucose tolerance test was performed in normal, diabetic control, plant extract treated normal and diabetic groups and tolbutamide also treated normal and diabetic groups. Results: The alcoholic extract of *A. speciosa* showed significant ($P<0.05$) dose dependent percentage blood glucose reduction in normal (26.42% at 250 mg/kg, 28.50% at 500 mg/kg and 34.25% at 750 mg/kg body weight) and in diabetic rats (24.72% at 250 mg/kg, 31.10% at 500 mg/kg and 40.47% at 750 mg/kg body weight) respectively at 8 h. Conclusion: The hypoglycemic and antihyperglycemic effect of *A. speciosa* was compared with the reference standard drug tolbutamide (40 mg/kg).

Key words: *Argyreia speciosa*; Diabetes; Tolbutamide.

1. Introduction

Diabetes mellitus is a chronic disease characterized by high blood glucose levels due to absolute or relative deficiency of circulating insulin levels. Diabetes is the world's largest endocrine disease involving metabolic disorders of carbohydrate, fat and protein. According to WHO projections, the prevalence of diabetes is likely to increase by 35%. Currently, there are over 150 million diabetics worldwide and this is likely to increase to 300 million or more by the year 2025. Statistical projection about India suggests that the number of diabetics will rise from 15 million in 1995 to 57 million in the year 2025 making it the country with the highest number of diabetics in the world [1,2]. Therefore, it is necessary to look for new solutions to manage this health problem. Although, many drugs and interventions are available to manage diabetes, in most instances these are expensive (like insulin, thiazolidinediones) for a developing country like

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India and have adverse effects (like hypoglycemia). Because of perceived effectiveness, minimal side effects in clinical experience and relatively low cost, herbal drugs are widely prescribed even when their biologically active compounds are unknown [3]. Recently, the search for appropriate hypoglycemic agents has been focused on plants used in traditional medicine partly because of leads provided by traditional medicine to natural products that may be better treatments than currently used drugs [4]. India is a country with a vast reserve of natural resources and a rich history of traditional medicine [5]. In the indigenous 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 were isolated [6-11]. However, search for newer antidiabetic drugs continues.

Argyreia speciosa Sweet (Convolvulaceae), commonly known as Vryddhadaru in sanskrit, is a woody climber found throughout India and has been used as a 'rasayana' drug in the traditional Ayurvedic system of medicine [12, 13]. The roots of this plant have been regarded as alterative and tonic and are said to be useful in rheumatism and diseases of the nervous system. Natives use the leaves as a local stimulant and rubefacient in skin diseases [14]. The seeds are a rich source of ergoline alkaloids while the roots are reported to be a tonic, aphrodisiac, bitter, diuretic and used in rheumatism, gonorrhaea, chronic ulcer and diseases of nervous system [6,13,]. Previous studies have shown the plant seed oil to possess anti-bacterial and anti-fungal properties [15]. Phytochemical screening of the plant has shown the presence of lipids, flavonoids, triterpenes and phenylpropanoids [15, 16]. Recent reports on the presence of a disubstituted tetrahydrofuran, aliphatic, and aryl esters and a coumarin in this species are available [17, 18]. There are no available reports on the effect of *A. speciosa* stem in diabetes. Therefore, we undertook the present investigation to evaluate the hypoglycemic and antihyperglycemic effects of *A. speciosa* stem extract in normal and alloxan induced diabetic rats.

2. Materials and Methods

2.1. Plant material

The plant *Argyreia speciosa* was collected in the month of august from Punyagiri hills, located at S. Kota, nearer to Visakhapatnam, Andhra Pradesh, South India. Dr. M. Venkaiah, Associate Professor, Department Of Botany, Andhra University, Visakhapatnam, identified the herb. A voucher specimen of (AS-07) was deposited in the herbarium of our department.

2.2 Alcoholic extraction

Shade dried powdered stem (1 kg) of *A. speciosa* was extracted with methanol (99.8% v/v) by continous hot (Soxhlation). The crude extract was evaporated to dryness in a rotary film evaporator (5.5 % w/w).

2.3. Drugs

Alloxan monohydrate was purchased from sigma chemicals (St Louis, USA). All other chemicals used for this study were analytical grade.

2.4. Animals

Laboratory bred Sprague Dawley rats of either sex weighing 200-225 g were employed for the study. All animals were procured from National Institute of Nutrition, Hyderabad. The rats were maintained under standard laboratory conditions at 25 ± 2°C, relative humidity 50 ± 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 [Regd no.516/01/A/CPCSEA].

2.5. Induction of diabetes

Animals were allowed to fast 18 h and were injected with alloxan monohydrate dissolved in sterile normal saline at a dose of 150 mg/kg body weight intraperitoneally [19]. After 2 week, diabetic rats (250---350 mg/dl) were used for the experiment.

2.6. Experimental design

In the experiment a total number of 60 rats (30 normal and 30 diabetic surviving rats) were used. The rats were divided into ten groups (5 normal and 5 diabetic), each group consisting of 6 animals. Group 1 normal rats treated with vehicle (1% Sodium CMC) and served as normal control, and Group 2, 3 and 4 normal rats were treated with methanolic extract of *Argyreia speciosa* at a doses of 250, 500 and 750 mg/kg respectively, Group 5 normal rats treated with tolbutamide (40 mg/kg) served as standard reference, Group 6 diabetic rats treated with vehicle and served as diabetic control. Group-7, 8, and 9 diabetic rats were treated with methanolic extract of *A. speciosa* at doses of 250, 500 and 750 mg/kg p.o respectively; Group 10 diabetic rats were treated with 40 mg/kg P.O dose of Tolbutamide.

The rats were fasted for 18 h and blood samples were collected by puncture of retro-orbital plexus immediately with capillary tube under ether anesthesia into glass vials containing a small quantity of a mixture of potassium oxalate and sodium fluoride as an anticoagulant at 0 h (before treatment) and 1, 2, 4, 6, 8, 12, 16, 24 h (after treatment). The plasma blood glucose levels were determined by using GOD—POD method [20].

2.7 Oral glucose tolerance test

After overnight fasting, an zero-min blood sample (0.2ml) was taken from the rats in normal, diabetic control, normal + plant extract treated group, diabetic + plant extract treated group, diabetic + tolbutamide treated groups by orbital sinus puncture [21]. Glucose solution (2 g/kg) was administered orally immediately. Four more samples were taken at 30, 60, 90 and 120 min after glucose administration [22].

2.8. Statistical analysis

All values were expressed as Mean ± SEM. A comparision of effects for normal, diabetic and OGT by one way ANOVA test was done. This was done as the maximum blood glucose reduction was observed at 8th hour for normal and diabetic and 60min for OGT for all the groups. The calculated $F_{4,25}$ value for normal is 76.11, diabetic 16.81 and OGT 399.34. The table $F_{4,25}$ value is 2.76. There is a significant difference among the groups. Further, to establish which among the groups are significantly different from the control Dunnet's Multille comparision test was done. P values <0.05 were considered as significant.

3. Results

3.1. Effect on normal rats

The percentage blood glucose reduction with 250, 500 and 750 mg/kg doses of *A. speciosa* at 8 h were 26.42%, 28.50% and 34.25% respectively and tolbutamide 40 mg/kg dose produced 29.68% blood glucose reduction in normal rats and the results are shown in table 1.

3.2. Effect on alloxan induced diabetic rats

The percentage blood glucose reduction with 250, 500 and 750 mg/kg dose of *A. speciosa* at 8 h were 24.72% mg/kg, 31.10% and 40.47% respectively. Tolbutamide 40 mg/kg dose produced 35.06% blood glucose reduction in alloxan induced diabetic rats and the results are shown in table 2.

3.3. Effect on oral glucose tolerance test

Table 3 shows the changes in the levels of blood glucose in normal, diabetic control and experimental groups after oral administration of glucose (2g/kg; 40%w/v).

Table 1. Effect of methanolic extract of *A. speciosa* on fasting blood glucose levels in normal rats

Group	Dose (mg/kg)	Blood glucose levels (mg/dl) at different hours after the treatment								
		0 h	1 h	2 h	4 h	6 h	8 h	12 h	16 h	24 h
Control	--	94.7 ± 1.8	92.6 ± 2.3 (2.2)	91.8 ± 2.1 (3.0)	89.3 ± 0.9 (5.7)	88.6 ± 1.2 (6.4)	87.6 ± 0.7 (7.5)	86.3 ± 1.4 (8.8)	85.6 ± 1.5 (9.6)	84.8 ± 0.4 (10.45)
A. speciosa	250	98.4 ± 1.3	91.9 ± 0.9 (6.60)	86.3 ± 1.4 (12.30)*	82.8 ± 1.7 (15.85)*	74.7 ± 0.8 (24.1)*	72.4 ± 1.1 (26.42)*	85.4 ± 0.4 (13.21)*	91.4 ± 0.6 (7.1)	93.1 ± 1.9 (5.38)
A. speciosa	500	96.8 ± 2.8	89.4 ± 1.7 (7.6)	82.6 ± 0.5 (14.66)*	78.6 ± 1.3 (18.8)*	72.1 ± 0.7 (25.52)*	69.2 ± 0.6 (28.5)*	81.3 ± 1.8 (16.0)*	89.7 ± 0.9 (7.3)	90.6 ± 0.2 (6.4)
A. speciosa	750	97.5 ± 0.8	87.8 ± 0.5 (9.9)	74.1 ± 0.8 (24.0)*	68.1 ± 1.3 (30.15)*	65.2 ± 0.6 (33.12)*	64.1 ± 0.5 (34.25)*	80.1 ± 2.7 (17.84)*	88.7 ± 1.1 (9.0)	91.6 ± 2.1 (6.05)
Tolbutamide	40	99.7 ± 1.4	90.7 ± 0.3 (9.02)	80.6 ± 0.5 (19.1)*	76.0 ± 0.7 (23.7)*	74.0 ± 0.1 (25.7)*	70.1 ± 0.8 (29.68)*	80.2 ± 0.6 (21.7)*	87.3 ± 0.5 (12.4)*	89.1 ± 1.3 (10.63)

All values are expressed as Mean ± SEM. Values given in the parenthesis are percent blood glucose reduction.
*Statistically significant P<0.05 compared to 0 h of their respective group. Percentage blood glucose reduction values are in parenthesis
At 8th hour $F_{4,25}$ value for normal is 76.11, table $F_{4,25}$ value is 2.76.

Table 2. Effect of methanolic extract of *A. speciosa* on fasting blood glucose levels in alloxan induced diabetic rats.

Group	Dose (mg/kg)	Blood glucose levels (mg/dl) at different hours after the treatment								
		0 h	1 h	2 h	4 h	6 h	8 h	12 h	16 h	24 h
Control	--	283.5 ± 11.3	279.7 ± 12.8	268.3 ± 9.8	264.13 ± 7.8	263.6 ± 3.3	269.4 ± 17.2	275.4 ± 7.8	277.8 ± 5.9	279.43 ± 2.9
A. speciosa	250	263.7 ± 3.8	248.9 ± 4.7 (5.60)	230.9 ± 1.9 (11.30)	211.7 ± 4.8 (19.7)*	205.4 ± 3.7 (22.22)*	198.5 ± 2.6 (24.72)*	217.5 ± 5.3 (17.52)*	222.4 ± 4.7 (15.66)*	237.5 ± 3.8 (9.97)
A. speciosa	500	277.8 ± 4.6	261.4 ± 7.7 (5.90)	257.4 ± 2.6 (7.30)	234.9 ± 3.7 (15.44)*	218.6 ± 4.7 (21.31)*	191.4 ± 3.3 (31.10)*	218.3 ± 7.1 (21.41)*	227.9 ± 4.3 (17.9)*	238.3 ± 4.6 (14.22)*
A. speciosa	750	298.7 ± 11.3	277.8 ± 9.7 (6.99)	256.0 ± 8.4 (14.29)*	223.7 ± 12.3 (25.11)*	209.3 ± 6.9 (29.93)*	177.8 ± 5.5 (40.47)*	229.7 ± 4.4 (23.1)*	244.5 ± 3.1 (18.14)*	256.1 ± 4.9 (14.26)*
Tolbutamide	40	284.1 ± 4.3	256.4 ± 8.4 (9.75)	242.3 ± 3.5 (14.71)*	231.1 ± 7.5 (18.66)*	209.5 ± 8.7 (26.26)*	184.5 ± 8.1 (35.06)*	211.3 ± 2.7 (25.62)*	239.5 ± 5.7 (15.69)*	247.7 ± 5.2 (12.81)*

All values are expressed as Mean ± SEM. Values given in the parenthesis are percent blood glucose reduction.
*Statistically significant P<0.05 compared to 0 h of their respective group. Percentage blood glucose reduction values are in parenthesis.
At 8th hour calculated $F_{4,25}$ value for diabetic 16.81, table $F_{4,25}$ value is 2.76.

Table 3. Oral glucose tolerance test in normal and experimental animals

Treatments	Blood glucose levels (mg/dl)				
	0 min	30 min	60 min	90 min	120 min
Normal	94.3 ± 1.8	187.4 ± 6.1*	168.7 ± 5.2*	138.4 ± 2.7*	107.8 ± 1.8
		(98.72)	(78.89)	(46.76)	(14.31)
Normal + A. speciosa	89.7 ± 4.3	138.6 ± 3.7*	126.3 ± 7.3*	113.6 ± 3.1*	94.3 ± 2.1
		(54.51)	(40.80)	(26.6)	(5.12)
Diabetic control	258.4 ± 3.8	338.6 ± 3.7*	387.5 ± 4.3*	364.4 ± 8.5*	333.3 ± 3.9*
		(31.03)	(49.96)	(41.02)	(28.98)
Diabetic + A. speciosa	132.7 ± 2.7	217.06 ± 3.7*	176.8 ± 3.8*	159.7 ± 6.3*	141.7 ± 4.8
		(63.97)	(33.23)	(20.34)	(6.78)
Diabetic + Tolbutamide	123.7 ± 8.1	203.3 ± 3.8*	189.7 ± 3.8*	139.4 ± 4.9*	128.3 ± 1.5
		(64.35)	(53.35)	(12.69)	(3.72)

All values are expressed as Mean ± SEM. *Statistically significant P<0.05 compared to 0 min of their respective group. Percentage increases of blood glucose levels are in parenthesis.
At 60 min calculated $F_{4,25}$ value for OGT 399.34, table $F_{4,25}$ value is 2.76.

The diabetic rats showed that significant increase in the blood glucose at 60 min and 90 min. In *A. speciosa* treated group, blood glucose level was significantly (P<0,05) decreased after 60 min and 90 min. *A. speciosa* treated animals tend to bring the values to near normal. *A. speciosa* (750 mg/kg) were more effective then Tolbutamide (40mg/kg).

4. Discussion

The present study was conducted to evaluate the hypoglycemic and antihyperglycemic activity of *A. speciosa*, a very new herbal drug first time identified by us to get a berth in the group of antidiabetic herbal drugs. In this study the methanolic extract of *A. speciosa* produced a dose dependent percentage blood glucose reduction in normal and diabetic groups. In normal treated groups a significant percentage blood glucose reduction was observed up to 12 h where as in diabetic groups a significant reduction in blood glucose was maintained up to 24 h. The percentage blood glucose reduction produced by the extract in normal rats did not show any dose dependent manner. The percentage blood glucose reduction produced by the extract in diabetic group is greater than the percentage reduction observed in normal treated groups and *A. speciosa* 750 mg/kg dose produced better percentage blood glucose reduction than the tolbutamide (40 mg/kg) in normal and diabetic groups. In the present study, the results are clearly indicating that the hypoglycemic and antihyperglycemic activities of *A. speciosa* in normal and alloxan induced diabetic rats.

Different mechanisms of action to reduce blood glucose levels with the help of plant extracts already exist. Some plants exhibit properties similar to the well-known sulfonylurea drugs like tolbutamide; they reduce blood glucose in normoglycemic animals [23, 24]. Some other plants act like biguanides such as metformin, which is an antihyperglycemic compound; they do not affect blood glucose in normal state [25-28]. We hypothesized that *A. speciosa* could have a sulfonylurea-like mechanism since it decreased blood glucose in normoglycemic rats such as tolbutamide and mechanism of action may be due to subsequent increased insulin secretion or by enhanced glucose utilization by peripheral tissue. Further work is in progress to identify the possible mechanisms of action for its hypoglycemic and antihyperglycemic activities.

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