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Hypoglycemic effect of *Argyreia nervosa* root extract in normal and streptozotocin-diabetic rats

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Abstract

This study reports hypoglycemic effect of alcoholic extract of Argyreia nervosa roots (500 mg/kg body weight orally) in normal, glucose loaded and streptozotocin (STZ) induced diabetic rats. The extract produced decrease in blood glucose level in normoglycaemic rats (82.6 ± 2.6 vs 61.3 ± 2.8 mg/dl at 6th hr). In oral glucose loaded rats, it reduced blood glucose levels from 118.4 ± 5.4 to 96.4 ± 4.2 mg/dl 2h after oral glucose load. When given orally for 7 days in STZ diabetic rats, it produced significant antihyperglycemic effect and also reversed the changes in total hemoglobin and glycosylated hemoglobin content.

Keywords: *Argyreia nervosa*, Blood glucose levels, Hypoglycemic effect, Streptozotocin

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INTRODUCTION

Diabetes mellitus, a chronic metabolic disorder, has now become an epidemic, with a worldwide incidence of 5 % in the general population [1]. The problem of diabetes is particularly relevant to India, as several studies have clearly documented an increased ethnic susceptibility to diabetes in-migrant Asian Indians. Indeed, according to the recent Diabetes Atlas produced by International Diabetes Federation (IDF), India is home to the largest number of people with diabetes in the world, 40.9 million diabetic subjects in 2007, and these numbers are predicted to increase to 69.9 million by 2025 [2].

The increased prevalence is attributed to the aging population structure, urbanization, the obesity epidemic and physical inactivity [3]. Currently available therapies for diabetes include insulin and various oral antidiabetic agents such as sulfonylureas, biguanides, α-glucosidase inhibitors and glinides. In developing countries such as India, these products are expensive and not easily accessible, hence plants have been used as source of drugs for the treatment of diabetes. Many indigenous Indian medicinal plants, which are readily available and having no side effects are found to be useful in successful management of diabetes.

Argyreia nervosa, commonly known as Elephant creeper in English (Family, Convolulaceae), is a woody climbing shrub found throughout India. It has been used as a Rasayana drug in the traditional Ayurvedic system of medicine [4]. Traditionally, the roots of *Argyreia nervosa* are used in treatment of ulcers, cough, bronchitis, hemorrhoids, obesity, diabetes, anemia, tuberculosis and arthritis [5]. It has been reported to possess anti-inflammatory [6] and immunomodulatory properties [7]. To our best knowledge, the antidiabetic effect of this plant has not been scientifically documented. Therefore, the objective of the present study was to determine the effect of alcoholic extract of *Argyreia nervosa* roots on blood glucose levels in normal, oral glucose loaded and streptozotocin induced diabetic rats.

MATERIALS AND METHODS

2.1. Plant material

Argyreia nervosa roots collected from surrounding fields of Belgaum, Karnataka, India, were authenticated in Department of Botany, R.L. Science College, Belgaum. A voucher specimen has been deposited in the herbarium of the college.

2.2. Preparation of extract

The *Argyreia nervosa* roots (ANR), shade dried and powdered were soxhlet extracted with 70 % ethanol. The extract was filtered with Whatman no. 1 filter paper and then solvent evaporated at reduced pressure by using Rotavapor apparatus to get a viscous mass, which was then stored at 4^0C until used. The % yield of the extract obtained was 3.9 %. For animal use, the extract was dissolved in 2 % gum acacia. The fresh extract was subjected to standard phytochemical screening tests for various phytoconstituents [8].

2.3. Chemicals

Streptozotocin (STZ) was obtained from Sigma Chemical co., St. Louis, MO, USA. Glibenclamide was obtained as gift sample from Swiss Pharma Private Ltd., Ahmedabad, India. All other chemicals were of analytical grade.

2.4. Animals

Male wistar rats (125-165 gm) were obtained from Central Animal House, J.N. Medical College, Belgaum and housed in a group of six animals for one week in a 12:12 hour light and dark cycle in a temperature and humidity controlled room. The animals were given free access to food and water. After one-week adaptation period, the healthy animals were used for the study. The Institutional Animal Ethics Committee, KLE University, Belgaum approved the experimental protocol. (IAEC permission number: 627/02/a/CPCSEA-1/13/2007)

2.5. Acute oral toxicity study

The acute oral toxicity test of the extract was carried out by using albino rats of either sex weighing between 150-200 g as per revised OECD (Organisation for Economic Coopertaion and Development) guidelines 423. The treated animals were monitored for 14 days, for mortality and general behaviour. No death was observed till the end of the study. The extract was found to be safe up to the dose of 5000 mg/kg, hence $1/10^{th}$ of the tested dose, 500 mg/kg dose was chosen as the experimental dose.

2.6. Hypoglycemic effect in normal rats

The overnight fasted rats were divided into 3 groups of six animals each. Control rats (group I) were given vehicle only (5 ml/kg of 2% gum acacia), while group II and III received 500 mg/kg of alcoholic extract of ANR and 200 µg/kg of glibenclamide respectively. Blood samples were

collected retro-orbitally before and after 1, 2, 4 and 6 h of treatment and subjected to determination of blood glucose level by glucose oxidase method.

2.7. Hypoglycemic effect in oral glucose loaded rats

The oral glucose tolerance test was carried out on overnight fasted rats. The 3 different groups of rats were given treatment as above and 30 min later; glucose (10 g/kg) was administered orally to all the rats. Blood samples were collected from retro orbital sinus at-30, 0, 30, 60 and 120 min and blood glucose levels were estimated.

2.8. Hypoglycemic effect in STZ induced diabetic rats.

Diabetes was induced in rats by a single intraperitoneal administration of STZ (55 mg/kg) dissolved in 0.1 M citrate buffer, pH 4.5. Forty eight hours later, blood samples were collected and glucose levels were estimated to confirm the development of diabetes. The rats that showed hyperglycemia (blood glucose level > 250 mg/dl) were selected for experimental study. The diabetic rats were divided into three groups of six animals each and treated orally with vehicle (5 ml/kg of 2% gum acacia), alcoholic extract of ANR at the dose of 500 mg/kg/day or glibenclamide 200 mg/kg/day everyday for seven days. One more group of normal non-diabetic rats was also included for the study. At the end of 7th day, rats were fasted for 16 hrs and blood glucose levels were estimated. The plasma was separated by centrifugation and was analyzed for concentration of total hemoglobin and glycosylated hemoglobin [9].

2.9. Statistical analysis

The experimental data are expressed as mean ± S.E.M. The difference between test and controls were evaluated by ANOVA followed by Dunnett's multiple comparison test. Values of $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

3.1. Hypoglycemic activity in normal rats

Figure 1 shows the effect of treatment with alcoholic extract of ANR and glibenclamide on blood glucose concentration in normal rats. The oral administration of ANR extract (500 mg/kg) induced a significant decrease in blood glucose level in normoglycaemic rats.

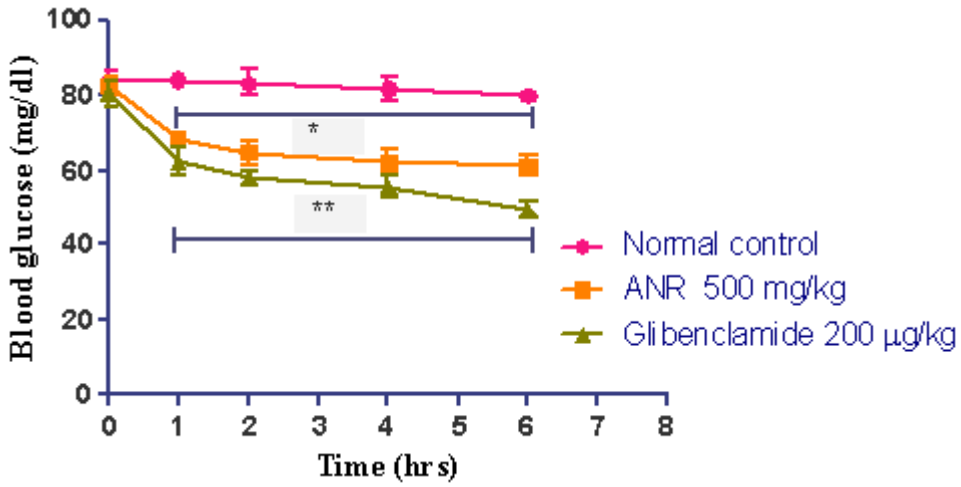

Fig. 1. Effect of *Argyreia nervosa* root extract (500 mg/kg) on blood glucose in normal rats

*Values are mean ± S.E.M, N = 6 in each group. *P < 0.05, **P < 0.01 vs control*

The glycaemia varied from 82.6 ± 2.6 to 64.8 ± 3.4 mg/dl ($p < 0.01$) 2 hr after the oral administration of extract. This effect persisted up to 6 hrs. Oral administration of vehicle (5 ml/kg of 1% Tween 80) did not change significantly the level of basal blood glucose (83.8 ± 2.6 vs. 80.4 ± 2.0 mg/dl). After 2 hrs, glibenclamide significantly decreased the blood glucose from 80.6 ± 3.2 to 60.2 ± 2.0 mg/dl.

3.2. Hypoglycemic activity in oral glucose loaded rats.

Figure 2 shows the effect of alcoholic extract of ANR and glibenclamide on blood glucose concentration in oral glucose loaded normal rats Oral glucose administration (10 g/kg) to normal fasted rats increased blood glucose from 82.6 ± 3.4 to 134.8 ± 2.8 mg/dl after 30 min of glucose load. The extract treated rats showed significantly ($p < 0.05$) reduced level of blood glucose at 30, 60 and 120 min after glucose administration. The glibenclamide significantly ($p < 0.001$) decreased the blood glucose at 30, 60 and 120 min after glucose administration.

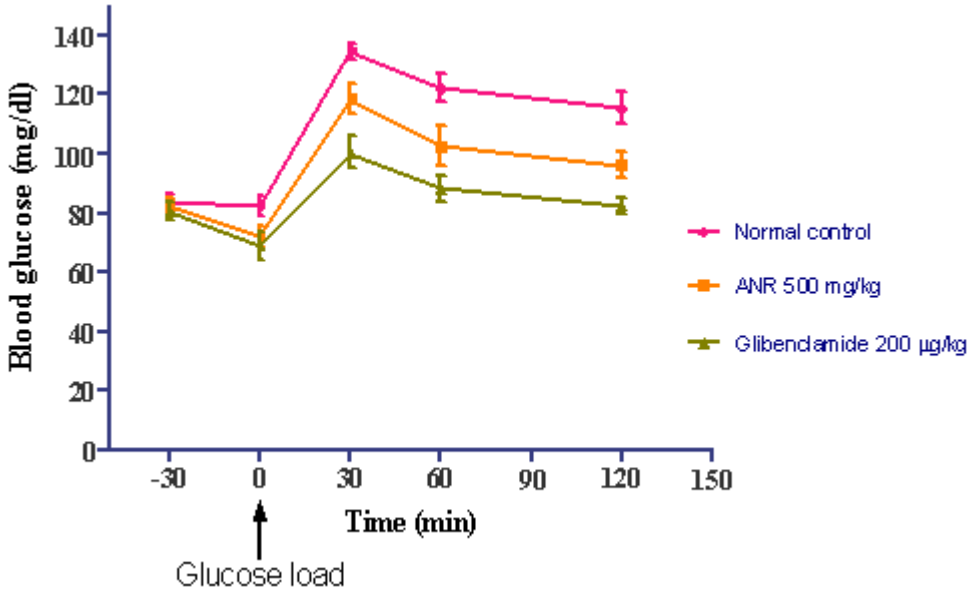

Fig 2. Effect of *Argyreia nervosa* root extract (500 mg/kg) on blood glucose in oral glucose loaded normal rats

3.3. Effect on STZ induced diabetic rats

Figure 3 shows the effect of treatment with alcoholic extract of ANR and glibenclamide on blood glucose concentration in STZ diabetic rats The vehicle treated diabetic rats did not show significant changes in blood glucose levels during 7 days of treatment (280.6 ± 4.5 vs 288.2 ± 5.6 mg/dl). Diabetic rats treated with 500 mg/kg of *Argyreia nervosa* extract showed a significant ($p < 0.001$) reduction in blood glucose from 302.4 ± 6.6 mg/dl on day 1 to 100.4 ± 4.2 mg/dl on day 7. The glibenclamide treatment also showed a significant ($p < 0.001$) antihyperglycemic effect (312.6 ± 5.3 mg/dl on day 1 to 92.4 ± 6.6 mg/dl on day 7).

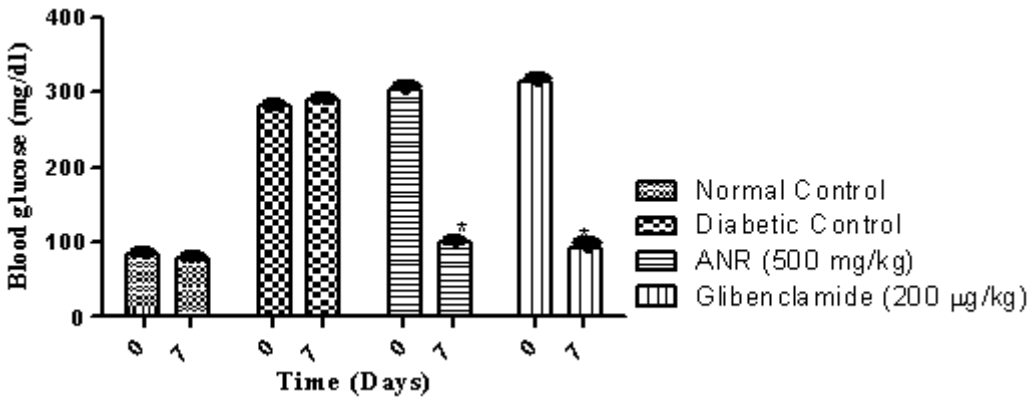

Fig. 3. Effect of *Argyreia nervosa* root extract (500 mg/kg) on blood glucose in STZ diabetic rats
*Values are mean ± S.E.M, N = 6 in each group. *P < 0.001 vs control.*

In uncontrolled or poorly controlled diabetes, the excess glucose present in the blood reacts with hemoglobin. Therefore, the total hemoglobin level is decreased and glycosylated hemoglobin is increased in diabetic rats. The diabetic rats showed decrease in level of total hemoglobin (7.42 ± 0.68 vs 11.64 ± 0.42 g/dl) and an increase in level of glycosylated hemoglobin (5.34 ± 0.38 vs 1.62 ± 0.24 g/dl) as compared to normal rats. Administration of *Argyreia nervosa* extract and glibenclamide to diabetic rats restored the changes in level of total hemoglobin (9.80 ± 0.68 and 10.90 ± 0.42 g/dl) and glycosylated hemoglobin (3.20 ± 0.55 and 1.82 ± 0.17) to near normal values. This could be due to the result of improved glycemic control produced by the treatment with extract and glibenclamide. The phytochemical screening of the *Argyreia nervosa* root extract showed the presence of flavonoids and polyphenolic compounds, which could be attributed to its hypoglycemic activity.

CONCLUSION

The present study concludes the beneficial effect of *Argyreia nervosa* roots in the control of blood glucose level in normal and diabetic rats. The study confirms the rational basis for its use in traditional medicine for the treatment of diabetes. Further phytochemical and pharmacological investigations are underway to characterize active phytoconstituents(s) and to establish exact mechanism of its hypoglycemic action.

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