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Original Article

ANTIDIABETIC ACTIVITY OF THE AQUEOUS EXTRACT OF *ANNONA SQUAMOSA* IN STREPTOZOTOCIN INDUCED- HYPERGLYCEMIC RATS

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ABSTRACT

The aqueous extract of roots of *Annona squamosa* L. at a dose of 250 mg/kg and 500 mg/kg body weight respectively was tested for antidiabetic activity in Streptozotocin (STZ) - induced hyperglycaemic rats. The blood glucose levels were measured at 0, 2h, 4h and 6h after the treatment. The aqueous extract reduced the blood glucose in STZ- induced diabetic rats from 285.52 to 208.81 mg/dl, 6h after oral administration of extract ($P<0.01$). The antidiabetic activity of aq. Extract of *Annona squamosa* was compared with glibenclamide, an oral hypoglycaemic agent (3mg/kg).

Key -words; Antidiabetic activity, *Annona squamosa,* Streptozotocin, Glibenclamide.

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1.0 INTRODUCTION

Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia. 1.3 % of the population suffers from this disease throughout the world [1]. Currently available synthetic antidiabetic agents like sulfonyl ureas, biguanides, α glucosidase inhibitors etc besides being expensive produce serious side effects [2]. Further their use is not safe during pregnancy [3]. Thus due to an increase in demand by patients to use natural products with antidiabetic activity, investigations on hypoglycaemic agents derived from medicinal plants have gained popularity in recent years. Laboratories are conducting research on these medicinal plants in a scientific manner for the development of alternative drugs and strategies for better management of diabetes.

Annona squamosa Linn. (Family; Annonaceae) commonly known as custard apple, is a woody, semi deciduos tree grown throughout India in rocky terrain with shallow and well drained soils. The plant reported to contain acetogenins [4, 5], aporphine alkaloids viz. anonaine, carydine, norcarydine, isocarydine etc. [6], sterols and volatile oils [7].

Annona squamosa has been traditionally used in treatment of ulcer [8], chronic tumors [9] and skin diseases [10]. The other reported pharmacological activities include antiplasmodial and antibacterial activity [11]. Antidiabetic activity of fruit pulp and leaf extract of *Annona squamosa* has already been reported [12,13,14]. In the present study the antihyperglycemic activity of aqueous extract of *Annona squamosa* roots is investigated in a scientific manner in STZ – induced hyperglycaemic rats.

2.0 Materials and Methods

2.1 Collection of Plant material

The roots of the plant *Annona squamosa* were collected from Khari Baoli market, New Delhi, India and authenticated by the taxonomist of Department of Botany, Faculty of Science, Hamdard University. The voucher specimen was deposited in the herbarium of university for future reference (JHFP-2005).

2.2 Preparation of Aqueous root extract

The aqueous extract was prepared by cold maceration of 100 g of shade dried root powder in 500 ml distilled water for 5 days. The extract was filtered, concentrated, dried in vacuo (yield 30 g) and the residue stored in a refrigerator at 2-8 ^{0}C for use in experiment [13].

2.3 Animals

The antidiabetic activity was carried out on Wistar rats of either sex and approximately the same age, weighing about 150-200 g, supplied by Central animal house facility of Jamia Hamdard, New Delhi (Registration no. 173/CPCSEA). They were maintained in a 12 h light/dark cycle at 25 $\pm$2 ^{0}C. They were allowed free access to standard pellet diet (Amrut Laboratory Rat Feed, Navamaharashtra, Pune, India) and water *ad*

libitum. The study was approved by institutional animal ethics committee and ethical norms were strictly followed during all experimental procedures.

2.4 STZ induced hyperglycemia in rats

Diabetes was induced experimentally in rats by a single intraperitoneal injection of a freshly prepared solution of STZ (Sigma, USA) at a dose of 50mg/kg body weight in 0.1 M cold citrate buffer of pH 4.5. After 72 h, blood was collected from tail vein of the rats under ether anesthesia and blood glucose levels were estimated using a glu-oxidase peroxidase reactive strips and glucometer one touch basic plus. The animals were considered to be diabetic if the blood glucose values were above 250 mg/dl, and those animals alone were used for the study. Control rats were injected with citrate buffer alone.

2.5 Experimental design for antidiabetic activity

The rats were divided into five groups comprising 6 animals in each group as follows

Group I: Normal control rats given only buffer.

Group II: Diabetic controls (STZ, 50 mg/kg body weight).

Group III: Diabetic rats treated with glibenclamide (3mg/kg b.w.)

Group IV and V: Diabetic rats treated with *Annona squamosa* (250 mg/kg and 500 mg/kg b.w., respectively)

The blood glucose levels of experimental animals were determined at 0, 2, 4 and 6 h after feeding the plant extract by using glu-oxidase peroxidase reactive strips and glucometer (one touch basic plus).

2.6 Statistical analysis

Values are expressed as mean±S.E.M. (n=6). Statistical significance was determined by one way analysis of variance (ANOVA) followed by Dunnet's *t* test [15]. $P<0.01$ and $P<0.05$ were considered statistically significant when compared with diabetic control.

3.0 RESULTS

Oral treatment with aqueous extract of *A. Squamosa* roots (250 and 500 mg/kg b.w.) to STZ induced diabetic rats produced dose dependant reduction of blood glucose levels particularly 6 h after treatment (n=6, $p<0.01$) (-59.6 to –76.71 g/dl, respectively) compared to diabetic control group (Table 1). Aqueous extract at a dose of 250 mg/kg b.w. and 500 mg/kg b.w. reduced the elevated level of blood glucose from 277.65 to 221.75 and 285.52 to 208.81 g/dl, respectively) 6 h after treatment. Glibenclamide (3 mg/kg b.w) also produced a significant reduction in blood glucose levels compared to control group (289.95 to 187.53 g/dl, $P<0.01$).

Table 1: Effect of *A. squamosa* on plasma glucose levels in Streptozotocin –induced diabetic rats

S. No.	Group	Treatment	Blood glucose level in mg/dl after			
			0	2h	4h	6h
1	I	Normal control	71.73±2.48	73.34±2.82	76.21±2.15	74.86±2.42
2	II	Diabetic control	284.53±8.74	280.11±7.62	278.25±7.54	281.71±8.07
3	III	Diabetic control +Standard (3mg/kg)	289.65±7.42	238.46±6.15*	202.74±6.86*	187.53±5.37*
4	IV	Diabetic control +Extract (250 mg/kg)	277.65±7.64	264.23±8.72	246.36±6.24**	221.75±5.58*
5	V	Diabetic control +Extract (500 mg/kg)	285.52±8.12	241.59±6.86*	223.38±5.85*	208.81±5.57*

Values are mean ±S.E.M. (n=6).
*P<0.01; **P<0.05 Vs Diabetic control. One way analysis followed by Dunnet's *t* test.

4.0 Discussion

The present work has detected the antidiabetic activity of *A. squamosa* root extract in STZ-induced hyperglycemia in rats. STZ induced diabetes mellitus and insulin deficiency lead to increased blood glucose level. When *A. squamosa* root extract was administered to diabetic rats, hypoglycaemia was observed after 2 hrs, with the maximum effect being seen at 6 h.

From the results it is assumed that the root extract could be responsible for stimulation of insulin release and observed restoration of blood glucose level. Further, the observed decreased blood glucose lowering effect of the extract in STZ-induced diabetic rats could also possibly be due to increased peripheral glucose utilization. It has been reported that using medicinal plant extract to treat STZ-induced diabetic rats results in activation of β-cells and insulinogenic effects [16]. The antihyperglycemic activity of the Aq. extract of *Annona squamosa* roots was comparable with glibenclamide, a standard hypoglycaemic drug.

REFERENCES

1. Raghunathan M and Raghunathan N. Diabetes Mellitus and Vitamin D. Nutrition News. 1992; 13, 4-6.
2. Suba V, Murgean T, Arunachalan G, Mandal SC, Sahu BP. Antidiabetic potential of *Barleria lupine* extract in rats. Phyto Med. 2004 ; 11, 202-205

3. Rahman Q, Zaman K. Medicinal plants with hypoglycaemic activity. J. Ethnopharmacol. 1989; 26:1-55.
4. Fujimoto Y, Eguchi T, Kakinuma K, Ikekawa N, Sahai M and Gupta YK. Squamocin, a new cytotoxic bis tetrahydrofuran containing acetogenins from *Annona squamosa*. Chem. Pharm Bull. 1988; 36, 4802-4806.
5. Sahai M, Singh S, Singh M, Gupta YK, Akashi S, Yaji R, Hirayama K, Asaki H, Araya H, Hara N, Eguchi T, Kakinuma K and Fujimoto Y. Annonaceous acetogenins from the seeds of *Annona squamosa* adjacent bis tetrahydro furanoic acetogenins. Chem. Pharm Bull. 1994; 42, 1163-1174.
6. Bhakuni DS, Tewari S, Dhar MM. Aporphine alkaloids of *Annona squamosa*. Phytochemistry. 1972; 11, 1819-1822.
7. Rao RVK, Satyanarayana T. Occurrence of Kaurenoic acid in *Annona squamosa*. Ind J Nat Products. 1986; 2:2, 8-9.
8. Bhatnagar LS, Singh VK, Pandey G. Medico botanical studies on the flora of ghatigaon forests, Gwalior, M.P. J. Res Ind. Med. 1973; 88:2, 67-100
9. Shah GL, Menon AR, Gopal GV. An account of the ethnobotany of Saurasthra in Gujrat state (India). J Econ Tax Bot. 1981; 2, 173-182.
10. Upadhyay OP, Kumar K, Tiwari RK. Ethnobotanical study of skin treatment uses of medicinal plants of Bihar. Pharmaceutical Biology. 1998; 36, 167-172
11. Sharma PC, Murti KS, Bhat AV, Narayanappa D, Kishore P. Medicinal lores of Orissa- I, Skin diseases. Bull Med Ethnobot Res. 1985; 6, 93-101.
12. Kaleem M, Asif M, Ahmad QU, Bano B. Antidiabetic and antioxidant activity of *Annona squamosa* extract in STZ-induced diabetic rats. Singapore Med Journal. 2006; 47:8, 670-675.
13. Shirwaiker A, Rajendran K, Dinesh Kumar C. Oral antidiabetic activity of *Annona squamosa* leaf alcohol extract in NIDDM rats. Pharm Biol. 2004; 42, 30-35.
14. Atique A, Iqbal M, Ghouse AKM. Use of *Annona squmosa* and *Piper nigrum* against diabetes. Fitoterapia. 1985; 56, 190-192.
15. Woolson RF. Statistical methods for the analysis of biomedical data. John Wiley and Sons Inc. New York.1987.
16. Padmini K, Chakrabarti CH. Effects of Bittergourd (*Momordica charantia*) seed and glibenclamide in streptozotocin-induced diabetes mellitus. Indian J Exp Biol. 1982; 20, 232-235.