

**ANTIDIABETIC AND CYTOPROTECTIVE EFFECT OF *Annona Squamosa* LEAVES ON DIABETIC WISTAR ALBINO RATS.**

**Manjari Mittal<sup>1\*</sup>, Vijay Juyal<sup>2</sup>, Anita Singh<sup>3</sup>**

<sup>1</sup>Dept. of Pharmacy, Sunrise Academy, Raipur Road, Dehradun (Uttarakhand)

<sup>2</sup>Dept. of Pharmaceutical Sciences, Kumaon University, Bhimtal (Uttarakhand)

<sup>3</sup>Dept. of Pharmacy, Devasthali Vidhyapeeth Rudrapur (Uttarakhand)

\*Corresponding author's E-mail: [manjarig21@rediffmail.com](mailto:manjarig21@rediffmail.com)

**Summary**

*Annona squamosa* or sugar apple is a used in various disorders like hyperthyroidism, antimicrobial and insecticidal, uterotonic, antifertility, antitumour, antioxidant, antispasmodic, analgesic, antiinflammatory, antiulcer, abortifacient etc. The present study is aimed to evaluate the antihyperglycemic activity of ethanolic extract of *Annona squamosa* leaves in alloxan induced diabetic rats. Administration of Ethanolic extract of *Annona squamosa* leaves in diabetic rats showed dose dependent reduction in hyperglycemia. The levels of Serum cholesterol, triglyceride, blood urea and serum creatinine were decrease very significantly in diabetic rats as compared with diabetic control rats.

**Key Words:** Diabetes, *Annona Squamosa*, wistar rats

**Introduction**

Diabetes mellitus (DM) comprises a group of common metabolic disorders that share the phenotype of hyperglycemia. Several distinct types of DM exist and are caused by a complex interaction of genetics, environmental factors, and life-style choices. DM is the leading cause of end-stage renal disease, nontraumatic lower extremity amputations, and adult blindness. With an increasing incidence worldwide, DM will likely continue to be a leading cause of morbidity and mortality for the foreseeable future<sup>1</sup>.

Diabetes was discovered as early as 700-200 BC; until the time insulin was invented, this disorder was managed principally by the traditional practices by using medicinal plants<sup>2</sup>. The Pharmaceutical Drugs are either too expensive or have undesirable side effects. Treatment with sulphonylureas and biguanides are also associated with side effects<sup>3</sup>.

Many indigenous Indian medicinal plants have been found to be useful to successfully manage diabetes and some of them have been tested and their active ingredient isolated<sup>2</sup>. The world health organization (WHO) has also recommended the evaluation of the plant's effectiveness and conditions where we lack safe modern drugs<sup>4</sup>. In India, indigenous remedies have been used in the treatment of DM since the time of Charaka and Sushruta (6th century BC).

Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. The ethnobotanical information reports about 800 plants that may possess anti-diabetic potential<sup>5</sup>.

***Annona squamosa***

*Annona squamosa* is commonly known as sugar apple, custured apple and Sharifa (Hindi) and belongs to the family Annonaceae. A small, tropical tree growing up to 15-25 feet tall. The leaves are thin, oblong while the flowers are greenish yellow. The fruit with a purple knobby skin is very sweet and eaten fresh. The fruit is juicy and creamy-white. Chemically contain annonaine an alkaloid, flavonoids, glycosides, aporphine alkaloids, tetrahydro isoquinoline alkaloids, terpens etc<sup>6</sup>. *Annona squamosa* used in hyperthyroidism, antimicrobial and insecticidal, antidiabetic, uterotonic, antifertility, antitumour<sup>7,8</sup>, antioxidant<sup>9</sup>, antispasmodic, analgesic, antiinflammatory, antiulcer, abortifacient etc<sup>10</sup>.



**Matrial And Methods**

**Collection & authentication:**

The matured crude drugs of *Annona squamosa* leaves were collected in April to May 2009 carefully from local areas of Dehradun, Uttarakhand, India. These crude drugs were authenticated at Botanical Survey of India (BSI), Dehradun by botanist Dr. J. R. Sharma. Voucher specimens of the plants were deposited in the Botanical Survey of India herbarium. The given voucher no for *Annona squamosa* is BSD-112713.

**Plant material:**

After proper identification by taxonomists, the leaves of *Annona squamosa* were dried in shade at room temperature and coarsely powdered by using mechanical grinder. The powdered drug was then extracted successively with petroleum ether, chloroform, ethanol and water in a soxhlet assembly and extract were concentrated under reduced pressure in rota evaporator the dried extracts were stored in the air tight container. Ethanolic extract of leaves of *Annona squamosa* is selected for animal screening because it contains maximum phytoconstituents.

**Animal:**

Male whister albino rats (weighting 150-200g) were housed in spacious cage and allowed 1 week to adapt to their new environment. The animals were maintained in an environment of controlled temperature ( $25 \pm 2$  C°) under a 12 h light–dark cycle. For rats, standard rodent chow and water were provided throughout the experimental period. All animal procedures used were in strict accordance with the committee for the purpose of control and supervision of experiments on animals (CPCSEA) and all experimental protocols were approved by the institutional ethics committee.

**Induction of Diabetes:**

All male wistar rats were divided into five groups with six animals in each group. Group- I was normal Rats and used as normal control. Group- II, III, IV and V were made diabetic by a single intraperitoneal injection of alloxan monohydrate 120mg/kg (Rolex chemical Limited, Mumbai) and served as diabetic control, standard and treatment groups respectively. Alloxan was first weighed individually for each animal according to the weight and then solublized with 0.2 ml saline just prior to injection. Three days after alloxan injection, rats with plasma glucose levels of >150 mg/dl were included in the study. Treatment with standard drug glimepiride 1mg/kg body weight (procured from Intas Pharmaceuticals Ltd) and plant extracts were started 72h after alloxan injection<sup>11</sup>. Blood samples were drawn at weekly intervals till the end of study. Fasting blood glucose estimation and body weight measurement ware done on day 0, 10, 20 and 30 of the study. On 30<sup>th</sup>, blood was collected through retro-orbital and tail under mild ether anesthesia.

**Estimation of biochemical parameter**

Serum glucose, serum cholesterol, serum triglycerides, and serum creatinine, blood urea, AST, ALT, ALP were estimated by commercially available kits (Span diagnostic Pvt. Ltd. Surat, India) and body weight is also recorded.

**Statistical Analysis**

All the values of body weight, blood sugar, and biochemical estimations were expressed as mean  $\pm$  S.E.M. (Standard Error Mean) and analyzed with SigmaStat software for ANOVA & Tukey's test. Differences between groups were considered significant at  $P < 0.05$  levels.

**Results & discussion**

Diabetes is possibly world's fastest growing metabolic disease, so does now there is an urgent need for more appropriate therapies<sup>12</sup>. India being country with rich plant diversity, its herbs can be used as curative remedies against diabetes. Diabetes mellitus has been treated orally with herbal remedies based on folk medicine since ancient times in india.<sup>13</sup>

Our study supports that Ethanolic extract of *Annon squamosa* leaves exhibited antidiabetic property. The antihyperglycemic effect of the Ethanolic extract of *Annona squamosa* leaves (100, 200 & 300mg/kg body wt.) compared with glimepiride (1mg/kg body wt.) in diabetic rats are shown in the Table-I. Administration of Ethanolic extract of *Annona squamosa* leaves in diabetic rats showed dose dependent reduction in hyperglycemia and statistically significant ( $p < 0.05$ ).

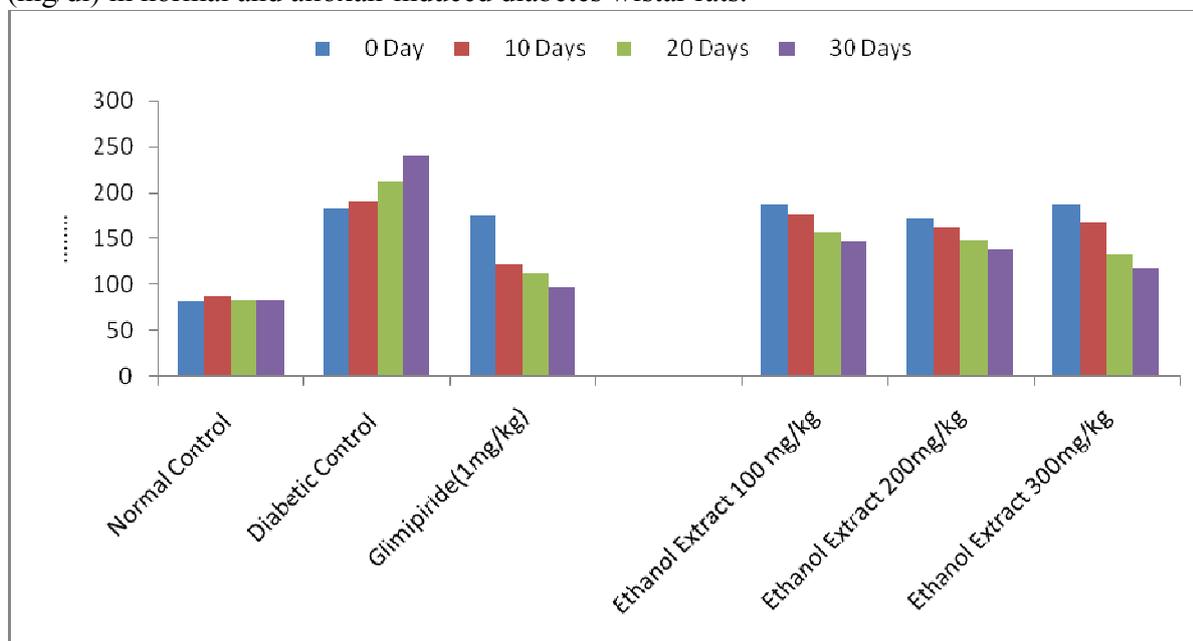
The Blood glucose was more significantly reduce at a dose level of 300mg/kg as compared with 100mg/kg body wt. of Ethanolic extract of *Annona squamosa* leaves on 0, 10, 20, 30<sup>th</sup> day after treatment when compared to diabetic control group.

The level of AST, ALT, ALP, cholesterol, triglyceride, blood urea and serum creatinine were raised in diabetic rats after 30 days when compared with control group but the levels of cholesterol, triglyceride, blood urea and serum creatinine were controlled significantly with 300mg/kg body wt. dose of Ethanolic extract in treated group. The levels of Serum cholesterol, triglyceride, blood urea and serum creatinine were decrease very significantly in diabetic rats as compared with diabetic control rats.

**Table-I:** Effect of Ethanolic extracts of *Annona squamosa* leaves on fasting blood glucose level (mg/dl) in normal and alloxan-induced diabetes wistar rats. Values are given as mean  $\pm$  Standard Error Mean (S.E.M.) for groups of six animals. Values are statistically significant at \*  $p < 0.05$ .

Groups	0 Day	10 Days	20 Days	30 Days
Normal Control	82.03 $\pm$ 4.68	87.40 $\pm$ 4.48	84.40 $\pm$ 4.48	83.80 $\pm$ 3.05
Diabetic Control	183.40 $\pm$ 3.79	191.20 $\pm$ 2.93	211.80 $\pm$ 3.78	241.00 $\pm$ 3.25
Glimepiride (1mg/kg)	176.20 $\pm$ 1.62	123.40 $\pm$ 3.09*	112.60 $\pm$ 3.87*	97.05 $\pm$ 3.38 *
Ethanol Extract 100 mg/kg	187.80 $\pm$ 3.76	177.60 $\pm$ 3.62	156.20 $\pm$ 2.30*	147.80 $\pm$ 3.22*
Ethanol Extract 200mg/kg	173.46 $\pm$ 4.16	162.80 $\pm$ 2.94*	148.00 $\pm$ 1.64*	139.60 $\pm$ 3.94*
Ethanol Extract 300mg/kg	187.56 $\pm$ 2.71	167.47 $\pm$ 3.63*	134.43 $\pm$ 2.36*	118.59 $\pm$ 3.31*

**Fig-II:** Effect of Ethanolic extracts of *Annona squamosa* leaves on fasting blood glucose level (mg/dl) in normal and alloxan-induced diabetes wistar rats.



**Table-II:** Effect of alcoholic extract of *Annona squamosa* leaves on serum profile in alloxan (120 mg/kg i.p.) induced diabetic wistar rats after 30 days of treatment. Values are given as mean ± Standard Error Mean (S.E.M.) Values are statistically significant at \* p< 0.05.

Parameter	Control	Diabetic control	A. squamosa extract 100mg/kg	A.squamosa Extract 200mg/kg	A.squamosa Extract 300mg/kg
Body weight (gm)	167.34±1.40	172±2.00	166.23±0.73*	165.48±1.24*	166.53±0.95
Blood urea (mg/dl)	23.66±0.98	61.0±1.59	31.35±0.75*	27.65±1.36*	24.31±1.07*
S.Creatinine(mg/dl)	0.52±1.26	1.35±1.18	0.87± 1.21	0.75±0.58	0.65±0.91
AST (U/L)	47.90±1.38	136.10±1.19	141.83±2.17	139.53±2.20	137.43±1.72
ALT (U/L)	44.48±0.91	142.37±1.40	131.07±1.52	126.07±0.84	121.73±1.48
ALP(U/L)	96.32±2.27	197.41±2.09	185.15±1.24	187.70±2.95	179.08±1.68
T.C.(mg/dl)	98.76±0.55.	164.31±0.71	153.29±1.17*	133.29±0.76*	112.94±1.38*
T.G.(mg/dl)	84.26±0.77	134.23±2.74	122.04±0.79*	126.34±1.13*	103.07±1.49*

**References**

1. Harrison's Principles of Internal medicine, 15<sup>th</sup> edition. Vol-II 2001;Mc Graw Hill Medical Publishing Division. pp 2109.
2. Grover JK, Yadav S, Vats V. Medicinal plants of India with antidiabetic potential. *Journal of Ethnopharmacology* 2002; 81: 81-100.
3. Rang and Dale's pharmacology, 6<sup>th</sup> edition. 2007; Churchill Livingstone Elsevier. pp 4.
4. Day C. Traditional plant treatment for diabetes mellitus: pharmaceutical foods. *British Journal of Nutrition* 1998; 80: 5-6.
5. Alarcon-Aguilara FJ, Roman-Ramos R, Perez-Gutierrez S, Aguilar-Contreras A, Contreras- Weber CC, Flores-Saenz JL. Study of the anti-hyperglycemic effect of plants used as antidiabetics. *Journal of Ethnopharmacology* 1998; 61(2): 101-110.
6. Gupta R K, Kesari A N, Watal G, MurthyP S, Chandra R, Maithal K, Tandon V. Hypoglycaemic and antidiabetic effect of aqueous extract of leaves of *Annona squamosa* (L.) in experimental animal. *Current Science* 2005; 88(8): 1244-1254.
7. Gupta R K, Kesari A N, Diwakar S, Tyagi A, Tandon V, Chandra R, Watal G. In vivo evaluation of anti-oxidant and anti-lipidimic potential of *Annona squamosa* aqueous extract in type 2 diabetic models. *Journal of Ethnopharmacology* 2008; 118(1): 21-25.
8. Shirwaikar A, Rajendran K, Kumar C D, Bodla R. Antidiabetic activity of aqueous leaf extract of *Annona squamosa* in streptozotocin-nicotinamide type 2 diabetic rats. *Journal of Ethnopharmacology* 2004; 91: 171-175.
9. Shirwaikar A, Rajendran K, Kumar C D. In vitro antioxidant studies of *Annona squamosa* Linn.Leaves. *Indian Journal of Experimental Biology* 2004; 42: 803-807.
10. Chavani M J,Shinde D B,Major volatile constituents of *Annona squamosa* L.bark. *Natural Product Research*2006; 20(8): 754-757.
11. Semwal B C, Shah K, Chauhan NS, Bhade R, Divakar K. Anti-diabetic activity of stem bark of *berberis aristata* D C. in alloxan induced diabetic rats. *The Internet journal of pharmacology* 2008; 6: 1.
12. Ashok T K, Madhushudana R J. Diabetes mellitus and multiple therapeutic approach of phytochemicals: present status and future prospects. *Current Science* 2002; 83(1): 30-38.
13. Modak M, Dixit P, Lodhe j, Ghaskadbi s, Devasagayam TPA. Indian herbs and herbal drugs used for the treatment of diabetes. *Journal of clinical Biochemistry & Nutrition* 2007; 40: 163-173.