ORIGINAL ARTICLE

Antidiabetic activity of *Annona squamosa* Linn. in alloxan - induced diabetic rats

Ranveer Singh Tomar, Siddharaj Singh Sisodia
Department of Pharmacology, Bhupal Nobles' College of Pharmacy, Udaipur, Rajasthan, India

Background: Diabetes mellitus is characterised by rise in blood sugar levels resulting from insulin dysfunction or insulin insufficiency. **Aim:** The aim of the present investigation is to evaluate antidiabetic activity of hydroalcoholic extract of *Annona squamosa* Linn (*A. squamosa* Linn) in alloxan-induced diabetic rat model. **Materials and Methods:** Diabetes is induced by a single-dose intraperitoneal injection (i.p.) of alloxan (120 mg/kg) to albino rats. **Results and Discussion:** Treatment with *A. squamosa* Linn. extract at a dose of 350 mg/kg and 700 mg/kg and glibenclamide at a dose of 5mg/kg for 28 days, after induction of diabetes by alloxan, caused significant reduction in blood serum glucose and serum lipid profiles like total cholesterol and triglycerides but significant increase in body weight and serum high density lipoproteins (HDL) level in diabetic rats compared to untreated group. Histological study of the pancreas of diabetic rat treated with *A. squamosa* extract also showed partial regeneration of beta cells. The antidiabetic activity of this extract is found comparable to glibenclamide. Thus, leaves of *A. squamosa* Linn. can be used as potential antidiabetic drug.

Key words: *Annona squamosa*, antidiabetic, glibenclamide

INTRODUCTION

Medicinal plants are used in many countries to control diabetes mellitus. The hypoglycemic action of these medicinal plants is being studied.[1] Diabetes is a chronic disorder of carbohydrate, fat and protein metabolism characterised by increased fasting and post-prandial blood sugar levels resulting from either insulin insufficiency or insulin dysfunction.[2] *Annona squamosa* Linn. (*A. squamosa* Linn.) belongs to the family Annonaceae, commonly known as sitaphal (Hindi) and custard apple or sugar apple in English. It is a native of West Indies and is now cultivated throughout India.[3] Fruits of *A. squamosa* Linn. are normally eaten fresh. Leaves of the plant have been used as insecticide, anthelmintic, styptic.[4] Unripe and dried fruit work as antidysentric, and its bark is used as tonic, powerful astringent, antidysentric and vermifuge. Powdered seeds are used to kill head lice[4] The plant is reported to contain glycosides, flavonoids, phenolic compounds, proteins, tannins, etc.[5] Flavonoids are reported to possess antidiabetic activity.[6] Phytochemical analysis of leaves of *A. squamosa* Linn. revealed the presence of flavonoids.[7] Hence, the aim of the present study is to evaluate the antidiabetic activity of hydroalcoholic extract of leaves of *A. squamosa* Linn. in rat model.

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MATERIALS AND METHODS

Chemicals

Alloxan (Hi-Media Lab Pvt. Ltd., Mumbai), Ethanol, Diagnostic Kits (Roche Diagnostics India Pvt. Ltd., Mumbai).

Animals

Albino rats of either sex weighing 150–200g were used for this experiment. The rats were housed in a group of six in polypropylene cages at controlled room temperature 25°C ± 2°C and relative humidity 55% and 12 h light-dark cycle. They were fed with standard chow diet and water *ad-libitum* during the experiment. All the study protocols were approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and cleared by Institutional Animal Ethical Committee (IAEC) at Bhupal Nobles' College of Pharmacy, Udaipur (Rajasthan) wide no. 37/SSS/BNCP-09/IAEC.

Plant Material and Preparation of Extract

Leaves of *A. squamosa* Linn. were collected from the local region near Udaipur between the month of October–November, 2008 and authenticated by Dr. M. S. Rathore, Head, Department of Botany, Bhupal Nobles' P.G. College, Udaipur (Rajasthan). Hydroalcoholic extract was prepared using the cold maceration method.[8] Leaves of *A. squamosa* Linn.

Addess for correspondence: Dr. Ranveer Tomar – Lecturer, B N College of Pharmacy, Old station road, Udaipur 313 002, Rajsthan.
E-mail: qlohitaksh_2012@rediffmail.com
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were shade-dried at room temperature for one week. The dried leaves were crushed to fine powder using a mixer grinder. The powder was weighed and used for extraction using 70% alcohol as solvent. The solvent was then evaporated at room temperature to obtain a reddish brown extract. The extract was stored at 2°C-8°C till further use.

Experimental Induction of Diabetes

Animals were fasted for 18 h before induction of diabetes.[9] Diabetes was induced by a single intraperitonial (i.p.) injection of alloxan (120mg/kg) prepared in ice-cold normal saline. The observed mortality after alloxan injection is 40%. After 72 h of alloxan administration, blood was collected from the retro-orbital plexus[1] of survived rats using heparinised capillaries, and centrifuged at 3000 rpm for 20 min to separate serum. Fasting blood sugar was estimated by glucose peroxidase method[10] using Roche diagnostic kit. Rats having fasting blood serum glucose more than 250 mg/dl were used for the experiment.[11]

Experimental Design

Rats were divided into five groups. Group 1 remained non-diabetic, group 2 consisted of untreated diabetic rats and groups 3, 4 and 5 consisted of treated diabetic rats.

Group 1

Rats received vehicle (1ml/kg 0.5% carboxymethylcellulose, CMC) orally for 28 days.

Group 2 (Untreated)

Rats received a single i.p. dose of alloxan in ice-cold normal saline (120mg/kg).

Group 3

Rats received a single i.p. dose of alloxan (120mg/kg) in ice-cold normal saline along with suspension of *A. squamosa* (AS) Linn. extract (350mg/kg in 0.5% CMC) orally for 28 days.

Group 4

Rats received a single i.p. dose of alloxan (120 mg/kg) in ice-cold normal saline along with suspension of AS Linn. extract (700mg/kg in 0.5% CMC) orally for 28 days.

Group 5

Rats received single i.p. dose of alloxan (120 mg/kg) plus suspension of glibenclamide (5 mg/kg in 0.5% CMC) orally for 28 days.

Rats were fasted overnight, and blood was collected from the retro-orbital plexus before the experiment (0 day), after 72 h and after 28 days. The serum was separated by centrifugation and used for estimation of biochemical parameters like fasting blood glucose[10] and lipid profile (Roche diagnostic kits; semi-automatic auto analyzer Microlab 300) wide total cholesterol, triglycerides and high density lipoproteins (HDL). Physical parameters like body weight were observed before the experiment (0 day), after 72 h and after 28 days of treatment.[12]

Statistical Analysis

Results of biochemical estimations are reported as mean ± SD of six animals in each group. Data were subjected to one-way analysis of variance (ANOVA) followed by Scheff's/Dunnett's test applied for determining statistical significance of difference in blood serum glucose and other parameters. The level of significance was $P < 0.05$.

RESULTS

Effect of AS Linn. Extract on Body Weight of Diabetic Rats

There was significant reduction ($P < 0.01$) in the body weight of untreated diabetic rats compared to normal control rats by single i.p. dose of alloxan (120mg/kg) administered. Treatment of diabetic rats with AS Linn. extract for 28 days caused dose-dependent increase in the body weight of diabetic rats. Glibenclamide-treated diabetic rats also showed significant ($P < 0.05$) increase in body weight after 28 days of treatment as shown in Table 1.

Effect of AS Linn. Extract on Fasting Blood Sugar Level of Diabetic Rats

There was significant ($P < 0.01$) elevation in fasting blood sugar level of alloxan-induced diabetic rats compared to normal control rats as shown in Table 2. Treatment of diabetic rats with AS Linn. extract for 28 days caused marked reduction in fasting blood sugar level compared to diabetic control rats. The extract resulted in dose-dependent reduction in fasting blood sugar

Table 1: Effect of *Annona squamosa* Linn. extract on body weight of alloxan induced diabetic rats

Group treatment	Body weight (g)		
	0 day (initial)	After 72 h	29th day (final)
Normal control 0.5% CMC 1 ml/kg/day orally	170.00±20.00	170.00±20.00	174.16±17.72
Diabetic control alloxan (120 mg/kg) in a single i.p. dose	190.83±9.17	110.00±14.49+	110.00±20.24+
Diabetic+AS Linn. extract (350mg/kg/day) orally	180.00±18.16	102.50±14.40+	121.66±16.32*
Diabetic+AS Linn. extract (700mg/kg/day) orally	177.50±17.53	127.50±17.53+	148.33±20.16*
Diabetic+glibenclamide 5mg/kg/day orally	182.50±14.05	121.66±14.37+	145.83±14.63*

i.p. – Intraperitoneal, AS – *Annona squamosa*, CMC – Carboxymethylcellulose. Values were expressed as mean±SD, n=6, P values: +P<0.01 compared to normal control group, *P<0.05 compared to diabetic control group

level after 28 days of treatment. Glibenclamide-treated diabetic rats also showed marked reduction in fasting blood sugar level compared to diabetic control rats after 28 days of treatment.

Effect of AS Linn. Extract on Serum Total Cholesterol Level in Diabetic Rats

There was significant ($P < 0.01$) elevation in serum total cholesterol in alloxan-induced diabetic rats compared to normal control rats. Treatment of diabetic rats with AS Linn. extract for 28 days caused marked reduction in serum total cholesterol level compared to diabetic control rats, as shown in Table 3. The AS Linn. extract showed its effect on serum total cholesterol level in a concentration-dependent manner. Glibenclamide-treated diabetic rats also showed significant reduction ($P < 0.01$) in serum total cholesterol after 28 days of treatment.

Effect of AS Linn. Extract on Serum Triglycerides level in Diabetic Rats

Alloxan (120mg/kg) administered i.p. to albino rats caused significant ($P < 0.01$) elevation in serum triglycerides in diabetic rats compared to normal rats. Treatment of diabetic rats with AS Linn. extract for 28 days caused marked reduction in serum triglycerides level in a dose-dependent manner compared to diabetic control rats, as shown in Table 4. Glibenclamide-treated diabetic rats also showed marked reduction in serum triglycerides level compared to diabetic control rats.

Effect of AS extract on serum High Density Lipoproteins level in Diabetic Rats

There was marked reduction in serum HDL level in alloxan-induced diabetic rats compared to normal control rats. The AS Linn. extract treated diabetic rats showed significant elevation ($P < 0.05$) in serum HDL level compared to diabetic control rats after 28 days of treatment. The AS Linn. extract-treated diabetic rats showed elevation in serum HDL level in a dose-dependent manner. Glibenclamide-treated diabetic rats also showed significant elevation ($P < 0.05$) in serum HDL level compared to diabetic control rats after 28 days of treatment as shown in Table 5.

Histological Studies of Pancreas

Histology of the pancreas of normal rat showed islets cells with normal acini and abundant of cytoplasm, as shown in Figure 1. Pancreas of untreated diabetic control rat showed atrophy with degeneration and necrosis of pancreatic tissue and invasion of connective tissues in the parenchyma of pancreatic islets, as shown in Figure 2. The pancreas of AS

Table 2: Effect of *Annona squamosa* Linn. extract on fasting blood sugar level in alloxan induced diabetic rats

Group treatment	Fasting blood serum glucose (mg/dl)		
	0 day (initial)	After 72 h	29th day (final)
Normal control 0.5% CMC 1 ml/kg/day orally	90.16±11.51	90.16±11.51	84.83±11.65
Diabetic control alloxan (120 mg/kg) in a single i.p. dose	93.83±14.49	289.83±14.74+	278.00±26.92+
Diabetic+AS Linn. extract (350mg/kg/day) orally	95.00±13.97	296.83±14.38+	192.33±18.90**
Diabetic+AS Linn. extract (700mg/kg/day) orally	95.83±8.28	279.00±15.81+	165.66±18.83**
Diabetic+glibenclamide 5mg/kg/day orally	93.66±14.92	276.00±18.50+	145.50±14.46**

i.p. – Intraperitoneal, AS – *Annona squamosa*, CMC – Carboxymethylcellulose. Values were expressed as mean±SD, *n*=6, *P* values: +*P*<0.01 compared to normal control group, ****P*<0.01 compared to diabetic control group

Table 3: Effect of *Annona squamosa* Linn. extract on serum total cholesterol level in alloxan induced diabetic rats

Group treatment	Serum total cholesterol (mg/dl)		
	0 day (initial)	After 72 h	29th day (final)
Normal control 0.5% CMC 1ml/kg/day orally	79.83±14.16	79.66±14.30	74.66±12.5
Diabetic control alloxan (120mg/kg) in a single i.p. dose	95.33±17.11	174.33±19.60+	170.33±17.82+
Diabetic+AS Linn. extract 350mg/kg/day orally	97.16±11.94	174.16±21.67+	132.50±17.64**
Diabetic+AS Linn. extract 700mg/kg/day orally	84.66±21.62	175.00±12.11+	114.83±13.77**
Diabetic+glibenclamide 5mg/kg/day orally	104.16±6.94	173.00±16.01+	114.00±13.85**

i.p. – Intraperitoneal, AS – *Annona squamosa*, CMC – Carboxymethylcellulose. Values were expressed as mean±SD, *n*=6, *P* values: +*P*<0.01 compared to normal control group, ****P*<0.01 compared to diabetic control group

Table 4: Effect of *Annona squamosa* Linn. extract on serum triglycerides level in alloxan induced diabetic rats

Group treatment	Serum triglycerides (mg/dl)		
	0 day (initial)	After 72 h	29th day (final)
Normal control 0.5% CMC 1 ml/kg/day crally	75.66±15.22	75.16±15.68	68.83±16.53
Diabetic control Alloxan (120mg/kg) in a single i.p. dose	73.33±15.47	160.16±6.79+	154.00±4.77+
Diabetic+AS Linn. extract 350mg/kg/day orally	86.66±17.37	175.00±17.94+	112.00±16.73**
Diabetic+AS Linn. extract 700mg/kg/day orally	78.83±19.02	170.16±16.61+	101.00±14.05**
Diabetic+glibenclamide 5mg/kg/day orally	94.83±11.82	173.83±17.29+	107.66±9.87**

i.p. – Intraperitoneal, AS – *Annona squamosa*, CMC – Carboxymethylcellulose. Values were expressed as mean±SD, *n*=6, *P* values: +*P*<0.01 when compared with normal control group, ***P*<0.01 compared to diabetic control group

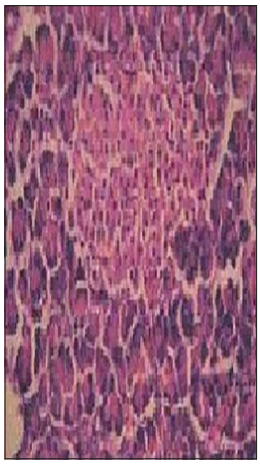

Figure 1: Histology of pancreatic section of normal healthy rat

Figure 2: Histology of pancreatic section of diabetic control rat

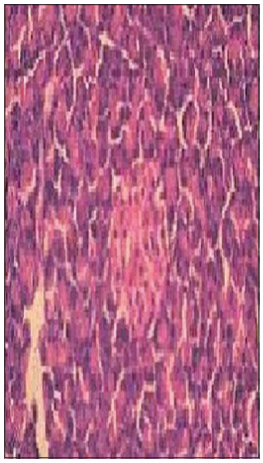

Figure 3: Histology of pancreatic section of diabetic rat treated with *Annona squamosa* (AS) Linn. extract (350mg/kg)

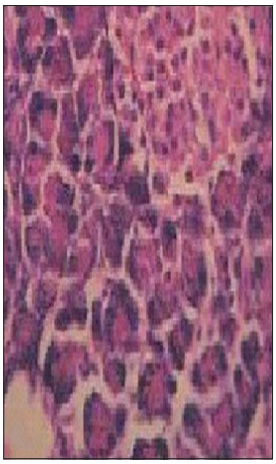

Figure 4: Histology of pancreatic section of diabetic rat treated with AS Linn. extract (700mg/kg)

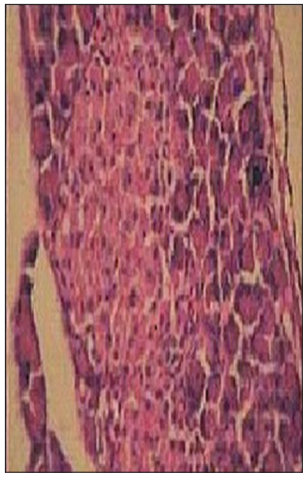

Figure 5: Histology of pancreatic section of diabetic rat treated with glibenclamide (5 mg/kg)

Linn. extract-treated diabetic rat showed slight regeneration of pancreatic cells with normal islet cells, as shown in Figures 3 and 4. The pancreas of glibenclamide-treated diabetic rat revealed partial regeneration of islet cells with presence of numerous beta cells in pancreatic islets, as shown in Figure 5.

Table 5: Effect of *Annona squamosa* Linn. extract on serum high density lipoproteins level in alloxan induced diabetic rats

Group treatment	Serum HDL (mg/dl)		
	0 day (initial)	After 72 h	29th day (final)
Normal control 0.5% CMC 1 ml/kg/day orally	49.16±9.45	48.50±10.05	46.83±10.00
Diabetic control alloxan (120mg/kg) in a single i.p. dose	48.16±5.67	29.83±5.81+	34.16±5.63+
Diabetic+AS Linn. extract 350mg/kg/day orally	48.00±7.69	26.83±4.95+	38.50±3.33*
Diabetic+AS Linn. extract 700mg/kg/day orally	47.16±6.30	26.50±3.27+	43.33±4.03*
Diabetic+glibenclamide 5 mg/kg/day orally	49.66±7.73	30.83±3.65+	44.16±5.03*

i.p. – Intraperitoneal, AS – *Annona squamosa*, CMC – Carboxymethylcellulose. Values were expressed as mean±SD, n=6, P values: +P<0.01 when compared with normal control group, *P<0.05 compared to diabetic control group. HDL – High density lipoproteins

DISCUSSION

The present investigation revealed the antidiabetic activity of the hydroalcoholic extract of the leaves of AS Linn. in alloxan-induced diabetic model.

Chemically, alloxan (2,4,5,6-tetraoxypyrimidine) is an oxygenated pyrimidine derivative. It is a well-known diabetogenic agent that is used to induce type 1 diabetes in experimental animals. In the present study, alloxan caused significant reduction in body weight and marked increase in fasting serum glucose level and serum lipid profile like total cholesterol and triglycerides in diabetic rats. Diabetes mellitus is a metabolic disorder of carbohydrate, lipid and protein metabolism. The disturbed lipid metabolism associated with diabetes mellitus results in hyperlipidemia which in turn may lead to chronic complications like atherosclerosis, myocardial infaraction, etc.[13] Treatment of diabetic rats with AS Linn. extract at a dose of 350 mg/kg and 700 mg/kg for 28 days caused significant reduction in fasting blood serum glucose in a dose-dependent manner compared to diabetic control group. The extract-treated diabetic rats also showed marked improvement in their body weight. There was remarkable improvement in the condition of damaged beta cells as revealed in histological study of the pancreas of AS Linn. extract-treated diabetic rats. The AS Linn. extract also showed beneficial effect on disturbed serum lipids parameters. The AS Linn. extract considerably decreased the elevated levels of serum total cholesterol and triglycerides whereas significantly elevated the level of serum HDL in diabetic rats compared to diabetic control rats. However, the AS Linn. extract did not restore the disturbed biochemical parameters to normal value in diabetic rats. Hence, the extract can be used in combination with other established antidiabetic drugs or herbal formulations for more effective outcomes.

CONCLUSION

The above findings revealed that the hydroalcoholic extract of the leaves of *A. squamosa* possesses potent antidiabetic and antihyperlipidemic activities. Thus, it can be useful in the treatment of lipid abnormalities associated with diabetes mellitus. However, the exact mechanism of the antidiabetic and antihyperlipidemic effects of AS extract is unknown. Hence, future studies are required to study its mechanism of action.

REFERENCES

1. Tenpe CR, Yeole PG. Comparative evaluation of antidiabetic activity of some marketed polyherbal formulation in alloxan induced diabetic rats. J Pharma Tech Res 2009;1:43-9.
2. Modak M, Dixit P, Londhe J, Ghaskadbi S, Devasagayam TP. Indian herbs and herbal drugs for the treatment of diabetes. J Clin Biochem Nutr 2007;40:163-73.
3. Porwal M, Sharma K and Malik P. Anticovulsant Effect of *Annona squamosa* Linn. Leaves in Mice. Pharmacologyoline 2011; 2:44-52.
4. Shah R. Pharmacognosy and Pharmacology of *Annona squamosa*: A review. Int J Pharm Life Sci 2011;2:1183-9.
5. Pandey N, Barve D. Phytochemical and Pharmacological Review on *Annona squamosa* Linn. Int J Res Pharm Biomed Sci 2011;2:1404-8.
6. Lukacinova A, Mojzis J, Benacka R, Keller J, Kurila P, Vasko L, *et al*. Preventive effects of flavonoids on alloxan induced diabetes mellitus rats. Acta Vet Brno 2008;77:175-82.
7. Vanitha V, Umadevi KJ, Vijaylakshmi K. Determination of bioactive components of *Annona squamosa* L. Leaf by GC-MS Analysis. Int J Pharm Sci Drug Res 2011;3:309-12.
8. Sivaiah K, Reddy GA. Evaluation of anti-hyperlipidemic activity of hydro alcoholic extract of moringa oleifera seeds in high fat diet induced rat model. Int J Pharma Screen Meth 2012;2:72-6.
9. Rathnakar UP, Hashim SD, Pemminatti S, Shenoy A, Gopalkrishna HN, Siddique F, *et al*. Hypoglycaemic activity of a polyherbal product in alloxan induced diabetic rats. Drug Int Today 2011;3:1-2.
10. Hugo WB, Russel AD. Pharmaceutical microbiology. 3rd ed. Blackwell Science Publication; 1984. p. 179-200.
11. Puranik N, Kararashah FK, Sheela D. Anti-diabetic activity of *Tinospora cordifolia* (Wild.) in Streptozotocin diabetic rats; does it act like Sulfonylureas. Tur J Med Sci 2010;40:265-70.
12. Srividya AR, Dhanabal SP, Satish Kr MN, Kumar P, Baradia H. Antidiabetic and antioxidant activity of *Alpinia galanga*. Int J Pharm Phytochem Res 2010;3:6-12.
13. Obaidullah L, Ikramullah KL, Zafar I, Wilayat A, Shah NM. Study of hypolipidemic effect of herbal and homeopathic antidiabetic drugs in alloxan induced diabetic rabbits. Int J Pharma Med Chem 2005;2:183-7.

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