ISSN 0976 – 3333

Available Online at www.ijpba.info

International Journal of Pharmaceutical & Biological Archives 2012; 3(6):1492-1495

ORIGINAL RESEARCH ARTICLE

Antidiabetic Activity Of *Annona squamosa* L. In Experimental Induced Diabetic Rats

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Received 24 Sep 2012; Revised 11 Dec 2012; Accepted 23 Dec 2012

ABSTRACT

The principle aim of present investigation is to evaluate antidiabetic activity of hydroalcoholic extract of *Annona squamosa* Linn. in experimentally induced diabetic rat model. Treatment with *Annona squamosa* extract and Glibenclamide at a dose of 350mg/kg and 5mg/kg respectively for 28 days, after induction of diabetes by Streptozotocin, caused significant reduction in blood serum glucose, lipid profiles like serum cholesterol and triglycerides but significant increase in HDL and body weight in diabetic rats compared to untreated group. Furthermore, the extract showed significant reduction in blood serum glucose after glucose loading compared to control group in oral glucose tolerance test performed in normal rats. The antidiabetic activity of extract is found comparable than Glibenclamide. Thus, leaves of *Annona squamosa* Linn. can be used as potential antidiabetic drug.

Key words: *Annona squamosa*, Glibenclamide, Antidiabetic.

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 characterized by increased fasting and post prandial blood sugar levels resulting from either insulin insufficiency or insulin dysfunction [2]. *Annona squamosa* Linn. belongs to family Annonaceae, commonly known as sitaphal (Hindi) and custard apple or sugar apple is a native of West- Indies and is now cultivated through out India [3]. Fruits of *Annona 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 bark is used as tonic and also as powerful astringent, antidysentric and vermifuge [4]. Powdered seeds are used to kill headlice [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 *Annona squamosa* Linn. revealed the presence of flavonoids [7]. Hence, the aim of present study is to evaluate antidiabetic activity of hydroalcoholic extract of leaves of *Annona squamosa* Linn. in rat model.

MATERIALS AND METHODS

Chemicals

Streptozotocin (Hi media labs, Mumbai), Glucose (Chemdyes corporation, Mumbai),
Diagnostic Kits (Roche, Mumbai), Ethanol.

Animals

Albino rats of either sex weighing between 150 – 200 gm were used for the experiment. Animals were housed in a group of six in polypropylene cages at controlled room temperature 25 ± 2ºC, relative humidity 55% and 12 hrs. light: dark cycle. They were fed with standard chow diet and water *ad libitum* during the experiment. All the study protocols were approved by CPCSEA and cleared by Institutional Animal Ethical Committee (IAEC) at Bhupal Nobles' College of Pharmacy, Udaipur (Rajasthan).

Plant Material and Preparation of Extract

Leaves of *Annona squamosa* Linn. were collected from local regions near Udaipur city in the month of October - November and authenticated by Dr. M. S. Rathore, Assistant Professor and Head, Department of Botany, Bhupal Nobles' PG College, Udaipur (Rajasthan). Extract was prepared using cold maceration method. Leaves of *Annona squamosa* L. were shade dried at room temperature for one week. Dried leaves were crushed to fine powder by using mixer grinder.

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Powder was weighed and used for extraction using 70% alcohol as solvent. The solvent is then evaporated at room temperature to obtain reddish brown extract. The extract was stored at 2 - 8ºC till further use.

Oral glucose tolerance test (OGTT) in normoglycemic rats

Albino rats weighing between 150 - 200gm were used in the test. Animals were fasted overnight and divided into 3 groups. Each group contained six rats. Blood is withdrawn from the retro- orbital plexus from the rats of each group and the fasting serum glucose is determined -

Group 1: Served as control received 0.5% Carboxy methyl cellulose (1ml/kg).

Group 2: Received *Annona squamosa* Linn. extract (350mg/kg) in 0.5% CMC orally.

Group 3: Received standard drug Glibenclamide in a dose of 5mg/kg in 0.5% CMC orally. After 30 minutes of treatment, rats of each group were given glucose (5gm/kg) in distilled water orally and blood is withdrawn at 0, 30, 60 and 120 minutes from rats of each group and blood serum glucose is determined using Glucose peroxidase method [8,9].

Experimental Induction of Diabetes

Animals were fasted for 18 hrs. before induction of Diabetes [10]. Diabetes is induced by single Intraperitonial (i. p.) injection of Streptozotocin (55mg/kg) in citrate buffer pH 4.5. After 48 hrs. of streptozotocin injection, blood is withdrawn from retro orbital plexus [1] and centrifuged at 3000 rpm for 20 minutes to separate serum and sugar were estimated using glucose peroxidase method. Rats having fasting blood serum glucose above 250 mg/dl were used for the experiment [11].

Experimental Design

Rats were divided in four groups group 1 remained non-diabetic and groups 2 consist diabetic and group 3 and 4 consisting diabetic plus treated -

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

Group 2: Rats received single dose of Streptozotocin in citrate buffer pH4.5 (55 mg/kg b.w.) i.p.

Group 3: Rats received single dose of Streptozotocin in citrate buffer pH 4.5 (55 mg/kg b.w.) i.p. plus suspension of *Annona squamosa* Linn. extract (350mg/kg b.w. in 0.5% CMC) orally for 28 days.

Group 4: Rats received single dose of Streptozotocin in citrate buffer (55 mg/kg b.w.) i.p. plus suspension of Glibenclamide (5mg/kg b.w. in 0.5% CMC) orally for 28 days.

Rats were fasted overnight and blood is withdrawn from retro orbital plexus before the treatment (initial) and after 28 days of treatment. Serum was separated by centrifugation and used for estimation of biochemical parameters like blood glucose [9] and lipid profile (Roche diagnostic kits and using semiautomatic auto analyzer- micro lab 300) wide total cholesterol, triglycerides and high density lipoprotein (HDL). Physical parameter like body weight is observed initially and after the 28 days of treatments [12]

Statistical analysis

Results of biochemical estimations are reported as mean $\pm$ SD of six animals in each group. The data were subjected to one-way analysis of variance (ANOVA) followed by Scheff's/Dunnett's test was applied for determining statistical significance of difference in blood serum glucose and other parameters. The level of significance was $p<0.05$ [13].

RESULTS AND DISCUSSION

The present investigation revealed the antidiabetic activity of hydroalcoholic extract of *Annona squamosa* Linn. leaves. Streptozotocin at a dose of 55mg/kg body weight caused significant elevation of blood serum glucose [8] in untreated groups when compared to control group was shown in (**Table 2**). There was also significant elevation in serum lipids like cholesterol and triglycerides and significant decrease in serum high density lipoproteins in diabetic rats compared to control group after 28 days. Diabetes mellitus is a metabolic disorder of carbohydrate, lipid and protein metabolism. The disturbed lipid metabolism results in hyperlipidemia which in turn may lead to chronic complications like atherosclerosis, myocardial infarction etc. [14]. *Annona squamosa* L. extract at a dose of 350 mg/kg b.w. and Glibenclamide at a dose of 5 mg/kg b.w. caused significant decrease in blood serum glucose was shown in (Table 2) and serum lipids like cholesterol and triglycerides and significant increase in serum HDL levels after 28 days of treatment when compared to diabetic control group was shown in (**Table 3**).There was also significant decrease in body weight of diabetic rats compared to control group after 28 days which is significantly increased compared to diabetic control group after 28 days treatment with extract and Glibenclamide was shown in (**Table 4**). In oral glucose tolerance test performed on normal rats was shown in (**Table 1**), there is

significant decrease in rise of fasting blood serum glucose at 30, 60 and 120 minutes after glucose loading by *Annona squamosa* Linn. extract and Glibenclamide respectively when compared with control group. Literature evidence suggest that oxidative stress play a major role in pathogenesis of diabetes mellitus [12] and flavonoids from plants are reported to possess antidiabetic [6] and free radical scavenging activity [15]. Since, leaves of *Annona squamosa* Linn. contain large amount of flavonoids, so flavonoids may be responsible for its antidiabetic activity. More detailed investigation is required with respect to the activity of flavonoids and scavenging of free radicals. Antidiabetic effect of this plant can also try with combination with marketed existing antidiabetic drugs and it may cause more effective in combination with marketed preparation and may be beneficial with respect severe another side effects of allopathic drugs.

Table1: Effect of *Annona squamosa* Linn. extract and Standard drug Glibenclamide on oral glucose tolerance in normal rats

Group	Fasting Blood Serum Glucose (mg/dl)				
	Initial	After glucose loading			
		At 0 min	At 30 min	At 60 min	At 120 min
Control (0.5% CMC 1ml/kg) orally	97.50 ± 11.30	98.00 ± 13.34	138.83 ± 23.02	140.33± 21.89	139.33± 20.73
Test group *Annona squamosa*(350mg/kg) orally	93.00 ± 33.70	93.33 ± 37.37	118.16 ± 16.36**	108.00±15.20**	104.16± 15.91**
Standard Gliben (5mg/kg) orally	90.66 ± 14.33	91.00 ± 13.79	110.83 ± 10.14**	95.50±11.61**	92.33± 11.67**

All values are represented as Mean ± SD (n=6).
P Values: ** < 0.05 when compared with normal control animals.

Table2: Effect of *Annona squamosa* extract and standard drug Glibenclamide on fasting blood serum glucose in Streptozotocin induced diabetic rats after 28 days of treatment in rats

Group	Fasting Blood Serum Glucose (mg/dl)		Percentage reduction (Fasting blood glucose)
	Initial (Before treat.)	Final (After treat.)	
Control (0.5% CMC orally) 1ml/kg/day	75.33 ± 38.35	69.00 ± 36.19	8.40
Diabetic control (untreated)	289.33 ± 35.42	263.16 ± 34.69++	9.04
Test *Annona squamosa* (350mg/kg/day orally)	298.66 ± 30.50	149.00± 25.86**	50.11
Standard Glibenclamide (5mg/kg/day orally)	281.33 ± 20.10	124.66 ± 20.60**	56

All values are represented as Mean ± SD (n=6)
P Values: ++< 0.05 When compared with normal control animals; ** < 0.05 When compared with diabetic control animals

Table3: Effect of *Annona squamosa* Linn. extract and standard drug Glibenclamide on serum lipid profiles in Streptozotocin induced diabetic rats after 28 days of treatment in rats

Group	Cholesterol (mg/dl)		Triglycerides (mg/dl)		High Density Lipoproteins (mg/dl)	
	Initial	Final	Initial	Final	Initial	Final
Control (0.5% CMC) 1ml/kg/day ,orally	90.00 ± 11.94	81.50 ± 13.78	78.16 ± 23.00	72.16 ± 22.69	75.33 ± 13.64	74.33 ± 16.41
Diabetic control (untreated)	169.50 ± 21.68	162.50 ± 20.25++	155.33 ± 32.22	142.82 ± 35.52++	29.16 ± 4.87	32.33 ± 2.94++
Extract treated *Annona squamosa* (350mg/kg orally)	171.16 ± 18.66	108.50 ± 6.28**	183.00 ± 12.72	94.83 ± 19.05**	18.50 ± 4.23	44.50 ± 6.53**
Standard Glibenclamide (5mg/kg/day) orally	177.16 ± 19.92	112.00 ± 7.23**	180.50 ± 14.32	111.33 ± 21.19**	29.5 ± 8.04	43.66 ± 5.12**

All values are represented as Mean ± SD (n=6)
P Values: ++< 0.05 when compared with normal control animals; **< 0.05 when compared with diabetic control animals

Table 4: Effect of *Annona squamosa* Linn. extract and standard drug Glibenclamide on body weight of Streptozotocin induced diabetic rats after 28 days of treatments

Group	Body weight (gm)		% change in body weight
	Initial (Before treatment	Final (After Treatment)	
Control (0.5% CMC orally)1ml/kg/day	171.67±14.38	173.33 ± 19.40	0.96
Diabetic control (untreated)	120.00 ± 13.03	111.67 ± 8.16++	-33.13
Test *Annona squamosa* (350mg/kg/day orally)	124.16 ± 15.94	148.33 ± 13.29**	19.46
Standard Glibenclamide (5mg/kg/day orally)	121.60 ± 14.37	163.33 ± 11.69**	34.31

All values are represented as Mean ± SD (n=6).
P Values: ++ < 0.05 when compared with normal control animals; **< 0.05 when compared with diabetic control animals

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