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Hypoglycemic and antidiabetic effect of ethanolic extract of leaves of *Annona squamosa* L. in experimental animals

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Abstract

The ethanolic extract of *Annona squamosa* L. (Annonaceae) leaves was administered orally at different doses to normal as well as streptozotocin (STZ)-induced diabetic rats and alloxan-induced diabetic rabbits. The dose of 350 mg/kg body weight (bw) reduced the fasting blood glucose (FBG) level by 6.0% within 1 h, whereas, the peak blood glucose at 1 h during glucose tolerance test (GTT) was reduced by 17.1% in normal rats. The same dose of ethanolic extract reduced FBG by 26.8% and improved glucose tolerance by 38.5 and 40.6% at 1 and 2 h, respectively, during GTT in alloxan-induced diabetic rabbits. In STZ-diabetic rats, a fall of 13.0% in FBG and an improvement in glucose tolerance by 37.2 and 60.6% at 1 and 2 h, respectively, was observed during GTT. The dose of 350 mg/kg bw of ethanolic extract in 10-day treatment of a group of STZ-diabetic rats produced 73.3% fall in FBG level and no sugar was observed in fasting urine. Treatment of severely-diabetic rabbits for 15 days with a dose of 350 mg/kg of extract reduce FBG by 52.7% and urine sugar by 75%. It brought about fall in the level of total cholesterol (TC) by 49.3% with increase of 30.3% in high-density lipoprotein (HDL) and decrease of 71.9 and 28.7% in low-density lipoprotein (LDL) and triglycerides (TG) levels, respectively.

Keywords: *Annona squamosa*; Diabetes; Alloxan; Streptozotocin; Glucose tolerance test

1. Introduction

Diabetes is a metabolic disorder characterised by fast elevation of blood sugar level. The incidence of diabetes mellitus is on rise all over the world, especially in Asia. Many oral hypoglycemic agents, such as biguanides and sulfonylurea are available along with insulin for the treatment of diabetes mellitus (Holman and Turner, 1991), but these synthetic agents can produce serious side effects, and in addition, they are not suitable for use during pregnancy (Larner, 1985; Rao et al., 1997; Valiathan, 1998). Therefore, search for safe and more effective agents has continued to be an important area of active research. Since ancient times, diabetes has been treated orally with several medicinal plants or their extracts based on folklore medicine. These herbal remedies are apparently effective, produce minimal or no side effects in clinical experience and are of relatively low costs as compared to oral synthetic hypoglycemic agents. Furthermore, after the recommendation made by WHO on diabetes mellitus, investigations on hypoglycemic agents from medicinal plants have become more important (WHO, 1980).

Annona squamosa L. (Annonaceae), commonly known as custard apple, is a native of West Indies and is now cultivated throughout India, mainly for its edible fruit. This plant is re-

Abbreviations: BGL, blood glucose level; bw, body weight; FBG, fasting blood glucose; GTT, glucose tolerance test; HDL, high density lipoprotein; LDL, low density lipoprotein; PPG, postprandial glucose; S.D., standard deviation; STZ, streptozotocin; TC, total cholesterol; TG, triglyceride; US, urinary sugar; VLDL, very low density lipoprotein

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puted to possess several medicinal properties (Asolkar et al., 1992). Various phytochemical and biological activity studies have been carried out with the plant (Vohora et al., 1975; Nonfon et al., 1990). From the leaves of *Annona squamosa*, several flavonoids (Seetharaman, 1986) and a tetrahydroisoquinoline alkaloid with cardiotonic activity (Wagner et al., 1980) have been isolated. Many workers have reported its use as an insecticidal agent (Cheema et al., 1985). Partially purified flavonoids of aqueous *Annona squamosa* leaves extract possess antimicrobial and insecticidal activity (Kotkar et al., 2002). Methanolic leaves extract was tested for mosquitocidal effect against *Culex quinquefasciatus* (Jaswanth et al., 2002), and the ethanolic leaves and stem extract are reported to have an anti-cancerous activity (Bhakuni et al., 1969). Ayurvedic practitioners use stem and leaves extract as an indigenous uterotonic drug (Mishra et al., 1966). The aqueous extract of *Annona squamosa* seeds do not interfere with the reproductive performance of pregnant rats (Damasceno et al., 2002). Post-coital anti-fertility activity (Mishra et al., 1979) is reported in the seed extract of *Annona squamosa*, while the aerial parts are inactive.

The aqueous leaves extract has also been reported to ameliorate hyperthyroidism (Sunanda and Anand, 2003), which is often considered as a causative factor of diabetes (Williams, 1997). The tribals and villagers of Aligarh district (Atique et al., 1985) and Chotanagpur divison (Topno, 1997) in India extensively use the young leaves of *Annona squamosa* along with seeds of *Piper nigrum* for the management of diabetes. The hot water extract of *Annona squamosa* leaves possess hypoglycemic and antidiabetic activity (patent filed Tandon et al., 2003). Antidiabetic activity of cold aqueous extract has also been reported in STZ-nicotinamide type 2 diabetic rats (Shirwaikar et al., 2004). The objective of this investigation was to ascertain the scientific basis for its use in the treatment of diabetes. The present paper reports the hypoglycemic and antihyperglycemic activity of the ethanolic extract of *Annona squamosa* leaves on which there are no previous studies.

2. Material and methods

2.1. Chemicals

Alloxan and streptozotocin (STZ) were purchased from Sigma–Aldrich Co., USA. Glucose, total cholesterol (TC), high-density lipoprotein (HDL) and triglyceride (TG) were assayed using standard kits from Ranbaxy Diagnostics, New Delhi, India. One-touch gluco-meter (Accu-chek sensor) of Roche Diagnostics, Germany, and Uristix of Bayer Diagnostics India Ltd. were used in the experiment.

2.2. Animals

Wistar strain of rats, weighing about 150–200 g and albino rabbits, weighing about 1–1.25 kg, were obtained from the National Institute of Communicable Diseases (NICD), Delhi, India and used in the experiments. Animals were kept in our animal house at room temperature of 25–30 °C and at 45–55% relative humidity for 12 h, each of dark and light cycle. Animals were fed on pellet diet of Golden feed, Delhi, India and water ad libitum. The study was approved by the Animal Ethical Committee of the Institute.

2.3. Preparation of the plant extract

Leaves of *Annona squamosa* were collected in the month of April and May from the gardens of Indian Agriculture Research Institute, New Delhi, India and was identified by Prof. C.R. Babu, Taxonomist, Department of Botany, University of Delhi, Delhi, India. A voucher specimen has been kept at the herbarium of the University. The leaves were washed with water and shade-dried. About 500 g of crushed leaves were extracted twice with 5 l of boiling ethanol for 6 h. The resulting extract was cooled and filtered. The filtrate was evaporated in vaccum to give a residue (yield: 8.2% w/w).

2.4. Biochemical parameters

Blood glucose, total cholesterol, HDL-cholesterol and triglyceride levels in serum were measured spectrophotometrically by methods prescribed by the manufacturer (Allain et al., 1974; Buccolo and David, 1973). Urine sugar was detected by reagent-based uristrix from Bayer.

2.5. Induction of experimental diabetes

A freshly prepared solution of STZ (50 mg/kg) in 0.1 M citrate buffer, pH 4.5 was intraperitoneal injected to overnight fasted rats (Brosky and Logothelopoulos, 1969). Similarly, intravenous injection of alloxan (80 mg/kg) was used for inducing diabetes in rabbits. FBG level was estimated at the time of induction of diabetes and postprandial blood glucose (PPG) was checked regularly up to stable hyperglycemia, usually after one week with STZ and two weeks with alloxan. Depending on their glucose levels, the animals were allotted in three groups:

(i) Sub-diabetic animals with nearly normal FBG of 80–120 mg/dl but showing abnormal glucose tolerance.
(ii) Mild-diabetic animals with FBG of 120–250 mg/dl.
(iii) Severely-diabetic animals showing FBG above 250 mg/dl.

2.6. Experimental design

Initial screening of the extract for the hypoglycemic activity was done in normal healthy rats by conducting glucose tolerance test (GTT). The antidiabetic effect was assessed in diabetic animals by two methods: (i) by studying the effect of different doses of the ethanolic extract on blood glucose levels of sub-diabetic and mild-diabetic rabbits during GTT. The most effective dose was again tested on STZ-induced

sub-diabetic rats for glucose tolerance, and (ii) by giving the extract (350 mg/kg) daily for 10 days to STZ-diabetic rats and for 15 days to severely-diabetic rabbits and studying their effects on FBG, postprandial blood glucose (PPG) level and urine sugar. Total lipid profile was estimated to assess the effect of ethanolic extract on abnormal lipid profile seen in severely-diabetic rabbits. An attempt was made to determine the LD_{50}.

2.6.1. Assessment of hypoglycemic activity in normal healthy rats

Five groups of six rats in each were used in the experiment. Group 1 served as untreated control and animals of groups 2, 3, 4 and 5 received different doses of plant extract (200, 300, 350 and 400 mg/kg, respectively). In overnight fasted rats, initial fasting blood samples were taken and then different doses (200, 300, 350, 400 mg/kg) of the ethanol extract were given orally to different groups of animals and their effect on FBG was studied hourly up to 2 h. The animals were then orally administered 3 g/kg of glucose solution and glucose tolerance was studied at 1 h intervals for another 3 h. Thus, the total period of blood collection was up to 5 h.

2.6.2. Assessment of antidiabetic activity by GTT in diabetic rabbits and rats

Different doses (200, 300, 350 and 400 mg/kg) of extract and the standard drug tolbutamide (350 mg/kg) were assessed to find out the effective dose. GTT-based activity testing was carried out on sub-diabetic and mild-diabetic rabbits (Babu et al., 1988) and STZ-diabetic rats, in which the same animals served as their own control. Five groups of six rabbits each were used in the experiment. In overnight-fasted diabetic rabbits, the initial fasting blood samples were drawn from ear vein (for FBG) and then water was orally given. After 90 min, blood was drawn again that gives the '0 h' value for blank GTT. The animals were then given a glucose solution (2 g/kg) orally and blood samples were drawn at 1, 2 and 3 h after glucose administration to get the GTT pattern of the untreated-diabetic rabbits (control). After a week, same animals were again fasted overnight. Fasting blood samples were drawn and different graded doses (200, 300, 350 and 400 mg/kg) of plant extract and tolbutamide were administered to different groups. After 90 min, blood samples were drawn again. This serves as '0 h' sample of the treated-diabetic rabbits. The animals were given glucose solution (2 g/kg) orally and blood samples were drawn at 1, 2 and 3 h after glucose administration to get the GTT pattern of the treated-diabetic rabbits (same animals). Glucose tolerance studies were also carried out with a dose of 350 mg/kg of the extract in STZ-induced diabetic rats.

2.6.3. Treatment of diabetic rats

Three groups of five rats each were used in the experiment. Group 1 served as normal healthy control group and group 2 as diabetic-untreated control. Group 3 was treated daily with a dose of 350 mg/kg extract for 10 days. At the beginning and end of the experiment, FBG and urine sugar (US) were estimated. Postprandial blood glucose (PPG) was estimated daily during the treatment period.

2.6.4. Treatment of severely-diabetic rabbits

Three groups of six rabbits each were used in the experiment. Group 1 served as normal healthy control group and group 2 as diabetic-untreated control. Group 3 was treated daily with a dose of 350 mg/kg extract for 15 days. At the beginning and end of the experiment, blood and urine were collected from fasted animals. FBG, PPG, TC, HDL cholesterol and TG were estimated, and LDL and VLDL cholesterol were calculated. Urine sugar was also assessed.

2.7. LD_{50} experiment

Four groups of rats of both sex (6 animals per group, 3 females and 3 males), weighing about 150–180 g were orally administered by a single dose of 875 mg/kg, 1.750, 3.5 and 5.250 g/kg of ethanolic extract of *Annona squamosa* leaves. Then rats were observed for gross behavioural, neurologic, autonomic, and toxic effects continuously. Food consumption, faeces and urine were also examined at 2 h and then at 6 h intervals for 24 h.

2.8. Statistical calculations

Data were expressed as mean ± S. D. for all experiments and significant differences between groups were calculated according to Student's two-tailed t-test. Values corresponding to $p < 0.05$ were considered statistically significant.

3. Results

3.1. Effect in normal healthy rats

In order to know the optimum effective dose of the ethanolic extract of *Annona squamosa* on FBG and glucose tolerance, different doses of the ethanolic extract were given to overnight-fasted healthy rats. The extract at doses of 200 and 300 mg/kg did not had any appreciable effect on the FBG, but there was a slight improvement in glucose tolerance of about 10.6 and 13.8% at 1 h peak value (3 h in Table 1), respectively. A dose of 350 mg/kg ethanol extract brought about a slight fall (6.0%) in FBG after 1 h of the extract administration, and improved glucose tolerance by 17.1% at the 1 h peak value (3 h in Table 1) when compared to control rats which received equal volume of water instead of extract. The higher dose of 400 mg/kg had more or less the same effect as that of 350 mg/kg. It therefore appears that 350 mg/kg of the ethanol extract of *Annona squamosa* leaves is the most effective dose on FBG and GTT of healthy rats. One of the reasons why even with the optimal dose of 350 mg/kg, the fall was only 17.1% could be that normal regulatory mechanisms operate to prevent hypoglycemia in normal animals.

Table 1
Effect of different doses of *Annona squamosa* leaves ethanol extract on the fasting blood glucose and glucose tolerance in normal healthy rats (mean ± S.D.)

Group ($n = 6$)	Treatment (mg/kg)	Blood glucose level (mg/dl)					
		0 h	1 h	2 h[a]	3 h	4 h	5 h
1	Normal control	76.0 ± 9.6	74.5 ± 7.6	77.0 ± 7.4	108.0 ± 6.7	96.0 ± 7.1	77.0 ± 5.9
2	200	77.0 ± 7.9	75.5 ± 7.8	78.0 ± 7.9	96.5 ± 6.5	87.0 ± 6.9	75.0 ± 6.3
3	300	77.0 ± 7.5	73.0 ± 7.1	78.0 ± 6.3*	93.0 ± 6.1*	84.0 ± 6.7*	71.0 ± 6.8
4	350	76.5 ± 8.9	70.0 ± 6.6*	72.5 ± 5.4*	89.5 ± 6.3*	81.0 ± 6.9*	69.0 ± 5.9
5	400	75.0 ± 9.3	69.5 ± 8.0	74.0 ± 7.8*	91.5 ± 6.8*	82.0 ± 6.4*	68.0 ± 6.8

[a] 3 g/kg of glucose solution given orally.
* $p < 0.01$ when compared with control group.

The maximum fall in FBG was after 1 h and maintained up to 2 h during fasting. So in further experiments, effect of the extract on FBG was observed after 90 min of the extract administration considering that it takes a minimum of 1 h to act and then GTT was performed by giving glucose solution.

3.2. Effect on diabetic rabbits and rats during GTT

In order to choose the optimum dose for the diabetic animals, different doses of ethanolic extract (200, 300, 350 and 400 mg/kg) were evaluated on glucose tolerance in diabetic rabbits along with the standard drug tolbutamide (350 mg/kg). The rabbits were treated with the extract and improvement in GTT was assessed by comparing the blood glucose level (BGL) before and after the treatment. A dose of 200 and 300 mg/kg of ethanolic extract reduced FBG by 9.8 and 18.2% (Table 4) within 90 min of the extract administration (0 h of GTT), while after 1 h of glucose administration (1 h of GTT), the BGL was reduced by 14.5 and 31.2%, respectively. A reduction of 16.4% was observed in 2 h of GTT with 200 mg/kg, while this fall increased further to 28.3% with 300 mg/kg of extract. A dose of 350 mg/kg significantly ($p < 0.001$) reduced FBG by 26.8% at 0 h and 38.5 and 40.6% at 1 and 2 h, respectively (Table 4). The higher dose of 400 mg/kg had about the same effect as that of 350 mg/kg. It therefore appears that 350 mg/kg of the ethanol extract of *Annona squamosa* is the effective dose on FBG and GTT of alloxan-induced diabetic rabbits. Therefore, this dose was assessed by GTT in STZ-induced diabetic rats. A significant ($p < 0.001$) reduction of 37.2% and 60.6% during 1 and 2 h of GTT was observed with the dose of 350 mg/kg (Fig. 1). A dose of 350 mg/kg of tolbutamide reduced FBG by 22.8, 38.2 and 33.8% at 0, 1 and 2 h respectively, during GTT.

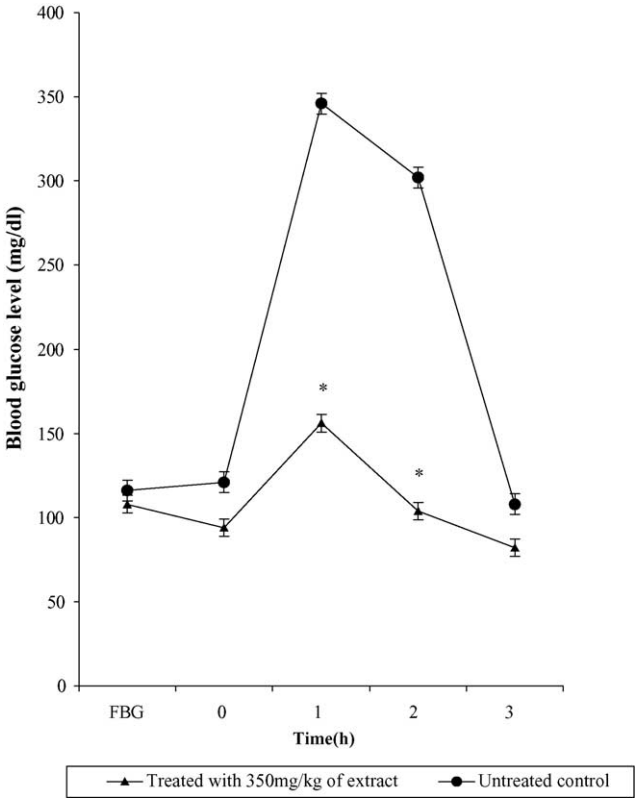

Fig. 1. Effect of a single oral dose of *Annona squamosa* leaves extract (350 mg/kg) on blood glucose levels during glucose tolerance test in streptozotocin-diabetic rats. * $p < 0.01$ when compared with control group.

3.3. Effect on BGL and urine sugar of diabetic rats

After 10-day treatment of diabetic rats with extract, there was a 73.3% fall in FBG. The animals returned to normal

Table 2
Effect of 10-day treatment with ethanol extract of *Annona squamosa* leaves on the fasting blood glucose (FBG), postprandial glucose (PPG) and urine sugar (US) of streptozotocin-diabetic rats

Groups	Initial			Final			% change		
	FBG	PPG	US	FBG	PPG	US	FBG	PPG	US
Normal control	78.0 ± 6.8	108.0 ± 7.8	0	77.0 ± 5.9	105.0 ± 7.6	0	0	0	0
Diabetic control	238.0 ± 5.6	437.0 ± 5.2	++	298.0 ± 5.4	515.0 ± 6.2*	++	0	0	0
Diabetic treated	255.0 ± 5.8	440.0 ± 5.6	++	68.0 ± 9.6*	255.0 ± 7.8*	0	73.3	42.0	50.0

$n = 5$ in each group.
* $p < 0.001$ when compared with initial (before treatment) values.

Table 3
Effect of 15-day treatment with ethanol extract of *Annona squamosa* leaves on the fasting blood glucose (FBG), postprandial glucose (PPG), urine sugar (US) and lipid profile of alloxan induced severely-diabetic rabbits

Parameters	Normal control		Diabetic control		Diabetic treated		% change
	Initial	Final	Initial	Final	Initial	Final	
FBG (mg/dl)	72.4 ± 5.2	76.2 ± 4.4	277.4 ± 5.8	305.8 ± 6.2	364.5 ± 6.0	172.0 ± 5.2	52.7
PPG (mg/dl)	105.2 ± 8.0	106.5 ± 8.5	512.4 ± 5.6	436.7 ± 5.4	548.0 ± 6.2	257.0 ± 5.8	53.1
US (g/l)	0	0	+4	+4	+4	+1	75.0
TC (mg/dl)	42.0 ± 5.2	46.0 ± 4.6	176.0 ± 4.6	174.0 ± 5.2	221.0 ± 6.2	112.0 ± 5.8*	49.3
HDL (mg/dl)	17.0 ± 4.9	19.0 ± 4.6	40.0 ± 4.8	21.0 ± 5.4	32.4 ± 6.2	46.5 ± 5.4*	30.3
LDL (mg/dl)	8.2 ± 5.2	11.0 ± 4.8	108.4 ± 4.6	122.6 ± 4.2	159.4 ± 5.2	44.7 ± 4.8*	71.9
VLDL(mg/dl)	16.8 ± 4.8	16.0 ± 4.4	27.6 ± 4.4	30.4 ± 5.0	29.2 ± 5.9	20.8 ± 5.7*	28.7
TG (mg/dl)	84.0 ± 5.4	80.0 ± 5.1	138.0 ± 5.2	152.0 ± 5.5	146.0 ± 6.4	104.0 ± 7.8*	28.7

$n = 6$ in each group; values are presented as mean ± S.D.
* $p < 0.001$ when compared with initial (before treatment) values.

Table 4
Effect of the ethanolic extract of *Annona squamosa* leaves (200–400 mg/kg) and tolbutamide (350 mg/kg) on the glucose tolerance in sub-diabetic and mild-diabetic rabbits

Group	Extract dose (mg/kg)	Blood glucose level (mg/dl)				
		FBG	0 h	1 h	2 h	3 h
Control	0	93.0 ± 5.8	90.0 ± 6.1	220.0 ± 6.8	181.0 ± 6.3	119.0 ± 6.9
Treated	200	91.0 ± 4.6	82.0 ± 5.4	188.0 ± 6.6**	168.0 ± 5.8*	112.0 ± 7.5
Control	0	128.0 ± 5.2	132.0 ± 6.6	314.0 ± 7.4	212.0 ± 5.2	144.0 ± 6.4
Treated	300	132.0 ± 4.8	108.0 ± 4.2	216.0 ± 5.8**	152.0 ± 4.7**	129.0 ± 6.8
Control	0	132.0 ± 5.9	141.0 ± 3.8	306.0 ± 6.9	192.0 ± 4.8	104.0 ± 5.6
Treated	350	138.0 ± 3.7	101.0 ± 4.2	188.0 ± 5.4*	114.0 ± 5.8*	96.0 ± 4.8
Control	0	118.0 ± 4.8	122.0 ± 4.1	298.0 ± 5.4	191.0 ± 5.6	138.0 ± 5.8
Treated	400	124.0 ± 4.6	105.0 ± 5.2	194.0 ± 6.2**	152.0 ± 4.8*	114.0 ± 4.7
Control	0	108.0 ± 5.2	104.0 ± 6.1	252.0 ± 6.7	148.0 ± 5.1	112.0 ± 5.9
Treated	350 (Tolbutamide)	116.0 ± 4.5	91.0 ± 5.3	158.0 ± 6.4*	112.0 ± 4.1*	96.0 ± 3.6

Values are presented as mean ± S.D. FBG: Fasting blood glucose.
* $p < 0.001$ compared with their control.
** $p < 0.01$ compared with their control.

blood sugar level (from 255 ± 5.8 mg/dl to 68 ± 9.6 mg/dl). To check the effect of extract on the postprandial glucose (PPG) level of diabetic rats, PPG was estimated regularly during the treatment period. A 42% fall in PPG was observed after the treatment and also no urine sugar was observed, while initially, it was 1.5 g/l (Table 2).

3.4. Effect on FBG, lipid profile and urine sugar of severely-diabetic rabbits

It was intended to assess the effect of long-term treatment on BGL, urine sugar and associated abnormal lipid profile in alloxan-induced severely-diabetic rabbits. Rabbits were treated with 350 mg /kg of ethanol extract once a day in the morning for 15 days. At the end of the treatment, the animals were compared with their own initial values and significant reduction ($p < 0.001$) of 52.7, 53.1 and 75% in FBG, PPG and urine sugar (US), respectively (Table 3) was observed. The various parameters of blood lipid profile of severely-diabetic rabbits were tested before and after the treatment. The enhanced levels of TC, LDL, VLDL cholesterol and TG were brought down significantly ($p < 0.001$) after the treatment period. A 49.3% fall in TC, 71.9% decrease in LDL cholesterol and 28.7% fall in TG was observed in treated-diabetic rabbits. There was also an increase of 30.3% in HDL cholesterol in the treated-diabetic rabbits (Table 3). At the end of the experiment, food intake and body weight were normal.

3.5. LD_{50}

Experiment was carried out on normal healthy rats. The behaviour of the treated rats appeared normal. No toxic effect was reported at doses up to 10 and 15 times of effective dose of the ethanol extract and there was no death in any of these groups. The consumption of food was increased by 20% in 10 and 15 times doses treatment within 2 h and it became normal afterwards; body weight was also normal.

4. Discussion

The present study for the first time reports the hypoglycemic and antidiabetic effects of an ethanolic extract of *Annona squamosa* leaves. Antidiabetic effect of hot aqueous

extract of its leaves was observed by our research group for the first time in 2002 and got the patent filed in 2003 (Tandon et al., 2003). Subsequently, antidiabetic effect was also observed in its cold aqueous extract (Shriwaikar, 2004).

The study reveals that the maximum hypoglycemic effect was produced within 1 h during GTT (Table 1). This indicates that it takes about 1 h for the active ingredient(s) or its (their) metabolites in the ethanol extract to enter into the circulation and target tissues to bring about hypoglycemic effect, which is maintained for at least 3 h. Different doses of the ethanol extract were assessed on BGL of alloxan-diabetic rabbits during GTT, and the most effective dose was found to be 350 mg/kg. The effect of 350 mg/kg of ethanolic extract was better than the effect of the same dose of the standard drug tolbutamide during GTT. Maximum improvement in glucose tolerance, as indicated by reduction in peak blood glucose levels was observed at 1 and 2 h during GTT. Treatment of STZ-induced diabetic rats for 10 days (Table 2) and alloxan-induced diabetic rabbits (Table 3) for 15 days brought down the elevated blood glucose levels ranging from 250 to 350 mg/dl to nearly normal range.

Alloxan not only destroys the pancreatic β-cells but also damages the kidney. The effect is however reversible. STZ selectively destroys pancreatic insulin-secreting β-cells (Goldner and Gomori, 1943; Hofteizer, 1973) causing diabetes close to type 2 diabetes of humans. The elevated blood glucose levels in the diabetic animals used by us were in the range of 150–350 mg/dl which resembles both type 2 diabetes (150 to about 250 mg/dl) with partially functional pancreas, as well as type 1 (above 300 mg/dl) with considerable pancreas damage. This shows that the ethanol extract of *Annona squamosa* might be useful in both type 1 and 2 diabetes. This is an additional advantage of the extract over the existing sulfonylurea drugs, which act only when there is functional pancreas (Sharma et al., 1997; Ivorra et al., 1989). It is well known that in uncontrolled type 2 diabetes mellitus, there will be an increase in TC, LDL and VLDL cholesterol and triglyceride with decrease in HDL cholesterol which contribute to the coronary artery disease (Arvind et al., 2002; Palumbo, 1998). From this point of view, it is encouraging that the 15-day treatment of ethanol extract brought down the elevated levels of TC, LDL and VLDL cholesterol and TG in diabetic animals to nearly normal level (Table 3). There was increase in HDL cholesterol also, which was a desirable feature.

The LD_{50} of the extract is high (no death even with 15 times of effective dose) indicating high margin of safety. These results clearly indicate the possible usefulness of ethanolic extract of *Annona squamosa* leaves in diabetes mellitus.

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