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Original Research Article

ANTIDIABETIC POTENTIAL OF ALCOHOLIC EXTRACT OF *ANNONA SQUAMOSA* LINN. BARK IN NORMAL AND ALLOXAN INDUCED DIABETIC RATS

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Abstract: The present investigation aims to examine the diabetic potential of the plant sareefa in normal and alloxan induces diabetic rats. The barks were successively macerated with Pet. Ether (60-80), chloroform, ethyl acetate, ethanol and water. The ethanolic extract showing the more potent chemical screening results were selected and prepared through Maceration. Alcoholic extract of bark of *Annona squamosa* at dose of 300 mg/Kg was given to normal and alloxan induced diabetic rats and blood samples taken from the retero-orbital plexus vein were analyzed for blood glucose level as per standard protocol with available haemoglucostrips of TOUCH blood glucometer. The comparison of blood sugar levels as per model schedule shows that in normal group the ethanolic extract, at a dose of 300 mg/Kg intra-peritoneal, the blood glucose lowering was shown which conclude, the ethanolic extract of *Annona squamosa* reflected anti-diabetic potential through its glucose lowering activity in experimental animals. It supported the folklore claim of anti-dibetic activity of plant.

Keywords: *Annona squamosa*, antidiabetic, herbal remedy, sareefa,

Introduction: Diabetes mellitus elevate glucose in plasma and ketoacidosis, excessive urine excretion, include excessive thirst, glucosuria, polyuria, lipemia and hunger. If not treated patient suffer with fatal ketoacidosis.(1) Diabetes insipidus and brittle diabetes are other form of diabetes.(2) diabetes mellitus patient having fasting glucose level in excess of 140 mg/dL and plasma glucose level in excess of 200 mg/dL at two times as glucose tolerance test (GTT), one within hours of two hours of ingestion of glucose.(3) The oxidative stress is present due to increased production of oxygen free radicals and a sharp reduction of antioxidant defenses. (4) first appearance of diabetes in Indian literature was in the work of the physician Susruta (6th century BC) and in Charaka Samhita(5) Diabetes required lot of attention in India Earlier known as "rich man's disease", as India will become capital of world by 2025, as 2000 about 24 million person were

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affected and this will reach 57.02 million by 2015.(6) now a day about 15-20 % of the patient's with NIDDM diagnosis shows very little or no response to sulphonyl ureas and about 3% of patients develops adverse effect.(7) However, large number of herbal having literature support showing hypoglycemic/anti-hyperglycemic properties available in very low cost, and safe and faithful.(8) Diabetes mellitus (especially Type 2 diabetes) affects a considerable section, Traditional or folk medicinal practitioners, use a variety of medicinal plant parts to treat this disease,

General Information: *Annona squamosa* Linn. (Annonaceae) originated from West Indies and South America, Cultivated in Thailand and found in India.(9) Mainly grown in gardens for its fruits and ornamental value. It is mainly known as Seetaphalam, Sudha in Sanskrit, Sharifa, Ata in Hindi, Athachakka in Malyalam and Ghista in Arabic.(10-12) *A. squamosa* is beneficial for cardiac disease, diabetes hyperthyroidism and cancer. The root is found drastic purgative and dried unripe fruit powder is used to destroy vermin. The seeds are acrid and poisonous.(13,14)

Materials and methods

Drugs and chemicals used: Bovine serum albumin (Sigma chemical St. Louis, MO, USA), thiobarbituric acid, nitro blue tetrazolium chloride (NBT), hemoglobin (Loba Chemie, Mumbai, India), trichloro acetic acid (Merck Ltd, Mumbai, India), 5,5'-dithio bis-2-nitrobenzoic acid (DTNB) were used. All the solvents were of analytical grade and purchased from local market.

Animals: Wistar albino rats of either sex, weighing 180-250 g. The selected animals were housed in acrylic cages in standard environmental conditions (25-30 °C). They were allowed free access to standard dry pellet diet and water ad libitum. All experiments were carried out as per the guidelines of the Institutional Animal Ethical Committee of Scientific and Applied Reserch Center, East marredpally, secunderabad, India (Approval No: SIP/CPCSEA/IAEC/2013/02).

Collection and authentication of plant material

The plant material was collected from wild sources around Babhnan, Gonda, authenticated by National Botanical Research Institute (Council of Scientific and Industrial Research) Rana Pratap Marg Lucknow (Ref No: NBRI/CIF/413/2013) and the voucher specimens were deposited in department herbarium for future reference. The bark was shade dried at room temperature; the dried bark were subjected to size reduction to coarse powder by using dry grinder (Philips, India) and passed through the sieve before stored in a closed vessel for further use.

Preparation of extract: The powdered plant material (400 g) was defatted with petroleum ether (60-80 °C) and then extracted with 1.5 litre of ethanol (95%) in a soxhlet apparatus. The solvent was removed under reduced pressure by rotary vacuum evaporator, which obtained a greenish-brown sticky residue (yield: 11.6% w/w with respect to dried plant material). Aqueous extracts were prepared by using distilled water as solvent for the experiment. The dried extract was stored in a desiccator till further study.

Preliminary phyto-chemical screening: The weeds extracts of *Annona squamosa* were subjected to qualitative tests for the identification of various active constituents viz. carbohydrate, glycoside, alkaloid, amino acids, flavonoids, fixed oil, tannins, gum and mucilage and phyto-sterols using standard test procedure. (15,16)

Acute toxicity study: the acute toxicity studies were conducted using Wistar albino rats of either sexes taking the weed extract at various dose levels (5, 50, 300, 2000 mg/Kg), by adopting fixed dose method as per the OECD guidelines. (17) The animal were observed continuously for 2 hours and then occasionally for further 4 hours and finally overnight mortality/survival was recorded and LD_{50} was extapoated graphically.(18)

Screening for ant diabetic activity: The method of Joy and Kuttan was followed (19). The acclimatized animals were kept fasting for 24 h with water ad libitum and injected intraperitoneally a dose of 150 mg/kg of alloxan monohydrate in normal saline. After one hour, the animals were provided feed ad libitum. The blood glucose level was checked before and 72 h after alloxan injection. The animals were considered diabetic when the blood glucose level was raised beyond 300 mg/dl of blood. This condition was observed at the end of 72 h after alloxan injection.

Effect on oral glucose tolerance in rats: After overnight fasting, a 0-min blood sample was taken from the tip of the tail of each rat of different groups under mild ether anesthesia. Without delay a glucose solution (2 g/kg) was administered by a gavage. Four more samples were taken at 30, 60, 90 and 120 min after glucose administration (20). All blood samples were taken for the estimation of the blood glucose. Estimation of blood glucose was carried out with the haemoglucostrips supplied by M/s Lifescan, Inc. USA with the help of a Johnson & Johnson ONE TOUCH blood glucometer.

Single dose study: The animals were segregated into five groups of six rats in each. Group I and II rats were randomly selected from normal rats that received only distilled water and the extract (300 mg/kg, p.o.) respectively. Group III to Group V animals were selected from the alloxanised rats. Group III animals served as diabetic control. Group IV animals received glibenclamide (600 µg/kg) and group V was treated with the extract (300 mg/kg) in a similar manner. Blood samples were collected from the tip of tail of each rat under mild ether anesthesia at 0 h, 1 h, 2 h and 4 h after the administration of test samples and tested for glucose concentration as above.

Multidose study: For multidose study, administration of test samples was continued for 10 days, once daily through oral route. Blood samples were collected from the tip of tail and the estimation of blood glucose was carried out as above on the 1, 3, 7 and 10 day of the drug administration. Body weights of all the animals were recorded just prior to and on the 10th day of the study to determine the change in the body weight, if any.

Statistical analysis: Statistical significance was determined by one way analysis of variance (ANOVA) followed by Dunnet's t-test. $P<0.05$ indicates significant difference between group means.

Results and Discussion: Table 1 shows the blood glucose level of normal and experimental animals after oral administration of glucose (2 g/kg). Extract as well as standard drug treated animals showed more significant decrease in peak blood glucose level after 1 h. After 2 h, the extract treated animals tended to bring the values near normal. The results of Table 2 reveals that the extract produced significant decrease in the blood glucose level when compared with the controls in a lloxan induced hyperglycaemic rats in the single dose experiment at the tested dose level and is comparable with the standard drug glibenclamide. In the multi dose study (Table 3), the test extract constantly maintained significant reduction of the glucose level in diabetic rats throughout the experimental period suggesting the antihyperglycaemic property of the extract. Diabetes mellitus causes failure to use of glucose for energy that leads to increased utilization and decreased storage of protein responsible for reduction of body weight essentially by depletion of the body proteins (21). In the present study, it was observed that the ethanolic extract reversed the weight loss of the diabetic rats and they returned to near normal. During diabetes the excess glucose present in the blood reacts with hemoglobin to form glycosylated hemoglobin. The rate of glycosylation is directly proportional to concentration of blood glucose and with improvement of glycemic control glycosylated hemoglobin also decreases (22). Hence the estimation of glycosylation of hemoglobin is a

well established parameter useful in the management and prognosis of the disease (23). Our study gave a clear view that the ethanolic extract prevented significant elevation of glycosylated hemoglobin in vitro, with IC50 value being 11.25 µg/ml that is comparable with the reference drug α- tocopherol (Table 4). Further, since the non-enzymatic glycosylation of hemoglobin is an oxidative reaction (24). Literature evidence suggest that oxidative stress play a major role in pathogenesis of diabetes mellitus (25) and flavonoids from plants are reported to possess antidiabetic(26) and free radical scavenging activity(27). Since, Bark of *Annona squamosa* contain 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 antidibetic drugs and it may cause more effective in combination and may be beneficial with respect severe another side effects of allopathic drugs.

Table 1. Effect of ethanolic extract of *Annona squamosa* (300 mg/kg, p.o.) on oral glucose tolerance test (OGTT) in normal and alloxan induced diabetic rats.

S.N.	Groups	Treatment	Groups Treatment Blood sugar level (mg/dl)				
			Fasting	30 min	60 min	90 min	120 min
1	I	Normal	77.00 ±0.83	149.92±3.18	172.61 ± 2.19	121.33 ±2.69	84.56±2.05
2	II	Normal + Extract	74.93±0.74 ns	146.59±1.54ns	180.43±2.72ns	128.32±3.33ns	89.16±3.2ns
3	III	Diabetic control (Alloxan only)	249.45±3.93*	328.51±3.18*	369.11±4.83*	312.62±3.23*	311.58±2.53*
4	IV	Diabetic + Exrtact	74.32±1.88*	147.40±2.74*	173.63±3.90*	125.35±2.59*	84.72±1.50*
5	V	Diabetic + Glibenclamide	75.96±3.89*	159.59±3.63*	185.51±2.30*	123.72±2.62*	86.23±1.62*

Values are mean ± SEM for n=6; *P < 0.05 = significant; NS = Not significant; Group II and III are compared with group I while Group IV and V are compared with group III.

Table 2. Effect of single dose treatment of ethanolic extract of *Annona squamosa* (300 mg/kg, p.o.) on blood glucose level in normal and alloxan induced diabetic rats.

S.N.	Groups	Treatment	Blood glucose level (mg/dl)			
			Basal Value	1 h	2 h	4h
1	I	Normal	75.28 ±0.52	74.08±0.51	74.17±0.33	74.52±0.83
2	II	Normal + Extract	76.13±0.29ns	75.42±0.73ns	75.04±0.26ns	74.24±0.62ns
3	III	Diabetic control (Alloxan only)	346.73±2.77*	349.55±2.31*	346.72±2.61*	346.19±2.21*
4	IV	Diabetic + Glibenclamide	343.17±4.11ns	319.49±5.11*	300.72±3.82*	290.82±3.50*
5	V	Diabetic + Exrtact	338.56±3.18ns	272.12±4.33*	266.72±3.23*	248.72±3.12*

Values are mean ± SEM for n=6; *P < 0.05 = significant; NS = Not significant; Group II and III are compared with group I while Group IV and V are compared with group III.

Table 3. Effect of multiple dose treatment of ethanolic extract ofAnnona squamosa (300 mg/kg, p.o., once daily) on blood glucose level and change in body weight after 15 days in normal and alloxan induced diabetic rats

S.N.	Groups	Treatment	Blood glucose level (mg/dl)					
			Basal Value	Day 1	Day 3	Day 7	Day 10	Change in body weight (g)
1	I	Normal	75.28 ±0.52	76.09±0.25	75.38±0.92	76.35±0.62	76.32±0.56	(+)10.09±1.57
2	II	Normal + Extract	76.13±0.29ns	75.00±0.83ns	75.52±0.38ns	74.15±0.63ns	73.16±0.27*	(+)10.00±0.38ns
3	III	Diabetic control (Alloxan only)	346.73±2.77*	351.41±2.59*	356.24±3.67*	355.12±3.44*	351.22±3.49*	(-)7.42±0.79*
4	IV	Diabetic + Glibenclamide	343.17±4.11ns	273.14±3.04*	238.59±3.71*	212.13±4.58*	209.55±3.62*	(+)9.90±0.89*
5	V	Diabetic + Exrtact	338.56±3.18ns	214.27±3.03*	218.44±3.38*	209.31±2.68*	189.17±1.42*	(+)9.42±1.38*

Values are mean ± SEM for n=6; *P < 0.05 = significant; NS = Not significant; Group II and III are compared with group I while Group IV and V are compared with group III.

Table 4. Effect of ethanolic extract of *Annona squamosa* on percent inhibition of hemoglobin glycosylation in vitro.

S. No.	Groups	Treatment	Blood glucose level (mg/dl)			
			Basal Value	1 h	2 h	4h
1	I	Normal	75.28 ±0.52	76.09±0.31	75.13±0.62	75.97±0.84
2	II	Normal + Extract	76.13±0.29ns	75.69±0.44ns	75.92±0.57ns	73.35±0.83ns
3	III	Diabetic control (Alloxan only)	346.73±2.77*	350.22±2.36*	347.70±2.67*	349.09±2.36*
4	IV	Diabetic + Glibenclamide	343.17±4.11ns	323.13±3.47*	294.70±3.78*	272.81±3.41*
5	V	Diabetic + Exrtact	338.56±3.18ns	289.88±3.63*	262.18±3.06*	258.12±2.62*

Values are Mean ± S.D. for n=3; r = regression co efficient.

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