

Biochemical study on the hypoglycaemic effects of extract and fraction of *Acacia catechu* Willd in alloxan-induced diabetic rats

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Abstract

Various extracts including petroleum ether, chloroform, acetone, ethanol, aqueous and crude aqueous of barks of *Acacia catechu* (*A. catechu*) Willd (Leguminosae) and the two fractions of ethanolic extract were tested for antihyperglycaemic activity in glucose-loaded hyperglycaemic rats. The effective extract and fraction of *A. catechu* were subjected to anti-diabetic study in alloxan-induced diabetic rats at two dose levels, 200 and 400 mg/kg, respectively. Biochemical parameters, including glucose, urea, creatinine, serum cholesterol, serum triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL), haemoglobin and glycosylated haemoglobin were also assessed. The ethanolic extract of *A. catechu* and the water insoluble fraction of ethanolic extract exhibited significant anti-hyperglycaemic activity and produced dose-dependent hypoglycemia in fasted normal rats. Treatment of diabetic rats with ethanolic extract and water-insoluble fraction of this plant restored the elevated biochemical parameters significantly ($p < 0.05$) to the normal level. Comparatively, the water insoluble fraction of ethanolic extract was more effective than the ethanolic extract and the activity was comparable to that of the standard, glibenclamide (5 mg/kg).

Keywords: *Acacia catechu* Willd, antidiabetic activity, hypoglycaemic activity, alloxan, lipid profile.

Introduction

Diabetes mellitus, a chronic metabolic disorder, has now become an epidemic with a worldwide incidence of 5% in the general population. The number of people suffering from diabetes has soared to 246 million and the disease now kills more people than AIDS.¹ Decreased physical activity, increasing obesity, stress and changes in food consumption have been implicated in its increasing prevalence over the past two decades.² Overt diabetes affects 2–3% of the total world population. In conventional therapy, type 1 diabetes is treated with exogenous insulin and type 2 with oral hypoglycaemic agents (sulphonylureas, biguanides).³ Though different types of oral hypoglycaemic agents are available along with insulin for the treatment of diabetes, there is an increase demand by patients to use the natural products with antidiabetic activity.⁴ Since time immemorial, patients with non-insulin requiring diabetes have been treated orally in folk medicine with a variety of plant extracts. In India, a number of plants are mentioned in ancient literature (Ayurveda) for the cure of diabetic conditions.

Acacia catechu Willd (Cutch tree) belonging to the family

Leguminosae is commonly used by many traditional healers in most of the herbal preparations for diabetes.⁵ Traditionally it is used as a thermogenic, digestive, appetizer, aphrodisiac, hepatoprotective, haemostatic, anthelmintic, depurative and tonic agent. It is also used in toothache, ulcerations, soreness of gums,⁶ asthma and bronchitis.⁷ It has been reported to possess antipyretic, antiarrhoeal, hypoglycaemic (wood), hepatoprotective,⁸ anthelmintic,⁹ antimicrobial¹⁰ and antioxidant activities.¹¹ The constituents reported in this plant are acacatechin, quercetin, quercitrin, pseudotannin, phlobotannin,⁸ epicatechin, catechin, catechutannic acid, tetramer, dicatechin, galocatechin, kaempferol, taxifolin, isorhamnetin and afzelechinn.⁶

Only the aqueous extract of barks of this plant is used in traditional herbal preparations. Moreover, researchers focus mainly on ethanol and aqueous extracts for diabetes, but considerable number of studies stated that the petroleum ether, benzene and chloroform extracts were also active against diabetes.¹²⁻¹⁴ Knowing the effective extract and isolating the active fraction from the effective extract is important in the development of new drugs. The standard fraction of an active extract may prove better therapeutically than the extract, less toxic and inexpensive compared to pure isolated compounds. Keeping these facts in mind, the present study was aimed to identify the active antidiabetic extracts of the above-mentioned plant prepared using various solvents, to identify the active antidiabetic fraction of the active extract and to study the effect of *A. catechu* in diabetes-associated complications.

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Materials and Methods

Plant material

Barks of *A. catechu* were collected in March 2006 from Tamil Nadu, India. The taxonomical identification of the plant was done by Dr. H.S. Chatree, Botanist, Government Arts and Science College, Mandasaur, India. The voucher specimen (BRNCP/A/008/2006) was deposited in the Herbarium of Department of Pharmacognosy, B. R. Nahata College of Pharmacy, Mandasaur.

Preparation of extracts

Dried and powdered plant material (500 g) was successively Soxhlet extracted with petroleum ether (60–80^o), chloroform, acetone, ethanol and water for 72 h each. Crude aqueous extracts of this plant was prepared separately by boiling the plant material (25 g) with 200 ml of water for 15 min. The obtained extracts were evaporated in vacuum to give residues. Percentage yield of various extracts are given in Table 1.

Fractionation of ethanolic extract

Fractionation of ethanolic extract was done using its solubility profile.^{15–17} Fifteen grams of dried ethanol extract was placed in a stoppered flask containing 200 ml of water and shaken mechanically for 1–2 h in a flask shaker. The ethanolic extract was not completely soluble in water. The water insoluble portion of the ethanolic extract was separated using filtration and both fractions (water-soluble and water-insoluble) were dried and their percentage yield with respect to ethanolic extract was determined (Table 1). The extracts and fractions that were not soluble in water were suspended in 1% Tween 80 before administration to rats.

Preliminary phytochemical screening

In order to determine the presence of alkaloids, glycosides, flavones, tannins, terpenes, sterols, saponins, fats and sugars, a preliminary phytochemical study (colour reactions) with various plant extracts and fractions was performed.^{18,19}

Experimental animals and treatment

Healthy Wistar rats of either sex (150–180 g) with no prior drug treatment were used for the present study. The animals were fed with commercial pellet diet (Kamadenu Agencies, Bangalore, India) and water *ad libitum*. The animals were acclimatized to laboratory hygienic conditions for 10 days before starting the experiment. Animal study was performed in the Division of Pharmacology, B. R. Nahata College of Pharmacy, Mandasaur, with approval from Institutional Animal Ethics Committee (Registration number: 918/ac/05/CPCSEA).

Acute toxicity studies

The acute toxicity test of the extracts and fractions was determined according to the OECD guidelines No. 420 (Organization for Economic Co-operation and Development). Female Wistar rats (150–180 g) were used for this study. After the sighting study, starting dose of 2,000 mg/kg of the test samples was given, *per os*, to various groups containing 5 animals in each group. The treated

animals were monitored for 14 days for mortality and various responses like behavioural, neurological and autonomic responses. No death was observed up to the end of the study. The test samples were safe up to the dose of 2,000 mg/kg and from the results, 400 mg/kg was chosen as the maximum dose for further experimentation.

Anti-hyperglycaemic activity in glucose-loaded hyperglycaemic animals

Antihyperglycaemic activity was studied in glucose-loaded hyperglycaemic rats.²⁰ Animals were divided into various treatment groups (n = 5) as mentioned in Tables 2 and 3. Glibenclamide (5 mg/kg) was used as the reference standard and the negative control group animals received only the vehicle. The remaining groups of rats were treated with 400 mg/kg of various extracts and fractions of the plant suspended in 1% Tween 80. Blood sugar level was determined from overnight fasted animals at 0 h. After 30 min of the drug treatment, animals were fed with glucose (4 g/kg) and blood glucose was determined 1/2, 1, 2, and 3 hours after glucose load. Blood glucose concentration was estimated by the glucose oxidase enzymatic method using a commercial glucometer and test-strips (Accu-chek ActiveTM Test meter, Basel, Switzerland).

Hypoglycaemic activity

Animals were classified into 6 groups (n = 5). Group 1 was kept as control and received a single dose of 0.5 ml/100 g of the vehicle, group 2 was treated with glibenclamide (5 mg/kg) as hypoglycaemic reference drug. Groups 3 to 6 were treated with ethanolic extract and water insoluble fraction of ethanolic extract at two dosage levels (200 and 400 mg/kg) as mentioned in Table 4. Blood samples were collected from the tail tip at 0 (before oral administration), 1/2, 1, 2, and 3 h after vehicle, samples and drug administration.²¹ The blood glucose level was measured using Accu-chek ActiveTM Test strips in Accu-chek ActiveTM Test meter.

Antidiabetic activity in alloxan-induced diabetic rats

Alloxan-induced diabetic model was selected to confirm the utility of active antihyperglycaemic extract and fraction in diabetic conditions. Diabetes was induced by injecting 120 mg/kg of alloxan monohydrate intraperitoneally in 0.9 % w/v NaCl to overnight-fasted rats. 10% glucose solution bottles were kept in their cages for the next 24 h to prevent hypoglycemia. After 72 h of injection, fasting blood glucose level was measured. Animals which did not develop more than 300 mg/dl glucose levels, were rejected.^{22,23} Diabetic animals were divided into 6 groups (n = 5) and one more group of normal non-alloxanised animals was also added in the study. Group 1 was kept as normal control (non-alloxanised rats), received a single dose of 0.5 ml/100 g of the vehicle, group 2 was kept as negative control, alloxan-induced and received a single dose of 0.5 ml/100 g of the vehicle, group 3, diabetic, was treated with glibenclamide (5 mg/kg) as reference drug. Groups 4 to 7, diabetic-induced were treated with ethanolic extract and water insoluble fraction of ethanolic extract that exhibited antihyperglycaemic and hypoglycaemic activity at two dosage levels (200 and 400 mg/kg) as mentioned in Table 5. Treatment was continued orally for 7 consecutive days. At

Table 1: Preliminary phytochemical studies and percentage yield of various extracts and fractions of *A. catechu*

Plant extracts	% yield (w/w)	Constituents
AC-P	0.90	Fats
AC-C	1.02	Steroids
AC-A	6.20	Tannins
AC-E	7.40	Carbohydrates, alkaloids, tannins, flavonoids and saponins
AC-Aq	9.50	Carbohydrates, tannins, flavonoids and saponins
AC-CAq	12.88	Carbohydrates, tannins, flavonoids, and saponins
E- WSF	57.20	Carbohydrates, tannins, flavonoids and saponins
E- WISF	41.00	Alkaloids, tannins and flavonoids

AC-*Acacia catechu*, P-Petroleum ether (60-80⁰), C-Chloroform, A-Acetone, E-Ethanol, Aq-Aqueous, CAq-Crude aqueous, WSF-Water soluble fraction, WISF-Water insoluble fraction.

Table 2: Effect of various extracts of *Acacia catechu* in glucose loaded hyperglycemic rats

Treatments	Dose mg/kg	Blood glucose concentration (mg/dl)				
		0 th h	1/2 h	1 h	2 h	3 h
Gluc. control	--	89.80 ± 3.02	144.40 ± 4.85	150.60 ± 4.01	124.40 ± 3.32	105.20 ± 4.77
Glibenclamide	5	94.20 ± 3.59	105.20 ± 3.49**	92.20 ± 4.60**	78.40 ± 4.20**	66.20 ± 3.68**
AC-P	400	86.80 ± 4.41	124.20 ± 8.23	112.20 ± 10.19	108.60 ± 10.60	101.00 ± 7.70
AC-C	400	95.00 ± 4.15	126.60 ± 6.80	88.40 ± 3.69**	89.60 ± 6.80**	92.40 ± 5.16
AC-A	400	90.80 ± 5.18	120.40 ± 10.20	132.60 ± 9.60	106.20 ± 8.20	98.20 ± 8.90
AC-E	400	95.80 ± 5.80	124.40 ± 8.80	87.20 ± 5.09**	83.20 ± 4.04**	79.20 ± 2.70**
AC-Aq	400	84.60 ± 6.42	128.60 ± 7.24	116.80 ± 4.22	99.40 ± 2.22*	92.80 ± 2.51
AC-CAq	400	88.00 ± 3.20	130.20 ± 8.24	110.60 ± 5.22	100.20 ± 3.22*	94.40 ± 3.55

Each value represents the mean ± S.E.M. of five observations. *P < 0.05, **P < 0.01 Vs control. AC-*Acacia catechu*, P-Petroleum ether (60-80⁰), C-Chloroform, A-Acetone, E-Ethanol, Aq-Aqueous, CAq-Crude aqueous, WSF-Water soluble fraction, WISF-Water insoluble fraction.

Table 3: Effect of fractions of ethanolic extract of *Acacia catechu* in glucose loaded hyperglycemic rats

Treatments	Dose mg/kg	Blood glucose concentration (mg/dl)				
		0 th h	1/2 h	1 h	2 h	3 h
G. control	--	85.80 ± 3.20	131.00 ± 4.46	110.00 ± 4.22	85.40 ± 2.20	83.80 ± 4.20
Glibenclamide	5	82.40 ± 2.15	85.40 ± 1.93**	71.80 ± 2.49**	63.80 ± 1.85**	58.80 ± 1.85**
AC-E	400	86.20 ± 2.35	90.20 ± 5.42**	89.20 ± 3.40**	76.00 ± 2.09*	79.20 ± 3.60*
AC- WSF	400	80.60 ± 3.80	130.40 ± 7.52	116.40 ± 2.24	85.20 ± 1.85	82.60 ± 2.80
AC- WISF	400	82.40 ± 3.44	87.60 ± 4.63**	83.40 ± 5.20**	65.00 ± 3.78**	68.20 ± 3.24**

Each value represents the mean ± S.E.M. of five observations. *P < 0.05, **P < 0.01 Vs control. AC-*Acacia catechu*, E-ethanol, WSF-Water soluble fraction, WISF-Water insoluble fraction

Table 4: Hypoglycaemic activity of active antihyperglycaemic ethanolic extract and water insoluble fraction of *A. catechu* in normal rats.

Treatments	Dose mg/kg	Blood glucose concentration (mg/dl)				
		0 th h	1/2 h	1 h	2 h	3 h
Normal control	--	84.40 ± 2.32	83.00 ± 1.80	81.80 ± 2.42	82.20 ± 1.70	80.20 ± 2.38
Glibenclamide	5	82.00 ± 2.15	45.20 ± 3.86**	36.60 ± 1.43**	33.20 ± 1.39**	35.00 ± 3.16**
ACE	200	83.80 ± 2.40	82.40 ± 2.24	81.20 ± 3.10	71.20 ± 2.28*	75.20 ± 2.88
	400	85.20 ± 3.01	82.40 ± 2.80	70.00 ± 3.14*	68.60 ± 3.37**	65.80 ± 2.32**
WISF	200	84.40 ± 2.10	82.20 ± 2.80	72.40 ± 2.80*	71.20 ± 2.28*	65.80 ± 2.32**
	400	83.20 ± 1.89	71.20 ± 2.80*	69.40 ± 3.10**	60.80 ± 4.02**	55.00 ± 3.32**

Each value represents the mean ± S.E.M. of five observations. *P < 0.05, **P < 0.01 Vs control (Dunnett's test), ACE-*Acacia catechu* ethanolic extract, WISF- Water insoluble fraction of ethanolic extract.

Table 5: Biochemical parameters of normal and experimental animals on 7th day post treatment

Parameters	Experimental groups						
	Normal control	Diabetic control	ACE		WISF		Glibencl
			200 mg/kg	400 mg/kg	200 mg/kg	400 mg/kg	5mg/kg
Blood glucose	81.4±3.2**	512.0±15.3	282.2 ± 9.1**	192.0±10.4**	150.0±12.2**	134.4 ± 10.2**	124.4±7.8**
S. urea	30.2±1.8**	279.0±14.0	93.4 ± 2.4**	77.2±5.1**	47.0±5.5**	38.6 ± 2.2**	32.8 ± 1.4**
S. creatinine	0.45±0.0**	1.9±0.4	1.5 ± 0.0	0.9±0.1*	0.5 ± 0.03**	0.5 ± 0.4**	0.4 ± 0.03**
S. cholesterol	34.0±1.7**	84.0 ±4.9	70.2 ± 3.5**	65.4±1.6**	44.8 ± 3.3**	38.2 ± 1.9**	32.2 ± 2.5**
S. triglyceride	33.4±3.5**	123.0±6.6	96.2 ± 3.5**	88.4±3.6**	55.8 ± 3.3**	47.0 ± 2.4**	38.2 ± 1.9**
HDL	24.6±1.7**	10.2±1.1	14.2 ± 0.6**	14.8±2.2**	18.0 ± 0.7**	21.0 ± 2.0**	25.8 ± 1.0**
LDL	22.0±2.1**	58.8±3.2	36.6 ± 2.4**	30.0±2.4**	29.2 ± 3.3**	24.4 ± 2.2**	23.6 ± 1.9**
Haemoglob	11.2±0.3**	6.9±0.4	8.80 ± 0.3*	9.0±0.4**	10.0 ± 0.4**	11.4 ± 0.3**	11.0 ± 0.5**
Gly. haemogl	1.9±0.2**	5.7 ± 0.4	4.20 ± 0.3*	3.0±0.2**	2.6 ± 0.2**	2.2 ± 0.2**	2.0 ± 0.2**

Each value represents the mean ± S.E.M. of five observations. * $P < 0.05$, ** $P < 0.01$ Vs diabetic control (ANOVA followed by Dunnett's test), ACE-*Acacia catechu* ethanolic extract, WISF-Water insoluble fraction of ethanolic extract, Glibencl-Glibenclamide.

the end of the 7th day the rats were fasted for 16 h and blood parameters were determined.

Collection of blood and estimation of biochemical parameters

The blood glucose level was measured using Accu-chek Active™ Test meter on blood from rat tail vein. For other plasma profiles, blood was collected from retro-orbital venous plexus of the rats under light ether anesthesia using capillary tubes into Eppendorf tubes containing heparin. The plasma was separated by centrifugation (5 min, 5000 rpm) and was analyzed for lipid profiles (serum cholesterol, serum triglyceride, HDL cholesterol, LDL cholesterol), serum creatinine, serum urea, haemoglobin and glycosylated haemoglobin. The plasma profiles were measured by standard enzymatic methods with an automatic analyzer¹⁴ and glycosylated haemoglobin by colorimetric method.

Statistical analysis

The values are expressed as mean ± SEM. The results were analyzed for statistical significance using one-way ANOVA followed by Dunnett's test. $p < 0.05$ was considered significant.

Results

Preliminary phytochemical screening

Phytochemicals and percentage yield of various extracts and fractions of *A. catechu* are given in Table 1.

Effect of extracts and fractions in glucose loaded hyperglycaemic animals

Tables 2 and 3 show the antihyperglycaemic effect in glucose-loaded hyperglycaemic rats, after administration of plant extracts and fractions at a dose of 400 mg/kg. Thirty minutes after the glucose load, there was a significant rise in the blood glucose levels of control animals and a decline at the end of 2nd h. The antihyperglycaemic activity of any extracts would be determined by its ability to lower the increasing blood glucose after a glucose load. The plant studied for the activity exhibits significant

antihyperglycaemic activity ($p < 0.05$) at 1, 2 and 3 h after the glucose load compared to control. Chloroform and ethanol extract of the plant exhibited significant antihyperglycaemic activity. Aqueous and crude aqueous extracts exhibit the effect only at 2nd h. The ethanolic extract produced hypoglycaemia at the end of 3rd h (Table 2). Among the fractions of ethanolic extract, only the water insoluble fraction was found to produce significant activity ($p < 0.01$) and it was also found to produce hypoglycaemia at the end of 3rd h. Comparatively the water-insoluble fraction of ethanolic extract of *A. catechu* was found to be more active than ethanolic extract (Table 3).

Effect of extract and fraction in fasted normal rats

Based on the antihyperglycaemic activity, the active ethanolic extract and water insoluble fraction were subjected to hypoglycaemic studies at two dose levels (200 and 400 mg/kg) and the results are given in Table 4. Both the ethanolic extract and its fraction exhibited significant ($p < 0.05$) hypoglycaemic activity and the activity was dose-dependent. Water-insoluble fraction showed hypoglycaemic activity after ½ h of its administration and ethanolic extract exhibited the activity after 1 h of its treatment. Comparatively, the fraction obtained from ethanolic extract was more active.

Effect of extract and fraction in alloxan-induced diabetic rats

The basal blood glucose levels of all the groups were statistically not different from each other. Three days after alloxan administration, blood glucose values were 5-folds higher in all the groups and were not statistically different from each other. After 7 days, values of blood glucose decreased in all the treated groups and the diabetic rats showed a slight increase in blood glucose level. The administration of plant extracts, fraction and glibenclamide to diabetic rats restored the level of blood glucose significantly ($p < 0.01$) (Table 5).

The level of total haemoglobin, glycosylated haemoglobin, serum urea, serum creatinine and lipid profiles of different experimental groups are also represented in Table 5.

Diabetic rats showed a significant decrease in the level of total haemoglobin and significant increase in the level of glycosylated haemoglobin. The administration of plant extract, fraction and glibenclamide to diabetic rats restored the changes in the level of total hemoglobin and glycosylated haemoglobin to near normal levels ($p < 0.05$).

Alloxan-induced diabetic rats showed significant hypercholesterolemia as compared with control. Treatment with plant extract and fraction showed a significant decrease in cholesterol levels ($p < 0.01$) at the same time increase in HDL-c. Hypercholesterolemia was associated with hypertriglyceridemia as compared with control animals. Hypertriglyceridemia was also significantly prevented by treatment with plant extract and fraction ($p < 0.01$). Diabetic control rats showed a significant increase in creatinine and urea levels as compared with control animals. Treatment with ethanolic extract and water-insoluble fraction of ethanolic extract of *A. catechu* significantly decreased these values ($p < 0.01$). Both the extract and the fraction were effective in alleviating diabetes and diabetes-related complications. There was no significant difference in the activity of 200 and 400 mg/kg doses of fraction and the activity at these two dose levels was better than that of the activity exhibited at 400 mg/kg of ethanolic extract. The activity of water-insoluble fraction was more active and the activity was comparable with that of the standard drug, glibenclamide (Table 5).

Discussion

This study was performed to find out the active antihyperglycaemic extract using various solvents and the active antihyperglycaemic fraction isolated from the active extract of this plant. Also the extracts obtained by successive solvent extraction method were compared with crude aqueous extract of the same plant prepared in a traditional manner. To draw out the mechanism behind their activity, the active antihyperglycaemic extract and fraction were subjected to hypoglycaemic studies to determine the effect of these extract and fraction in diabetes-associated complications. Biochemical parameters were also assessed.

Diabetes is a major health problem affecting major populations worldwide. Epidemiological studies and clinical trials strongly support the notion that hyperglycemia is the principal cause of complications. Effective blood glucose control is the key for preventing or reversing diabetic complications and improving quality of life in patients with diabetes. Thus sustained reduction in hyperglycemia will decrease the risk of developing microvascular complications and most likely reduce the risk of macrovascular complications.²⁴ On the basis of this statement we have selected the glucose-induced hyperglycaemic model to screen the anti-hyperglycaemic activity of the plants extracts. Any drug that is effective in diabetes will have the ability to control the rise in glucose level by different mechanisms and the ability of the extracts to prevent hyperglycaemia could be determined by glucose- loaded hyperglycaemic model.

In the glucose-loaded hyperglycaemic model, the plant tested for antihyperglycaemic activity exhibited significant antihyperglycaemic activity at a dose level of 400 mg/kg. Excessive amount of glucose in the blood induces insulin secretion. This secreted insulin will stimulate peripheral glucose consumption and controls the production of glucose through different mechanisms.²⁵ However, from the study (glucose control) it was clear that the secreted insulin requires 2-3 h to bring back the glucose level to normal. In the case of chloroform extract, ethanol extract, water-insoluble fraction of ethanol extract and drug-treated groups, the glucose levels have not exceeded more than the negative control group, giving an indication regarding the supportive action of the extracts, fraction and drug in glucose utilization. The effect of glibenclamide, the standard drug used in this study, on glucose tolerance has been attributed to enhanced activity of beta cells of the pancreas resulting in secretion of larger amounts of insulin. So the mechanism behind this antihyperglycaemic activity of plant extracts and fractions involves an insulin-like effect, probably, through peripheral glucose consumption or enhancing the sensitivity of beta cells to glucose, resulting in increased insulin release.²⁴ In these contexts, a number of other plants have also been reported to have hypoglycemic effects.²⁶ The ethanolic extract and water insoluble fraction exhibited the tested hypoglycaemic activity. The hypoglycaemic effect produced by the extract and fraction may be due to the increased insulin release resembling the mechanism of actions of sulphonylureas.^{27,28} Alloxan induces hyperglycaemia by selective cytotoxic effect on pancreatic beta cells. One of the intracellular phenomena for its cytotoxicity is through generation of free radicals demonstrated both *in vivo* and *in vitro*.²⁹ Our investigations indicate the efficiency of the plant in the maintenance of blood glucose levels in alloxan-induced diabetic rats may be possibly by the above mentioned mechanisms.

In uncontrolled or poorly controlled diabetes, there is an increased glycosylation of a number of proteins including haemoglobin. Glycosylated haemoglobin level is increased in patients with diabetes mellitus to approximately 16% and the amount of increase was found directly proportional to the fasting blood glucose level. During diabetes, the excess glucose present in blood reacts with haemoglobin. Therefore, the total haemoglobin level is decreased in alloxan diabetic rats.³⁰ Administration of ethanolic extract and fraction for 7 days prevented a significant elevation in glycosylated haemoglobin thereby increasing the level of total haemoglobin in diabetic rats. This could be due to the result of improved glycemic control produced by plant extract and fraction.

The levels of serum lipids are usually elevated in diabetes mellitus and such an elevation represents a risk factor for coronary heart disease. This abnormal high level of serum lipids is mainly due to the uninhibited actions of lipolytic hormones on the fat depots mainly due to the action of insulin. Under normal circumstances, insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides. However, in diabetic state lipoprotein lipase is not activated

due to insulin deficiency resulting in hypertriglyceridaemia.³¹ Also insulin deficiency is associated with hypercholesterolaemia. Insulin deficiency may be responsible for dyslipidaemia, because insulin has an inhibitory action on HMG-CoA reductase, a key rate-limiting enzyme responsible for the metabolism of cholesterol-rich LDL particles. The mechanisms responsible for the development of hypertriglyceridemia and hypercholesterolemia in uncontrolled diabetes in humans are due to a number of metabolic abnormalities that occur sequentially.³² In our study, diabetic rats showed hypercholesterolaemia and hypertriglyceridaemia and the treatment with plant extract and fraction significantly decreased both cholesterol and triglyceride levels. This implies that ethanolic extract of *A. catechu* barks and water insoluble fraction of ethanolic extract can prevent or be helpful in reducing the complications of lipid profile seen in some diabetics in whom hyperglycaemia and hypercholesterolaemia coexist quite often.³³ These findings also support the hypothesis that the activity of plant extract and fraction may be directly attributed to improvements in insulin levels upon treatment.³³

The diabetic hyperglycemia induced by alloxan produces elevation of plasma levels of urea and creatinine, which are considered as significant markers of renal dysfunction.³⁴ Our results also showed significant increase in the level of plasma urea and creatinine in the diabetic groups compared to control level. These results indicated that diabetes might lead to renal dysfunction. While, after treatment of alloxan-diabetic rats with ethanolic extract and water insoluble fraction, the level of urea and creatinine were significantly decreased compared to the mean value of diabetic group. This further confirms the utility of these plants in diabetes-associated complications.³⁵

Mostly it was believed that the formation of the artefacts during the preparation of crude aqueous extracts would be responsible for the biological activities.³⁶ In our study, the crude aqueous extract of *A. catechu* was active only at 2nd h after glucose administration. Though the main classes of active constituents are present in the aqueous and crude aqueous extract of this plant, the activity was not similar to the ethanolic extract and this may be due to the fewer amounts of active constituents present at 400 mg/kg of crude aqueous extracts when compared to 400 mg/kg of successively fractionated chloroform and ethanolic extracts or may be due to absence of steroids and alkaloids that are present in the chloroform and ethanolic extracts respectively. Hence, the antidiabetic activity of the extracts was caused by substances that naturally exist in the plant parts, and not due to transformations induced by heating. In our study the maximum activity was found in ethanolic extract and water insoluble fraction of ethanolic extract, which contains alkaloids along with flavonoids that were not found in any other extracts and fraction. Alkaloids are absent in the crude aqueous extract and water soluble fraction of ethanolic extract in which the activity was found to be minimum or nil. This confirms that it was the role of alkaloids in ethanolic extract and water insoluble fraction to

exhibit antidiabetic activity along with flavonoids and a lot of alkaloids are reported to have antidiabetic activity.^{37,38}

Conclusion

We conclude that the extract and fraction of the plant tested for antidiabetic activity have shown appreciable results in decreasing the serum glucose level and other complications associated with diabetes. This research supports the inclusion of this plant in traditional antidiabetic preparations and the formulations made using these identified effective extract and fraction of this plant could serve the purpose better than the existing formulations with crude aqueous extract.

References

1. Anonymous. Diabetes now a global threat gets own day. Sunday Times India 2006; 24: 11.
2. Shastri K. Comments on Charaka Samhita. Chanukah bharati: Varanasi, 1980: 22.
3. Pepato MT, Mori DM, Baviera AM, Harami JB, et al. Fruit of the Jambolan tree (*Eugenia jambolana* Lam.) and experimental diabetes. J Ethnopharmacol 2005; 96: 43-48.
4. Venkatesh S, Reddy GD, Reddy BM, Ramesh M, et al. Antihyperglycemic activity of *Carulluma attenuate*. Fitoterapia 2003; 74: 274-277.
5. Vaishali VA, Sangeeta SM, Mandar A, Kishore MP, et al. Antioxidant and trace element potential of Chyavanprash and some Ayurvedic preparations. Indian J Trad knowledge 2003; 2: 215-223.
6. Mradhu G, Tuhin KB, Shyamali S, Pratip KD. Therapeutic utilization of secretory products of some Indian medicinal plants – a review. Indian J Trad knowledge 2006; 5: 569-575.
7. Chandra PK, Pitamber PD, Bikram SS. Developing the medicinal plants sector in northern India: challenges and opportunities. J Ethnobiol Ethnomed online publication 15 July 2006; doi:10.1186/1746-4269-2-32.
8. Ray D, Sharatchandra K, Thokchom IS. Antipyretic, anti-diarrhoeal, hypoglycaemic and hepatoprotective activities of ethyl acetate extract of *Acacia catechu*. Indian J Pharmacol 2006; 38: 408-413.
9. Nongyao S, Kitja S. The effects of extracts from anti-diarrheic Thai medicinal plants on the invitro growth of the intestinal protozoa parasite: *Blastocystis hominis*. J Ethnopharmacol 2005; 98: 67-72.
10. Supayang V, Amornrat L, Wanpen J, Trechada S, Souwalak P, Thanomjit S. Effective medicinal plants against enterohaemorrhagic *Escherichia coli* O157:H7. J Ethnopharmacol 2004; 94: 49-54.
11. Naik GH, Priyadarsini KI, Satav JG, Banavalikar MM, Sohoni DP, Biyani MK, Mohan H. Comparative antioxidant activity of individual herbal components used in Ayurvedic medicine. Phytochemistry 2003; 63: 97-104.
12. Nagarajan NS, Muruges N, Thirupathy KP, Radha N, Murali A. Antidiabetic and antihyperlipidemic effects of *Cleome feline*. Fitoterapia 2005; 76: 310-315.
13. Nalamolu KR, Srinivasu N. Antidiabetic and renoprotective effects of chloroform extract of *Terminalia chebula* seeds in streptozotocin induced

- diabetic rats. BMC Complement Altern Med online publication 13 November 2006; doi: 10.1186/1472-6882-6-17.
14. Phuong ML, Ali BA, Aziz E, Abdellatif S, et al. The petroleum ether extract of *Nigella sativa* exerts lipid lowering and insulin-sensitizing action in the rats. J Ethnopharmacol 2004; 94: 251-259.
 15. Chattopadhyay RR. Possible Mechanism of Antihyperglycemic Effect of *Gymnema sylvestre* Leaf Extract, Part I. Gen Pharmac 1998; 31: 495-496.
 16. Shoei SL, Buh FT, Karin CL. Three Triterpene Esters From *Zizyphus Jujuba*, Phytochemistry 1996; 43: 847-851.
 17. Shoei SL, Jeng SW, Karin CSC. Chemical constituents of *Zizyphus Jujuba* Mill (var) *spinosa*. J Chinese Chem Soc 1995; 42: 77-82.
 18. Brain KR, Turner TD. The practical evaluation of Phytopharmaceuticals. Wright- Scientecnica: Bristol, 1975: 10-30.
 19. Khandelwal KR. Practical Pharmacognosy, 16th edition. Nirali Prakashan: Pune, 2005: 149-153.
 20. Babu V, Ganga DT, Subramonium A. Antihyperglycaemic activity of *Cassia kleinii* leaf extract in glucose fed normal rats and alloxan induced diabetic rats. Indian J Pharmacol 2002; 34: 409-413.
 21. Ekrem S, Mustafa A, Erdem Y, Shigeru I. Hypoglycaemic activity of *Gentiana olivieri* and isolation of the active constituent through bioassay-directed fractionation techniques. Life Sci 2005; 76: 1223-1238.
 22. Jamal AAB, Issa AAH, Mohammad HHA. Hypoglycaemic and antihyperglycaemic effects of *Trigonella foenum-graecum* leaf in normal and alloxan induced diabetic rats. J Ethnopharmacol 1997; 58: 149-155.
 23. Sabu MC, Subburaju T. Effect of *Cassia auriculata* Linn. on serum glucose level, glucose utilization by isolated rat hemidiaphragm. J Ethnopharmacol 2002; 80: 203-206.
 24. Muniappan L, Leelavinothan P, Sandhya S, Ramesh B. Insulin-secretagogue activity and cytoprotective role of the traditional antidiabetic plant *Scoparia dulcis* (Sweet Broomweed). Life Sci 2004; 75: 2003-2014.
 25. Andrew JK. Diabetes. New York: Churchill living stone, 2000; 1-9.
 26. Leila Z, Eliandra DS, Luisa HC, Anildo CJ, et al. Effect of crude extract and fractions from *Vitex megapotamica* leaves on hyperglycemia in alloxan-diabetic rats. J Ethnopharmacol 2007; 109: 151-155.
 27. Okine LKN, Nyarko AK, Osei-Kwabena N, Oppong IV, et al. The antidiabetic activity of the herbal preparation ADD-199 in mice: a comparative study with two oral hypoglycaemic drugs. J Ethnopharmacol 2005; 97: 31-38.
 28. Miura T, Itoh C, Iwamoto N, Aato M, Kawai M, Park SR, Suzuki I. Hypoglycemic activity of the fruit of the *Momordica charantia* in Type 2 diabetic mice. J Nutr Sci Vitaminol (Tokyo) 2001; 47: 340-344.
 29. Yadav S, Vats V, Dhunnoo Y, Grover JK. Hypoglycemic and antihyperglycemic activity of *Murraya koenigii* leaves in diabetic rats. J Ethnopharmacol 2002; 82: 111-116.
 30. Pari L, Amarnath SM. Antidiabetic activity of *Boerhaavia diffusa* L.: effect on hepatic key enzymes in experimental diabetes. J Ethnopharmacol 2004; 91: 109-113.
 31. Pushparaj PN, Low HK, Manikandan J, Tan BKH, et al. Anti-diabetic effects of *Cichorium intybus* in streptozotocin-induced diabetic rats. J Ethnopharmacol 2007; 111: 430-434.
 32. Murali B. Upadhyaya UM, Goyal RK. Effect of chronic treatment with *Enicostemma littorale* in non-insulin dependent diabetic (NIDDM) rats. J Ethnopharmacol 2002; 81: 199-204.
 33. Sharma S., Nasir A, Prabhu KM, Murthy PS, Dev G. Hypoglycaemic and hypolipidemic effect of ethanolic extract of seeds of *Eugenia jambolana* in alloxan-induced diabetic rabbits. J Ethnopharmacol 2003; 85: 201-206.
 34. Alarcon AFJ, Calzada BF, Hernandez GE, Ruiz AC, et al. Acute and chronic hypoglycaemic effect of *Ibervillea sonora* root extracts-II. J Ethnopharmacol 2005; 97: 447-452.
 35. El-Demerdash FM, Yousef MI, Abou El-Naga NI. Biochemical study on the hypoglycemic effects of onion and garlic in alloxan-induced diabetic rats. Food Chem Toxicol 2005; 43: 57-63.
 36. Mukherjee KP. Quality control of herbal drugs. New Business Horizons, New Delhi: Pharmaceutical Publishers, 2002: 379-425.
 37. Pulok KM, Kuntal M, Kakali M, Peter JH. Leads from Indian medicinal plants with hypoglycemic potentials. J Ethnopharmacol 2006; 106: 1-28.
 38. Mohamed B, Abderrahim Z, Hassane M, Abdelhafid T, et al. Medicinal plants with potential antidiabetic activity - A review of ten years of herbal medicine research (1990-2000). Int J Diabetes & Metabolism 2006; 14: 1-25.