Indian Journal of Experimental Biology
Vol. 46 September 2008, pp. 660-667

Antidiabetic activity of aqueous extract and non polysaccharide fraction of *Cynodon dactylon* Pers.

E E Jarald, S B Joshi & D C Jain

Department of Herbal Drug Research,

B.R. Nahata College of Pharmacy and Research Centre, Mhow Neemuch Road, Mandsaur 458 001, India

Received 20 February 2008; revised 15 July 2008

Petroleum ether (60°-80°C), chloroform, acetone, ethanol, aqueous and crude hot water extracts of the whole plant of *C. dactylon* and the two fractions of aqueous extract were tested for antihyperglycaemic activity in glucose overloaded hyperglycemic rats and in alloxan induced diabetic model at two-dose levels, 200 and 400 mg/kg (po) respectively. The aqueous extract of *C. dactylon* and the non polysaccharide fraction of aqueous extract were found to exhibit significant antihyperglycaemic activity and only the non polysaccharide fraction was found to produce hypoglycemia in fasted normal rats. Treatment of diabetic rats with aqueous extract and non polysaccharide fraction of the plant decreased the elevated biochemical parameters, glucose, urea, creatinine, serum cholesterol, serum triglyceride, high density lipoprotein, low density lipoprotein, haemoglobin and glycosylated haemoglobin significantly. Comparatively, the non polysaccharide fraction of aqueous extract was found to be more effective than the aqueous extract.

Keywords: Alloxan, Antidiabetic activity, *Cynodon dactylon*, Hypoglycaemic activity, Lipid profile

The number of people suffering from diabetes all over the World has soared to 246 million and the disease now kills more people than AIDS[1]. Diabetes leads to major complications such as diabetic neuropathy, nephropathy, retinopathy and cardiovascular diseases. In conventional therapy, type I diabetes is treated with exogenous insulin and type 2 with oral hypoglycaemic agents (sulphonylureas, biguanides etc)[2]. Though different types of oral hypoglycemic agents are available along with insulin for the treatment of type 2 diabetes, there is an increase demand by patients to use the natural products with antidiabetic activity. One such plant that is being used by the traditional practitioners to treat diabetes is *Cynodon dactylon* Pers belonging to the family Graminae. The common name of this plant is Bermuda grass and in Hindi it is known as *Doorva*[3]. Traditionally it is being used in diabetes[3], jaundice[4], kidney problems[5], urinary diseases, gastrointestinal disorders, constipation and abdominal pains[6]. It has been reported to possess antimicrobial[7], wound healing[8] and antioxidant activities[9]. The constituents reported in this plant are cynodin, hydrocyanic acid, triticin, proteins[10], carbohydrates, beta-carotene and minerals like calcium, phosphorous, iron and potassium[11].

Only the aqueous extract of this plant is being used in traditional herbal preparations. From plants not only commonly used ethanol and aqueous extracts are active but the petroleum ether, benzene and chloroform extracts were also found to be active against diabetes[12-14]. Therefore knowing the most effective solvent extract and isolating the active fraction from the most effective solvent extract would be useful in the development of new drugs from plants. Active solvent extracts of plants are commonly used because they may contain more than one active ingredient and less expensive than a purified single compound. Keeping these facts in view the present study has been undertaken to identify the active antidiabetic extract of *Cynodon dactylon* in diabetes associated complications and to identify the active antidiabetic fraction of the active extract.

Materials and Methods

Plant material—Cynodon dactylon was collected during March 2006 from Karungal, Tamil Nadu. The taxonomical identification of the plant was done by Dr. H.S. Chatree, Botanist, Government Arts and Science College, Mandsaur, Madhya Pradesh. The voucher specimen (BRNCP/C/003/2006) was deposited in the Herbarium of Department of

Telephone: 91-7422-255734
E- mail: jaraldpharm@rediffmail.com

Pharmacognosy, B. R. Nahata College of Pharmacy, Mandsaur.

Preparation of extracts—Dried and powdered plant material (500 g) was successively Soxhlet extracted with petroleum ether (60°-80°C), chloroform, acetone, ethanol and water for 72 hr each. Crude hot water extract of the 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.

Fractionation of aqueous extract—The aqueous extract (15 g) obtained through sequential extraction of *C. dactylon* was dissolved in 250 ml water and excess of ethanol was added till complete precipitation of polysaccharides[15]. Precipitate (polysaccharide fraction) was filtered and dried. Remaining non polysaccharide fraction of aqueous extract was also dried. Percentage yield of both the fractions (polysaccharide fraction and non polysaccharide fraction) was determined with respect to the total weight of extract. The extracts and fractions that were not soluble in water were suspended in 1% Tween 80 just before administration to rats.

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[16].

Animals and treatment—Healthy Wistar rats of either sex (150–180 g) with no prior drug treatment were used for the present studies. 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 Division of Pharmacology, B R Nahata College of Pharmacy, Mandsaur with due permission 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)[17]. Female and male Wistar rats (150–180 g) were used for this study. After the sighting study, starting dose of 2000 mg/kg (po) of the test samples were given to various groups containing 5 male and 5 female animals in each groups. The treated animals were monitored for 14 days for mortality and behavioural, neurological and autonomic responses. No abnormal behavioural, neurological, autonomic changes and death was observed till the end of the 14th day. The test samples were found to be safe up to the dose of 2000 mg/kg and from the results 400 mg/kg dose was chosen for further experimentation as the maximum dose.

Antihyperglycemic activity in glucose overloaded hyperglycemic rats—Antihyperglycaemic activity was studied in glucose overloaded hyperglycemic rats[18]. Animals were divided in to various treatment groups (n = 5) as mentioned in Tables 1 and 2. Glibenclamide (5 mg/kg) was used as the reference standard and the untreated control group animals received (po) only vehicle. Remaining groups were treated with 400 mg/kg (po) of various extracts and fractions of plant suspended in 1% Tween 80. Zero hour blood sugar level was determined from overnight fasted animals. After 30 min of the drug treatment animals were fed with glucose (4 g/kg) and blood glucose was determined after 1/2, 1, 2, and 3 hr of the glucose load. Blood glucose concentration was estimated by the glucose oxidase enzymatic method using a commercial glucometer and test-strips (Accu-chek Active™ Test meter).

Hypoglycaemic activity—Animals were classified in to 6 groups (n = 5). Group 1 control animals received a single dose of 0.5 ml/100 g of the vehicle, while those of group 2 with glibenclamide (5 mg/kg) as hypoglycemic reference drug. Animals of groups 3 to 6 were treated with aqueous extract and non polysaccharide fraction of aqueous extract at two dose levels (200 and 400 mg/kg), po as mentioned in Table 3. Blood samples were collected from the vein of the tail tip at 0 (before oral administration), ½, 1, 2 and 3 hr after vehicle, samples and drug administration[19]. The blood sugar level was measured as mentioned above.

Antidiabetic activity in alloxan induced diabetes model—Diabetes was induced in rats by injecting 120 mg/kg of alloxan monohydrate intraperitoneally in 0.9% w/v NaCl to overnight-fasted rats. The rats were then kept for the next 24 hr on 10% glucose solution bottles, in their cages to prevent hypoglycaemia. After 72 hr of injection, fasting blood glucose level was measured. Animals, which did not develop more than 300 mg/dl glucose levels, were rejected[20]. The selected diabetic animals were divided in to 6 groups (n = 5) and one more group of normal non-alloxanised animals was also added in the study. Group 1 (normal

control and non-alloxanised rats) and group 2 untreated diabetic control rats received a single oral dose of 0.5 ml/100 g of the vehicle, group 3 diabetic rats were treated orally with glibenclamide (5 mg/kg) as reference drug. Groups 4 to 7 diabetic rats were treated orally with aqueous extract and non polysaccharide fraction of aqueous extract at two dose levels (200 and 400 mg/kg), po as mentioned in Table 4. Treatment was continued for 7 consecutive days. At the end of 7th day, the rats were fasted for 16 hr and blood parameters were determined.

Collection of blood and estimation of biochemical parameters—The blood sugar level was measured using Accu-chek Active^{TM} glucose strips in Accu-chek Active^{TM} Test meter by collecting the blood from rat tail vein. For other plasma profiles blood was collected from retro-orbital plexus of the rats under light ether anaesthesia 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 total cholesterol, serum triglycerides, HDL cholesterol, LDL cholesterol), serum creatinine, serum urea, haemoglobin and glylosylated haemoglobin. The plasma profiles were measured by standard enzymatic methods with an automatic analyzer[14] and glycosylated haemoglobin by colorimetric method[21].

Statistical analysis—The values are expressed as mean±SE. 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—The dry weight of petroleum ether and chloroform extracts was 1 and 1.2% w/w respectively. Both the petroleum ether (60^{o}-80^{o}C) and chloroform extracts contained fats. But chloroform extract contained steroids also. Acetone extract (yield 2.40% w/w) contained flavonoids and steroids. Ethanol extract (yield 8.75% w/w) contained carbohydrates, flavonoids, alkaloids and saponins. Aqueous extract (yield 20.11% w/w), crude hot water extract (yield 19.80% w/w) and AqNPF (yield 52.00% w/w) contained carbohydrates, flavonoids and saponins. Bur AqPF (yield 40.05% w/w) contained only carbohydrates.

Effect of extracts and fractions in glucose loaded hyperglycaemic animals—Data in Tables 1 and 2 show antihyperglycaemic effect in glucose loaded hyperglycaemic rats after administration of plant extracts and fractions at a dose of 400 mg/kg. After ½ hr of the glucose load there was a significant rise in the blood glucose levels of control animals and at the end of 2nd hr the glucose level declined. The antihyperglycaemic activity of any extract would lower the increasing blood glucose after a glucose load. The plant studied for the activity was found to exhibit significant antihyperglycaemic activity ($P<0.05$) ($P<0.01$) at 1, 2 and 3 hr after the glucose load compared to control. The aqueous and crude hot water extracts of the plant exhibited more significant antihyperglycaemic activity than the extracts of the other solvents (Table 1). When the most active aqueous extract was fractionated and subjected to

Table 1—Effect of oral treatment of various extracts of *C. dactylon* in glucose loaded hyperglycemic rats
[Values are mean ± SE of 5 observations]

Treatment	Dose (mg/kg)	Blood glucose concentration (mg/dl)				
		0 hr	1/2 hr	1 hr	2 hr	3 hr
Gluc. control	--	89.80 ± 3.02	144.40 ± 4.85	150.60 ± 4.01	124.40 ± 3.32	105.20 ± 4.77
Glibenclamide	5	90.20 ± 3.59	105.20 ± 3.49**	92.20 ± 4.60**	78.40 ± 4.20**	66.20 ± 3.68**
CD-P	400	83.80 ± 5.32	131.80 ± 5.80	130.20 ± 7.44	98.00 ± 5.40	95.40 ± 3.88
CD-C	400	86.60 ± 4.20	111.60 ± 6.36	127.60 ± 8.10	98.20 ± 4.80	93.40 ± 4.36
CD-A	400	88.00 ± 4.50	127.40 ± 7.80	121.00 ± 6.96	95.40 ± 5.20	90.80 ± 2.81
CD-E	400	90.40 ± 4.23	128.60 ± 7.88	115.40 ± 4.50	107.20 ± 7.86	94.20 ± 6.21
CD-Aq	400	88.80 ± 3.48	110.00 ± 4.24*	107.00 ± 6.02**	89.40 ± 6.20**	86.40 ± 3.71**
CD-CAq	400	88.60 ± 4.02	124.20 ± 6.80	114.40 ± 4.70*	94.80 ± 3.22*	90.20 ± 4.02

Statistical significance using one-way ANOVA followed by Dunnett's test
$^{*}P < 0.05$, $^{**}P < 0.01$ vs control
CD= *Cynodon dactylon*, P= petroleum ether (60^{o}-80^{o}C), C= chloroform, A= acetone, E= ethanol, Aq= aqueous, CAq= crude aqueous

antihyperglycaemic studies, only the non-polysaccharide fraction of aqueous extract (Table 2) exhibited significant antihyperglycaemic activity. Comparatively, the non polysaccharide fraction of aqueous extract of *C. dactylon* was found to be more active than any other extracts and fraction of this plant.

Effect of aqueous extract and its fraction in fasted normal rats—Based on the antihyperglycaemic activity, the active aqueous extract and non polysaccharide fraction were subjected to hypoglycaemic studies at two dose levels (200 and 400 mg/kg) in normal rats and the results are given in Table 3. Only the non polysaccharide fraction exhibited significant ($P<0.05$) ($P<0.01$) hypoglycaemic activity and the activity was found dose dependant and the aqueous extract did not show any hypoglycaemic effect in normal rats.

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 (Table 4). 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 treatment of diabetic animals with plant extract, its fraction (AqNPF) and glibenclamide, values of blood glucose decreased significantly ($P<0.01$), while the untreated diabetic rats showed a slight increase (Table 4).

Table 2—Effect of oral treatment of polysaccharide and non polysaccharide fractions of aqueous extract of *C. dactylon* in glucose loaded hyperglycemic rats
[Values are mean ± SE of 5 observations]

Treatment	Dose (mg/kg)	Blood glucose concentration (mg/dl)				
		0 hr	1/2 hr	1 hr	2 hr	3 hr
Gluc. 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**
CD-Aq	400	82.80 ± 2.43	108.00 ± 4.42*	93.00 ± 4.22**	88.40 ± 3.20	80.60 ± 3.42
AqPF	400	83.60 ± 2.60	138.80 ± 3.40	121.20 ± 4.20	90.40 ± 4.22	85.40 ± 3.38
AqNPF	400	80.80 ± 2.40	90.20 ± 3.22**	85.40 ± 2.84**	85.80 ± 2.20	81.60 ± 3.60

Statistical significance using one-way ANOVA followed by Dunnett's test
*$P < 0.05$, **$P < 0.01$ vs control
CD= *Cynodon dactylon*, Aq= aqueous, AqPF= polysaccharide fraction of aqueous extract, AqNPF= non polysaccharide fraction of aqueous extract

Table 3—Effect of oral treatment of aqueous extract of *C. dactylon* and its non-polysaccharide fraction on blood glucose levels in normal rats
[Values are mean ± SE of 5 observations]

Treatment	Dose (mg/kg)	Blood glucose concentration (mg/dl)				
		0 hr	1/2 hr	1 hr	2 hr	3 hr
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**
CDAq	200	83.40 ± 3.21	82.60 ± 2.15	90.00 ± 3.80	85.40 ± 2.22	80.20 ± 3.21
	400	81.20 ± 1.89	80.20 ± 1.94	90.00 ± 2.99	82.40 ± 3.44	79.40 ± 2.80
AqNPF	200	82.40 ± 2.10	81.20 ± 2.80	73.40 ± 2.80*	71.20 ± 2.28*	65.80 ± 2.32**
	400	84.20 ± 1.89	71.80 ± 2.80*	69.40 ± 3.10**	65.80 ± 4.02**	60.00 ± 3.32**

\Statistical significance using one-way ANOVA followed by Dunnett's test
*$P < 0.05$, **$P < 0.01$ vs control
CDAq= *Cynodon dactylon* aqueous extract, AqNPF= non polysaccharide fraction of aqueous extract

Table 4—Biochemical parameters of experimental animals on day 0 (A) and 7th day (B) post treatment
[Values are mean ± SE of 5 observations]

Parameters		Normal control	Diabetic control	Experimental groups				Glibenclamide (5 mg/kg)
				CDAq		AqNPF		
				200 mg/kg	400 mg/kg	200 mg/kg	400 mg/kg	
Blood glucose	A	82.60 ± 1.24	84.40 ± 2.84	84.00 ± 1.20	83.80 ± 2.40	79.00 ± 1.22	82.00 ± 2.72	82.20 ± 2.10
	B	81.40 ± 3.22**	512.00 ± 15.29	354.00 ± 11.40**	278.20 ± 10.10**	281.60 ± 11.22**	216.40 ± 9.20**	124.40 ± 7.84**
S. urea	A	30.20 ± 1.77	32.80 ± 1.39	28.60 ± 2.15	31.60 ± 1.65	32.00 ± 2.36	29.40 ± 3.22	31.00 ± 2.40
	B	28.20 ± 1.87**	279.00 ± 14.01	185.20 ± 7.229**	153.80 ± 6.44**	126.40 ± 5.26**	74.40 ± 2.60**	32.80 ± 1.39**
S. creatinine	A	0.45 ± 0.03	0.44 ± 0.03	0.47 ± 0.44	0.46 ± 0.04	0.40 ± 0.03	0.46 ± 0.18	0.46 ± 0.18
	B	0.46 ± 0.03**	1.85 ± 0.36	1.10 ± 0.05	0.81 ± 0.03*	0.80 ± 0.03*	0.60 ± 0.24*	0.44 ± 0.03**
S. cholestrol	A	34.00 ± 1.74	32.20 ± 2.49	36.20 ± 1.93	33.20 ± 2.83	34.60 ± 3.20	37.00 ± 2.33	36.00 ± 2.23
	B	36.00 ± 1.44**	84.00 ± 4.94	79.20 ± 4.50	55.60 ± 2.63**	57.20 ± 3.30**	46.20 ± 1.83**	32.20 ± 2.49**
S. triglyceride	A	33.40 ± 3.45	30.20 ± 1.90	32.00 ± 2.42	34.00 ± 2.62	35.80 ± 2.30	34.20 ± 1.80	36.20 ± 1.60
	B	32.00 ± 2.45**	123.00 ± 6.63	117.20 ± 4.51	95.60 ± 4.61*	99.00 ± 3.80*	77.00 ± 2.84**	38.20 ± 1.90**
HDL	A	24.60 ± 1.47	25.80 ± 0.98	22.00 ± 1.98	24.00 ± 1.08	22.00 ± 0.90	24.60 ± 1.02	26.60 ± 1.02
	B	23.80 ± 1.67**	10.20 ± 1.12	14.80 ± 0.45**	16.80 ± 2.42**	16.20 ± 0.80**	18.00 ± 1.08**	25.80 ± 0.98**
LDL	A	22.00 ± 2.10	23.60 ± 1.90	24.40 ± 2.20	22.40 ± 1.20	20.00 ± 1.33	24.20 ± 2.80	22.20 ± 2.00
	B	22.80 ± 2.00**	58.80 ± 3.22	40.20 ± 2.24**	33.00 ± 1.41**	34.00 ± 2.33**	30.40 ± 2.80**	23.60 ± 1.90**
Haemoglobin	A	11.20 ± 0.33	12.00 ± 0.47	11.40 ± 0.33	11.20 ± 0.35	11.00 ± 0.57	11.80 ± 0.94	12.40 ± 0.84
	B	11.40 ± 0.23**	6.90 ± 0.41	8.30 ± 0.45*	8.80 ± 0.48*	9.00 ± 0.57**	10.60 ± 0.43**	10.99 ± 0.47**
Gly. haemoglobin	A	1.92 ± 0.17	2.00 ± 0.15	1.84 ± 0.15	2.04 ± 0.15	2.06 ± 0.24	1.80 ± 0.35	1.80 ± 0.35
	B	1.94 ± 0.15**	5.70 ± 0.37	4.40 ± 0.28*	4.80 ± 0.34**	4.00 ± 0.24**	3.00 ± 0.25**	2.00 ± 0.15**

Statistical significance using one-way ANOVA followed by Dunnett's test
The values are non significant ($P > 0.05$) vs normal control on 0 day (A). $^{*}P < 0.05$, $^{**}P < 0.01$ vs diabetic control on 7th day (B). CDAq= *Cynodon dactylon* aqueous extract, AqNPF= non polysaccharide fraction of aqueous extract.

The level of total haemoglobin, glycosylated haemoglobin, serum urea, serum creatinine and lipid profiles of different experimental groups of untreated and treated animals are represented in Table 4. The diabetic rats showed a significant decrease in the level of total haemoglobin and significant increase in the level of glycosylated haemoglobin. The administration of aqueous extract, its fraction and glibenclamide to diabetic rats restored the changes in the level of total hemoglobin and glycosylated haemoglobin to near normal levels ($P<0.05$) ($P<0.01$).

Alloxan induced diabetic rats showed significant hypercholesterolemia as compared with control. Treatment with plant extract and its fraction NPF showed a significant decrease in cholesterol levels ($P<0.01$) while there was increase in HDL-c. Hypercholesterolemia was associated with hypertriglyceridemia as compared with control animals. Hypertriglyceridemia also was significantly prevented by the treatment with plant extract and fraction ($P<0.05$) ($P<0.01$). Diabetic control rats showed a significant increase in creatinine and urea levels as compared with control animals. Treatment with aqueous extract and non polysaccharide fraction of aqueous extract of *C. dactylon* significantly decreased these values ($P<0.05$) ($P<0.01$). Treatment with 200 mg/kg of aqueous extract was found inactive in restoring the values of cholesterol, triglycerides and creatinine. Both the extract and the fraction were found effective in alleviating diabetes and diabetes related complications. Comparatively the activity of non polysaccharide fraction was better than that of the activity exhibited by aqueous extract (Table 4).

Discussion

In the glucose loaded hyperglycaemic model used in the present studies, the *C. dactylon* extracts exhibited significant antihyperglycaemic activity at a dose level of 400 mg/kg in 2 to 3 hr. It is well established that after glucose loading in glucose tolerance test, along with blood glucose blood insulin levels also increases. Insulin brings down blood glucose in about 2 to 3 hours by its utilization through different mechanisms. The present results with extracts and glibenclamide are in support of this. In the case of the aqueous extract, crude hot water extract, non polysaccharide fraction of aqueous extract and drug treated groups, the glucose levels were not increased like the control group (Tables 1 and 2), giving an indication regarding the supportive action of the extracts, fraction and drug in the glucose utilization. Glibenclamide is reported to enhance the activity of Beta cells of the pancreas resulting in secretion of larger amounts of insulin which in turn brings down blood glucose level[2]. When the active antihyperglycaemic extract and non polysaccharide fraction were tested for hypoglycaemic activity, only the non polysaccharide fraction exhibited hypoglycaemic activity. So the mechanism behind the antihyperglycaemic activity of extracts and fraction suggests an insulin-like effect[22]. However insulin levels were not estimated in the present study.

Sustained reduction in hyperglycaemia in diabetes mellitus will decrease the risk of developing micro- and macro- vascular complications[22]. In uncontrolled or poorly controlled diabetes, there is an increased glycosylation of a number of proteins including haemoglobin. Therefore, the total haemoglobin level is decreased and glycosylated haemoglobin is increased in alloxan diabetic rats[23]. The aqueous extract and its fraction significantly prevented elevation in glycosylated haemoglobin thereby increasing the level of total haemoglobin ($P<0.05$) ($P<0.01$) 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 decrease in the action 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 hypertriglyceridemia[24] and insulin deficiency is also associated with hypercholesterolemia due to metabolic abnormalities[25]. In the present study also the diabetic rats showed hypercholesterolemia and hypertriglyceridemia and the treatment with plant extract and its fraction significantly ($P<0.05$) ($P<0.01$) decreased both cholesterol and triglyceride levels (Table 4). This implies that the extract and its fraction can prevent or be helpful in reducing the complications of lipid profile seen in some diabetics in whom hyperglycaemia and hypercholesterolemia coexist quite often[26].

The results in Table 4 showed significant increase

in the level of plasma urea and creatinine which are markers of renal dysfunction[27] in the diabetic groups compared to control level. After treatment of alloxan-diabetic rats with aqueous extract and its fraction, the levels of urea and creatinine were significantly ($P<0.05$) ($P<0.01$) decreased compared to those in untreated diabetic group. This further confirms the utility of this plant in diabetes associated renal complications and also supports the usage of this plant by tribals in kidney diseases[5].

The aqueous extract and crude hot water extract of *C. dactylon* were less active than the partially purified non polysaccharide fraction probably due to the removal of many inactive fractions including polysaccharides of the extracts. Flavonoids and saponins are the main classes of compounds present in non polysaccharide fraction and aqueous extracts. Though acetone extract contains flavonoids it was found to be inactive, therefore the antidiabetic activity exhibited by *C. dactylon* and its partially purified non polysaccharide fraction may be due to the saponins. Most of the saponins are reported to have antidiabetic activity[28].

It can therefore be concluded that the aqueous extract (prepared in serial solvent extraction) and non polysaccharide fraction of the plant decrease the serum glucose level and other complications in alloxan diabetic animals. Further studies are necessary to see whether the values come to normal range and also to understand the mechanism of antihyperglycaemic effect.

References

1 Wild S, Roglic G, Green A, Secree R & King H, Global prevalence of diabetes estimates for the year 2000 and projections for 2030, *Diabetes Care*, 27 (2004) 1047.

2 Andrew J K, *Diabetes*, (Churchill Living Stone, New York), 2000, 1.

3 Kirtikar K K & Basu B D, *Indian medicinal plants*, (Lalit Mohan Publication, India), 1980, 2650.

4 Borah P K, Gogoi P, Phukan A C & Mahanta J, Traditional medicine in the treatment of gastrointestinal diseases in Upper Assam, *Indian J Trad Knowledge*, 5 (2006) 510.

5 Cheryl A L, Ethnomedicines used in Trinidad and Tobago for urinary problems and diabetes mellitus, *J Ethnobiol Ethnomed*, (2006) doi:10.1186/1746-4269-2-45.

6 Paolo M G, Traditional phytotherapy in Central Italy (Marche, Abruzzo, and Latium), *Fitoterapia*, 76 (2005) 1.

7 Premkumar V G & Shyamsundar D, Antimicrobial activity of *Cynodon dactylon*, *Indian Drugs*, 41 (2004) 748.

8 Biswas T K & Mukherjee B, Plant medicines of Indian origin for wound healing activity: a review, *Int J Low Extrem Wounds*, 2 (2003) 25.

9 Auddy B, Ferreira M, Blasina F, Lafon L, Arredondo F, Dajas F, Tripathi P C, Seal T & Mukherjee B, Screening of antioxidant activity of three Indian medicinal plants, traditionally used for the management of neurodegenerative diseases, *J Ethnopharmacol*, 84 (2003) 131.

10 Kao S H, Su S N, Huang S W, Tsai J J & Chow L P, Sub-proteome analysis of novel IgE-binding proteins from Bermuda grass pollen, *Proteomics*, 5 (2005) 3805.

11 George S, Basilios P, Christos D G & Nikos A G, An extreme temperature protector of phosphoenolpyruvate carboxylase from the C4-plant *Cynodon dactylon*, *Phytochemistry*, 46 (1997) 1331.

12 Nagarajan N S, Murugesh N, Thirupathy K P, Radha N & Murali A, Antidiabetic and antihyperlipidemic effects of *Cleome feline*, *Fitoterapia*, 76 (2005) 310.

13 Nalamolu K R & Srinivasu N, Antidiabetic and Renoprotecticve effects of chloroform extract of *Terminalia chebula* seeds in streptozotocin induced diabetic rats, *BMC Complement Altern Med*, (2006) doi: 10.1186/1472-6882-6-17.

14 Phuong M L, Ali B A, Aziz E, Abdellatif S, Yahia C & Pierre S H, The petroleum ether extract of *Nigella sativa* exerts lipid lowering and insulin-sensitizing action in the rats, *J Ethnopharmacol*, 94 (2004) 251.

15 Li S P, Zhang G H, Zeng Q, Huang Z G, Wang Y T, Dong T T X & Tsim K W K, Hypoglycemic activity of polysaccharide, with antioxidation, isolated from cultured *Cordyceps mycelia*, *Phytomedicine*, 13 (2006) 428.

16 Khandelwal K R, *Practical pharmacognosy*, ed 16, (Nirali Prakashan, Pune), 2005, 149.

17 OECD, *Guideline for testing of chemicals*, Acute oral toxicity, Environmental health and safety monograph series on testing and adjustment no 420, 2001.

18 Babu V, Ganga D T & Subramonium A, Antihyperglycaemic activity of *Cassia kleinii* leaf extract in glucose fed normal rats and alloxan induced diabetic rats, *Indian J Pharmacol*, 34 (2002) 409.

19 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*, 76 (2005) 1223.

20 Sabu M C & Subburaju T, Effect of *Cassia auriculata* Linn. on serum glucose level, glucose utilization by isolated rat hemidiaphragm, *J Ethnopharmacol*, 80 (2002) 203.

21 Maroo J, Vasu V T & Gupta S, Dose dependent hypoglycemic effect of aqueous extract of *Enicostemma littorale* Blume in alloxan induced diabetic rats, *Phytomedicine*, 10 (2003) 196,.

22 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*, 75 (2004) 2003.

23 Pari L & Amarnath S M, Antidiabetic activity of *Boerhaavia diffusa* L.: effect on hepatic key enzymes in experimental diabetes, *J Ethnopharmacol*, 91 (2004) 109.

24 Pushparaj P N, Low H K, Manikandan J, Tan B K H & Tan C H, Anti-diabetic effects of *Cichorium intybus* in

streptozotocin-induced diabetic rats, *J Ethnopharmacol*, 111 (2007) 430.

25 Murali B, Upadhyaya U M & Goyal R K, Effect of chronic treatment with *Enicostemma littorale* in non-insulin dependent diabetic (NIDDM) rats, *J Ethnopharmacol*, 81 (2002) 199.

26 Sharma S, Nasir A, Prabhu K M, Murthy P S & Dev G, Hypoglycaemic and hypolipidemic effect of ethanolic extract of seeds of *Eugenia jambolana* in alloxan-induced diabetic rabbits, *J Ethnopharmacol*, 85 (2003) 201.

27 Alarcon A F J, Calzada B F, Hernandez G E, Ruiz A C & Roman R R, Acute and chronic hypoglycaemic effect of *Ibervillea sonorae* root extracts-II, *J Ethnopharmacol*, 97 (2005) 447.

28 Pulok K M, Kuntal M, Kakali M & Peter J H, Leads from Indian medicinal plants with hypoglycemic potentials, *J Ethnopharmacol*, 106 (2006) 1.