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Antidiabetic activity of extracts and fraction of *Cassia auriculata* Linn.

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, Madhya Pradesh, India

Abstract

Various extracts, petroleum ether (60-80°), chloroform, acetone, ethanol, aqueous and crude aqueous, of flowers of *Cassia auriculata* Linn. (Caesalpiniaceae) and the fractions of chloroform and ethanolic extracts were tested for anti-hyperglycemic activity in glucose over loaded hyperglycemic rats. The effective anti-hyperglycemic extracts and water-soluble fraction of ethanolic extract were tested for their hypoglycemic activity at two-dose levels, 200 and 400 mg/kg, respectively. To confirm their utility in higher model the effective extracts and fraction of *C. auriculata* were subjected to antidiabetic study in alloxan induced diabetic model at two dose levels, 200 and 400 mg/kg, respectively. The chloroform extract, ethanolic extract and the water-soluble fraction of ethanolic extract were found to exhibit significant ($p<0.05$) ($p<0.01$) anti-hyperglycemic and hypoglycemia activities. Treatment of diabetic rats with chloroform extract, ethanolic extract and the water soluble fraction of ethanolic extract of this plant restored the elevated biochemical parameters, glucose, urea, creatinine, serum cholesterol, serum triglyceride, HDL, LDL, hemoglobin and glycosylated hemoglobin significantly ($p<0.05$) ($p<0.01$) to the near normal level. Comparatively the chloroform extract was found to be more effective followed by water-soluble fraction of ethanolic extract and ethanolic extract. The activity of chloroform extract was comparable with that of the standard drug, glibenclamide.

Key words: *Cassia auriculata* Linn., alloxan, antidiabetic activity, biochemical parameters, hypoglycemic.

1. Introduction

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia, hypertriglyceridaemia and hypercholesterol-aemia, resulting from defects in insulin secretion or action or both [1]. It is as old as mankind and its incidence is considered to be high (4-5%) all over the world. Oral hypoglycemic drugs like sulphonylureas and biguanides, have been used in the treatment of DM [2]. In spite of the introduction of hypoglycemic agents, diabetes and related complications continue to be a major medical problem. Since time immemorial, patients with non-insulin diabetes have been treated orally

* Corresponding author
Email: jaraldpharm@rediffmail.com

in folk medicine with a variety of plant extracts. In India, numbers of plants are mentioned in ancient literature (Ayurveda) for the cure of diabetic conditions known as 'madhumeha' and some of them have been experimentally evaluated and the active principles were isolated [3]. One such plant that is being used by the traditional practitioners to treat diabetes [4,5] is *Cassia auriculata* Linn. belonging to the family Caesalpiniaceae. Traditionally it is also being used in anthelmintic [6], diabetes [5], jaundice [7], urinary diseases [8], and diarrhea [9]. The constituents reported in this plant are alkaloids, tannins, glycosides [10], polysaccharides, flavonoids, and anthracene derivatives [11].

Only the aqueous extract of flowers of this plant is being used in traditional herbal preparations [11,12]. Herbal preparations containing the aqueous extract of flowers of *C. auriculata* as one of the ingredients were proven for their antidiabetic effect [12-14] and the aqueous extract of *C. auriculata* alone was also reported to have antidiabetic activity [15]. The researchers focus mainly in ethanol and aqueous extracts for diabetes, but considerable number of studies state that the petroleum ether, benzene and chloroform extracts were also found active against diabetes [16-18]. Therefore knowing the most effective solvent extract and isolating the active fraction from the most effective extract would be useful in the development of new drugs from plants. The standard fraction of an active extract may prove better therapeutically, less toxic and inexpensive compared to pure isolated compound drugs. Keeping these facts in mind the present study was undertaken to identify the active antidiabetic extract of *Zizyphus mauritiana* in diabetes associated complications and to identify the active antidiabetic fraction of the active extract.

2. Materials and methods

2.1 Plant material

C. auriculata (flowers) was 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, Mandsaur, India. The voucher specimen (BRNCP/C/004/2006) was deposited in the Herbarium of Department of Pharmacognosy, B. R. Nahata College of Pharmacy, Mandsaur.

2.2 Preparation of extracts

Dried and powdered plant material (500 g) was successively soxhlet extracted with petroleum ether (60-80°), chloroform, acetone, ethanol and water for 72 h each. Crude aqueous extract 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 were determined.

2.3 Fractionation of ethanolic and chloroform extracts

Fractionation of ethanolic extract was done using its solubility profile [19-21]. 15 g of dried ethanolic extract was taken 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 ethanolic extract was separated by filtration and both the fractions (water soluble and water insoluble) were dried and their percentage yield with respect to ethanolic extract was determined.

Fractionation of chloroform extract of *C. auriculata* was done by column chromatography. The solvent system for column chromatography, petroleum ether (60-80°): chloroform (1:1), was selected on the basis of separation achieved by thin layer chromatography (TLC).

Table 1. Effect of various extracts of *Cassia auriculata* in glucose loaded hyperglycemic rats.

Treatments	Dose mg/kg	Blood glucose concentration (mg/dl)				
		0th 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	90.20 ± 3.59	105.20 ± 3.49**	92.20 ± 4.60**	78.40 ± 4.20**	66.20 ± 3.68**
CA-P	400	86.50 ± 4.42	126.80 ± 3.44	125.20 ± 5.34	83.60 ± 8.42	85.60 ± 6.32
CA-C	400	93.60 ± 4.32	105.80 ± 8.20**	106.60 ± 8.02**	72.60 ± 6.22**	68.40 ± 4.20**
CA-A	400	95.00 ± 3.44	121.80 ± 4.44	129.20 ± 3.19	89.20 ± 9.90	90.80 ± 6.20
CA-E	400	85.60 ± 6.14	110.80 ± 4.13*	110.20 ± 4.17**	84.00 ± 6.70*	82.80 ± 4.80**
CA-Aq	400	91.60 ± 2.21	120.80 ± 5.74	129.00 ± 5.88	90.80 ± 6.82	88.80 ± 5.72
CA-CAq	400	87.80 ± 2.62	172.20 ± 15.54	133.60 ± 6.60	93.60 ± 2.49	88.80 ± 6.62

Each value represents the mean ± S.E.M. of five observations. $^{*}P < 0.05$, $^{**}P < 0.01$ Vs control. CA-*Cassia auriculata*, P-petroleum ether (60-80^{o}), C-chloroform, A-acetone, E-ethanol, Aq-aqueous, CAq-crude aqueous.

Table 2. Effect of fractions of chloroform and ethanolic extracts of *Cassia auriculata* in glucose loaded hyperglycemic rats.

Treatments	Dose mg/kg	Blood glucose concentration (mg/dl)				
		0th h	1/2 h	1 h	2 h	3 h
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**
CA-C	400	82.20 ± 3.80	90.40 ± 2.17**	84.80 ± 2.72**	73.00 ± 3.02**	64.00 ± 2.09**
$CACF_1$	400	85.20 ± 2.10	118.80 ± 4.72	96.40 ± 3.77	87.40 ± 3.50	84.20 ± 2.57
$CACF_2$	400	86.00 ± 3.51	108.80 ± 5.38*	96.60 ± 4.92	86.00 ± 2.47	83.40 ± 3.10
$CACF_3$	400	80.40 ± 3.24	115.40 ± 5.87	90.00 ± 3.88*	84.20 ± 2.57	82.60 ± 3.80
$CACF_4$	400	84.20 ± 2.82	128.40 ± 5.22	117.80 ± 3.56	93.20 ± 2.24	84.20 ± 3.80
CA-E	400	83.60 ± 2.20	94.60 ± 3.22**	90.20 ± 3.80*	83.20 ± 3.04	75.00 ± 1.02*
E-WSF	400	82.00 ± 2.80	91.40 ± 3.80**	84.40 ± 2.82**	68.80 ± 1.24**	65.20 ± 2.24**
E-WISF	400	80.40 ± 2.20	121.80 ± 3.90	100.40 ± 4.82	94.40 ± 3.88	84.60 ± 2.40

Each value represents the mean ± S.E.M. of five observations. $^{*}P < 0.05$, $^{**}P < 0.01$ Vs control. CA-*Cassia auriculata*, C-chloroform, E-ethanol, WSF- Water soluble fraction, WISF- Water insoluble fraction, $CACF_1$-$CACF_4$– fractions of *Cassia auriculata* chloroform extract F_1 to F_4

Table 3. Hypoglycemic activity of active anti-hyperglycemic extracts and fraction of *Cassia auriculata* in normal rats.

Treatments	Dose mg/kg	Blood glucose concentration (mg/dl)				
		0th 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**
CA-C	200	82.20 ± 1.15	81.80 ± 1.68	69.20 ± 1.68*	66.40 ± 2.71**	62.00 ± 5.32**
	400	81.60 ± 3.25	81.00 ± 1.76	62.20 ± 4.85**	63.40 ± 1.88**	57.80 ± 4.06**
CA-E	200	84.40 ± 2.88	86.40 ± 2.55	68.60 ± 4.94*	65.60 ± 3.80*	70.80 ± 2.88*
	400	83.00 ± 2.90	85.20 ± 2.74	61.00 ± 5.45**	60.80 ± 3.22**	64.20 ± 3.10**
E-WSF	200	82.20 ± 1.20	79.80 ± 1.80	67.40 ± 3.10**	66.80 ± 2.10**	64.00 ± 2.02**
	400	80.00 ± 2.39	80.20 ± 2.22	65.20 ± 2.12**	64.00 ± 2. 02**	63.20 ± 1.12**

Each value represents the mean ± S.E.M. of five observations. *P < 0.05, **P < 0.01 Vs control. CA-*Cassia auriculata*, C-chloroform, E-ethanol, WSF- Water soluble fraction.

Table 4. Biochemical parameters of normal and experimental animals on 7th day post treatment.

Parameters	Experimental groups								
	Normal control	Diabetic control	CA-C		CA-E		E-WSF		Glibenclamide
			200 mg/kg	400 mg/kg	200 mg/kg	400 mg/kg	200 mg/kg	400 mg/kg	5mg/kg
Blood glucose	81.40±3.22**	512.00±15.29	180.40±9.80**	164.40±11.80**	220.00±13.23**	206.00±10.10**	198.20±10.80**	172.00±9.62**	124.40±7.84**
S. urea	30.20±1.77**	279.00±14.01	56.40±2.63**	54.40±2.30**	83.40±3.40**	67.00±4.46**	58.20±2.02**	53.20±2.22**	32.80±1.39**
S. creatinine	0.45±0.03**	1.85±0.36	0.60±0.34**	0.59±0.28**	0.65±0.03**	0.55±0.03**	0.63±0.12**	0.58±0.18**	0.44±0.03**
S. cholestrol	34.00±1.74**	84.00±4.94	45.00±2.33**	42.80±1.03**	59.40±1.23**	55.80±2.30**	52.20±2.13**	50.00±2.43**	32.20±2.49**
S. triglyceride	33.40±3.45**	123.00±6.63	49.00±3.30**	38.20±2.60**	78.40±3.81**	56.80±3.80**	60.00±2.82**	58.00±1.90**	38.20±1.90**
HDL	24.60±1.47**	10.20±1.12	17.80±1.23**	20.00±1.10**	16.80±0.56**	19.00±0.80**	18.20±1.02**	17.80±1.22**	25.80±0.98**
LDL	22.00±2.10**	58.80±3.22	30.40±2.88**	28.20±2.60**	38.00±2.40**	30.20±2.34**	34.00±2.24**	24.00±1.60**	23.60±1.90**
Haemoglobin	11.20±0.33**	6.90±0.41	11.60±0.23**	10.80±0.84**	9.80±0.35**	10.30±0.37**	10.60±0.84**	10.40±0.64**	10.90±0.47**
Gly. haemoglobin	1.92±0.17**	5.70±0.37	3.00±0.25**	2.80±0.35**	4.00±0.36*	2.80±0.44**	3.20±0.25**	2.00±0.55**	2.00 ± 0.15**

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), CA-*Cassia auriculata*, C-chloroform, E-ethanol, WSF- Water soluble fraction.

Column was packed using wet packing technique using hexane as the solvent and silica gel (# 60-120) as the adsorbent. Extract (10 g) was loaded and the column was eluted using the chosen solvent till 90% of the extract loaded eluted out. 12 fractions (100-500 ml) were collected on the basis of the colour bands appeared and according to the total volume of solvent eluted. Fractions showing similar TLC pattern were pooled together and finally 4 fractions were obtained ($CACF_1$-$CACF_4$). Percentage yield of fractions was determined with respect to the total weight of the extracts.

2.4 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 [22].

2.5 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). The extracts and fractions that were not soluble in water were suspended in 1% Tween 80 just before administration to rats.

2.6 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, a starting dose of 2000 mg/kg (P.O.) of the test samples were given to various groups containing 5 animals in each groups. The treated animals were monitored for 14 d for mortality and general behavior. No death was observed till the end of the study. 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.

2.7 Anti-hyperglycemic activity in glucose overloaded hyperglycemic rats

Anti-hyperglycemic activity was studied in glucose overloaded hyperglycemic rats [23]. Animals were divided in to various treatment groups (n = 5) as mentioned in Table 1 and 2. Glibenclamide (5 mg/kg) was used as the reference standard and the negative control group animals received only vehicle. Remaining groups were treated with 400 mg/kg 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 ½, 1, 2, and 3 h 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).

2.8 Hypoglycemic activity

Animals were divided in to 8 groups (n = 5). Group 1 was kept as control, received a single dose of 0.5 ml/100 g of the vehicle, group 2 was treated with glibenclamide (5 mg/kg) as hypoglycemic reference drug. Groups 3 to 8 were treated with chloroform extract, ethanolic extract, and water soluble fraction of ethanolic extract at two dose levels, (200 and 400 mg/kg) as mentioned in Table 3. Blood samples were collected from the tail tip at 0 (before oral

administration), ½, 1, 2, and 3 h after vehicle, samples and drug administration [24]. The blood sugar level was measured using Accu-chek Active™ test strips in Accu-chek Active™ test meter.

2.9 Antidiabetic activity in alloxan induced diabetes model

Diabetes was induced to 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 h on 10% glucose solution bottles, in their cages 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 [25, 26]. The selected diabetic animals were divided in to 8 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 induced was treated with glibenclamide (5 mg/kg) as reference drug. Groups 4 to 9, diabetic induced were treated with chloroform extract, ethanolic extract, and water soluble fraction of ethanolic extract at two dose levels, (200 and 400 mg/kg) as mentioned in Table 4. Treatment was continued for 7 consecutive days (P.O.). At the end of 7th day the rats were fasted for 16 h and blood parameters were determined.

2.10 Collection of blood and estimation of biochemical parameters

The blood sugar level was measured using Accu-chek Active™ test strips in Accu-chek Active™ 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 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, hemoglobin and glylosylated hemoglobin. The plasma profiles were measured by standard enzymatic methods with an automatic analyzer [18] and glycosylated hemoglobin by colorimetric method.

2.11 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.

3. Results

3.1 Preliminary phytochemical screening

The dry weight of petroleum ether and chloroform extracts was 2.18 and 2.50% w/w respectively. The petroleum ether extract contained only fats and chloroform extract contained steroids. Acetone extract (yield 5.52% w/w) contained tannins. Ethanol extract (yield 7.50% w/w) contained carbohydrates, flavonoids, alkaloids, tannins and saponins. Aqueous extract (yield 10.10% w/w) contained carbohydrates, tannins, flavonoids and saponins. Crude aqueous extract (yield 13.80% w/w) contained carbohydrates, tannins, flavonoids, alkaloids and saponins. Water soluble fraction of ethanolic extract (yield 62.50% w/w) contained carbohydrates, flavonoids, alkaloids, tannins and saponins. Water insoluble fraction of ethanolic extract (yield 34.00% w/w) contained only alkaloids, flavonoids and saponins. All the four fractions of chloroform extract ($CACF_1$ – $CACF_4$) contained steroids and their percentage yield was found to be 11.20%, 32.00%, 22.30% and 28.20% w/w respectively.

3.2 Effect of extracts in glucose loaded hyperglycemic animals

The chloroform and ethanolic extracts exhibited significant anti-hyperglycemic activity ($p<0.05$) ($p<0.01$) at ½, 1, 2, and 3 h after the glucose load compared to control (Table 1). Comparatively the chloroform extract was found to be more effective and it was found to produce hypoglycemia at the end of 2nd h and 3rd h ($p<0.01$) (Table 1). Crude aqueous extract was found to increase the blood glucose level (Table 1). Among the fractions of chloroform and ethanolic extracts, only the water soluble fraction of ethanolic extract (Table 2) exhibited significant anti-hyperglycemic activity ($p<0.01$) at all the time intervals tested. The fractions of chloroform extract did not exhibit anti-hyperglycemic effect better than that of the chloroform extract (Table 2). Comparatively the chloroform extract of *C. auriculata* was found to be more active than any other extracts and fractions of this plant (Table 2).

3.3 Effect of extracts and fraction in fasted normal rats

Both the extracts (chloroform and ethanol) and the water soluble fraction, tested for hypoglycaemic activity, exhibited significant ($p<0.05$) ($p<0.01$) hypoglycemic activity and the activity was found dose dependant. Comparatively the chloroform extract was found to be more active, followed by water soluble fraction of ethanolic extract and ethanolic extract (Table 3).

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

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

Alloxan induced diabetic rats showed significant hypercholesterolemia as compared with normal control. Treatment with plant extracts 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 the treatment with plant extracts and fraction ($p<0.01$). Diabetic control rats showed a significant increase in creatinine and urea levels as compared with control animals. Treatment with extracts and fraction of ethanolic extract of *C. auriculata* significantly decreased these values ($p<0.01$). Comparatively the activity of chloroform extract was found to be better and the activity was comparable with that of the standard (Table 4).

4. Discussion

The present study was undertaken to examine the antidiabetic activity of various extracts of *C. auriculata* and to find out the active anti-hyperglycemic fraction of the active extract of this plant. In the glucose loaded hyperglycemic

model, the plant tested for anti-hyperglycemic activity exhibited significant ($p<0.05$) ($p<0.01$) anti-hyperglycemic activity at a dose level of 400 mg/kg. Excessive amount of glucose in the blood induces the insulin secretion and this secreted insulin will stimulate peripheral glucose consumption and control the production of glucose through different mechanisms [27]. 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 case of the chloroform extract, ethanolic extract, water soluble fraction of ethanolic extract and drug treated groups the glucose levels have not increased like the control group (Table 1) (Table 2), giving an indication regarding the supportive action of the extracts, fraction and drug in the glucose utilization. In our study, at the end of the 2nd h, the chloroform extract was found to produce hypoglycemia. The active anti-hyperglycemic chloroform extract, ethanolic extract and water soluble fraction when tested for hypoglycemic activity, all the three samples exhibited the tested hypoglycemic activity. So the mechanism behind this anti-hyperglycemic activity of plant extracts and fraction involves an insulin-like effect [28].

In uncontrolled or poorly controlled diabetes, there is an increased glycosylation of a number of proteins including hemoglobin. Therefore, the total hemoglobin level is decreased and glycosylated haemoglobin is increased in alloxan diabetic rats [29]. The extracts and fraction significantly prevented elevation in glycosylated hemoglobin thereby increasing the level of total hemoglobin ($p<0.01$) in diabetic rats.

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 hypertriglyceridemia [30] and insulin deficiency is also associated with hypercholesterolemia due to metabolic abnormalities [31]. In the present study also the diabetic rats showed hypercholesterolemia and hypertriglyceridemia and the treatment with plant extracts and fraction significantly ($p<0.01$) decreased both cholesterol and triglyceride levels. This implies that the plant extracts and water soluble fraction of ethanolic extract can prevent or be helpful in reducing the complications of lipid profile seen in some diabetics in whom hyperglycemia and hypercholesterolemia coexist quite often [32].

The results in Table 4 showed significant increase in the level of plasma urea and creatinine, which are markers of renal dysfunction in the diabetic groups compared to control level [33]. After treatment of alloxan-diabetic rats with extracts and fraction, the level of urea and creatinine were significantly ($p<0.01$) decreased compared to those in diabetic group. This further confirms the utility of this plant in diabetes associated complications [34].

Synergistic effect of steroids present in the chloroform extract may be responsible for the antidiabetic activity, because when these steroids were separated during fractionation the activity was not found equal or better than that of the chloroform extract. Many steroids are reported to have antidiabetic effect [35]. Though antidiabetic compounds like, alkaloids, flavonoids, tannins and saponins [35, 36] are present in ethanolic extract, aqueous extract, crude aqueous extract and water soluble fraction

of ethanolic extract, the activity was found only in ethanolic extract and its water soluble fraction. The activity of ethanolic extract was less than that of the fraction and this may be due to the fewer amounts of active constituents present at 400 mg/kg of ethanolic extract when compared to 400 mg/kg of fraction. Babayi et al. [37] suggested the same for the better activity of fractions compared to extracts. Pari and Latha [15] reported the antidiabetic activity of aqueous extract of *C. auriculata* flowers, contradictory to this, in our study (glucose loaded hyperglycemic model), the crude aqueous extract was found to produce hyperglycemia initially (½ h) and then there was a gradual decrease in glucose level at 1, 2 and 3 h (Table 1). This may be due to the amount of carbohydrates present in the crude aqueous extract or may be due to the difference in time duration used for extraction (the extraction was carried out for 6 h in their case) or the long term treatment of this extract may prevent diabetic complications. However, the plant contains potential antidiabetic agents, which is confirmed from our studies (Table 4).

5. Conclusion

We conclude that the chloroform extract, ethanolic extract and fraction of ethanolic extract 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 extracts and fraction of this plant could serve the purpose better than the existing formulations with crude aqueous extract.

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