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Effect of aqueous and ethanol extracts of *Cassia auriculata* L. flowers on diabetes using alloxan induced diabetic rats

F Lukmanul Hakkim [1], S Girija [1], R Senthil Kumar [2], MD Jalaludeen [2]

Department of Biotechnology, Bharathiar University, Coimbatore- 641 046, Tamilnadu, India,[1] *Department of Biochemistry, Mohammed Institute of Education and Technology, Tiruchirappalli, Tamilnadu, India.* [2]

Abstract

In the present study the antidiabetic potential of aqueous and ethanol extract of *Cassia auriculata* L. flowers was assessed in alloxan-induced diabetic rats. The phytochemical screening and antioxidant activity were made in these extracts. Antidiabetic agents (Flavonoids and phenolic acids) and free radical scavenging activity in water-soluble fraction of the ethanol extract was higher compared to that of aqueous extract. Diabetic rats showed increase in blood glucose ($p<0.01$) and decrease in plasma insulin ($p<0.01$) levels after 48 hrs of alloxan administration. The oral administration of water-soluble fraction of the ethanol extract at a dose of 0.25 and 0.5g/kg of body of weight, for 30 days exhibited a significant ($p<0.001$) reduction in the blood glucose level and a remarkable increase in plasma insulin level compared to the aqueous extract-treated rats and diabetic control. The level of serum triglycerides and total cholesterol were significantly ($p<0.01$) increased in diabetic rats. The marker enzymes of liver toxicity such as serum alanine transaminase (ALT), serum aspartate transaminase (AST), serum acid phosphatase (ACP), and serum alkaline phosphatase (ALP) were elevated significantly ($p<0.01$) in diabetic control. The liver glycogen and glycogen synthase levels were also increased significantly ($p<0.01$) in alloxan-induced diabetic rats. In addition changes in the body weight and food intake were also analyzed in diabetic and the extracts-treated rats. Treatment with water soluble fraction of ethanol extract and aqueous extract of *C. auriculata* flowers restored the above altered parameters significantly in diabetic animals. The water soluble fraction of the ethanol extract showed a more efficient antihyperglycemic effect compared to the aqueous extract.

Key words*: Cassia auriculata, Alloxan, Antidiabetic effect, Water soluble fraction, Aqueous extract*

Introduction

Diabetes mellitus is a metabolic disorder featured by hyperglycemia and alterations in carbohydrate, fat and protein metabolism associated with absolute or relative deficiency of insulin secretion and /or insulin action.[1] It is one of the oldest diseases affecting millions of people all over the world.[2] Although numerous oral hypoglycemic drugs exist alongside insulin, still there is no promising therapy to cure diabetes.[3] Over the last few decades the reputation of herbal remedies has increased globally due to its therapeutic efficacy and safety. Herbal drugs are widely prescribed today despite the fact that their biologically active compounds are unknown, due to its minimal adverse effects and low costs.[4] In recent years, numerous traditional medicinal plants were tested for their antidiabetic potential in the experimental animals.[5] In the present investigation, *Cassia auriculata* L. flowers were tested for their antidiabetic efficacy. *C. auriculata* (family: Cesalpinaceae) is a common plant in Asia, profoundly used in Ayurvedic medicine as a tonic, astringent and as a remedy for diabetes, conjunctivitis and opthalmia.[6] It is one of the principle constituents of 'Avaarai panchaga chooranam'- an Indian herbal formulation used in the treatment of diabetes to control the blood sugar level.[7] The antidiabetic activity of aqueous extract of *C. auriculata* flowers has been documented previously.[8] Our *in vitro* studies revealed that the water-soluble fraction of ethanol extract has more antioxidant potential than aqueous extract of *C. auriculata* flowers i.e. the potential of scavenging the free radicals by water-soluble fraction of ethanol extract has more efficient than the aqueous extract. It is known that free radicals formation is elevated in diabetes and its complications.[9] In view of the above consideration, the present study was designed to investigate the comparative antidiabetic efficacy of aqueous and ethanol extracts of *C. auriculata* flowers in alloxan-induced diabetic rats.

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Correspondence to: F.Lukmanul Hakkim, Research Scholar, Plant Biotechnology Division, Department of Biotechnology, Bharathiar University, Coimbatore – 46, Tamilnadu, India.email – clonehakkim@yahoo.co.in,
Ph - +091-44-25584292,
Fax: 091-422-2422 387

Materials and Methods

Plant material

C. auriculata flowers were freshly collected from the gardens of M.I.E.T Arts and Science College, Tiruchirappalli, Tamilnadu, India. The plant was identified and authenticated in Botanical Survey of India, Southern Circle, Coimbatore, Tamilnadu, India. A voucher specimen

(No.156) was deposited in the Department of Biochemistry, M.I.E.T Arts and Science College, Tiruchirappalli, Tamilnadu.

Preparation of plant extract and quantification of phytochemicals

The *C. auriculata* flowers were shade-dried at room temperature for seven days and the dried flowers were powdered in a dry grinder. 100 g of powdered *C. auriculata* flowers was packed in a Soxhlet apparatus and extracted twice with 95 % ethanol. The aqueous extract was prepared separately using 100 g powdered *C. auriculata* flowers extracted with water by the method of continuous hot extraction at 60°C for 6 hr.[8] The extracts were concentrated under reduced pressure in the Buchi Rotavapour R-114 and it was finally evaporated to dryness. The yield of ethanol and aqueous extracts were 60 % and 53 %, respectively. The residue was suspended in water and used for further study. The phytochemical screening was made in these extracts by the method of Das and Bhattacharjee.[10] The flavonoids were extracted according to the methods of Harborne[11] and Markham.[12] Phenolic acids were extracted according to the methods of Bate-Smith[13] and Ibrahim and Towers.[14] The flavonoids and phenolic acids were quantitatively estimated according to the method of Swain and Hillis.[15]

In vitro DPPH radical scavenging activity of extracts

The ability of the extracts to scavenge DPPH radicals was assessed.[16] Briefly 50 µl aliquot of the extract was mixed with 450 µl Tris-HCl buffer (50 mmol/l, pH 7.4) and 1.0 ml DPPH (2, 2-diphenyl-2-picrylhydrazyl) (Himedia, India) (0.1 mmol/l, in methanol). After a 30 min reaction period, the resultant absorbance was recorded spectrophotometrically (Shimadzu, Japan) at 515 nm. The control was performed in the absence of extract and percentage of inhibition was calculated from the following equation.

Percentage of inhibition = [(Abs $_{(control)}$) - (Abs sample $_{(extract)}$) / (Abs $_{(control)}$)] X 100

Animals

Male albino wistar rats weighing 180–200gm were used for the present study. The animals were housed in the Department of Animal Science, Bharathidasan University, Tiruchirapalli, Tamilnadu, India. The study was approved by the ethical committee, Bharathidasan University. All animals were fed with standard pellet diet (Gold Mohur lat feed; Ms Hindustan lever Ltd, Mumbai, India) and water *ad libitum*.

Acute toxicity studies

Healthy adult male albino rats were starved overnight and divided into seven groups (n = 7) and were orally fed with water soluble fraction of ethanol extract and aqueous extract at a dose levels of 100, 500, 1000, and 3000 mg/kg of body weight.[17] The rats were observed continuously for 2h for behavioral, neurological and autonomic profiles and after 24 and 72hrs for any lethality.[18]

Induction of experimental diabetes

Rats were induced diabetes by the administration of single intraperitoneal dose of alloxan monohydrate (150 mg/kg).[19] Two days after alloxan injection, rats screened for diabetes having glycosuria and hyperglycemia with blood glucose level of 300 – 400 mg/dl was taken for the study. All animals were allowed free access to water and pellet diet and maintained at room temperature in plastic cages.

Experimental Design

In the present investigation, a total of 42 rats (36 diabetic surviving rats and 6 normal rats) were taken and divided into seven groups of 6 rats each.

Group I: Normal untreated rats

Group II: Diabetic control rats

Group III: Diabetic rats given aqueous extract of *C. auriculata* flowers (0.25 g/kg body weight).

Group IV: Diabetic rats given aqueous extract of *C. auriculata* flowers (0.5 g/kg body weight).

Group V: Diabetic rats given water soluble fraction of ethanol extract of *C. auriculata* flowers (0.25 g/kg body weight).

Group VI: Diabetic rats given water soluble fraction of ethanol extract of *C. auriculata* flowers (0.5 g/kg body weight).

Group VII: Diabetic rats given reference drug glibenclamide (600 µg/kg of body weight). [20]

Biochemical Analysis

The animals were sacrificed at the end of the experimental period of 30 days by decapitation. Blood was collected in tubes containing potassium oxalate and sodium fluoride solution for the estimation of blood glucose. Plasma was separated for assay of insulin. For the serum analysis, the blood was collected in separate tubes and centrifuged at 2000 rpm for 10 min. Blood glucose was measured by the O-toluidine method.[21] Glucose levels were expressed as mg/dl. The plasma insulin level was assayed by Enzyme Linked Immunosorbant Assay (ELISA) kit using human insulin as a standard.[8] Serum Triglycerides levels [22] and Total cholesterol levels.[23] Serum AST and ALT was measured colorimetrically by utilizing the method of Mohun and Cook.[24] Serum alkaline phosphatase was measured by hydrolyzed phenol antipyrine method.[25] Serum acid phosphatase levels,[26] Liver glycogen levels,[27] Glycogen synthase levels,[28] changes in body weight and food intake were assessed in the diabetic animals treated with extracts and compared with other groups.

Statistical Analysis

The data were analyzed using Student's t – test statistical methods. For the statistical tests a p values of less than 0.01, 0.05 and 0.001 was taken as significant.

Results

The phytochemical screening of aqueous and water-soluble fraction of ethanol extract of *C. auriculata* flowers revealed the presence of flavonoids, phenolic acids, sterods/triterpenoids, alkaloids, tannins and anthocyanins. The flavonoid content of aqueous and water soluble fraction of ethanol extract were 7.93 and 13.21 mg/100 g of dry

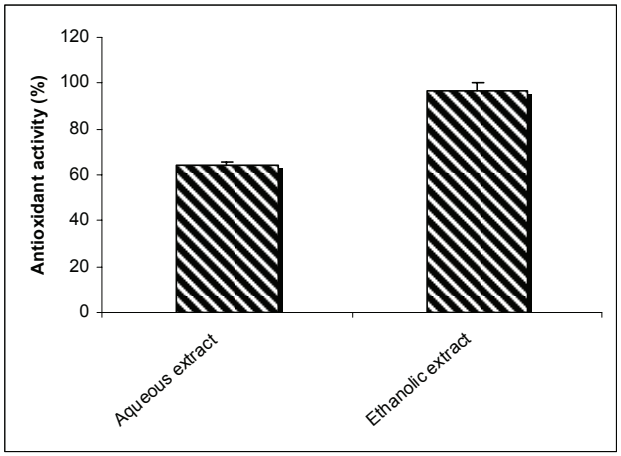

Figure 1: Antioxidant activity of aqueous and ethanol extract of *C. auriculata* flowers

Data were presented as mean ± SD of each three replicates

** - represents statistical significance Vs aqueous extract of *C. auriculata* flowers ($p<0.05$)

weight of the flower powder whereas, the phenolic acid content of aqueous and water soluble fraction of ethanol extract was 12.52 and 16.32 mg/100 g of dry weight of the flower powder respectively. Free radical scavenging activity of both water soluble fraction of ethanol extract and aqueous extract of *C. auriculata* flowers are depicted in Fig.1. Acute toxicity studies revealed the non-toxic nature of the ethanol extract of *C. auriculata* flowers. The intraperitonial injection of alloxan monohydrate 150 mg/kg of body weight to rats resulted in loss of body weight significantly from 206 ± 9.8 to 154 ± 13.6 ($p <0.01$) and increased food intake from 14.53 ± 0.7 to 18.62 ± 0.6 ($p <0.01$). The diabetic rats fed with aqueous extract showed increase body weight and decrease the food intake significantly at a dose of 0.25g (198 ± 18.3 and 17.7 ± 0.5, $p <0.05$) and 0.50 g (208 ± 10.3 and 17.2 ± 0.6, $p < 0.05$) per body wt. than diabetic control rats whereas, the diabetic rats fed with water soluble fraction of ethanol extract showed increase body weight and decrease the food intake more significantly at a dose of 0.25g (205 ± 16.2 and 16.2 ± 0.4, $p <0.001$) and 0.50 g (214 ± 12.7 and 15.59 ± 0.7, $p < 0.001$) per body wt. than aqueous extract treated rats (Table 1). The alloxan diabetic rats elicited significant rise in blood glucose from 88.4 ± 30.2 to 320.2 ± 10.6 ($p < 0.01$) and a significant decrease in plasma insulin level from 16.3 ± 1.0 to 4.3 ± 0.9 ($p < 0.01$). On the contrary, the diabetic rats fed with aqueous extract exhibited decrease blood glucose and increase the plasma insulin significantly at a dose of 0.25g (176.1 ± 15.3 and 10.4 ± 0.7, $p <0.05$) and 0.50 g (158.6 ± 17.2and 13.4 ± 0.6, $p < 0.05$) per body wt. than diabetic control rats whereas, the diabetic rats fed with water soluble fraction of ethanol extract showed decrease blood glucose and increase the plasma insulin more significantly at a dose of 0.25g (142.3 ± 10.2 and 12.1 ± 0.4, $p <0.001$) and 0.50 g (113.3 ± 11.3 and 14.2 ± 0.5, $p < 0.001$) per body wt. than aqueous extract treated rats (Table 2). In diabetic animals serum triglycerides increased from 43.5 ± 3.6 to 64.8 ± 1.4 ($p < 0.01$) and total cholesterol was also elevated from 73.0 ± 1.5 to 99.0 ± 4.1($p < 0.01$). Significant decline of both triglycerides and total cholesterol level was observed in the diabetic rats fed with aqueous ($p<0.05$) and water soluble fraction of ethanol extract ($p<0.001$) for 30 days at a dose of 0.25 and 0.50 g per body weight (Table 3). The diabetic rats showed significantly increased level of AST from 6.3 ± 0.2 to 24.4 ± 0.1 ($p < 0.01$) and level of ALT from 7.4 ± 0.1 to 18.2 ± 0.2 ($p < 0.01$). Diabetic animals fed with aqueous extract exhibited decreased AST and ALT significantly at a dose of 0.25g (18.4 ± 0.3 and 15.2 ± 0.4, $p <0.05$) and 0.50 g (16.3 ± 0.2 and 13.4 ± 1.2, $p < 0.05$) per body wt. than diabetic control rats whereas, the diabetic rats fed with water soluble fraction of ethanol extract showed decreased AST and ALT significantly at a dose of 0.25g (16.0 ± 0.2 and 12.8 ± 1.4, $p <0.001$) and 0.50 g (15.2 ± 0.3 and 11.4 ± 1.3, $p < 0.001$) per body wt. than aqueous extract treated rats (Table 4). Significantly elevated level of ACP (from 3.6 ± 0.4 to 7.2 ± 0.8, $p < 0.01$) and ALP (from 2.4 ± 0.72 to 22.8 ± 0.8, $p < 0.01$) were observed in the diabetic rats. The administration of aqueous extract to diabetic rats at a dose of 0.25 and 0.50 g / body wt. showed reduction in ACP (6.4 ± 0.7 and 5.6 ± 1.4, $p < 0.05$) and ALP (17.4 ± 1.4 and 14.6 ± 1.3, $P < 0.05$) levels whereas, the diabetic rats fed with water soluble fraction of ethanol extract showed increased ACP and ALP levels significantly at a dose of 0.25g (5.2 ± 1.7 and 14.1 ± 0.9, $p <0.001$) and 0.50 g (4.2 ± 1.5 and 13.4 ± 1.2, $p < 0.001$) per body wt. than aqueous extract treated rats (Table 5). A significant reduction in the level of liver glycogen (3.9 ± 0.6 to 1.6 ± 0.1, $p < 0.01$) and glycogen synthase (from 3.4 ± 0.2 to 1.0 ± 0.1, $p < 0.01$) were in alloxan induced diabetic rats. Diabetic rats fed with aqueous extract exhibited increased liver glycogen and glycogen synthase significantly at a dose of 0.25g (1.7 ± 0.3 and 1.9 ± 0.3, $p <0.05$) and 0.50 g (2.0 ± 0.2 and 2.4 ± 0.1, $p < 0.05$) per body wt. than diabetic control rats whereas, the diabetic rats fed with water soluble fraction of ethanol extract showed increased AST and ALT significantly at a dose of 0.25g (2.3 ± 0.1 and 2.7 ± 0.4, $p <0.001$) and 0.50 g (2.7 ± 0.3 and 3.1 ± 0.3, $p < 0.001$) per body wt. than aqueous extract treated rats (Table 6).

Discussion

The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health was widely observed.[29] Over 50 % of currently available drugs are derivatives of plant.[30] The present study was undertaken to assess the antihyperglycemic activity of ethanol extract of *C. auriculata* flowers. Previously it was reported that different parts of *C. auriculata* plant have antidiabetic efficacy.[31] This has been further supported by Latha and Pari using aqueous extract of *C. auriculata* flowers.[8] The preliminary investigation in our laboratory showed that water soluble fraction of ethanol extract of *C. auriculata* flowers has a potent antioxidant activity than the aqueous extract of the same. Under normal physiological conditions, a wide range of antioxidant defenses protect the body from the adverse effects of free radicals produced *in vivo* [32] but free radicals are generated more in diabetes.[33] The water soluble fraction of ethanol extract showed increased scavenging activity

Table 1: Effect of aqueous and ethanol extract of *C auriculata* flowers on body weight and food intake levels in diabetic rats.

Group	Body weight (g)	Food intake (g/day)
Normal control	206 ± 9.8	14.53 ± 0.7
Diabetic control	154 ± 13.6*	18.62 ± 0.6*
Aqueous extract (0.25g/kg of body wt.)	198 ± 18.3**	17.7 ± 0.5**
Aqueous extract (0.5g/kg of body wt.)	208 ± 10.3**	17.2 ± 0.6 **
Ethanol extract (0.25g/kg of body wt.)	205 ± 16.2***	16.2 ± 0.4***
Ethanol extract (0.5g/kg of body wt.)	214 ± 12.7***	15.59 ± 0.7***
Glibenclamide (600µg/kg of body wt.)	207 ± 13.4**	16.27 ± 0.5**

Values are given as mean ± S.D (n = 7), * - represents statistical significance Vs normal control ($p<0.01$), ** – represents statistical significance Vs diabetic control ($p<0.05$), *** - represents statistical significance Vs aqueous extract treated rats and diabetic control ($p<0.001$).

Table 2: Effect of aqueous and ethanol extract of *C. auriculata* flowers on Blood Glucose and Plasma Insulin levels in diabetic rats.

Group	Blood Glucose (mg/dl)	Plasma Insulin (µU/ml)
Normal control	88.4 ± 30.2	16.3 ± 1.0
Diabetic control	320.2 ± 10.6*	4.3 ± 0.9*
Aqueous extract (0.25g/kg of body wt.)	176.1 ± 15.3**	10.4 ± 0.7**
Aqueous extract (0.5g/kg of body wt.)	158.6 ± 17.2**	13.4 ± 0.6**
Ethanol extract (0.25g/kg of body wt.)	142.3 ± 10.2***	12.1 ± 0.4***
Ethanol extract (0.5g/kg of body wt.)	113.3 ± 11.3***	14.2 ± 0.5***
Glibenclamide (600µg/kg of body wt.)	127.4 ± 14.2**	12.7 ± 0.6**

Values are given as mean ± S.D (n = 7), * - represents statistical significance Vs normal control ($p<0.01$), ** – represents statistical significance Vs diabetic control ($p<0.05$), *** - represents statistical significance Vs aqueous extract treated rats and diabetic control ($p<0.001$)

Table 3: Effect of aqueous and ethanol extract of *C . auriculata* flowers on serum triglycerides and total cholesterol levels in diabetic rats

Group	Triglycerides (mg/dl)	Total cholesterol (mg /dl)
Normal control	43.5 ± 3.6	73.0 ± 1.5
Diabetic control	64.8 ± 1.4*	99.0 ± 4.1*
Aqueous extract (0.25g/kg of body wt.)	59.1 ± 1.8**	92.3 ± 3.2**
Aqueous extract (0.5g/kg of body wt.)	53.6 ± 2.6**	84.2 ± 2.2**
Ethanol extract (0.25g/kg of body wt.)	51.3 ± 2.3***	81.2 ± 2.7***
Ethanol extract (0.5g/kg of body wt.)	48.2 ± 2.4***	76.3 ± 3.1***
Glibenclamide (600µg/kg of body wt.)	59.3 ± 1.7**	91.3 ± 1.4**

Values are given as mean ± S.D (n = 7), * - represents statistical significance Vs normal control ($p<0.01$), ** – represents statistical significance Vs diabetic control ($p<0.05$), *** - represents statistical significance Vs aqueous extract treated rats and diabetic, control ($p<0.001$)

Table 4: Effect of aqueous and ethanol extract of *C. auriculata* flowers on Serum AST and ALT levels in diabetic rats

Group	AST (IU/L)	ALT (IU/L)
Normal control	6.3 ± 0.2	7.4 ± 0.1
Diabetic control	24.4 ± 0.1*	18.2 ± 0.2*
Aqueous extract (0.25g/kg of body wt.)	18.4 ± 0.3**	15.2 ± 0.4**
Aqueous extract (0.5g/kg of body wt.)	16.3 ± 0.2**	13.4 ± 1.2**
Ethanol extract (0.25g/kg of body wt.)	16.0 ± 0.2***	12.8 ± 1.4***
Ethanol extract (0.5g/kg of body wt.)	15.2 ± 0.3 ***	11.4 ± 1.3***
Glibenclamide (600µg/kg of body wt.)	17.1 ± 0.4**	14.7 ± 1.1**

Values are given as mean ± S.D (n = 7), * represents statistical significance Vs normal control ($p<0.01$), ** represents statistical significance Vs diabetic control ($p<0.05$), *** represents statistical significance Vs aqueous extract treated rats and diabetic control ($p<0.001$)

Table 5: Effect of aqueous and ethanol extract of *C. auriculata* flowers on serum ACP and ALP levels diabetic rats

Group	ACP (KAUnit)	ALP (KAUnit)
Normal control	3.6 ± 0.4	2.4 ± 0.72
Diabetic control	7.2 ± 0.8*	22.8 ± 0.8*
Aqueous extract (0.25g/kg of body wt.)	6.4 ± 0.7**	17.4 ± 1.4**
Aqueous extract (0.5g/kg of body wt.)	5.6 ± 1.4**	14.6 ± 1.3**
Ethanol extract (0.25g/kg of body wt.)	5.2 ± 1.7***	14.1 ± 0.9***
Ethanol extract (0.5g/kg of body wt.)	4.2 ± 1.5 ***	13.4 ± 1.2***
Glibenclamide (600µg/kg of body wt.)	5.4 ± 0.6**	12.7 ± 1.2**

Values are given as mean ± S.D (n = 7), * - represents statistical significance Vs normal control ($p<0.01$), ** – represents statistical significance Vs diabetic control ($p<0.05$), *** - represents statistical significance Vs aqueous extract treated rats and diabetic control ($p<0.001$), KAU – Karmen Unit

Table 6: Effect of aqueous and ethanol extract of *C. auriculata* flowers on liver glycogen and glycogen synthase levels in diabetic rats

Group	Liver Glycogen (mg/kg)	Glycogen Synthase (Unit/mg of protein/min)
Normal control	3.9 ± 0.6	3.4 ± 0.2
Diabetic control	1.6 ± 0.1*	1.0 ± 0.1*
Aqueous extract (0.25g/kg of body wt.)	1.7 ± 0.3**	1.9 ± 0.3**
Aqueous extract (0.5g/kg of body wt.)	2.0 ± 0.2**	2.4 ± 0.1**
Ethanol extract (0.25g/kg of body wt.)	2.3 ± 0.1***	2.7 ± 0.4***
Ethanol extract (0.5g/kg of body wt.)	2.7 ± 0.3 ***	3.1 ± 0.3***
Glibenclamide (600µg/kg of body wt.)	2.3 ± 0.2**	2.9 ± 0.2**

Values are given as mean ± S.D (n = 7), * - represents statistical significance Vs normal control ($p<0.01$), ** – represents statistical significance Vs diabetic control ($p<0.05$), *** - represents statistical significance Vs aqueous extract treated rats and diabetic control ($p<0.001$)

against free radicals than aqueous extract. It may be due to abundant presence of antioxidant active principles in water soluble fraction of ethanol extract than aqueous extract. This result provoked us to focus on the antidiabetic effect of ethanol extract of *C. auriculata* flowers. Several authors reported that flavonoids, sterols/terpenoids, phenolic acids are known to be bioactive antidiabetic principles.[34,35] Flavonoids are known to regenerate the damaged beta cells in the alloxan diabetic rats.[36] Phenolics are found to be effective antihyperglycemic agents.[37] In the present study, the phytochemical content of water soluble fraction of ethanol extract of *C. auriculata* flowers clearly points out the abundant presence of flavonoids and phenolic acids than aqueous extract. It denotes that the antidiabetic effect of ethanol extract of *C. auriculata* flowers may be due to the presence of more than one antihyperglycemic principles and their synergistic effects.

In the diabetic state, the body weight is reduced whereas food intake is increased and this recovers during the exposure of hypoglycemic treatment.[38] In this study, a reduced level of body weight and elevated level of food intake were observed in alloxan induced diabetic rats. The administration of water-soluble fraction of ethanol extract restored these levels significantly (p value <0.001) towards the normal control than the aqueous extract treated rats at the same level of doses. In all diabetic patients, treatment should aim to lower blood glucose to near normal level.[39]

The present investigation fulfills this statement by producing a significant (p value <0.001) fall in blood glucose levels in water soluble fraction of ethanol extract administered alloxan diabetic rats than the aqueous extract ($p<0.05$) treated rats. Earlier report states that insulin deficiency occurs in alloxan induced diabetic rats leading to alterations in the carbohydrate metabolism such as elevated blood glucose and reduced level of insulin.[40] In our study, it was observed that water soluble fraction of ethanol extract reversed these effects in diabetic animals. The possible mechanism by which water soluble fraction of ethanol extract brings about its antihyperglycemic action may be by induction of pancreatic insulin secretion from β cells of islets of Langerhans or due to enhanced transport of blood glucose to peripheral tissue. This was clearly evidenced from the data with significant (p value <0.001) increase in plasma insulin level of diabetic rats treated with water soluble fraction of ethanol extract than the aqueous extract ($p<0.05$) treated rats. In this study, the marked increase in serum triglycerides and cholesterol levels observed in diabetic rats agree with the findings of Nikkila and Kekki. [41] The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia.[42] Administration of the water soluble fraction of ethanol extract to the alloxan induced diabetic rats significantly (p value < 0.001) restored these parameters than the aqueous extract ($p<0.05$) treated rats. The observed hypolipidemic effect may be because of decreased cholesterogenesis and

fatty acid synthesis because of extract supplementation to the diabetic animals. Significant lowering of total cholesterol and triglycerides is a very desirable biochemical state for prevention of diabetes and its complications. [43]

The serum AST and ALT levels increase as a result of metabolic changes in the liver, such as administration of toxin, cirrhosis of the liver, hepatitis and liver cancer including diabetes.[44] Similarly in the present study, it was observed that the levels of serum AST and ALT in alloxan induced diabetic rats were elevated. It may be due to leaking out of enzymes from the tissues and migrating into the circulation by the adverse effect of alloxan.[45] AST and ALT were used as markers to assess the extent of liver damage in streptozotocin induced diabetic rats.[46] In this study, the administration of water soluble fraction of ethanol extract to alloxan-induced diabetic rats reduces AST and ALT levels (p value <0.001) efficiently than aqueous extract treated rats. In addition to the assessment of AST and ALT levels during diabetes, the measurement of enzymatic activities of phosphatases such as acid phosphatase (ACP) and alkaline phosphatase (ALP) is of clinical and toxicological importance as changes in their activities are indicative of tissue damage by toxicants.[47] In our study, serum ACP and ALP increased considerably in alloxan induced diabetic rats. Elevated level of these enzymes in diabetes may be due to extensive damage to liver in the experimental animals by alloxan. Treatment with water soluble fraction of ethanol extract in alloxan-induced diabetic rats produces a more significant (p value <0.001) decline in these levels than the aqueous extract treated rats. From the present observation, it was evident that water soluble fraction of ethanol extract protects the adverse effects of lipid peroxide mediated tissue damage in alloxan induced diabetic rats.

In diabetes, the glycogen content of the skeletal muscles and liver, markedly depleted [48] and the reduced level of hepatic glycogen is due to inadequate insulin secretion, which results in the inactivation of glycogen synthatase system.[49] In a similar way, in the present study decreased levels of glycogen and glycogen synthase were observed in diabetic control rats. It may be due to insufficient secretion of insulin in the diabetic state as stated earlier. It was reported that the treatment with insulin favours the accumulation of glycogen and its content rises to 300% of normal level within 24hr and this inordinate restoration of glycogen may account for up to 60 % of dry liver weight in diabetic animals.[50] In accordance to this, our study also showed significantly (p value <0.001) elevated levels of liver glycogen and glycogen synthase in the water-soluble fraction of ethanol extract administered diabetic rats than the aqueous extract (p<0.05) treated rats. This may be due to activation of glycogen synthatase system by the modulatory effects of constituents of water-soluble fraction of ethanol extract through induction of insulin secretion.

In conclusion, although numerous medicinal plants have been reported to possess antihyperglycemic activity, *C. auriculata* is gaining much importance in diabetic control as it has been used as a traditional medicine (Avaarai panchaga chooranam) for diabetes. The preliminary investigation on the antidiabetic efficacy of ethanol extract of *C. auriculata* flowers will be significant to proceed further in this path for the isolation of active principles responsible for antidiabetic activity. The present study emphasizes that the ethanol extract has more antidiabetic effect than aqueous extract and it contains potent and safe antihyperglycemic principles unlike synthetic drugs. Further studies will be carried out to elucidate the exact mechanism of action of water soluble fraction of ethanol extract of *C. auriculata* flowers on diabetes and its antiperoxidative effect.

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