Original Article

Antidiabetic Activity of Isolated Compound from *Coccinia indica*

Anupam Jamwal, Suresh Kumar*

Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala- 147 002, Punjab, INDIA.

ABSTRACT

Background and Objective: *Coccinia indica* Wight. and Arn (Ivy gourd, family Cucurbitaceae) has a long tradition of use in the treatment of various ailments. Ayurveda and Unani systems claim *C. indica* as antidiabetic agent. Therefore the present investigation was aimed to evaluate antidiabetic activity of isolated compound(s) from *C. indica*. **Materials and Methods:** Methanol extract of *C. indica* aerial parts was prepared by Soxhlet extraction method and ethyl acetate fraction was prepared by partitioning of methanol extract. Ethyl acetate fraction was subjected to antidiabetic activity-guided-fractionation using column chromatography for the isolation of antidiabetic compound(s). Various fractions and isolated compounds were screened for antidiabetic activity using streptozotocin-nicotinamide type-2 diabetic model. The glucose level in rats was determined using glucometer. The antidiabetic biochemical estimations were performed on bioactive isolated compound. **Results:** Bioactivity-guided-fractionation yielded a bio-flavonoid quercetin, which has been isolated for the first time from *C. indica* aerial parts. Quercetin (5 mg/kg, *p.o.*) significantly reduced the glucose levels in type-2 diabetic rats but did not produce hypoglycemic effects in oral glucose tolerance test on normal rats. Quercetin significantly increased insulin and serum HDL-cholesterol levels and showed better β-cell functions. Quercetin caused slight change in hepatic marker enzymes and kidney functions markers in diabetic rats. **Conclusion:** Quercetin is responsible for antidiabetic activity of *C. indica* aerial parts.

Key words: Antidiabetic, *Coccinia indica*, Cucurbitaceae, Quercetin, Streptozotocin.

INTRODUCTION

Diabetes is the world's largest endocrine disease and will be the 7th leading cause of death in 2030.[1-2] According to W.H.O. projection, the prevalence of diabetes is likely to increase by 35%. The global prevalence of diabetes in 2014 was estimated to be 9% among adults aged 18+ years.[3] An estimated 1.5 million deaths were directly caused by diabetes and more than 80% of diabetes deaths occur in low- and middle-income countries.[4] Recent estimates indicate there were 171 million diabetics worldwide in the year 2000 and this would increase to 366 million by the year 2030. The number of diabetic patients will be double in urban population in developing countries between 2000 to 2030. The diabetic epidemic will continue to increase in world population without increase in the occurrence of obesity.[5] India leads the world with largest number of diabetic subjects earning the dubious distinction of being termed the "**Diabetes capital of the world**". Statistical projections about India suggest that the number of diabetics will rise from 31 million in 2000 to 79 million in the year 2030 making the country with the highest number of diabetics in the world followed by China and then USA.[6]

Coccinia indica Wight. and Arn (Synonym - *Cephalandra indica* Naud), commonly known as Ivy Gourd or Little gourd belongs to family Cucurbitaceae. It is native of Africa and Asia (India). It is widely distributed as weed in all over India and widely cultivated in large areas.[7] It shows

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Correspondence:
Dr. Suresh Kumar,
Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala- 147 002, Punjab, INDIA.
Phone no: +91-9815543175
E-mail: thakur_pu@yahoo.com

www.ijper.org

presence of various chemical constituents such as alkaloids, carbohydrates, glycosides, phenolic compound, gums, mucilages, triterpenoids, flavonoids, anthraquinones and polysaccharides.[8] Ayurveda and Unani systems claim *C. indica* as antidiabetic agent and other traditional uses are anti-inflammatory, antipyretic, analgesic, antimicrobial, antibacterial, antidepressant and expectorant.[9-11] Sporadic pharmacological studies have supported traditional claims of *C. indica* in treating diabetes. But most of these studies have employed uncharacterized crude extract of the plant. A scrutiny of literature revealed that no systematic work has been conducted to isolate constituent(s) responsible for antidiabetic activity of the plant. Therefore, it was envisaged to carry out antidiabetic activity-guided-fractionation of bioactive crude fraction of *C. indica* with an objective to isolate constituent(s) responsible for antidiabetic activity.

MATERIAL AND METHOD

Plant Material

C. indica aerial parts were collected from Patiala and Chandigarh region in September, 2011. The plant was identified from the Botany Division, Forest Research Institute, Dehra Dun (Reference no 341/2011-Bot-15-1, dated 12/09/2011).

Solvents, Chemicals and Instruments

Solvents, viz. petroleum ether (60-80^{0}C), n-hexane, chloroform, methanol and ethyl acetate (E, Merck, India, LR grade) were used for preparation of extracts and fractions. Streptozotocin (Sigma chemicals, USA), sodium citrate (S.D. Fine Chemicals, Mumbai), nicotinamide (Himedia, Mumbai), metformin (Triko Pharmaceuticals, Rohtak, India), diagonstic kits (Erba, India) were used in present studies. Rotary vacuum evaporator (Buchi, Switzerland) was used for recovery of solvent under reduced pressure.

Extraction Method

C. indica aerial parts were dried under shade and powdered in a grinder. Dried powdered plant material (10 kg) was extracted successively in a Soxhlet apparatus using solvents (50 L each) in increasing order of polarity *viz.,* petroleum ether (60-80^{0}C), chloroform and methanol. The methanol extract (ME) was recovered under vacuum and kept in a desiccator. The ME (1kg) of plant material was suspended uniformly in water and taken in a separating funnel. To the contents, 500 ml *n*-hexane was added and shaken. The *n*-hexane layer was separated and discarded. The ME suspended in water was then placed in a round bottom flask and partitioned with ethyl acetate by heating at 50ºC for 30 min along with continuous stirring. This procedure of partitioning with ethyl acetate was repeated eight more times. All the separated layers of ethyl acetate were pooled and concentrated under reduced pressure to get ethyl acetate fraction (EAF) and water layer was also concentrated to get remaining methanol extract (RME).

Animals

Sprague Dawley (SD) rats, either sex, of body weight 250-350 g were purchased from IMTECH, Chandigarh. The animals were fed with normal laboratory pellet diet and water *ad libitum*. The approval was taken from Institutional Animal Ethics Committee of Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala before carrying out animal studies (107/199/CPCSEA-2012-47).

Induction of type-2 Diabetes Mellitus

A freshly prepared solution of streptozotocin (50 mg/kg. *i.p.*) in 0.1 M citrate buffer, pH 4.5 was administered intraperitoneally. The nicotinamide (100 mg/kg) was given intraperitoneally 15 min before STZ administration.[12] After 48 h of administration, rats with diabetes having glycosuria and hyperglycemia (>140 mg/dl) were taken for experimental studies.[13] Fasting glucose levels was estimated by using a glucose oxidase–peroxidase reactive strips and a glucometer (Contour, Bayer health-care, Japan).

OGTT in Normal and Diabetic Rats

The animals were fasted overnight and were loaded with 2 g/kg, p.o., D-glucose solution after 30 min of drug administration. Blood samples were collected from tail vein just prior to drug administration. Serum glucose levels are measured at 0, 30, 60 and 120 min after glucose loading.[14] Blood glucose level was measured immediately using glucometer (Contour, Bayer health-care, Japan).

Experimental Design

Three experimental protocols were designed. A total of 18 groups of animals were made and each group comprised six animals. The standard drug and test samples were administered orally to rats once daily at 9:00 AM for 10 days to diabetic rats. The blood glucose concentration was determined in all the groups on 0 day, 5th day and 10th day. The day of drug administration is considered as 0 day.

Experimental protocol I, comprising 12 groups, was designed to assess antidiabetic activity of fractions, sub fractions and isolates of *C. indica.*

Group 1 – Control group received vehicle (1 ml, *p.o.*).

Group 2 – Diabetic control received STZ-NA (50/100 mg/kg, *i.p.*).
Group 3 – Standard group received metformin (150 mg/kg, *p.o.*).
Group 4 – Test group received 9 mg/kg dose of F_1
Group 5 – Test group received 12 mg/kg dose of F_2
Group 6 – Test group received 15 mg/kg dose of F_3
Group 7 – Test group received 8 mg/kg dose of F_4
Group 8 – Test group received 3 mg/kg dose of $F_{3.1}$
Group 9 – Test group received 6 mg/kg dose of $F_{3.2}$
Group 10 - Test group received 4 mg/kg dose of $F_{3.3}$
Group 11 –Test group received 5 mg/kg dose of SA-1
Group 12 – Test group received 5 mg/kg dose of SA-2

Experimental protocol II, comprising three groups, was designed to assess oral glucose tolerance test (OGTT) of SA-2 in normal rats.
Group 1 - Control group received vehicle (1 ml, *p.o.*).
Group 2 - Standard group received metformin (150 mg/kg, *p.o.*).
Group 3 - Test group received 5 mg/kg dose of SA-2

Experimental protocol III, comprising three groups, was designed to assess oral glucose tolerance test (OGTT) of SA-2 in diabetic rats.
Group 1 – Diabetic control group received STZ-NA (50/100).
Group 2 - Standard group received metformin (150 mg/kg, *p.o.*).
Group 3 - Test group received 5 mg/kg dose of SA-2

BIOCHEMICAL STUDIES

Collection of blood and tissue sample

Animals were euthanized by diethyl ether and blood sample for biochemical estimation were collected through cardiac puncture. Blood samples were collected in tube containing EDTA for plasma analysis and without EDTA for serum analysis. Serum is obtained after centrifugation at 3000 rpm for 15 min and stored at -25^{0}C.

Determination of plasma glucose and insulin

Blood glucose was determined by the glucose oxidase-peroxidase (GOD-POD) method using a commercial kit (ERBA, Mumbai, India).[15] Serum insulin levels were determined by solid phase enzyme linked immunosorbent assay using commercial kit (ELISA Kit, Roche diagnostic Germany).[16] Homeostatic model assessment (HOMA-IR and HOMA-β) scores were calculated using serum insulin and blood glucose according to the following formula.[17]

HOMA-IR = [(Fasting serum insulin in U/L x Fasting blood glucose in mmol/L/22.5]
HOMA-β = (Fasting serum insulin in U/L x 20 / Fasting blood glucose in mmol/L- 3.5)

Determination of lipids

Triglycerides were determined by colorimetric Hantzsch condensation method using a commercial kit (ERBA, Mumbai, India).[18] Total cholesterol levels were estimated by single stable reagent method.[19] HDL cholesterol was estimated by PEG / CHOD-PAP Method using commercial kit (Coral/clinical System, India).[20] LDL-Cholesterol and VLDL-Cholesterol levels were calculated using following equation.[21]
VLDL-C =TG/5
LDL-C =Total cholesterol – (HDL-C + VLDL-C)

Determination of Hepatic Marker Enzymes

The hepatic marker enzymes *viz.*, AST (Aspartate Amino Transaminase) or SGOT (Serum Glutamate Pyruvate Transaminase), ALT (Alanine Amino Transaminase) or SGPT (Serum Glutamate Oxaloacetate Transaminase), ALP(AlkalinePhosphatase),LDH(LactateDehydrogenase) and ACP (Acid Phosphatase) levels were estimated by IFCC method using commercial kit (Coral/Clinical System, India).[22-26]

Determination of Kidney Function Markers

Creatinine level was estimated by compensated Jafee reaction method using commercial kit (Coral/clinical System, India).[27] Urea level was estimated by GLDH/UV-Kinetic Method using commercial kit (Coral/clinical System, India).

Determination of Total Protein, Albumin

Total protein levels were estimated by biuret spectrophotometric method. Albumin levels were estimated by spectrophotometric method using commercial kit (Coral/clinical System, India).[28-29]

Statistical Analysis

All the data are expressed as the mean ± S.D. The statistical analysis was carried out by using Sigma stat version 3.5. The obtained results were analyzed by one-way ANOVA followed by Student Newman Keul's test.[30]

RESULTS

The ethyl acetate fraction of methanol extract of *C. indica* aerial parts was found to be active at doses of 25 and 50 mg/kg.[31] Column chromatography of ethyl acetate fraction of *C. indica* aerial parts was performed using *n*-hexane, *n*-hexane-ethyl acetate, ethyl acetate,

ethyl acetate-methanol as mobile phases in different concentrations. A crystalline compound was obtained from fraction 1 (SA-1). EAF yielded four fractions (F_1-F_4), which were evaluated for antidiabetic activity using STZ-NA (type 2) diabetes model. It is evident from Table 1 that F_3 exhibited significant antidiabetic activity with respect to control and statistically equivalent to standard drug at the dose of 15 mg/kg. F_1, F_2 and F_4 could not able to produce antidiabetic effects comparable to the standard drug. The column chromatography of F_3 was performed using *n*-hexane, *n*-hexane-ethyl acetate, ethyl acetate, ethyl acetate-methanol as mobile phases in different concentrations and yielded three pooled sub fractions ($F_{3.1}$-$F_{3.3}$). The fraction $F_{3.2}$ yielded a pure crystalline compound (SA-2). All sub fractions ($F_{3.1}$-$F_{3.3}$) of F_3 were evaluated for antidiabetic activity using STZ-NA (type 2) diabetes model. The selection of doses of various fractions and sub fractions was made on the basis of their yields with respect to extract and fraction from which these were obtained. It is interesting to note here that none of the sub-fractions of the bioactive fraction (F_3) showed antidiabetic activity (Table 1). Two isolated compounds SA-1 and SA-2 were also subjected to antidiabetic activity in rats using STZ-NA induced (type 2) diabetes model. Only SA-2 exhibited significant antidiabetic activity at the dose of 5 mg/kg, *p.o.* (Table 1). SA-2 was characterized as quercetin by interpreting its UV, IR and NMR spectral data.

The blood glucose levels of control, isolated bioactive compound (quercetin) and standard drug (metformin) were estimated on 0, 30, 60, 90 and 120 min in the oral glucose tolerance test on normal rats (Table 2). At 120 min, quercetin after glucose load cause decrease in blood glucose level in similar pattern as observed with normal control and standard drug. Both quercetin and metformin did not show hypoglycaemic effects in OGTT in normal rats.

The blood glucose levels of diabetic rats were estimated during 0, 30, 60, 90 and 120 min after administration of vehicle, quercetin and metformin in the oral glucose tolerance test (Table 3). At 120 min, quercetin did not significantly reduce glucose levels in diabetic rats, whereas metformin significantly reduced the blood glucose level after 60 min, thus, produced antihyperglycaemic effects in diabetic rats.

The effects on the serum glucose and insulin levels were estimated after induction of STZ-NA (type 2) induced diabetes (Table 4). The homeostatic model assessment-insulin resistance (HOMA-IR) and HOMA-β (pancreatic β-cell function) scores were calculated. Quercetin showed increase in serum insulin levels and better β-cell function when compared with diabetic control. The serum lipid levels were estimated on 0 day and 10th day after induction of type 2 diabetes in rats. Quercetin and metformin caused decrease of total cholesterol, triglycerides, LDL-cholesterol, VLDL levels and increase in HDL-cholesterol levels (Table 5). Hepatic marker enzymes levels after induction of type 2 diabetes in rats were estimated on 10th day after administration of quercetin and metformin. Table 6 shows moderate decrease in levels of ALT, AST, LDH, ALP, ACP, Total Protein (TP) and albumin with quercetin and significant decrease in metformin treated groups. Kidney function markers levels after induction of type 2 diabetes in rats

Table 1: Effect of various fractions, sub-fractions and isolates of EAF on blood glucose level in diabetic rats.

Groups	Dose (mg/kg)	Blood glucose concentration (mg/dL)		
		0 day	5th day	10th day
Control	Vehicle	85.52 ± 8.36*[a]	86.49 ± 9.23*[a]	86.98 ± 8.05*
STZ-NA control	50/100	179.49 ± 15.42	192.23± 14.54[a]	200.46 ± 14.89[a]
Metformin	150	182.43 ± 16.32	140.23 ± 14.37*	96.63 ± 8.38*
F_1	9	185.07 ± 9.12	190.35 ± 11.82[a]	199.64 ± 12.81[a]
F_2	12	178.45 ± 10.76	200.49 ± 15.39[a]	205.26 ± 13.45[a]
F_3	15	183.75 ± 9.11	141.71 ± 11.68*	90.18 ± 8.29*
F_4	8	188.91 ± 9.52	196.61 ± 8.14[a]	201.34 ± 10.12[a]
$F_{3.1}$	3	192.14 ± 6.71	198.35 ± 9.81[a]	206.14 ± 8.53[a]
$F_{3.2}$	6	181.51 ± 5.16	195.34 ± 8.19[a]	217.19 ± 10.32[a]
$F_{3.3}$	4	194.11 ± 3.48	197.11 ± 11.68[a]	214.80 ± 9.19[a]
SA-1	5	187.29 ± 12.51	195.10 ± 12.31[a]	204.83 ± 10.18[a]
SA-2	5	186.68 ± 10.25	151.63 ± 11.82*	89.73 ± 11.21*

N (no. of animals) = 6; the data is expressed as Mean ± S.D.; *$P<0.05$ vs. STZ-NA control; [a]$P<0.05$ vs. Metformin (Standard drug); one-way ANOVA followed by Student Newman Keul's test.

Table 2: Effect of quercetin on oral glucose tolerance test on normal rats.

Groups	Dose (mg/kg)	Blood glucose concentration (mg/dL)			
		0 min	30 min	60 min	120 min
Control	Vehicle	75.33 ± 4.32	164.33 ± 5.20	126.33 ± 2.58	98.51 ± 2.88
Metformin	150	82.50 ± 3.39	163.33 ± 3.77	122.17 ± 3.48	95.33 ± 2.94
Quercetin	5	84.50 ± 4.13	163.83 ± 3.54	129.11 ± 2.81	99.50 ± 3.08

N (no. of animal) = 6; the data is expressed as Mean ± S.D.; $^{*}P<0.05$ vs. control; $^{a}P<0.05$ vs. Metformin (Standard drug); one-way ANOVA followed by Student Newman Keul's test.

Table 3: Effect of quercetin on oral glucose tolerance test on diabetic rats.

Groups	Dose (mg/kg)	Blood glucose concentration (mg/dL)			
		0 min	30 min	60 min	120 min
Diabetic control	50/100	185.33 ± 4.32	245.33 ± 5.20^{a}	196.33 ± 2.58^{a}	178.51 ± 2.88^{a}
Metformin	150	182.50 ± 3.39	143.33 ± 3.77*	114.17 ± 3.48*	90.33 ± 2.94*
Quercetin	5	189.52 ± 4.13	253.83 ± 3.54^{a}	181.17 ± 2. 81^{a}	159.50 ± 3.08^{*a}

N (no. of animal) = 6; the data is expressed as Mean ± S.D.; $^{*}P<0.05$ vs. Diabetic control; $^{a}P<0.05$ vs. Metformin (Standard drug); one-way ANOVA followed by Student Newman Keul's test.

Table 4: Effect of quercetin on serum glucose, insulin, HOMA-IR and HOMA-β in STZ-NA diabetic (type-2) rats.

Parameter	Day	Vehicle control	STZ-NA control	Quercetin (5 mg/kg)	Metformin (150 mg/kg)
Glucose (mg/dL)	0 day	88.67 ± 2.80*^{a}	185.67 ± 4.08	185.50 ± 4.41	181.33 ± 4.48
	10th day	91.33 ± *3.55**	225.17± *3.76*^{a}	101.50 ± 1.52*	96.33 ± *2.06**
Insulin (μIU/mL)	0 day	17.23 ± 0.42*^{a}	14.01 ± 0.71	12.24 ± 0.35	13.68 ± 0.55
	10th day	17.46 ± *0.56**	12.81 ± *0.11*^{a}	16.65 ± 0.27*	17.38 ± *0.61**
HOMA-IR	0 day	3.77 ± 0.24*^{a}	6.39 ± 1.13	6.24 ± 1.37	6.11 ± 0.21
	10th day	3.93 ± 0.65*	7.11 ± 0.39^{a}	3.65 ± 0.45*	3.94 ± 0.11*
HOMA-β	0 day	67.11 ± 1.98*^{a}	23.18 ± 0.42	20.19 ± 1.38	23.72 ± 0.56
	10th day	64.83 ± 1.54*	18.91 ± 0.37^{a}	48.82 ± 1.88*^{a}	64.65 ± 0.41*

N (no. of animal) = 6; the data is expressed as Mean ± S.D.; $^{*}P<0.05$ vs. STZ-NA control; $^{a}P<0.05$ vs. Metformin (Standard drug); one-way ANOVA followed by Student Newman Keul's test.

Table 5: Effect of quercetin on serum lipid levels in STZ-NA diabetic (type-2) rats.

Parameter	Day	Vehicle control	STZ-NA control	Quercetin (5 mg/kg)	Metformin (150 mg/kg)
Total cholesterol (mg/dL)	0 day	62.00 ± *1.41*^{*a}	112.33 ± 1.63	112.00 ± 3.27	114.00 ± 1.41
	10th day	67.16 ± *1.47**	123.83 ± *1.61*^{a}	81.50 ± 2.25*^{a}	71.66 ± *2.16**
Trigylcerides (mg/dL)	0 day	66.83 ± *2.22*^{*a}	206.83 ± 2.22	203.50 ± 3.98	218.66 ± 0.68
	10th day	64.16 ± 2.13*	211.66 ± *4.27*^{a}	87.50 ± 2.42*^{a}	66.50 ± *1.51**
HDL-cholesterol (mg/dL)	0 day	68.33 ± *2.50*^{*a}	31.50 ± 2.16	29.50 ± 1.87	32.00 ± 1.41
	10th day	66.50 ± *2.07**	22.00 ± *1.41*^{a}	47.00 ± 1.41*^{a}	71.00 ± *1.21**
LDL-cholesterol (mg/dL)	0 day	19.63 ± 2.84*^{a}	39.76 ± 2.31	41.25 ± 2.23	38.58 ± 1.48
	10th day	11.36 ± 2.27*	42.43 ± 0.29^{a}	17.26 ± 1.86*^{a}	12.90 ± 1.13*
VLDL (mg/dL)	0 day	13.30 ± 0.37*^{a}	41.23 ± 0.32	40.60 ± 3.58	43.76 ± 0.34
	10th day	12.86 ± 0.27*	42.43 ± 0.29^{a}	17.23 ± 0.58*^{a}	12.93 ± 0.37*

N (no. of animal) = 6; the data is expressed as Mean ± S.D.; $^{*}P<0.05$ vs. STZ-NA control; $^{a}P<0.05$ vs. Metformin (Standard drug); one-way ANOVA followed by Student Newman Keul's test.

Table 6: Effect of quercetin on hepatic marker enzymes levels in STZ-NA diabetic (type-2) rats.

Parameter	Day	Vehicle control	STZ-NA control	Quercetin (5 mg/kg)	Metformin (150 mg/kg)
ALT (U/L)	0 day	20.66 ± 1.36*[a]	53.00 ± 4.51	57.83 ± 2.31	55.33 ± 1.36
	10th day	21.00 ± *1.78**	66.00 ± *2.19*[a]	41.33 ± 2.16*[a]	20.50 ± *1.87**
AST (U/L)	0 day	48.00 ± 1.41*[a]	115.33 ± 3.20	122.50 ± 2.25	117.00 ± 2.36
	10th day	47.50 ± *1.87**	126.50 ± *2.25*[a]	101.83 ± 2.63*[a]	53.83 ± *2.99**
LDH (U/L)	0 day	1262.83 ± 4.07*[a]	2008.16 ± 5.67	2082.33 ± 9.60	2066.83 ± 6.43
	10th day	1269.66 ± *1.36**	2934.66 ± *5.16*[a]	1642.83 ± 3.43*[a]	1291.66 ± *3.83**
ALP (U/L)	0 day	30.33 ± 1.86*[a]	74.66 ± 2.94	72.50 ± 2.88	75.50 ± 3.89
	10th day	30.83 ± *1.16**	91.16 ± *2.04*[a]	53.16 ± 2.48*[a]	33.50 ± *1.87**
ACP (U/L)	0 day	9.45 ± 0.26*[a]	16.41 ± 0.39	15.82 ± 0.15	15.83 ± 0.11
	10th day	9.46 ± *0.37**	19.19 ± *0.19*[a]	12.46 ± 0.21*[a]	10.31 ± *0.14**
TP (g/dL)	0 day	7.47 ± 0.36*[a]	6.07 ± 0.04	6.37 ± 0.07	6.11 ± 0.03
	10th day	7.45 ± *0.34**	5.34 ± *0.26*[a]	6.75 ± 0.07*[a]	7.20 ± *0.12**
Albumin (g/dL)	0 day	3.33 ± 0.32*[a]	3.43 ± 0.14	3.72 ± 0.29	3.81 ± 0.04
	10th day	3.24 ± *0.14**	4.26 ± *0.03*[a]	3.42 ± 0.06*[a]	3.15 ± *0.08**

N (no. of animal) = 6; the data is expressed as Mean ± S.D.; *$P<0.05$ vs. STZ-NA control; [a]$P<0.05$ vs. Metformin (Standard drug); one-way ANOVA followed by Student Newman Keul's test.

Table 7: Effect of quercetin on kidney function markers levels in STZ-NA diabetic (type-2) SD rats.

Parameter	Day	Vehicle control	STZ-NA Control	Quercetin (5 mg/kg)	Metformin (150 mg/kg)
Creatinine (mg/dL)	0 day	0.28 ± 0.01*[a]	1.29 ± 0.04	1.35 ± 0.17	1.31 ± 0.04
	10th day	0.34 ± *0.02**	1.58 ± *0.03*[a]	1.02 ± 0.03*[a]	0.27 ± *0.02**
Urea (mg/dL)	0 day	31.04 ± 0.24*[a]	40.17 ± 0.06	41.21 ± 0.50	39.63 ± 0.96
	10th day	34.16 ± *0.08**	51.64 ± *0.37*[a]	39.33 ± 0.60*[a]	30.67 ± *0.37**
Uric acid (mg/dL)	0 day	2.17 ± 0.06*[a]	3.63 ± 0.06	3.53 ± 0.17	3.68 ± 0.03
	10th day	2.43 ± *0.16**	4.10 ± *0.05*[a]	3.13 ± 0.08*[a]	2.26 ± *0.18**

N (no. of animal) = 6; the data is expressed as Mean ± S.D.; *$P<0.05$ vs. STZ-NA control; [a]$P<0.05$ vs. Metformin (Standard drug); one-way ANOVA followed by Student Newman Keul's test.

were estimated on 10th day after administration of quercetin and metformin. Table 7 shows mild alteration in levels of creatinine, urea and uric acid in diabetic rat.

DISCUSSION

A scrutiny of literature reveals that few pharmacological reports on *C. indica* support its antidiabetic potential but these studies are too preliminary to validate its traditional claims. These pharmacological studies have employed crude extracts for antidiabetic activity studies. Thus it was envisaged to investigate *C. indica* aerial parts systematically with a view to isolate constituent responsible for antidiabetic activity.

The column chromatography of EAF yielded four fractions (F_1-F_4), which were evaluated for antidiabetic activity. F_3 exhibited significant antidiabetic activity at the dose of 15 mg/kg, with respect to positive control. F_1 yielded pure crystalline compound and designated as SA-1 (298 mg). Bioactive fraction F_3 was subjected to column chromatography to isolate bioactive constituents. F_3 yielded 3 sub-fractions ($F_{3.1}$-$F_{3.3}$) and a compound (transparent crystalline compound) was separated out from $F_{3.2}$ (56 mg), designated as SA-2. None of the sub-fractions of the bioactive fraction (F_3) showed antidiabetic activity. The isolated compounds (SA-1 and SA-2) were then evaluated for antidiabetic activity at the dose of 5 mg/kg, *p.o.* in rats. SA-2 (5 mg/kg, *p.o.*) exhibited significant antidiabetic activity which was equivalent to the standard drug, metformin. These observations suggested that only the isolated principle (SA-2) is responsible for antidiabetic activity

of plant. SA-2 was characterized as quercetin based on melting point, UV, IR and NMR spectral data. Quercetin has been isolated for the first time from *C. indica* aerial parts.

The type 2 diabetes is characterized with increased blood glucose level, alteration in lipid and protein metabolism.[32-33] The prevention and cure related to diagnosis of type 2 diabetes mellitus with new antidiabetic agents and therapies are decidedly depend upon on molecular basis of early defects in diabetes mellitus such as beta cell dysfunction, insulin resistance, insulin secretion, excess fatty acid and lipids, obesity and other root cause changes in normal homeostasis.[34-35] The biochemical estimations were performed to insight effects of quercetin on carbohydrate, protein and lipid metabolism.

Oral Glucose Tolerance Test (OGTT) is used as prime test for identification of diabetes development and glucose tolerance in patients.[36] Quercetin, isolated from *C. indica* aerial parts was subjected to oral glucose tolerance test on normal and diabetic rats. Quercetin (5 mg/kg) and metformin (150 mg/kg) did not significantly reduce blood glucose levels in normal rats whereas only metformin considerably reduced blood glucose levels in diabetic rats after 60 min of glucose load. These observations confirmed that quercetin and metformin do not possess hypoglycaemic effect in normal rats but metformin exhibits hypoglycaemic effect in diabetic rats. Our results are in conformity with literature reports where quercetin and metformin did not show hypoglycaemic effects in normoglycemic animals.[37-40] Flavonoids act as glucosidase inhibitor and exhibit their effect only on disaccharidases and regulate glucose homeostasis through **α-glucosidase activity**.[41] Quercetin causes α-glucosidase inhibition and decrease postprandial hyperglycaemia in T2DM patient loaded with maltose and shows no effect in glucose load.[42]

The effectiveness of antidiabetic agents in management of diabetes are judged with measurement of glucose levels in body.[43-44] Quercetin at the dose of 5 mg/kg significantly reduced glucose levels compared to diabetic control on 10th day. Quercetin exhibited a remarkable hypoglycaemic effect similar to metformin. Our results are in agreement with literature reports where quercetin has been reported as antidiabetic agent.[37,45-46] Homeostatic model assessment (HOMA) is a clinical tool for calculation based assessment of beta cell function (HOMA-β) and development of insulin resistance (HOMA-IR), which is derived from glucose and insulin concentrations.[47] Quercetin significantly reduced insulin resistance (HOMA-IR) and increased β-cell sensitivity (HOMA-β) in diabetic rats. These results are in agreement with available literature.[48-51]

Lipids and lipoproteins malfunctioning are associated with type 2 diabetes mellitus and development of insulin resistance. The lipid profile abnormalities in diabetes include elevated levels of Total Cholesterol (TC), Triglycerides (TG), Low Density Lipoprotein (LDL-cholesterol), Very Low Density Lipoprotein (VLDL) and reduced levels of High Density Lipoprotein (HDL-cholesterol).[52-55] Insulin resistance has prominent effects on size and concentration of lipoproteins (VLDL, LDL and HDL).[56] Quercetin treatment significantly lowered elevated levels of TC, TG, LDL-cholesterol, VLDL and increased HDL-cholesterol levels when compared with diabetic control. These results confirm potential of quercetin to reduce risk of cardiovascular disorder and atherosclerosis in diabetes mellitus. These results are in agreement with reports in which quercetin has shown effects such as decrease in serum lipids, improvement of lipid homeostasis and reduction in cardiovascular risk associated with diabetes.[57-59]

The liver has prominent role in metabolism of insulin and maintenance of glucose homeostasis in fasting and non-fasting conditions. The liver dysfunction results into hepatic insulin resistance and cause progression in diabetes.[55,60-61] The common hepatic marker enzymes include serum albumin and total proteins, Lactate Dehydrogenase (LDH), Acid Phosphatase (ACP) and aminotransferases *viz.,* Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP).[62] The mild and chronic elevation of aminotransferases is indicator of hepatic insulin resistance.[63] The increased levels of ALT and AST are clinical feature of metabolic syndrome in type 2 diabetic patients.[64-65] The metabolic syndrome is characterized with insulin resistance and diabetes.[65] In present study, alteration in hepatic marker enzymes is confirmed with increased levels of ALT, AST, LDH, ALP, ACP, albumin and decrease in level of total proteins in diabetic rats. Quercetin treatment caused moderate decrease in elevated levels of ALT, AST, LDH, ALP, ACP, albumin but no significant difference found in total proteins concentration when compared with diabetic control. Based on these observations, it is suggested that quercetin exhibits weak effects on hepatic marker enzymes in diabetic rat. Diabetic kidney disease (DKD) and end-stage renal disease (ESRD) development in diabetic patients are most common disorders in diabetes mellitus.[66-68] Quercetin treatment showed slight changes in the levels of creatinine, urea and uric acid in diabetic rats.

CONCLUSION

Proper implementation of rational strategy, i.e., bioactivity-directed-fractionation led to the isolation of

antidiabetic compound from ethyl acetate fraction of *C. indica*. The traditional claims of *C. indica* for anti-diabetic activity are attributed to a bioactive flavonoid compound, quercetin.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

ACP: Acid phosphatase; **ALP:** Alkaline phosphatase; **ALT:** Alanine amino transaminase; **ANOVA:** Analysis of variance; **AST:** Aspartate amino transaminase; **EAF:** Ethyl acetate fraction; **HDL:** High density lipoproteins; **HFD:** High-fat diet; **HOMA**: Homeostatic model assessment; **i.p.:** Intraperitoneally; **IR:** Infrared; **LDH:** Lactate dehydrogenase; **LDL:** Low density lipoprotein; **NA:** Nicotinamide; **NMR:** Nuclear Magnetic Resonance; **OGTT:** Oral glucose tolerance test; **p.o.:** Per oral; **SD:** Standard deviation; **SD rats:** Sprague Dawley rats; **STZ:** Streptozotocin; **TC:** Total cholesterol; **TG:** Triglycerides; **UV:** Ultraviolet; **VLDL:** Very Low Density Lipoprotein.

SUMMARY

Coccinia indica has been used in Ayurvedic and Unani practice in the Indian subcontinent for the treatment of various ailments especially in diabetes. Systematic phytochemical and antidiabetic activity studies were needed to validate its traditional claims. Thus, the present investigation was undertaken to fractionate bioactive ethyl acetate fraction (EAF) of *C. indica* aerial parts to isolate bioactive compound responsible for antidiabetic activity of the plant. Fractionation of bioactive EAF of *C. indica* aerial parts led to the isolation of a compound which was characterized as quercetin. Quercetin exhibited significant antidiabetic activity in STZ-NA type 2 diabetic model and normalized disturbed glucose lipid metabolism in diabetic rats.

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