Original Article

Antidiabetic and Antihyperlipidemic Activity of Alcoholic and Hydroalcoholic Extracts of *Cocculus orbiculatus* in Streptozotocin Induced Diabetic Rats

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ABSTRACT

Objective: The purpose of study was to determine the aerial parts of the plant *Cocculus orbiculatus* (Menispermaceae) for antidiabetic and antihyperlipidemic effects in streptozotocin induced diabetic rats.
Methods: *Cocculus orbiculatus* was subjected to soxhlet extraction. Aqueous, alcoholic and hydroalcoholic extracts were obtained. These extracts were tested for toxicity unto 2000 mg/kg as per OECD-423 guidelines. Antidiabetic activity was assessed by oral glucose tolerance test and streptozotocin induced model. In oral glucose tolerance test COAE and COHAE exhibited greater activity compared to aqueous extract. Hence the two extracts were further screened by streptozotocin induced model at 75, 150 and 300 mg/kg for 15 days.
Results: COAE and COHAE effectively lowered serum glucose, triglycerides, cholesterol, low density lipoproteins, very low density lipoproteins, aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), creatinine, urea and elevated high density lipoprotein (HDL) levels, liver glycogen levels and body weight.
Conclusion: COAE and COHAE elicited dose-dependent effect. The effect produced at the lower dose (COAE & COHAE) was not considerable. Hydroalcoholic extract (300 mg/kg) elicited greater antidiabetic and antihyperlipidemic activity compared to alcoholic extract (300 mg/kg).

Keywords: Antidiabetic, Antihyperlipidemic, Streptozotocin, Oral glucose tolerance test, *Cocculus orbiculatus*.

INTRODUCTION

Diabetes mellitus is characterized by raised blood glucose level which in turn is associated with increase in the risk for microvascular and macrovascular disease[1,2]. The overall world wide epidemic of diabetes is anticipated to double by 2025 attacking to about 5% of the adult population[3]. In the United States diabetes affects for more than 20 million persons[4]. According to the world wide survey more than 90% of diabetics belong to type II[5]. Death rate in people with diabetes is twice than that without diabetes[6]. Medicinal plants are gaining extensive importance, as they are rich sources for natural antidiabetic and antihyperlipidemic constituents, which lead to minimal side effects and inexpensive cost.

Cocculus orbiculatus (L.) DC belonging to family Menispermaceae is commonly called queen coral bead. It is a deciduous climber growing unto 4m. It flowers (monoecious) in August and seeds ripe in October[7]. Stem component of *Cocculus orbiculatus* possess the active constituents like (-)-sinococculine and (+)-isotrilobine that possess effective anticancer property[8]. Bisbenzyl isoquinoline alkaloids, amidic aporphines and berberine have been isolated from stems of *Cocculus orbiculatus*[9]. Bisbenzyl isoquinoline alkaloids were reported to possess antibacterial[8,10], cytotoxic, antimalarial, anticancer, hypotensive, anti-plasmodial, anti-cholinesterase activity. Leaves were used as food; bleached tendrils were used as ornamental infill in basket making[10,11]. Root is anodyne, anti-phlogistic, anti-rheumatic, carminative, depurative, diuretic, bactericidal[12] and vermifuge[13-15]. Distinct parts of plant have been claimed for their use in distinct diseases like anti-microbial[7], anticancer[8], asthma, bronchitis, paralysis, rheumatic arthritis, oedema, oliguria, intestinal parasite disorders[16], antifungal[9], antibacterial, antiamoebic and insecticidal activity[13,14]. *Cocculus orbiculatus*, the reported medicinal plant possess alkaloidal constituents like aporphines and berberine which have been claimed to produce antidiabetic activity. Scientifically studies were not reported regarding antidiabetic activity. Therefore the plant was choosen to assess antidiabetic and antihyperlipidemic activity.

MATERIALS AND METHODS

Aerial parts of *Cocculus orbiculatus* were collected from the ayurvedic center, vanasthalipuram, Hyderabad. It was authenticated by Dr. Madhavachetty, Associate Professor, Dept of botany, Sri Venkateshwara University, Tirupathi). A voucher specimen (N0: 581) was deposited in the college herbarium. Plant was shade dried, powdered and moved through sieve no. 60.

Preparation of extract

The powdered material was packed in soxhlet apparatus and extracted using alcohol, hydroalcohol (60:40) and water. The extract was filtered using whatman filter paper. The filtrate was concentrated using rotary evaporator at 40°C and stored in a dessicator.

Animals

Male wistar rats (250-300 g) were procured from Albino Research and Training Institute, Bachupally, Hyd). The rats were accomidated in polypropylene cages at standard laboratory conditions of temperature (23±2 °C), humidity (50-55%), light dark cycles (12 h: 12 h), standard rat pellet feed (Hindustan Lever Ltd.,) and water *ad libitum*. They were acclimatized for a period of 7 days prior to the experiment. Experimental protocol was accepted by the Institutional Animal Ethics committee (IAEC) guidelines of Albino Research and Training Institute comprising CPCSEA Regn No: 1722/PO/A/ 13/IAEC/CPCSEA EXP-049).

Chemicals

Streptozotocin (STZ) was procured from (loba chemie, Mumbai, India). Glibenclamide was purchased from (Apollo pharmacy, B. N Reddy Avanthi pharmaceuticals, Hyderabad). Glucose obtained from Qualigens fine chemicals, Mumbai. Kits for estimation of TG, CH, HDL were obtained from Span diagnostics pvt ltd, Surat, India. Estimations were carried out using ELICO semiauto CL 380.

Acute toxicity study

Acute toxicity study was conducted according to the OECD guidelines 423 using acute toxic class method. Animals were divided into groups containing three animals each. Doses of 300 mg/kg, 2000 mg/kg were given and observed for mortality and behavioural changes upto14 days.

Oral glucose tolerance test

This test is employed as preliminary screening model to evaluate antidiabetic activity. Overnight fasted rats were allocated to six groups possessing five each.

Group I: Normal control administered 1% sodium carboxymethyl cellulose.
Group II: Glucose control administered glucose (3 gm/kg).
Group III: Standard administered glibenclamide (10 mg/kg).
Group IV, V and VI: Received aqueous, alcoholic, and hydroalcoholic extracts at a dose of 100 mg/kg each to assess the effect of extracts on blood glucose levels. Blood samples were withdrawn from retro orbital plexus at intervals of 0, 30, 90 and 150 min for glucose estimation.

Induction of diabetes

Streptozotocin (50 mg/kg) was freshly formulated in 0.01M ice cold citrate buffer (PH 7.4) and administered intraperitoneally to overnight fasted rats. After 72 h rats possessing blood glucose level greater than 200 mg/dl were deemed as diabetic and assigned into the experimental study.

Experimental design

Rats were allocated into nine groups constituting five each.

Group I: Normal control administered 1% sodium carboxymethyl cellulose.
Group II: Diabetic control administered STZ (50 mg/kg, i.p).
Group III: Standard administered glibenclamide (10 mg/kg).
Group IV, V and VI: Received alcoholic extract of 75 mg/kg, 150 mg/kg and 300 mg/kg.
Group VII, VIII, and IX: Received hydroalcoholic extract of 75 mg/kg, 150 mg/kg and 300 mg/kg.

Treatment continued for 15 days. Samples withdrawn from retro orbital plexus under mild anaesthesia on 1st, 5th, 10th and 15th day. Serum separated by centrifugation (2000 r/m; 20 min) was used for estimating the parameters like glucose, triglycerides, cholesterol, HDL, LDL, VLDL, AST, ALT, ALP, creatinine and urea.

Statistical analysis

Results were expressed as mean ±SEM. Significance of data was evaluated by graph pad in stat version 3.2. P value of analysis less than 0.05 was considered to be statistically significant.

RESULTS

Acute oral toxicity study

Cocculus orbiculatus was non toxic upto 2000 mg/kg b.wt. Hence the dose selected for the study was 1/6th of LD_{50}. Gross behavioral changes were not observed during the studies.

Oral glucose tolerance test

All the three extracts (aqueous, alcoholic and hydroalcoholic) have shown reduction in blood glucose levels when compared to glucose control group. Among these, hydroalcoholic extract showed a greater decrease than alcoholic and aqueous extracts.

Antidiabetic activity

Administration of vehicle in normal group did not elicit significant change in blood glucose levels. Administration of Glibenclamide have shown significant reduction (71.75%; $P<0.01$) in glucose levels when compared to normal group. COAE and COHAE at doses of 75, 100 and 300 mg/kg produced reduction of (39.54%, 44.62%, 55.83%, 42.44%, 49.85%, 61.64%). Among the groups COHAE at a dose of 300 mg/kg elicited greater antihyperglycemic activity.

Antihyperlipidemic activity

Diabetic control rats have shown an elevation in total cholesterol, serum triglycerides, low density lipoproteins, very low density lipoproteins and decline in HDL compared to normal group. Alcoholic and hydroalcoholic extracts of *Cocculus orbiculatus* (75, 100 and 300 mg/kg) showed a decrease in triglycerides, total cholesterol, low density lipoproteins, very low density lipoproteins and an increase in high density lipoproteins when compared to diabetic control group. At lower dose COAE and COHAE produced very less effect. COHAE at a dose of 300 mg/kg exhibited greater antihyperlipidemic activity.

Effect of extract on AST, ALT & ALP levels

Increase in AST, ALT & ALP levels were found in diabetic control group (47.13%, 38.89%, 31.30%) when compared to normal group. Alcoholic and hydroalcoholic extract treated groups (75, 100 and 300 mg/kg) have shown decrease in the AST levels (13.04%, 20%, 29.39%, 16.52%, 20%, 36.7%); ALT levels (7.20%, 22.02%, 29.77%, 5.47%, 18.15%, 35.70%); and ALP levels (19.14%, 23.52%, 29.22%, 17.1%, 16.64%, 27.51%).

Effect of extract on creatinine and urea levels

Diabetic control group have shown increase in creatinine and urea levels (78.45%, 49.65%). Glibenclamide, Alcoholic and Hydroalcoholic extract treated group have shown decrease in creatinine levels (79.26%, 62.60%, 67.06%, 67.88%, 53.25%, 63.41%, 89.83%) and urea levels (49.97%, 21.01%, 31.71%, 42.81%, 24.17%, 30.53%, 48.72%).

Effect of extract on body weight

Normal rats have shown increase in body weight on 15th day (8.63%). A significant decrease in the body weight (6.94%) was observed in diabetic control group. Alcoholic and hydroalcoholic extract treated groups (150 and 300 mg/kg) have shown increase in the body weight (8.35%, 7.65%, 7.03%, 8.41%). Increase in the body weight at a dose of 75 mg/kg was insignificant for both COAE and COHAE.

Effect of extract on liver glycogen levels

Diabetic control group manifested decline in (50%) liver glycogen levels on 15th day. Glibenclamide, Alcoholic and Hydroalcoholic extract treated groups (75,150 and 300 mg/kg) have shown increase in liver glycogen levels (30.55%, 41.86%, 44.44%, 34.21%, 40.47%, 46.80%).

DISCUSSION

In OGTT aqueous, alcoholic and hydroalcoholic extracts improved glucose tolerance at 90 min and 150 min suggesting peripheral utilization of glucose. COHAE (100 mg/kg) was more potent when compared to COAE (100 mg/kg). Aqueous extract (COAqE) exhibited decreased glucose

tolerance effect. Therefore aqueous extract is not evaluated further. Streptozotocin is extensively employed to screen natural products for their insulinomimetic, insulinotropic and other antihyperglycemic activities[17-22]. Streptozotocin induced hyperglycemia by cytotoxic action on pancreatic beta cells[23,24]. COAE and COHAE elicited antihyperglycemic activity in a dose-dependent manner. At lower dose (75 mg/kg); COAE and COHAE produced lesser antidiabetic effect. At higher dose (300 mg/kg); COAE and COHAE produced greater antidiabetic effect. Antidiabetic effect of COHAE (300 mg/kg) was comparatively more than COAE (300 mg/kg). Both the extracts produced less antidiabetic activity than Glibenclamide Extract was unable to restore glucose level to the base line value. This indicates *Cocculus orbiculatus* should be employed with alternatives like diet or hypoglycemic agents for diabetes control. Glibenclamide, a sulfonyl urea derivative elicited antidiabetic activity by stimulating beta cells of pancreas. Mechanism related *in vitro* studies were crucial to assess the mode of action. Hyperglycemia produced is accompanied by an increase in serum triglycerides, total cholesterol, low density lipoproteins and decreased high density lipoproteins. Administration of COHAE and COAE normalized blood glucose levels along with restoration of serum triglycerides and cholesterol levels. Hence both extracts were considered to possess antidiabetic activity and antihyperlipidemic activity. Antidiabetic activity elicited might be related to the existence of active alkaloidal constituents; aporphine and berberine[9]. Aporphine produced antidiabetic affect by inhibiting intestinal glucose uptake[25]. Scientific investigation revealed that berberine modulated glucose and lipid metabolism through a multiple pathway methodology of AMP-activated protein kinase (AMPK); P38 MAPK-glut4, JNK pathway and PPAR α pathway[26]. Berberine also elevated insulin sensitivity in insulin resistant rat models[27]. In diabetic control group, the typical loss of body weight is probably because of impairment in insulin action in the conversion of glucose into glycogen and catabolism of fats[28]. Treatment with extract (COAE & COHAE), substantially prevented loss of body weight due to reversal of gluconeogenesis or release of insulin. Extract treated groups increased glycogen content might be due to decreased endogenous glucose output from liver. Derangements in metabolic processes during the progression are frequently associated with alteration in serum enzyme activities. Hence the estimation of serum enzymes has become prominent in diabetes. In diabetes, the increased amino acids; which tend to be active in absence of insulin were responsible for increased formation of gluconeogenesis and ketogenesis[29]. COAE & COHAE have shown reduction in the level of AST, ALT and ALP which might be by inhibiting gluconeogenesis process.

CONCLUSION

Results of the experimental study reveal that alcoholic and hydroalcoholic extracts of *Cocculus orbiculatus* possess promising antidiabetic and antihyperlipidemic activity in dose-dependent manner. COHAE manifested enhanced antidiabetic activity compared to COAE. However investigation was essential to isolate bioactive principles and to illuminate the accurate antidiabetic mechanism of action.

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Conflict of interest

None declared.

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Table 1. Phytochemical screening of *Cocculus orbiculatus*

Phyto constituents	Alcoholic	Hydroalcoholic	Aqueous
Alkaloids	++	++	--
Glycosides	++	++	++
Carbohydrates	++	++	++
Flavonoids	++	++	--
Saponins	++	++	++
Tannins	++	++	--
Terpenoids	++	++	++
Polyphenols	++	++	--
Starches	--	++	++

Note: Present (++); Absent (--)

Table 2. Effect of *Cocculus orbiculatus* on oral glucose tolerance test in normal control rats (mean ± SEM)

Group	Treatment (mg/kg b.wt)	Serum glucose (mg/dl)			
		Initial	30 min	90 min	150 min
I	Normal Control	66.30±2.79	65.48±1.36	67.98±2.48	68.10±3.06
II	Control + 10 g/kg glucose	68.22±1.23	109.80±3.10**	117.68±4.20**	130.44±3.22**
III	Glibenclamide(10)	71.36±2.58	83.42±2.81**	77.44±4.39**	72.46±2.90**
IV	Ethanolic (100)	69.64±2.54	85.76±3.51**	82.52±2.64**	79.75±2.95**
V	Hydroalcoholic (100)	70.26±2.32	85.54±2.15**	80.56±3.89**	73.40±1.75**
VI	Aqueous (100)	74.43±1.25	92.40±3.04**	90.76±5.0**	87.64±2.59**

n=5; Group II was compared with Group I. Groups III- VI were compared with Group II. *P<0.05. **P<0.01.

Table 3. Effect of *Cocculus orbiculatus* on serum glucose levels in STZ induced diabetic rats (mean ± SEM)

Group	Treatment (mg/kg b.wt)	Serum glucose (mg/dl)				
		Initial	1st day	5th day	10th day	15th day
I	Normal Control	72.2±2.110	67.60±2.80	69.00±2.30	70.20±2.90	75.50±3.20
II	Diabetic Control	73.0±1.20	260.90±5.24**	27.350±5.20**	281.60±6.20**	297.50±6.94**
III	Glibenclamide (10)	68.8±0.95	244.30±3.30*	141.30±4.70**	88.30±3.60**	69.00±3.20** (71.75%)
IV	COAE (75)	71.45±3.15	241.05±2.97**	196.34±2.83**	187.78±2.61**	145.73±4.10** (39.54%)
V	COAE (150)	72.7±2.23	242.60±4.90**	190.60±3.50**	180.90±3.20**	134.34±3.30** (44.62%)
VI	COAE (300)	72.7±8.15	259.00±3.11	169.40±4.70**	128.40±2.40**	114.40±4.20** (55.83%)
VII	COHAE (75)	73.57±2.73	243.65±3.55**	192.45±3.97**	180.57±3.11**	140.23±3.34** (42.44%)
VIII	COHAE (150)	70.5±3.63	245.50±3.09*	185.20±3.0**	170.40±3.90**	123.10±6.80** (49.85%)
IX	COHE (300)	71.3±2.45	250.00±3.63	167.30±3.50**	102.90±2.70**	95.90±1.94** (61.64%)

n=5; Group II was compared with Group I. Groups III- IX were compared with Group II. *P<0.05. **P<0.01

Table 4. Effect of *Cocculus orbiculatus* on serum TC, TG, HDL, LDL, VLDL levels on 15th day (mean ± SEM)

Group	Treatment (mg/kg b.wt)	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
I	Control	143.10±3.1	74.60±0.8	35.50±1.2	92.60±1.3	14.90±0.11
II	Diabetic Control	240.90±4.5** (40.59%)	147.50±4.2** (49.24%)	30.50±0.3** (14.08%)	180.90±2.4** (48.41%)	29.50±0.19** (49.49%)
III	Glibeclamide (10)	145.40±3.0** (39.64%)	83.50±1.9** (43.39%)	35.70±1.0** (14.56%)	93.30±1.7** (48.42%)	16.70±0.08** (43.38%)
IV	COAE (75)	187.31±4.1** (22.45%)	112.15±3.0** (23.97%)	30.35±0.27 (0.49%)	134.53±2.56 (25.63%)	22.43±0.47** (23.96%)
V	COAE (150)	178.30±3.9** (25.98%)	93.40±2.3** (36.67%)	32.10±0.33 (4.98%)	127.50±2.0** (29.51%)	18.60±0.25** (36.94%)
VI	COAE (300)	164.20±3.3** (31.83%)	88.80±2.2** (39.79%)	33.20±0.4* (8.13%)	113.10±1.6** (37.47%)	17.70±0.27** (40%)
VII	COHAE (75)	179.17±3.9** (25.62%)	109.23±3.1** (25.95%)	33.41±0.35* (8.71%)	123.91±2.61 (31.50%)	21.85±0.77** (25.93%)
VIII	COHAE (150)	184.00±4.0** (23.62%)	99.00±2.2** (32.88%)	35.20±0.5** (13.52%)	130.00±2.3** (28.13%)	19.80±0.29** (32.88%)
IX	COHAE (300)	149.00±3.3** (38.14%)	81.40±2.4** (44.81%)	36.80±0.53* (17.12%)	95.80±1.7** (47.04%)	16.20±0.25** (45.08%)

n=5; Group II was compared with Group I. Groups III- IX were compared with Group II.
*P<0.05. **P<0.01.

Table 5. Effect of *Cocculus orbiculatus* on animal body weight (mean ± SEM)

Group	Treatment (mg/kg b.wt)	Animal body weight (g)			
		Initial	5th day	10th day	15th day
I	Normal Control	275.00±5.34	280.40±3.21	289.00±5.38	301.40±6.69
II	Diabetic Control	282.40±5.42	276.20±5.77	268.00±3.64*	262.80±4.45** (6.94%)
III	Glibenclamide (10)	284.60±3.11	294.40±3.99*	298.20±4.80**	306.20±3.93** (7.05%)
IV	COAE (75)	288.45±4.15	300.50±4.10**	296.40±2.10**	310.50±3.55** (7.10%)
V	COAE (150)	285.20±3.12	294.00±4.92*	302.00±5.40*	311.20±4.92** (8.35%)
VI	COAE (300)	287.00±5.50	298.20±3.30**	305.20±3.49**	310.80±5.59** (7.65%)
VII	COHAE (75)	289.67±5.30	305.65±3.15**	310.56±3.11**	315.32±4.15** (8.13%)
VIII	COHAE (150)	290.60±4.86	303.20±3.73**	307.00±3.83**	312.60±6.96** (7.03%)
IX	COHAE (300)	285.20±3.85	294.90±4.39*	302.90±5.97**	311.40±5.10** (8.41%)

n=5; Group II was compared with Group I. Groups III- IX were compared with Group II.
*P<0.05. **P<0.01.

Table 6. Effect of *Cocculus orbiculatus* on AST, ALT and ALP

Group	Treatment (mg/kg b.wt)	Serum (U/L)		
		AST	ALT	ALP
I	Normal Control	15.20±0.60	13.40±1.10	36.20±1.60
II	Diabetic Control	28.75±2.00** (47.13%)	21.93±0.90** (38.9%)	52.7±2.53 ** (31.30%)
III	Glibenclamide (10)	16.30±1.30** (43.3%)	14.60±0.82 ** (33.42%)	37.4±2.70** (29.03%)
IV	COAE (75)	25.0±1.73 (13.04%)	23.51±1.70 (7.20%)	44.56±2.31 (19.14%)
V	COAE (150)	23.0±1.50 (20.0%)	17.1±0.83 *(22.02%)	40.3±2.20** (23.52%)
VI	COAE (300)	20.3±1.70** (29.39%)	15.4±1.20** (29.77%)	37.30±1.80** (29.22%)
VII	COHAE (75)	24.0±1.90 (16.52%)	20.73±0.92 (5.47%)	47.19±3.15 (17.1%)
VIII	COHAE (150)	23.0±1.57 (20.0%)	17.95±0.20 *(18.15%)	43.93±1.02* (16.64%)
IX	COHAE (300)	18.2±0.90 ** (36.7%)	14.1±0.80** (35.70%)	38.20±1.90** (27.51%)

n=5; Group II was compared with Group I. Groups III- IX were compared with Group II.
*P<0.05. **P<0.01.

Table 7. Effect of *Cocculus orbiculatus* on serum creatinine and serum urea levels on 15th day (mean ± SEM)

Group	Treatment (mg/kg b.wt)	Serum Creatinine (mmol/L)	Serum Urea (mg/dl)
I	Normal Control	0.53±0.11	31.44±2.50
II	Diabetic Control	2.46±1.0** (78.45%)	62.44±4.86** (49.65%)
III	Glibenclamide (10)	0.51±0.13** (79.26%)	31.24±2.33** (49.97%)
IV	COAE (75)	0.92±0.14** (62.60%)	49.32±1.62*(21.01%)
V	COAE (150)	0.81±0.12*(67.07%)	42.64±2.75*(31.71%)
VI	COAE (300)	0.79±0.13*(67.88%)	35.71±2.87** (42.81%)
VII	COHAE (75)	1.15±0.13 (53.25%)	47.35±2.63** (24.17%)
VIII	COHAE (150)	0.9±0.10*(63.41%)	43.38±3.03** (30.53%)
IX	COHAE (300)	0.25±0.14** (89.83%)	32.02±2.26** (48.72%)

n=5; Group II was compared with Group I. Groups III- IX were compared with Group II.
*P<0.05. **P<0.01.

Table 8. Effect of *Cocculus orbiculatus* on liver glycogen levels on 15th day (mean ± SEM)

Group	Treatment (mg/kg b.wt)	Liver glycogen levels (mg/gm of wet tissue)
I	Normal Control	50±3.210
II	Diabetic Control	25±2.310** (50%)
III	Glibenclamide (10)	48±5.50** (47.9%)
IV	COAE (75)	36±3.20 (30.55%)
V	COAE (150)	43±2.35*(41.86%)
VI	COAE (300)	45±5.20** (44.44%)
VII	COHAE (75)	38±4.56 (34.21%)
VIII	COHAE (150)	42±3.00* (40.47%)
IX	COHAE (300)	47±4.80** (46.80%)

n=5; Group II was compared with Group I. Groups III- IX were compared with Group II. *P<0.05. **P<0.01.