RESEARCH PAPER

Accepted 20 October 2000
Revised 14 October 2000
Received 3 April 2000
Indian J. Pharm. Sci. 2001, 63(1) 30-35

Studies on Hypoglycemic and Cardiotonic Effects of Roots of *Cocculus hirsutus*

K. SATYANARAYANA, V. MANGATHAYARU, J. SREEKANTH, V. VENKATESWARLU* AND C.K. KOKATE
Faculty of Pharmaceutical Sciences
Kakatiya University, Warangal-506 009

Methanol extract of roots of *Cocculus hirsutus* was tested for its hypoglycemic and cardiotonic effects on diabetic rats and isolated perfused frog heart respectively. The methanol extract exhibited significant hypoglycemic activity on diabetic rats and cardiotonic activity on normal and hypodynamic frog heart preparation. Activity-guided fractionation of methanol extract was carried out. Butanol fraction of methanol extract of roots of *C. hirsutus* was found to have significant cardiotonic activity comparable to that of ouabain. Total alkaloid fraction prepared from methanol extract showed considerable hypoglycemic activity on alloxan-induced diabetic rats. The TLC profile of total alkaloid fraction showed the presence of four alkaloids, whereas the TLC profile of butanol fraction showed the presence of steroids and/or triterpenoids.

The plant *Cocculus hirsutus*, Linn, Diels (syn: *C. villosus*, family:minispermaceae) is a climbing shrub with dense hairy young parts growing in the tropical and subtropical regions of India, Africa, South China, Arabia and Ceylon. In India *C. hirsutus, C. laurifolius* and *C, macrocarpus* plants are available[1]. Root has been used as a laxative, diuretic, antiperiodic for fever, nervine tonic and for the treatment of rheumatism, bilious dyspepsia and abdominal ache in children. The juice of the leaves in water has been used in skin diseases and acute gonorrhea[1-3]. *Cocculus hirsutus* is commonly known as Jal-Jammi in India because of the fact that an aqueous extract, obtained by crushing fresh leaves of the plant in water, sets into a gel on standing[4]. Rao and Row reported the isolation of d-trilobine and dl-coclaurine from the roots of this plant[5]. Previous investigations on various parts of the plant resulted in the isolation of β-sitosterol, ginnol, a monomethyl ether of inositol[6], isotrilobine, coclaurine, magnoflorine[7], hirsudiol, nonacosan-10-ol[8], shaheenine[9], cohirsinine[10], hirsutine[11] and cohirsutinine[12]. The water soluble fraction of the ammonical ethanol extract of dried stems and roots of *Cocculus hirsutus* have been found to have central sedative, hypotensive, bradycardiac, cardiotonic and spasmolytic actions[13]. Masilamani *et al.* reported that the leaf juice of *Cocculus hirsutus* is effective in the treatment of eczema[14]. A recent study has reported upon the elaboration of antitumor metabolites by *Cocculus hirsutus* and *Cocculus pendulus* in tissue culture and high alkaloid producing cell lines have been established[15].

The present report covers the preliminary screening of extracts of roots of *Cocculus hirsutus* for hypoglycemic activity in alloxan-induced diabetic rats and cardiotonic activity on the isolated frog heart. The local tribes have been using the roots of this plant in the treatment of diabetes and a previous report revealed the presence of cardiotonic principles in the roots of *Cocculus hirsutus*[13].

MATERIALS AND METHODS

Plant Material:

Roots of *Cocculus hirsutus* were collected in the vicinity of Kakatiya University campus, Warangal, Andhra Pradesh and specimen samples were deposited in the Pharmacognosy Laboratory of the University College of Pharmaceutical Sciences, Kakatiya University. The plant

*For Correspondence

is a climbing, straggling, scandent, undershrub. Its leaves are alternate, dorsiventral, petiolate, exstipulate, ovate, ovate-oblong or sub-lanceolate. Roots of the plant are dark brown when wet and yellowish brown on drying with numerous irregular swellings on the surface.

Preparation of Methanol extract and Fractions:

The air-dried roots of *Cocculus hirsutus* (5 Kg) were powdered and exhaustively extracted with methanol. After evaporation of the solvent using a rotary flash evaporator, a dark brown viscous residue was dried in a desiccator to get a semisolid residue (350 g). About 100 g of methanol extract (ME) was loaded onto column packed with silica gel C and successively eluted with each of 1L of toluene, chloroform, ethyl acetate, n-butanol, methanol and methanol:water (1:1). All the fractions were concentrated using a rotary flash evaporator and per cent yields were recorded.

Isolation of total Alkaloids (TA):

The methanol extract (100 g) was extracted with aqueous 1% acetic acid (2 x 1L) and was kept overnight and filtered. The aqueous acid extract was extracted with chloroform (4x500 ml). The aqueous layer was rendered alkaline with ammonia (pH 9-10) and extracted with chloroform (4x500 ml). The pooled chloroform layers were evaporated to dryness to obtain total alkaloids (8.2 g).

Thin Layer Chromatography:

Butanol fraction (BF) of methanol extract and total alkaloid fraction (TA) were subjected for TLC on precoated silica plates (Si F, E. Merck, Darmstadt) using ethyl acetate:methanol:water (81:11:8) solvent system and toluene:ethyl acetate: diethylamine (70:20:10) solvent system respectively. Vanillin-sulphuric acid reagent and Dragendorff's reagent were used for the detection of spots on TLC plates of BF and TA respectively.

Male adult, healthy albino rats weighing between 200-250 g procured from National Institute of Nutrition, Hyderabad were used in these experiments. The animals were kept in an air-conditioned animal room and were offered a balanced rat feed and allowed tap water *ad libitum*. Frogs were purchased from a local animal supplier, Warangal. They were housed in a pond until use.

Preparation of Diabetic Rats and Evaluation of Hypoglycemic Activity:

Rats were made diabetic by injecting alloxan monohydrate intraperitoneally at a dose of 150 mg/kg body weight[16]. One week after injection, blood was collected from the tail vein of all surviving rats and the blood sugar levels were determined by the o-toluidine method of Fings *et al.*[17] Rats with blood sugar levels of 200-400 mg/100 ml were considered as diabetic and were employed in the study.

The rats were randomly divided into groups of six animals each. Rats in group 1 (untreated diabetic) were received a 2% gum tragacanth mucilage. The treated group received a suspension of the methanol extract (methanol extract was suspended in 2% gum tragacanth mucilage just before administration to rats) orally at a dose of 200 mg/kg body weight. The treatment was continued for 7 days administering methanol extract or 2% gum tragacanth mucilage daily in the morning. Blood was collected from tail vein of rats 2 h after oral administration of extract or 2% gum tragacanth mucilage and blood sugar levels were estimated daily.

All the fractions prepared from methanol extract of roots of *Cocculus hirsutus* were screened for their hypoglycemic activity at a dose of 200 mg/kg of body weight. The procedure followed was same as described above, except that the hypoglycemic activity of the fractions was assessed only on the seventh day.

Total alkaloid fraction was screened for its hypoglycemic activity at a dose of 200 mg/kg of body weight and the procedure followed was same as described above.

Effect on Isolated Perfused Frog Heart Preparation:

The experiments were conducted both on normal and hypodynamic frog heart[18]. The heart was made hypodynamic by perfusing frog-Ringer physiological solution containing half the concentration of calcium chloride used in normal frog-Ringer solution, delivered from another reservoir through a Cyme's canula.

The calcium content of methanol extract and butanol fraction was determined by flame photometry (Systronics Flame Photometer: Mediflame 127) prior to the experimentation. The weighed quantities of ME and BF were dissolved in normal frog-Ringer and frog-Ringer containing half-calcium. The experiments were carried out on 8 isolated frog hearts. The heart rate, force of contraction and persistence of effect were recorded on a smoked drum using a student's kymograph. The cardiac out put from the isolated frog heart is collected every

minute and measured. Various doses of ME and BF were tried to find out the dose-activity relationship both on normal and hypodynamic frog hearts. The cardiotonic effect of BF was compared with that of ouabain (G-Strphanthin, Sigma Chemical Company, St. Louis, USA) at the dose levels of 30,100 and 500 µg.

RESULTS

Fractionation of methanol extract of roots of *Cocculus hirsutus* by column chromatography yielded various fractions, of which methanol and methanol:Water (1:1) fractions contribute major portion (Table 1). The thin layer chromatography of methaol extract showed a number of spots, majority of which are alkaloids, steroids/ triterpenoids in addition to other unidentified compounds. The TLC profile of the butanol fraction showed presence of four compounds with vanillin-sulphuric acid reagent. The Rf values were 0.54 (green), 0.67 (voilet), 0.81 (blue and 0.89 (blue-voilet). The TLC profile of total alkaloidal fraction showed presence of four alkaloids with Dragendorff's reagent. The Rf values were 0.31, 0.44, 0.53 and 0.66. In the present study, activity-guided fractionation was followed to identify the active compounds.

TABLE 1: PERCENT YIELDS OF CHROMATOGRAPHIC FRACTIONS*

Solvent	% Yield
Toluene	2.6
Chloroform	3.7
Ethylacetate	10.9
n-Butanol	15.8
Methanol	26.5
Methanol : Water (1:1)	28.2

* Methanol extract of roots of *Cocculus hirsutus* was subjected to column chromatography and eluted with various solvents.

Effect of Methanol extract (ME) on Diabetic Rats:

Oral administration of ME at a dose of 200 mg/kg reduced the blood sugar levels of diabetic rats significantly. Reduction in blood sugar levels was observed on all seven days. The untreated diabetic rats showed higher blood sugar levels (Table 2). Various fractions prepared from ME were screened for hypoglycemic activity in diabetic rats. Chloroform and ethyl acetate fractions showed significant hypoglycemic activity. Chloroform fraction reduced the blood sugar levels from 338.6±21.9 to 280.0±18.9 mg/100 ml, where as, the ethyl acetate fraction reduced blood sugar levels of rats from 315.8±28.9 to 290.5±29.4 mg/100 ml. All other fractions tested did not exhibit considerable hypoglycemic activity (Table 3).

Effect of Total Alkaloids (TA) on Diabetic Rats:

Total alkaloids isolated from ME exhibited significant hypoglycemic activity in alloxan-induced diabetic rats. Reduction in blood sugar levels were observed for seven days with daily oral administration of TA. Blood sugar levels of the rats treated with TA were decreased significantly when compared to that of untreated rats (Table 4).

Effect of ME on Isolated Perfused Frog Heart Preparation:

The calcium content of methanol extract and butanol fraction as determined by flame photometry were 2.4 and 0.24 mg/g, respectively. The effect of ME on normal and hypodynamic frog heart preparation was studied at various dose levels. ME exhibited positive inotropic and negative chronotropic effect on hypodynamic frog heart.

TABLE 2: EFFECT OF METHANOL EXTRACT ON BLOOD GLUCOSE LEVELS OF DIABETIC RATS

Group	Initial level	Blood glucose (mg/dl)**						
		1	2	3	4	5	6	7
Unstreated	318.8 (26.4)*	314.3 (24.9)	313.7 (25.9)	318.5 (25.1)	316.6 (28.9)	309.8 (30.5)	310.9 (22.8)	305.7 (30.6)
Methanolic Extract	326.5 (28.9)	269.5 (24.7)	259.7 (34.7)	255.7 (36.2)	252.5 (44.4)	250.2 (43.5)	256.2 (35.9)	261.5 (31.9)

* Values in parenthesis indicate standard deviation. ** Blood glucose levels (mg/100 ml) 2 h after administration of methanol extract on different days.

TABLE 3: EFFECT OF FRACTIONS METHANOL EXTRACT ON BLOOD GLUCOSE LEVELS OF DIABETIC RATS

Fraction	Blood glucose (mg/dl)	
	Initial	2 h after administration*
Toluene	320.3±26.1**	326.1±24.9
Choloroform	338.6±21.9	280.6±18.9
Ethylacetate	315.8±28.9	290.5±29.3
Butanolic	296.6±19.6	289.4±21.6
Methanolic	312.6±26.1	316.1±24.9
Methanol:Water	326.4±27.8	321.3±23.8

*Blood glucose level in mg/100 ml 2 h after administration of methanol extract of roots of *cocculus hirsutus* on seventh day. ** Each value is mean ± standard deviation of 6 observations.

TABLE 4: EFFECT OF TOTAL ALKALOID FRACTION OF METHANOLIC EXTRACT ON BLOOD GLUCOSE LEVELS OF DIABETIC RATS

Group	Initial	Blood glucose (mg/dl) 2 h after administration**						
		1	2	3	4	5	6	7
Untreated	302.2 (28.4)*	306.2 (29.5)	306.2 (21.7)	317.3 (24.9)	311.5 (29.9)	298.8 (18.5)	300.7 (19.3)	310.5 (15.4)
Total Alkaloids (TA)	297.7 (26.5)	235.7 (39.4)	197.7 (21.6)	193.8 (43.9)	182.5 (31.9)	195.5 (28.3)	199.8 (32.9)	200.3 (27.9)

*Values in parenthesis indicate standard deviation. ** Blood glucose level in mg/100 ml 2 h after administration of TA on day.

It also exhibited positive inotropic effect on normal frog heart. The effect lasted for several minutes after administration of ME. However, cardiac out put was unaffected by ME. These results are shown in (Table 5).

Effect of BF of ME on Isolated Perfused Frog Heart Preparation:

The effect of the various fractions of ME on isolated frog heart was studied to find out the fraction(s) responsible for activity. Toluene, chloroform and ethylacetate fractions did not show any effect. Butanolic, metanolic and methanol-water fractions of ME showed positive inotropic effect. The effect of BF was more pronounced than the other two fractions. Hence, dose-response relationship of BF was studied on isolated frog heart rendered hypodynamic. It is evident from the Table 6 that BF has positive inotropic and negative chronotropic effect on isolated frog heart from 30 µg onwards whereas ME showed positive inotropic effect only from 100 µg onwards. From Table 6, it can be observed that the positive inotropic effect and negative chronotropic effect of ouabain is greater than that of BF at equal doses. The persistence of effect (the time taken for the heart to recover to normal) is greater for ouabain. At a dose of 500 µg the inotropic effect of BF is greater than that of ouabain.

DISCUSSION

Methanol extract (ME) of roots of *Cocculus hirsutus* exhibited significant hypoglycemic activity in alloxan-induced diabetic rats and cardiotonic activity on normal and hypodynamic isolated frog heart preparation. Hence, fractionation of ME was carried out with a battery of solvents of increasing polarity. Chloroform and ethylacetate fractions showed considerable hypoglycemic activity in

TABLE 5: EFFECT OF METHANOL EXTRACT (ME) ON ISOLATED PERFUSED FROG HEART PREPARATION

Dose (µg)	Change in HR (Beats/min)	Change in CO (ml/min)	Duration (min)	Increase in contraction (mm)
NORMAL HEART				
100	0	0	0	0.0
300	0	0	0	0.0
600	0	0	3.0	3.6
1000	-1.2	+1.5	3.5	7.8
HYPODYNAMIC HEART				
100	0	0	3.3	1.3
300	0	0	5.3	3.8
600	-2.2	+1.5	7.4	4.5
1000	-3.0	+1.5	9.3	9.5

HR = Heart Rate, CO = Cardiac Output. Average of 8 experiments

TABLE 6: CARDIAC EFFECTS OF BUTANOL FRACTION ON ISOLATED FROG HEART

Dose (µg)	Change in HR (Beats/min)	Change in CO (ml/min)	Duration (min)	Increase in contraction (mm)
BF*				
10	0.0	0.0	4.5	1.2
30	-2.3	0.0	5.3	2.5
50	-2.5	0.0	5.3	5.5
100	-2.9	+1.3	8.1	14.6
200	-3.3	+1.5	8.5	24.5
500	-3.4	+1.6	9.3	32.1
Ouabain and BF**				
30 (Ouabain)	-4.1	0	7.1	13.1
30 (BF)	-2.2	0	5.2	4.4
100 (BF)	-3.3	+1.2	6.1	6.3
100 (Ouabain)	-5.2	0	8.3	16.2
500 (BF)	-3.3	+1.2	7.4	47.3
500 (Ouabain)	-7.2	0	12.3	35.2

HR = Heart Rate, CO = Cardiac Output, Average of 8 experiments. * Effect of butanol fraction (BF) on hypodynamic frog heart. ** Comparison of the cardiotonic activity of butanol fraction (BF) with that of ouabain.

diabetic rats. The TLC of both fractions showed the presence of alkaloids. Presence of alkaloids prompted us to isolate total alkaloids (TA) for study. TA reduced the blood sugar levels of diabetic rats significantly. Hence, the alkaloids present in roots of *Cocculus hirsutus* are responsible for hypoglycemic activity. TLC of TA revealed the presence of four alkaloids. Isolation of individual alkaloids and evaluation of their activity may lead to the identification of the alkaloid(s) responsible for the activity. Structure elucidation of the compound(s) responsible for hypoglycemic activity will certainly be useful in understanding the mechanism of action and identification of new molecule.

The methanol extract (ME) of roots of *Cocculus hirsutus* showed positive inotropic and negative chronotropic effect on hypodynamic frog heart, indicating the presence of cardiotonic principles in it. The ME is subjected for fractionation with various solvents of increasing polarity and were screened for their effect on isolated frog heart to find out the fraction with cardiotonic activity. Of all the fractions screened, butanol fraction (BF) of ME exhibited significant cardiotonic activity both on normal and hypodynamic frog heart comparable with that of ouabain. The calcium content of BF was found to be 0.24 mg/g of BF and hence, the observed positive inotropic effect could not be attributed to the presence of calcium, which is too low in a dose of 500 µg of BF to show a positive inotropic effect. The TLC profile of BF showed the presence of four spots which reacted with vanillin-sulfuric acid reagent indicating the presence of steroids and/or triterpenoids. The effect of BF on hypodynamic isolated frog heart resembles qualitatively to that of ouabain. Hence, the compound(s) present in BF may be steroids/triterpenoids with cardiotonic activity. Isolation of individual compounds present in BF and evaluation of their cardiotonic activity will lead to the identification of the compound(s) responsible for the activity. The structure elucidation and pharmacological evaluation of the pure compound(s) with cardiotonic activity will certainly help us in the identification of new molecules with cardiotonic activity and their mechanism of action.

REFERENCES

1. Kirtikar, K.R. and Basu, B.D., In; The Indian Medical Plant. Vol. 1, L.M. Basu, Allahabad, 1973, 86.
2. Nadakarni, K.M., In; Indian Materia Medica, Vol. 1, Popular Prakashan., Mumbai, 1976, 362.
3. Chunekar, K.C., In; Bhav Prakash Nighantu Commentary, Chaukhamba Sanskrit Series, Varansi, 1960, 378.
4. Chopra, R.N., Chopra, I.C., Handa, K.L. and Kapoor, L.D., In; Indigeneous Drugs of India, V.N. Dhar and Sons Pvt. Ltd., Calcutta., 1958, 501.
5. Jagannadha Rao, K.V. and Ramachandra Row, L., **J. Sci. Ind. Res.**, 1961, 20B, 125.
6. Merchant, J.R., Naik, K.M. and Hirwe, S.N., **J. Indian Chem. Soc.**, 1962, 39, 411.
7. Tripathi, V.J.,Ray, A.B. and Dasgupta, B., **Indian J. Chem.**, 1976, 14B, 62.
8. Ahmad, V.U., Mohammad, F.V. and Rasheed, T., **Phytochemistry**, 1987, 26, 793.
9. Rasheed, T., Khan, M.N.I. and Zhadi, S.S.A., **Fitotherapia**, 1991, 62, 157.
10. Ahmed, V.U., Rasheed, T. and Iqbal, S., **Phytochemistry**, 1991, 30, 1350.
11. Rasheed, T., Khan, M.N.I., Zhadi S.S.A. and Durrani, S., **J. Nat. Prod.**, 1991, 54, 582.
12. Ahmed, V.U. and Iqbal, S., **J. Nat. Prod.**, 1992, 55, 237.
13. Das, P.K., Nath, V., Gode, K.D. and Sanyal, A.K., **Indian J. Med. Res.**, 1964, 52, 300.
14. Masilamani, G., Shokat Ali. and Subbulakhmi, V., **J. Research in Ayurveda and Siddha**, 1981, 2, 109.
15. Ramawat, K.G., Bhardwaj, L. and Tewari, M.N., **Bionature.**, 1992, 12, 33.
16. Cantharine, S.R. and Paul, G., **J. Nutrition.**, 1949, 39, 529.
17. Fings, C.S., Tatliff, C.R., Dunn, R.T., Toro, C. and Ackerman, P.G., In; Clinical Chemistry, Little Brown and Company, Boston, 1970, 115.
18. Burn, J.H., In; Practical Pharmacology, Blackwell Scientific Publication, Oxford, 1952, 58.