Diuretic and Antibacterial Activity of Aqueous Extract of *Cleome rutidosperma* D.C.

A. BOSE*, J. K. GUPTA[1], G. K. DASH, T. GHOSH, S. SI AND D. S. PANDA
Institute of Pharmacy and Technology, Salipur, Cuttack - 754 202, [1]Department of Pharmaceutical Technology, Jadavpur University, Kolkata - 700 032, India.

Crude aqueous extract of *Cleome rutidosperma* was investigated for diuretic and antibacterial activity. The diuretic activity was tested in rats at 400 and 600 mg/kg, orally and compared with furosemide (20 mg/kg, intraperitoneally) as the standard. The antibacterial activity was assessed by disc diffusion method against *Bacillus subtilis*, *Bacillus laterosporus*, *Staphylococcus aureus*, *Micrococcous luteus*, *Pseudomonas aeruginosa*, *Vibrio cholerae*, *Escherichia coli* and *Salmonella typhi* at concentrations of 100, 200 and 400 µg/disc respectively. Ciprofloxacin (5 µg/disc) was used as reference control for the antibacterial study. The extract was found to possess significant dose dependent diuretic activity and also effective against both gram-positive and gram-negative bacteria in a concentration dependent manner.

Cleome rutidosperma (family: Capparidaceae) is a low growing herb, up to 70 cm tall, found in waste grounds and grassy places with trifoliate leaves and small, violet-blue flowers, which turn pink as they age. The elongated capsules display the asymmetrical, dull black seeds. The plant is native to West Africa, from Guinea to Nigeria, Zaire and Angola. It has become naturalized in various parts of tropical America as well as Southeast Asia[1,2]. According to traditional use, the different parts like leaves, roots, and seeds of the plants of *Cleome* genus are used as stimulant, antiscorbutic, anthelmintic, rubifacient, vesicant and carminative[3]. The antiplasmodial activity of the chloroform-methanol (1:1) extract of leaves were reported earlier[4,5]. In the present study, we report the diuretic and antibacterial activity of aqueous extract of the entire plant of *Cleome rutidosperma*.

***For correspondence**
E-mail: anindyabose_in@yahoo.com

The plant material (whole plant) was collected from North 24-Pargana district of West Bengal, India during Aug 2003 and was authenticated at Botanical Survey of India, Shibpur, Howrah, West Bengal, India and a voucher specimen (C.R.-1) has been kept in our research laboratory for future reference. The fresh plant material was washed under running tap water to remove adhered dirt, followed by rinsing with distilled water, shade dried and pulverized in a mechanical grinder to obtain coarse powder.

The dried powdered plant material (350 g) was refluxed

with 800 ml of distilled water for 3 h. Following filtration and concentration under vacuum, a dark brown sticky residue was obtained (yield: 20.83% w/w with respect to dry plant material), which was preserved in a desiccator for further use. Standard methods[6,7] were used for preliminary phytochemical screening of the aqueous extract to know the nature of phytoconstituents present in it.

The method of Lipschitz *et al.*[8] was employed for the assessment of diuretic activity. The experimental protocols have been approved by the institutional Animal Ethical Committee, In this method, male rats weighing between 150-200 g, deprived of food and water for 18 h prior to the experiment were divided in four groups of six rats in each. The first group of animals, serving as control, received normal saline (25 ml/kg, p.o.); the second group received furosemide (20 mg/kg, i.p.) in saline; the third and fourth groups received the aqueous extract at doses of 400 and 600 mg/kg, respectively, in normal saline. Immediately after administration, the animals were placed in metabolic cages (2 per cage), specially designed to separate urine and faeces, kept at 20±0.5°. The volume of urine collected was measured at the end of 5 h. During this period, no food and water was made available to animals. The parameters taken were the body weight before and after test period, total urine volume, concentration of Na^+, K^+ and Cl^- in the urine.

Na^+ and K^+ concentrations was determined by flame photometer[9] and Cl^- concentration was estimated by titration[10] with silver nitrate solution (N/50) using 3 drops of 5% potassium chromate solution as indicator. All results were expressed as mean±standard error. The data was analyzed for statistical significance and the level of probability was set at $P \leq 0.05$ (Table 1).

The antibacterial activity of the extract was performed by disc diffusion method on nutrient agar plates[11]. The extract was dissolved in DMSO (10 mg/ml) and discs were prepared at concentrations of 100, 200 and 400 µg/disc respectively. Standard antibiotic discs of ciprofloxacin (5 µg/disc) were used for comparison. Solvent control (DMSO) was also maintained throughout the experiment. The selected microorganisms included *Bacillus subtilis*, *Bacillus laterosporus*, *Staphylococcus aureus*, *Micrococcus luteus, Pseudomonas aeruginosa*, *Vibrio cholerae*, *Escherichia coli* and *Salmonella typhi* respectively. The plates were incubated at 37° for 48 h. The zone of inhibition was calculated by measuring the minimum dimension of the zone of no microbial growth around the disc. For each data, an average of three independent determinations was recorded (Table 2).

The preliminary phytochemical screening of the extract showed the presence of tannins, saponins, flavonoids and

TABLE 1: DIURETIC ACTIVITY OF WATER EXTRACT OF *CLEOME RUTIDOSPERMA*

Experimental group	Dose	Urine volume (ml/kg)	Total sodium (mEq/l)	Total potassium (mEq/l)	Total chloride (mEq/l)	Na^+/K^+ ratio
Control (Normal Saline)	25 ml/kg, p.o.	3.7±0.2	125±12	76±11	321±58	1.65
Standard (Frusemide)	20 mg/kg, i.p.	44.3±2.4*	653±28*	203±22*	1117±87*	3.22
AECR	400 mg/kg, p.o.	3.4±0.5	550±17*	221±65	1157±17*	2.49
AECR	600 mg/kg, p.o.	10.1±1.7*	1226±96*	452±65*	1128±42*	2.71

AECRis the aqueous extract of *Cleome rutidosperma*, results indicate Mean?SEM (n=6), *indicates significant difference at $P<0.05$ when compared to control.

TABLE 2: ANTIBACTERIAL ACTIVITY OF AQUEOUS EXTRACT OF *CLEOME RUTIDOSPERMA*

Organism	Diameter of zone of Inhibition (mm)			
	Aqueous Extract			Standard (Ciprofloxacin) 5 µg/disc
	A	B	C	
Bacillus subtilis	8.3±0.6	9.7±0.6	13.3±0.6	25.0±0.0
Bacillus laterosporus	10.7±0.6	11.7±0.6	15.0±1.0	45.3±0.6
Staphylococcus aureus	10.0±0.0	11.0±1.0	12.7±0.6	27.7±0.6
Micrococcous luteus	7.3±0.6	10.0±0.0	10.3±0.6	23.0±0.0
Pseudomonas aeruginosa	8.0±0.0	11.0±1.0	12.3±0.6	35.3±0.6
Vibrio cholerae	7.7±0.6	8.3±0.6	10.0±0.0	23.3±0.6
Escherichia coli	9.7±0.6	11.7±0.6	12.7±0.6	48.0±1.0
Salmonella typhi	6.0±0.0	7.0±0.0	7.3±0.6	44.7±0.6

All values indicate mean±SD (n = 3), the concentrations were A: 100 µg/disc, B: 200 µg/disc and C: 400 µg/disc. DMSO had not shown any antibacterial activity against any of the organisms.

carbohydrates in the aqueous extract of *Cleome rutidosperma*. The aqueous extract at 400 and 600 mg/kg p.o. showed significant increase in excretion of sodium, potassium and chloride ions in the urine in a dose dependent manner. The obtained effect was comparable to that of furosemide (20 mg/kg, i.p.). At the same time, the tested extract increased the urinary output to a significant level only at the higher dose tested (600 mg/kg, p.o.). It was also observed that the extract increases the ratio of concentration of excreted sodium and potassium ion compared to the control. The antibacterial activity of the extract was found to be concentration dependent on the test organisms. The study also revealed that the extract was effective against both gram-positive and gram-negative bacteria.

The preliminary study supported the presence of effective diuretic constituents in the aqueous extract of *C. rutidosdperma*. It is reported previously that the flavonoid glycosides are endowed with diuretic activity[12]. It may therefore be presumed here that the diuretic activity is due to presence of flavonoids in the test extract. The data in the Table 1 allowed with the conclusion that the extract acts as a diuretic because of increased urinary electrolyte concentration with significant increase in the urinary output[13]. The increase in the ratio of concentration of excreted sodium and potassium ion for the tested extract, compared to control, indicates that the extract increases sodium ion excretion to a greater extent than potassium, which is a very essential quality of a good diuretic with lesser hyperkalaemic side effect.

As reported earlier secondary metabolites like tannins, saponins, flavonoids are likely responsible for the observed antibacterial activity of plants[14-16]. The presence of the said constituents in the aqueous extract of *Cleome rutidosperma* as found in the phytochemical tests, may be responsible for the observed antibacterial activities. Attempts for further purification and isolation with the target of obtaining substances with potent diuretic, antibacterial activities are under process in our institution.

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