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Studies on diuretic and laxative activity of ethanolic extract and its fractions of *Cleome rutidosperma* aerial parts

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ABSTRACT - Crude ethanolic extract and fractions of *Cleome rutidosperma* (family: Capparidaceae) was investigated for diuretic and laxative activity in albino rats that was compared with standard drugs Furosemide (10mg/kg, p.o.) and Agar agar (300mg/kg, p.o.), respectively. The extract was found to produce significant diuretic as well as laxative activity in dose dependant manner. Fractions of the extract potentiated the observed activities. The activities may be contributed to the phytoconstituents present.

KEYWORDS - Cleome *rutidosperma*; Acute toxicity study; Diuretic activity; Laxative activity.

INTRODUCTION

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 (1-3). According to traditional use, the different parts like leaves, roots and seeds of *Cleome* genus are used as stimulant, antiscorbutic, anthelmintic, rubifacient, vesicant and carminative (4). The antiplasmodial, analgesic, locomotor, antimicrobial and anthelmintic activities of *Cleome rutidosperma* were reported earlier (5-8). In the present study, we report the diuretic and laxative activity of ethanolic extract and its fractions of the aerial parts of *Cleome rutidosperma*.

MATERIALS AND METHODS

Plant material

The Plant material (whole plant) was collected from North 24-Pargana district of West Bengal, India during Aug 2005 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 aerial parts were 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.

Preparation of Extract

The aerial parts were extracted with 90% ethanol using Soxhlet apparatus. The solvent was removed under reduced pressure, which gave a greenish-black coloured sticky residue (yield- 11.6% w/w on dried material basis). A portion of dried ethanolic extract was suspended in water and fractionated successively with petroleum ether (40-60°C), diethyl ether, ethyl acetate and n-butanol. All the fractions were dried by distillation under reduced pressure. Standard methods (9,10) were used for preliminary phytochemical screening of the ethanolic extract and its fractions to know the nature of phytoconstituents present in it (Table 1).

Animals

Male Swiss albino mice, weighing 20–25 g, and Wistar albino rats, weighing 120-150 g, were used for acute toxicity study and evaluation of pharmacological studies. Animals were housed in standard environmental conditions and fed with standard rodent diet and water *ad libitum*. The Institutional Animals Ethics Committee approved all the experimental protocols.

Acute toxicity study

The test was carried out as suggested by Ganapaty *et. al*. (11). Swiss albino mice of either sex weighing between 25–30 g were divided into different groups comprising six animals each. The control group received normal saline (2 ml/kg, p.o.). The other groups received 100, 200, 300, 600, 800, 1000, 2000, 3000 and 4000 mg/kg of the test extract respectively, as well as, extract fractions up to 2000 mg/kg, in a similar manner. Immediately after dosing, the animals

were observed continuously for the first 4 hours for any behavioral changes. Thereafter, they were then kept under observation up to 14 days after drug administration to find out the mortality if any.

Diuretic activity

The method of Lipschitz *et al*, 1943 (12, 13) was employed for the assessment of diuretic activity. In this method, male albino rats weighing between 120-150 g, deprived of food and water for 18 hours prior to the experiment, were divided in eight 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 (10 mg/kg, p.o.) in saline (14); other groups received doses of extract (200 and 400mg/kg) or extract fractions (200mg/kg each), in normal saline. Immediately after admistration, the animals were placed in metabolic cages (2 per cage), specially designed to separate urine and faeces, kept at 20°C±0.5°C. 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 total urine volume, concentration of Na^+, K^+ and Cl^- in the urine.

Na^+ and K^+ concentrations were determined by flame photometer (15) and Cl^- concentration was estimated by titration (13, 16, 17) with silver nitrate solution (N/50) using 3 drops of 5% potassium chromate solution as indicator.

Laxative activity

The test was performed according to Capasso *et. al*. (18) on rats of either sex, fasted for 12 h before the experiment, but with water provided ad libitum. The animals were divided into eight groups of six in each. The animal groups were administered orally either with vehicle (1% Tween-80 solution in normal saline, 25 ml/Kg), reference standard drug, agar-agar (300 mg/kg, p.o.) in saline (11) or doses of extract (200 and 400mg/kg) or extract fractions (200mg/kg each). Immediately after dosing, the animals were separately placed in cages suitable for collection of faeces. After 8h of drug administration, the faeces were collected and weighed. Thereafter, food and water were given to all rats and faecal outputs were again weighed after a period of 16 h.

Statistical Analysis

All results are expressed as mean ± standard error.

The data was analyzed using two ways of analysis of variance (ANOVA). The statistical significance of the difference of the means was evaluated by Dunnet's t-test.

RESULTS

The results of the preliminary phytochemical screening of the ethanolic extract and its fractions are given in Table 1. In acute toxicity study, it was found that the extract induced sedation, diuresis, purgation, and temporary postural defect at all tested doses. However, there was no mortality at any of the tested doses till the end of 14 days of observation.

The ethanolic extract was found to produce significant increase in excretion of sodium, potassium and chloride ions at the higher dose tested (400 mg/kg p.o.). The order of activity of increase of urinary output was diethyl ether fraction > n-butanol fraction > ethyl acetate fraction > petroleum ether fraction. The order of activity of increase of urinary electrolyte excretion was found to be n-butanol fraction > diethyl ether fraction > ethyl acetate fraction > petroleum ether fraction.

In the evaluation of laxative activity, the ethanolic extract was found to produce significant dose dependant activity at both the tested level of doses (200 and 400 mg/kg, p.o.). The effect was superior to that of the standard tested at 400mg/kg, p.o. dose level. The order of activity for the ethanolic extract fractions was diethyl ether fraction > ethyl acetate fraction > n-butanol fraction > petroleum ether fraction.

DISCUSSION

The present study revealed that, ethanolic extract of *Cleome rutidosperma* significantly increased the urinary output as well as urinary electrolyte concentration at a higher dose tested (400mg/kg, p.o.). The fractionation of ethanolic extract potentiated the activity. The diethyl ether fraction was found to be the most potent in increasing the urinary output; the effect was comparable to that of the standard drug, whereas, the petroleum ether fraction was found to be least potent.

Determination of urinary electrolyte concentration revealed that, n-butanol fraction was most effective in increasing urinary electrolyte concentration for all the three ions tested (Na^+, K^+, Cl^-). All fractions except petroleum ether fraction caused similar type of increase of urinary electrolyte concentration, but to a lesser extent. Petroleum ether fraction did not increase urinary electrolyte concentration.

Table No.1- Phytochemical screening of extracts of C. rutidosperma aerial parts

Extract	Phytoconstituents present
Ethanolic extract	Lipids, steroids, terpenoids, flavonoids, tannins, saponins, sugars
Pet-ether fraction	Lipids, steroids, terpenoids
Diethyl ether fraction	Steroids, terpenoids, flavonoids
Ethyl acetate fraction	Flavonoids, tannins, saponins
n-Butanol fraction	Flavonoids, tannins, saponins

Table No.2- Diuretic Activity of Ethanolic Extract and its fractions of Cleome rutidosperma

Treatment	*Dose*	Urine Volume (ml)	Concentration of ions (mEq/l)			Na^+/K^+ ratio
			Na^+	K^+	Cl^-	
Control	25 ml/kg	2.85 ± 0.14	52.12 ± 2.86	141.72 ± 2.68	87.85 ± 3.88	0.37
Furosemide	10mg/kg	10.5 ± 0.54**	108.13 ± 3.71**	187.55 ± 1.98**	130.61 ± 3.69**	0.58
Ethanolic extract	200 mg/kg	2.97 ± 0.06	51.47 ± 3.05	139.55 ± 3.14	94.88 ± 4.77	0.37
	400 mg/kg	5.1 ± 0.21*	67.85 ± 4.22	147.50 ± 2.49	104.95 ± 1.69*	0.46
Pet ether Fraction	200 mg/kg	4.18 ± 0.12	54.30 ± 2.09	143.32 ± 1.83	85.70 ± 2.59	0.37
Diethyl ether Fraction	200 mg/kg	9.13 ± 0.79**	72.13 ± 1.47**	151.07 ± 3.35	108.87 ± 2.96**	0.48
Ethyl acetate Fraction	200 mg/kg	4.15 ± 0.49	78.18 ± 2.69	152.01 ± 2.44*	123.05 ± 5.59**	0.51
n-Butanol fraction	200 mg/kg	6.62 ± 0.92**	101.05 ± 2.42**	182.55 ± 3.05**	164.83 ± 6.89**	0.55

*Values are expressed as mean ± S.E. (n = 6). * P<0.05 and * * P<0.01 compared with vehicle control (ANOVA followed by Dunnet's t-test).*

Table No.3 - Laxative Activity of Ethanolic Extract and its fractions of Cleome rutidosperma

Treatment	*Dose*	Faecal Output (g)	
		8h	8 -16h
Control	-	0.87 ± 0.013	0.312 ± 0.035
Agar-agar	300mg/kg	1.069 ± 0.038* *	0.303 ± 0.033
Ethanolic extract	200 mg/kg	0.389 ± 0.008	0.200 ± 0.014
	400 mg/kg	1.252 ± 0.079* *	0.306 ± 0.042
Pet ether Fraction	200 mg/kg	0.278 ± 0.041	0.295 ± 0.032
Diethyl ether Fraction	200 mg/kg	1.149 ± 0.112* *	0.180 ± 0.043
Ethyl acetate Fraction	200 mg/kg	0.779 ± 0.057* *	0.400 ± 0.065
n-Butanol fraction	200 mg/kg	0.513 ± 0.049	0.107 ± 0.018* *

Values are expressed as mean ± S.E. (n = 6).
** * P<0.01 compared with vehicle control (ANOVA followed by Dunnet's t-test).*

Ethyl acetate fraction although did not increase urinary output significantly, it increased urinary electrolyte concentration significantly. The increase in the ratio of concentration of excreted sodium and potassium ions 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.

Ethanolic extract of *Cleome rutidosperma* was found to produce significant laxative activity, in a dose dependent manner up to 8h of drug administration. The effect was found to be superior to that of the standard drug. Fractionation of the extract potentiates the activity. Diethyl ether fraction was found to be most active and petroleum ether fraction was found to be least active.

Presence of phytoconstituents like terpenoids, saponins, flavonoids have been previously found to be responsible for diuretic and laxative activities in plants (19-23). The presence of the said constituents in ethanolic extract and its fractions of *Cleome rutidosperma* may be responsible for the observed diuretic and laxative activities. Attempts for isolation of active constituents responsible are under process in our laboratory. Further studies are necessary to understand the exact mechanism of action.

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Phcog.net was started on July 6, 2004

Development and launch of Website – www.phcog.net

Initiation of Discussion forum – http://groups.yahoo.com/group/phcog/

Started a forum – www.phcog.net/forum.php

Started a New Online peer reviewed magazine – **Pharmacognosy Magazine** (PHCOG MAG).

Editorial team was finalized for the term of three years (2004-2007).

Release of four issues in 2005.

Project Phcog Refbase started in the month of May 2005.

Release of 7th issue of Pharmacognosy Magazine in July 2006.

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