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Comparative Study of *Cuscuta reflexa* and *Cassytha filiformis* for Diuretic Activity

Sharma Sakshy, Hullatti K.K*, Prasanna S.M., Kuppast I.J[1] and Sharma Paras

Department of Pharmacognosy, National College of Pharmacy, Shimoga-INDIA
[1]Department of Pharmacology, National College of Pharmacy, Shimoga-INDIA

***Correspondence:** *email: kkhullatti@gmail.com , Phone: 0091 9448800184*

ABSTRACT

Aqueous and alcoholic extract of *Cuscuta reflexa* and *Cassytha filiformis* were investigated for diuretic activity in Wister rats. The extracts were administered once orally at a dose of 300mg/kg. Frusemide (20mg/kg) was used as standard reference drug and normal saline (25 ml/kg) was used as control. Total urine volume and concentration of Na^+, K^+ and Cl^- excreted in urine were estimated. Aqueous and alcoholic extract of *Cuscuta reflexa* and *Cassytha filiformis* exhibited significant diuretic activity and caused marked increase in Na^+ and K^+ excretion, when compared to saline treated controls. However the diuretic activity of *Cassytha filiformis* extract was higher than that of *Cuscuta reflexa*.

Keywords: *Cassytha filiformis, Cuscuta reflexa*, Diuretic activity, Electrolytes.

INTRODUCTION

The plant *Cuscuta reflexa* Roxb. Coron is a perennial herb of Convolvulaceae family, commonly known as Akashbela in Hindi. The plant is distributed throughout India, Ceylon and Malaya. The *Cuscuta reflexa* has been investigated for antispasmodic, heamodynamic, bradycardia (1), antisteroidogenic (2), antihypertensive, muscle relaxant, cardiotonic (3), psychopharmacological (4), and antiviral and anticonvulsant (5) activities. Many chemical constituents have been isolated from *Cuscuta reflexa* such as, cuscutin, amarbelin, beta-sterol, stigmasterol, kaempferol, dulcitol, myricetin, qurecetin, coumarin and oleanolic acid (6).

Cassytha filiformis Linn., is perennial, parasitic, herbaceous and leafless plant belonging to family Lauraceae. This plant is distributed throughout India and is used for medicinal purpose in China, Indochina, Madagascar and South Africa. *Cassytha filiformis* is used as antiplatelet agent, vesorelaxant (7), alpha-adrenoreceptar antagonist (8) and antitrypnosomal agent (9). Some of the isolated compound from this plant are aporphine alkaloid, oxo-aporphine alkaloid, cassyformine, filiformine, cathaformine, lignan, actinodophine, and octenine (10).

In ayurveda, *Cassytha filiformis* is used as substitute for *Cuscuta reflexa*. Literature review reveals that *Cuscuta reflexa* and *Cassytha filiformis* are also used as diuretics in traditional medicinal practice (11). Since the diuretic activity of these plants has not been scientifically investigated, the present study was designed to evaluate the diuretic activity of *Cuscuta reflexa* and *Cassytha filiformis* in a rodent model.

MATERIAL AND METHODS

Plant material

The aerial part of *Cuscuta reflexa* was collected from the field of Charthaval, U. P. and identify by Mr. Mohan Kumar, Dept of Botany, R.K.P.G. College, Shamli, U.P. India.

The aerial part of *Cassytha filiformis* was collected from the field of Tirupati, Andhra Predesh and authenticated by Dr. K. Madhva Chetty, Dept of Botany. Vankateswara University, Tirupati, A.P. India.

Extraction

The shade dried and course powdered plant material of *Cuscuta reflexa* and *Cassytha filiformis* were subjected to extraction with ethanol and then with water.

Preliminary Phytochemical Screening

Petroleum ether, chloroform, ethanol, and aqueous extracts of both *Cuscuta reflexa* and *Cassytha filiformis* were subjected to preliminary qualitative phytochemical investigations. All the extracts were screened for the presence of secondary metabolites such as steroids, alkaloids, flavonoids and tannins using standard methods.

Animals

Swiss albino mice (18 – 22 g) and Wister rats (100 – 200 g) of either sex were obtained from Central Animal House, NCP, Shimoga. All the animals were housed under standard condition (27±2°C, 55±5 % humidity and 12h light/dark cycle) (12). The animals were allowed free access to water and standard laboratory rat food. The experimental procedures described were approved by the Institutional Animal Ethical Committee (NCP/IAEC: Clear 05-4/07-08).

Acute toxicity study

The acute toxicity of alcoholic and aqueous extract of both plant *Cuscuta reflexa* and *Cassytha filiformis* was determined using Swiss albino mice. The animals were divided into 5 different groups. The group 1 received normal saline (25ml/kg) and served as control. The group 2 and 3 received 3000 mg/kg BW of alcoholic and aqueous extract of *Cuscuta reflexa* respectively. The group 4 and 5 received 3000 mg/kg BW of alcoholic and aqueous extract of *Cassytha filiformis* respectively. After oral administration of these extract, the animals were observed continuously for the behavioral changes for the first two to four hours and then observed for mortality if any, up to 24 h (13).

Diuretic activity

The method by Lipschitz *et al.* was employed for the assessment of diuretic activity (14). Healthy albino Wistar rats of either sex were divided into six groups of six animals each. Ethanolic and aqueous extracts, of both *Cuscuta reflexa* and *Cassytha filiformis* were evaluated for diuretic activity. Frusemide (20mg/kg) was used as standard reference drug. Before the experiment, the rats were fasted for 18 hours with free access to water. On the day of experiment, the animals of group 1 were administered saline orally (2.5ml of 0.9% NaCl/100 g body weight) (15) and this group served as control. Group 2 rats were treated with standard drug frusemide (20mg/kg) formulated in saline solution. Group 3 and Group 4 rats received ethanolic and aqueous extracts (300mg/kg body weight, orally) of *Cuscuta reflexa* respectively. $1/10^{th}$ dose of the maximum dose tried in the acute toxicity studies was selected for evaluating the diuretic effects. The extracts were formulated in saline solution. Similarly the Group 5 and Group 6 rats received ethanolic and aqueous extracts (300mg/kg body weight, orally) of *Cassytha filiformis* respectively. Immediately after the treatment, the animals were individually placed in metabolic cage (16). The urine was collected in measuring cylinder up to 5h for all control and treated groups. During this period no food or water was made available to the animals. The volumes of urine, electrolytes (Na^+, K^+, Cl^-) content were estimated in the urine for assessment of diuretic activity. Na^+, K^+ estimation was carried out using flame photometry (17) and Cl^- was estimated by titration (18). The diuretic action of tested drug was calculated by using the following formula:

$$\text{Diuretic action} = \frac{\text{Urinary excretion in test drug}}{\text{Urinary excretion in control}}$$

Statistical analysis

The results are expressed as the mean±SEM (n=6). Unless otherwise specified, differences between vehicle control and treatment groups were tested using one way Analysis of Variance (ANOVA). A value of $P<0.001$ was considered statistically significant.

RESULTS

The preliminary phytochemical analysis revealed the presence of steroids, saponins, triterpenes and flavonoids in *Cuscuta reflexa* and steroid, triterpene, flavonoids and alkaloids in *Cassytha filiformis* in petroleum ether,

Table 1. Preliminary Phytochemical screening

	Cuscuta reflexa				*Cassytha filiformis*			
	PE	CE	EE	AE	PE	CE	EE	AE
Steroid	+	+	-	-	+	+	-	-
Triterpenoids	+	+	-	-	+	+	-	-
Tannins	-	-	-	-	-	-	-	-
Alkaloids	-	-	-	-	-	-	+	-
Saponins	-	-	+	+	-	-	-	-
Flavanoids	-	-	+	+	-	-	+	+

+: Present, -: Absent, PE: pet. Ether fraction, CE: chloroform fraction, EE: ethanolic fraction, AE: aqueous fraction.

chloroform, ethanol and aqueous extracts. The results are summarized in table -1

Acute toxicity study with a dose of 3000mg/kg did not result in any mortality or visible adverse effects.

Effect on urine volume

Results are summaried in Table 2. The ethanolic extracts of the roots of *Cuscuta reflexa* at a dose of 300 mg/kg show marked diuresis during the 5 h of the test duration (*Cuscuta reflexa* ethanolic extract 7.5±0.32 ml versus control 2.3±0.56 ml; $P < 0.001$). A similar diuretic activity was observed with that of aqueous extract (*Cuscuta reflexa* aqueous extract 9.0±0.41 mL versus control 2.3±0.56 ml; $P < 0.001$). Where as both ethanolic and aqueous extracts of *Cassytha filiformis* extracts significantly increased urinary output compared to that of the control (ethanolic extract 11.3±0.33 ml and aqueous extract 13.6±0.43 ml versus control 2.3±0.56 ml; $P < 0.001$) but the effect was much less than that of furosemide (21.3±0.45 ml versus control 2.3±0.56 ml). At 5 hrs the animals were found normal and no evidence of dehydration was observed.

Effect on urinary electrolyte excretion

The effect of single doses of furosemide (20 mg/kg) and the ethanolic and aqueous extracts of *Cuscuta reflexa* and *Cassytha filiformis* (300 mg/kg) on urinary electrolyte (Na^+ and K^+) concentration at 5 h post administration in represented in Table 3. All the four extracts enhanced the excretion of the electrolytes ($P < 0.001$) to an extent similar to that of the furosemide. The Na^+ / K^+ excretion ratio was uniform (2.1 to 2.5) in all the groups studied.

DISCUSSION

The aim of this study was to investigate the comparative diuretic activity of ethanolic and aqueous extracts of *Cuscuta reflexa* and *Cassytha filiformis*. These plant materials are used as sources of "Akashbel" an important Ayurvedic drug which is used in treatment of urinary related disorders. In this study the ethanolic and aqueous extracts were tested at 300 mg/kg. The diuretic response was compared with that produced by furosemide, a widely used loop diuretic in clinical practice. The effect on electrolyte balance was also determined along with diuretic response.

The ethanolic and aqueous extracts of *Cuscuta reflexa* (300 mg/kg) showed comparatively milder diuretic activity during the 5 h of the test duration (Diuretic action – 2.67 and 3.21). The ethanolic and aqueous extracts of *Cassytha filiformis* increased urinary output significantly of that of control (Diuretic action – 1.6 and 1.3) but less than that of furosemide (Diuretic action –

Table 2. Effect of *Cuscuta reflexa* and *Cassytha filiformis* on excretion of urine

Sl. no	Drug	Dose (mg/kg)	Vol. of Urine Collected (in ml)					Diuretic Action
			After 1hr	After 2hr	After 3hr	After 4hr	After 5 hr	
1	Control	-	2.0±0.40	2.0±0.40	2.3±0.40	2.3±0.56	2.3±0.56	1.00
2	Standard	20	14.5±0.48*	17.3±0.18*	20±0.24*	20±0.24*	21.3±0.45*	9.87
3	CREE	300	2.0±0.32	2.9±0.32	5.1±0.24*	7.5±0.32*	7.5±0.32*	2.67
4	CRAE	300	2.0±0.33	3.1±0.12*	7.0±0.18*	8.3±0.29*	9.0±0.41*	3.21
5	CFEE	300	2.6±0.36	6.0±0.22*	10±0.24*	11.3±0.33*	11.3±0.33*	4.03
6	CFAE	300	3.3±0.13*	7.3±0.18*	8.6±0.29*	10.6±0.26*	13.6±0.43*	4.78

n = 6 in each group;

*$p < 0.001$ vs. Control group

CREE: *C. reflexa* ethanolic extract, CRAE: *C. reflexa* aqueous extract

CFEE: *C. filiformis* ethanolic extract, CFAE: *C. filiformis* aqueous extract

Table 3. Urinary electrolyte Concentration of *C. reflexa* and *C. filiformis*

Group	Dose (mg/kg)	Vol. of urine (ml)	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mEq/L)	Na^+/k^+ ratio
control	-	2.3±0.56	139.4±0.098	55.7±3.02	99.8±1.131	2.5
Standard	20	21.3±0.45*	149.6±0.13	71.2±3.11*	99.2±0.636	2.1
CREE	300	7.5±0.32*	154.8±0.056*	70.3±3.40*	98.7±0.777	2.2
CRAE	300	9.0±0.41*	150.2±0.021	65.3±2.62*	100.2±0.35	2.3
CFEE	300	11.3±0.33*	153.2±0.070	66.6±3.89*	98.2±0.494	2.3
CFAE	300	13.6±0.43*	148.7±0.098	67.5±4.75*	97.9±0.353	2.2

n = 6 in each group;

*$p < 0.001$ vs. Control group

CREE: *C. reflexa* ethanolic extract, CRAE: *C. reflexa* aqueous extract

CFEE: *C. filiformis* ethanolic extract, CFAE: *C. filiformis* aqueous extract

4.03 and 4.78). Urine output continued to be enhanced throughout the study period and the cumulative urinary excretion was significantly higher compared to that of the control.

Furosemide is reported to increase urinary output and urinary excretion of sodium by inhibiting $Na^+/K^+/Cl^-$ transporter system in the thick ascending loop of Henley (19). In saline primed rats a dose of *Cassytha filiformis* (300 mg/kg) extracts caused a significant increase in the urine output, beginning in the first hour and lasting untill the fourth hour. *Cuscuta reflexa* (300 mg/kg) extracts also induced an increase in urinary output, however the diuretic activity was milder compared to that of *Cassytha filiformis*. Although a significant increase in urinary excretion of Na^+ and K^+ ions was observed.

The pattern of results shows that the probable mechanism of action of tested extracts may be similar to that of Furosemide. Of the four extracts tested, aqueous extract of *Cassytha filiformis* (300mg/kg) had the highest diuretic activity. The secondary metabolites such as flavonoids, saponins are known to be responsible for diuretic activity (20,21). The preliminary phytochemical investigation has shown the presence of flavonoids, commonly present in both the plants. The diuretic activity of these two plants could be due to these flavonoids, but the presence of alkaloids in *Cassytha filiformis* cannot be ruled out. Since it has shown higher level of diuretic action compared to *Cuscuta reflexa*. However the exact constituents responsible for the diuretic activity of the extracts studied needs to be evaluated in the future studies.

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REFERENCES

1. Hassan Gilani A.U. and Aftab K., (1992), Pharmacological action of *Cuscuta reflexa*. *Int. J. Pharmacog.*, **30**, 296
2. Gupta M., Mazumdar U.K, Pal D.K and Bhattacharya S., (2003), Anti-Steroidogenic activity of methanolic extract of *Cuscuta reflexa* roxb. stem & *Corchorus olitorius* Linn. seed in mouse ovary, *Ind. J. Exp. Biol.* **41** (6), 641.
3. Singh G. S and Garg K. N, (1973), Some pharmacological studies on *Cuscuta reflexa* plant, *Ind. J. Pharmacog.*, **5**(2), 344–345
4. Pal D., Panda C., Sinhababu S., Dutta A. and Bhattacharya S., (2003), Evaluation of Psychopharmacological effect of petroleum ether extract of *Cuscuta reflexa* Roxb. Stem in mice, *Acta. Pol. Pharm.*, **60**(6), 481–486
5. Gupta M., Mazumdar U.K., Bhattacharya S. and Chakrabarty S., (2003), Studies on brain biogenic amines in methanolic extract of *Cuscuta reflexa* Roxb. & *Corchorus olitorius* Linn. seed treated mice, *Acta. Pol. Pharm.*, **60**(3), 207–210
6. Mohammad Ali V., (2004), Studies in the chemical constituents of *Bombax ceiba* and *Cuscuta reflexa*, University of Karachi/H.E.J Research Institute of Chemistry. (Thesis)
7. Wu, Yang-Chang, Chang Fang-Rong, Chao Ya-Chief and Teng Che-Ming, (1998), Antiplatelet and vasorelaxing action of aporphinoids from *Cassytha filiformis*., *Phytochem. Res.*, **12** (SI), 539–541
8. Hoet S., Stévigny C., Block S., Opperdoes F., Colson P. and Baldeyrou B., (2004), Alkaloid from *Cassytha filiformis* & related aporphines: Antitrypnosomal activity, cytotoxicity & interaction with DNA & topoisomerases. *Planta medica*, **70**, 407–413
9. Chang C. W, Ko F. N, Su M. J, Wu Y. C and Teng C. M, (1997), Pharmacological evaluation of ocoteine, isolated compound from *Cassytha filiformis* in rat thoracic aorta. *Jp. J. Pharmcol.*, **73**(3), 207
10. Chang Fang-Rong, Chao Ya-Chief, Teng Che-Ming and Wu Chang Yang, (1998), Chemical constituents from *Cassytha filiformis*. *J. Nat. Prod.*, **61**(7), 863–866
11. Kirtikar K. R and Basu B. D., (1991) *Indian Medicinal Plants*. 2nd ed. Vol-**3**, p1741 and 2163.
12. Vogel G., (2002), *Drug discovery and evaluation: Pharmacological assay*, 2nd ed. p.323
13. Turner R.A., (1965), The organization of screening. In: *Screening method in pharmacology*, Vol-**1**, New York and London, Academic press, p21
14. Mukherjee P. K., (2002), *Pharmacological screening of Herbal Drugs in Quality control of Herbal drugs*, 1st ed., p.537
15. Wiebelhaus V. D., Weinstock J., Maass A. R., Brennan F. T., Sosnowski G., and Larsen T. (1965), The Diuretic and Natruretic Activity of Triamterene and Several Related Pteridines in The Rat, *J. Pharmacol. Exp. Therap.*, Vol. **149** (3), 397–403
16. Kuppast I. J. and Nayak P.V., (2005), Diuretic activity of *Cordia dichotoma* forester. F. fruits, *Ind. J. Pharm. Edu. Res.*, **39**(4), 186–187
17. Jeffery G. H, Basset J., Mundhan J. and Denny R., (1989), *Vogel's Text book of quantitative Chemical analysis*, 5th ed., Addison Wesley Longman Ltd., England, p. 801
18. Beckette A.H. and Stanlake J.B., (1997), *Practical pharmaceutical chemistry*, part-1, 1st ed., CBS publisher and distributor, New Delhi, p.197
19. Jackson E.K, Drugs affecting renal and cardiovascular function, In: Hardman J.C, Gilman A.G, Limbird L.E, (Eds.), (1996), *Goodman and Gilman's the Pharmacological Basis of Theraputics*, 9th ed. Pergmon press, New York, p 685–713
20. Sood A.R, Bajpai A. and Digits M., (1985), Pharmacological and biological studies on saponins, *Ind. J. Pharmacol.*, **17** (3), 178–79
21. Chodera A., Dabrowska K., Sloderbach A., Skrzypczak L. and Budzianowski J. (1991), Effect of flavonoid fractions of *Solidago virgaurea* L. on diuresis and levels of electrolytes. *Acta Pol. Pharm.* **48**, 35–37
22. Massod A., Siltanual-haq and Saxena S. K, (1985), Effect of some plant extract on the Larval hatching of methodology incognita chit wood. *Indian forester* **111**(10), 841–845