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In-vivo Antitussive Activity of *Cressa cretica Linn.* using Cough Model in Rodents

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ABSTRACT

Cressa cretica Linn. Voigt. (Convolulaceae), has also been extensively used to get relief from asthma and cough by the indigenous people of India. In the present study the antitussive effect of the plant was evaluated in two different experimental models. The antitussive effect of aerosols of two different concentrations (2.5%w/v, 5%w/v)of methanolic extract of *Cressa cretica Linn.* (CME), codeine(0.03g/ml), and normal saline were tested by counting the numbers of coughs produced due to aerosols of citric acid 10 min after exposing the male guinea pigs to aerosols of different solutions (n=6). In another set of experiment CME was investigated for its therapeutic efficacy on a cough model induced by sulfur dioxide gas in mice. The results showed significant reduction of cough number obtained in the presence of both concentrations of CME and codeine. The antitussive effect on guinea pigs of higher concentration of CME was significantly ($p<0.01$) greater than those of lower concentration and the prototype antitussive agent codeine phosphate ($p<0.01$). It exhibited significant anti tussive activity as that of codeine phosphate, when compared with control in a dose dependent manner in sulfur dioxide gas induced cough model. The extract at 100, 200 and 400 mg/kg, p.o. showed inhibition of cough by 22.1, 34.35 and 55.44 % within 90 min of performing the experiment.

KEY WORDS: Antitussive activity, methanolic extract, *Cressa cretica Linn.* Voigt., Citric acid aerosol, Sulpher dioxide gas.

INTRODUCTION

Cressa cretica L. (Convolulaceae), popularly known as 'Rudanti' in Hindi is a widely grown halophytic plant. Different parts of the plant have been claimed to be valuable in a wide spectrum of diseases (1, 2, 3). In earlier studies *Cressa cretica* Linn flowers exhibited cytotoxic and anti-inflammatory activity (4). *Cressa cretica* is reported to be antibilous, antituberculosis, and expectorant (5). Shahat et al. yielded five flavonoids (quercetin, quercetin-3-O-glucoside, kaempferol- 3-O-rhamnoglucoside, and rutin) from the aerial parts of *Cressa cretica* (6). It is also reported the fruits of *Cressa cretica* is a potential source of edible oil. The oil of *C. cretica* was free from any undesirable components and could safely be recommenced for human consumption. Also the oil is of similar in composition with respect to individual fatty acids of commercial oils. (7). To substantiate its activity against cough and cold attack the present study was designed to investigate the effect of *Cressa cretica* Linn. extract on irritant aerosol induced coughing in male guinea pigs (8) and sulpher dioxide induced cough reflex in mice (9).

MATERIALS AND METHODS

Plant material and extraction

Cressa cretica was collected from Nalban island of Chilika lake, Orissa and was identified by Dr. H. O. Brahmam, Senior Scientist, Natural product division, Institute of Mineral and Material Technology. A voucher specimen has been kept in our laboratory for future reference. The whole plant was shade-dried, powdered, passed through a 40-mesh sieve and finally subjected to extraction with methanol in a soxhlet apparatus. The solvent was removed under vacuum and a solid mass (16.73% w/w with respect to dry

starting material) so obtained was stored in a desiccator and used for further experimental studies. The methanol extract of the whole plant was subjected for preliminary phytochemical screening to show the presence of steroid, alkaloid, glycoside, tannin, triterpenoid, carbohydrates and reducing sugar.

Experimental animals

The experiments were carried out in male guinea pigs (400-450 g) and Swiss albino mice of either sex weighing 400-450g and 30-40g respectively. Animals were kept in the departmental animal house at 26±2°C at relative humidity 44–55% and light dark cycles of 10 and 14 h, respectively. Animals were provided with rodent diet and water *ad-libitum*. The animal experiment was performed according to the institute's ethical committee approval and guidelines Reg no. 621/02/ac/CPCSEA of Birla Institute of Technology, Mesra, India.

Protocol for irritant aerosol induced antitussive evaluation

Male guinea pigs, five in each group were used in the study (body weight 500-600 g). The method has been described by Forsberg et al. (8). Unanaesthetized unrestrained animals were placed individually in a transparent test chamber, dimensions 30 cm × 20 cm × 20 cm and exposed to a nebulized aqueous solution of i.e. 0.1 g/ml of citric acid for 7min. The output of nebulizer was 0.65 ± 0.04 ml solution per minute and continued for 7 min. The same nebulizer was used throughout the experiment. During the last 5 min of the exposure, the animals were watched continuously by a trained observer, and the numbers of coughs were determined. Coughs could easily be distinguished from sneeze since there is a clear difference in sound as well as in behaviour of the animals (8).

The above protocol was performed 10 min after exposing animals to aerosols of the following solutions for a period of 7 min (n=5):

i) Normal saline (baseline measurements).
ii) Codeine solution (0.03 g/ml, positive control).
iii) CME (2.5%, w/v).
iv) CME (5%, w/v).

All the experiments were performed randomly with 2 h resting period between each two experiments

SO_2 induce antitussive evaluation

Antitussive effect against SO_2-induced cough was evaluated by the method as described by Miyagoshi et al (9). The experimental model is shown in Figure 1, where V_1 is 500 mL three-necked flask containing aqueous saturated solution of sodium hydrogen sulphite.

By opening the stopcock of a burette V_2, the concentrated sulphuric acid was introduced to generate SO2 gas. The chemical reaction that occurred in the flask A is

$$2NaHSO_3 + H_2SO_4 = 2SO_2 \uparrow + Na_2SO_4 + 2H_2O$$

Previously, SO_2 gas was filled in V_1 and V_3 gas reservoirs, and then by opening the cocks 3 and 2, pressure in the gas reservoir V_3 was elevated which was recorded by the water manometer V_4. Then the stopcock 2 was closed and stopcock 4 was opened slightly till the pressure in V_4 (11 mm i.d.) reached 75mm water, when the stopcock was closed. The procedure was operated in a draught.

The mice were divided into five groups, each containing 10 mice. One group served as a control group receiving only 2% v/v aqueous Tween 80 solution (10 ml/kg, p.o.). Three groups were used for methanol extract of *C. cretica* (100, 200 and 400 mg kg^{-1} p.o.) and the remaining group was used for standard drug codeine phosphate (10, 20 and, 40mg kg^{-1} p.o.). Both the extract and codeine phosphate were suspended separately in 2% v/v aqueous Tween 80 solution. Initially, the cough responses of all groups of animals were observed (0 min) by placing the animals individually in a desiccator V_5. The cocks 3, 6 and 5 were opened in order and when the pressure in V_4 became 0 mm of water, all the cocks were closed immediately. A certain amount of SO_2 gas (5ml which was kept constant throughout the experiment) was introduced in the desiccator in this way. After 1 min of introduction of the gas, the mice were taken out of the desiccator and the frequency of cough was observed for 5 min in an open-ended filter funnel with a stethoscope at the tip in which the mice were confined. In this way the frequency of cough was observed for all animal groups at 0 min (before the drug administration) and at 30 – 60 – 90 – 120 – min interval (after the drug administration).

Statistical Analysis

The frequency of cough produced by irritant-aerosol in guinea pigs were analyzed by one way ANOVA followed by Bonferroni's multiple comparison tests to compare all pairs of columns. The results are expressed as means ± standard mean errors (S.E.M.). The data obtained from sulfur dioxide-induced experiment were analyzed by one-way ANOVA followed by Dunnett's test for comparing between the control group and the various groups. Statistical significance was assumed at the 0.05 levels.

RESULTS

Aerosol induced antitussive evaluation

In the present study the antitussive effects of extracts from *C. Cretica* were evaluated using a standard method used previously by several investigators (8, 10). The results are depicted in Figure 2. Both concentrations of CME (2.5%w/v, 5%w/v) and the prototype drug, codeine phosphate (0.03 g/ml) caused significant reduction in cough number compared to base line value (p<0.001, Figure 2).The anti tussive effects 5% w/v CME also significantly greater than that of codeine(p<0.01, Figure 2) but, 2.5% w/v CME was not significant. In addition the antitussive effects of higher concentration of methanolic extract of *C. cretica* was significantly greater than those of lower concentration (p<0.01).

Sulpher dioxide induced antitussive evaluation

The effect of *C. cretica* extract on sulpher dioxide induced cough in experimental animals is shown in Table 1. It was observed that on exposure of the experimental animals (mice) to sulpher dioxide gas, the frequency of cough of control group remains more or less constant, i.e. it varies between 58.4 ± 1.21 and 62.67 ± 0.89 (mean ± SEM). But both in case of codeine phosphate and extract of *C. cretica* on oral administration, frequency of cough decreased in a dose- related manner. It was found that both the standard drug and extract (at different doses) produce maximum inhibition of cough reflex at 90 min after drug administration. A dose- dependent inhibition of cough was observed with CME and results were also comparable with the effect produced by codeine phosphate, a prototype antitussive agent. The results obtained with 100 - 200 - and 400 mg/kg doses of extract were statistically significant (p<0.01) through out the time span of the experiment. The highest inhibition of cough (55.44%) was produced by the extract of the 400 mg/kg dose level at 90 min of the experiment, where as codeine phosphate (40mg/kg) showed maximum 72.56% inhibition. The percentage inhibition of cough in codeine phosphate was 62.82 – 60.19% between 30 min to 120 min at the high (40 mg/kg) concentration which was comparatively higher than other two groups (10mg/kg, 20mg/kg) i.e. 22.93 – 38.69%, 42.56 – 58.11 % respectively, where as CME at 400mg/kg caused 45.75 - 47.09% cough inhibition between 30 – 120mins. The other two concentrations of extracts (100 mg/kg, 200mg/kg) inhibited 16.54 – 1.71 % and 27.71 – 26.4% respectively.

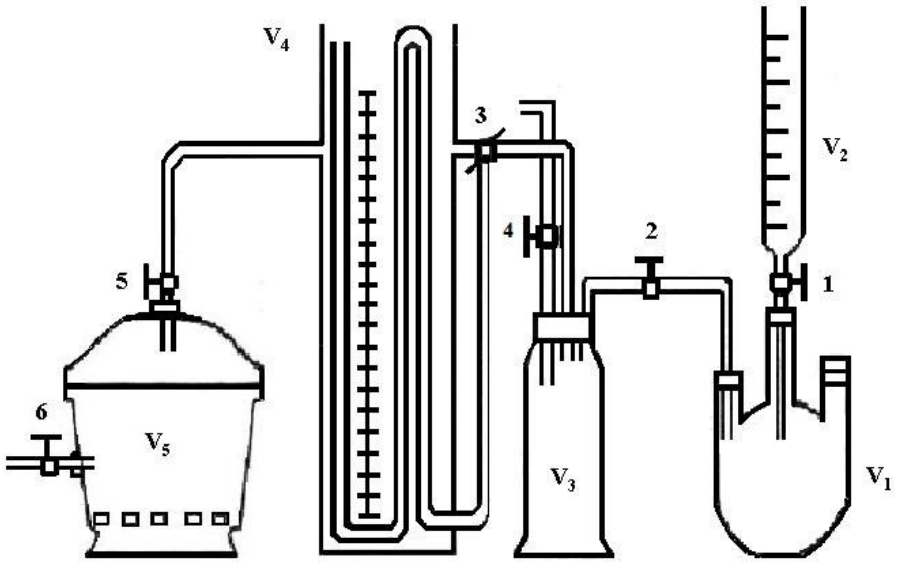

Figure 1: Apparatus for antitussive evaluation by sulfur dioxide gas production. V_1: Saturated $NaHSO_3$ solution in 500mL flask. V_2: Conc. H_2SO_4 in burette. V_3: Gas cylinder. V_4: Water manometer. V_5: Desiccator.

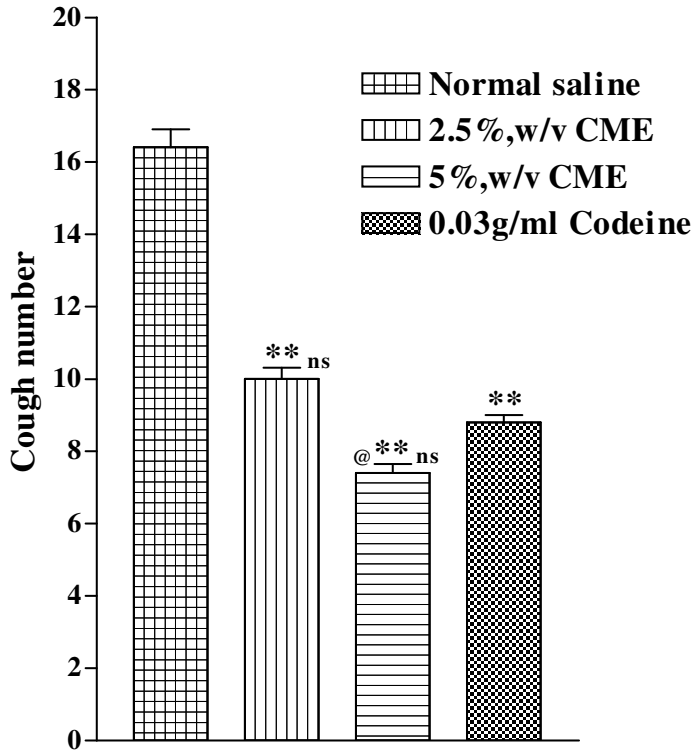

Figure 2: Cough number obtained in the presence of low (2.5%,w/v) and high (5%,w/v) concentrations of methanolic extract of Cressa cretica Linn. compared to those obtained in the presence of saline (baseline) and codeine phosphate (0.03g/ml). Statistical differences between plant extracts, standard (codeine) with baseline values; ** p < 0.001. Statistical differences between cough number obtained in presence of plant extracts with that of codeine; ns: non significant difference. Statistical difference between both the extract concentrations; [@]: p < 0.01.

Table 1: Effects of Cressa cretica Linn. methanolic extract (CME) and codeine phosphate on the cough induced by sulfur dioxide gas in mice.

Treatment	Dose	Frequency of cough (mean ±SEM)				
		0min	30min	60min	90min	120min
Saline control	10ml/kg	60.2±0.291	62.67±0.89	61.6±0.45	58.8±0.44	58.4±1.21
Codeine phosphate	10mg/kg	60.9±0.348 [ns]	48.3±1.76 [a] (22.93)	39.8±0.49 [a] (35.38)	35.7±0.87 [a] (39.28)	35.8±0.84 [a] (38.69)
Codeine phosphate	20mg/kg	60.4±0.163 [ns]	36.0±0.57 [a] (42.56)	25.8±0.77 [a] (58.11)	25.6±1.12 [a] (56.46)	25.9±0.46 [a] (55.6)
Codeine phosphate	40mg/kg	59.9±0.322 [ns]	23.3±1.2 [a] (62.82)	16.9±0.43 [a] (72.56)	23.2±0.78 [a] (60.54)	18.6±0.56 [a] (60.15)
CME	100mg/kg	60.1±0.180 [ns]	52.3±0.8 [a] (16.54)	50.6±0.82 [a] (17.85)	45.8±1.04 [a] (22.1)	53.4±0.54 [a] (01.71)
CME	200mg/kg	60.1±0.277 [ns]	45.3±1.8 [a] (27.71)	44.7±0.54 [a] (27.43)	38.6±0.9 [a] (34.35)	43.0±0.47 [a] (26.4)
CME	400mg/kg	60.3±0.213 [ns]	34.0±0.58 [a] (45.75)	33.1±0.82 [a] (46.26)	26.2±0.68[a] (55.44)	30.9±0.59 [a] (47.09)

The frequency of cough was counted for 5min after the sulphur dioxide gas challenge; Data are expressed in mean ± S.E.M. (n=10); ns : not statistically significant; [a] : p< 0.01; figures in parentheses indicate percentage inhibition of cough reflex.

DISCUSSION

Coughing is a normal physiological response to an irritation of the laryngo-tracheo-bronchial system caused by mechanical or chemical stimulation. It may be painful and fatiguing and require suppression by antitussive drugs. In animals, coughing has been elicited by mechanical (11) or chemical irritation (12) and by electrical stimulation (13) of tracheal mucosa or by nerve stimulation (14). Of all these methods, chemical or mechanical stimulation are more similar to the physiological event and also the experimental models generally used in man.

In the present study, the antitussive activity of CME has been compared with that of codeine against coughing induced in two different animal species by chemicals stimulation (irritant citric acid aerosol and SO_2 gas) stimulation. The extracts showed marked antitussive effect. The extract showed significant inhibition of cough like the standard drug (codeine phosphate) in dose-dependent manner; thus the extract might be acting via the central nervous system, but the exact mechanism of action can not be withdrawn from the preliminary study.

So from this study it can be concluded that on preliminary screening the extract of *C. cretica* produced a significant antitussive effect and thus established the claim of using the plant as an anti-cough agent in ancient folklore medicine. Further work relating to isolation and characterization of the active constituents present in the the plant and studies on various pharmacological evaluations, as well as evaluation of the mechanism of action for antitussive effect, is under way by our research team in our laboratory.

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