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RESEARCH ARTICLE

Evaluation of Anti-tussive Potential of *Acorus calamus* Linn on Ammonium Hydroxide Induced Cough Reflex in Mice

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ABSTRACT

Cough is a protective physiologic reflex of the respiratory system. Its function is to remove foreign object and abnormal excessive secretions from the respiratory tract. Cough also can pathologic and is a symptom of many underlying diseases. In adults in the United States, it is the fifth most common reason for visits to ambulatory care units, accounting for 30 million visits yearly. It can be due to a variety of pulmonary conditions such as asthma, chronic obstructive pulmonary disease, and lung cancer and adds to the adverse effect; those diseases have on quality of life. Cough is also a side effect of certain medications, with angiotensin converting enzyme inhibitors being the most familiar example. This study aimed to evaluate the anti-tussive potential of *Acorus calamus* on ammonium hydroxide-induced cough reflex in mice. The methods involved the administration of the ethanolic extract of *A. calamus* at a dose of 500 mg/kg to the mice, followed by induction of cough with ammonium hydroxide. The number of coughs was counted and the results were compared to the control groups. The results of the study showed that the ethanolic extract of *A. calamus* significantly reduced the number of coughs induced by ammonium hydroxide when compared to the control groups. This finding suggests that *A. calamus* has anti-tussive potential and could be a potential candidate for the development of new anti-tussive agents. In conclusion, the study highlights the potential of natural products, such as *A. calamus*, in the development of new anti-tussive agents. The findings also underscore the importance of developing effective therapies for the management of cough, a common symptom of various underlying diseases. Further research is necessary to confirm these findings and elucidate the mechanism of action of *A. calamus* in reducing cough.

Keywords: *Acorus calamus* rhizomes, ammonium hydroxide model, anti-tussive activity

INTRODUCTION

Cough is a common symptom and one of the main signs of respiratory tract diseases. Cough is a protective reflex mechanism which removes foreign material and secretions from the bronchi and bronchioles of the airways. It can be in various situations inappropriately stimulated; for example, by inflammation in the respiratory tract or neoplasia. In these cases, the cough has a pathological character and it is necessary to use cough-suppressing agents. It is also one of the most frequent reasons for children's visits to pediatricians or primary care physicians. Cough is helpful in getting rid of infectious material with the help of mucous from the airway, it should not be stopped. It is very important to remember that cough usually manifests in a common cold, but it may be the initial manifestation of serious illness such as pulmonary hypertension, pneumonia, tuberculosis, or asthma.[1,7,9]

MATERIALS AND METHODS

Collections of Plants

Acorus calamus, Rosc. (Rhizomes) were purchased from the local market, Indore, Madhya Pradesh, India.

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Preparation of Extracts

The powdered material was subjected to extraction using distilled water and alcoholic (Ethanol) solvents. The aqueous extract was prepared using reflux condenser and alcoholic extracts were prepared using soxhlet apparatus. The extracts were filtered, concentrated on water bath, dried in vacuum, and stored in refrigerator for subsequent experiments.[2,4,5]

Pharmacological Study

Animals

Swiss albino mice of either sex (22–30 g) were used in experiments. The animals were housed in polypropylene cages under standard conditions (12 h light; 12 h dark cycle; 25 ± 5°C; 35–60% of humidity).

Anti-tussive Activity

Ammonium hydroxide-induced cough

Mice of either sex weighing 21–24 g were divided randomly, ten mice per group. The negative control of animals was treated with distilled water orally, and the positive control was treated with dextromethorphan, the remaining groups treated were with aqueous and ethanolic extract, respectively. Anti-tussive activity was investigated on a classical mice cough model induced by ammonia liquor Briefly, each mouse was placed in a 300 mL special glass chamber and exposed to 40 µL 25% NH_4OH. The cough frequency produced during 2 min exposure period was counted. In the second assay for alkaloids, cough frequency and latent period of cough were recorded [Figure 1].[8,13] The chemical reaction which occurred in flask A is as follows:

$$2\ NaHSO_3 + H_2SO_4 \rightarrow 2\ SO_2 + Na_2SO_4 + 2\ H_2O$$

Flask A and gas cylinder C were filled with ammonium hydroxide (NH_4OH) gas. Cocks c and b were opened to elevate pressure in gas cylinder C, which was recorded by water manometer D. Stop-cock b was then closed, and stop-cock d was opened slightly until pressure in D (11 mm, i.d.) reached 75 mm H_2O, when stop-cock d was closed. The procedures were conducted in a draught.

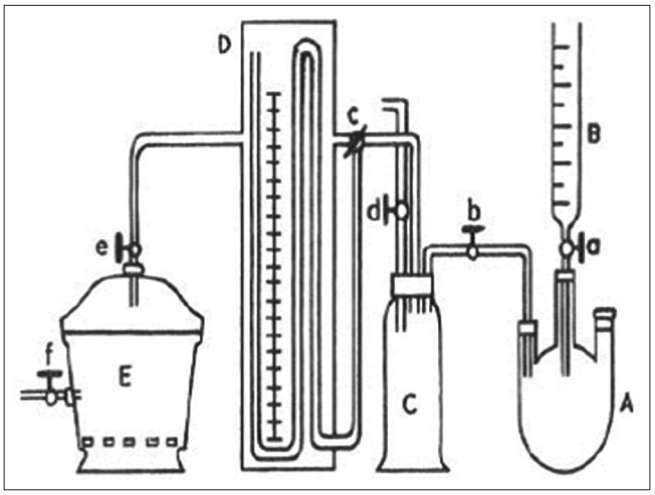

Figure 1: Apparatus used in ammonium hydroxide gas-induced cough model
A: Saturated NaHSO3 solution in 500 mL flask, B: Conc. H2SO4 in burette, C: Gas cylinder, D: Water manometer, E: Desiccator, and a, b, c, d, e, and f are stop cocks

The animals were divided into ten groups, each containing ten mice.

One served as control group, test drug at 250 mg/kg and 500 mg/kg aqueous and ethanolic extract administered in test Groups. The remaining group is treated with the standard drug dextromethorphan and control group received extracts. Cough responses of all the groups are observed (0 min) by placing the animals in desiccators E. The cocks c, f, and e are opened in order and when the pressure in D became 0 mm of H_2O; all the cocks are closed immediately. A certain amount, 5 mL sulfur dioxide gas is induced into the desiccator and this way. After a minute of introducing the gas, the animal is taken out of the dedicator and frequency of cough is observed for 5 min in an unended filter funnel with a stethoscope at the tip in which mice are confined. All groups were used in the same procedure.

The frequency of cough is observed for all the animal groups at 0 min., and at 30 min., 60 min., 90 min., and 120 min., (after drug administration) intervals.

It is usually observed that on exposure of the experimental mice to sulfur dioxide gas, the frequency of cough of control group remains more or less constant, that is, it varies between 57.3 ± 3.5 and 60.3 ± 3.3 (Mean ± standard error of mean [SEM]).

However, both in cases of dextromethorphan and test drug on oral administration, frequency of cough should decrease in dose-related manner if the drug is having anti-tussive properties.

Statistical Analysis

The experimental results were expressed as mean ± SEM. Significance was evaluated using the one-way ANOVA followed by Dunnet's test. Values of $P < 0.01$ versus control group implied significance.

RESULTS

Acute Toxicity Study

No toxic effects were observed at a higher dose of extracts and standard herbal drug. Hence, there were no lethal effects in any other groups.

Ammonium hydroxide-induced cough

The effect of ethanol and aqueous extracts of *A. calamus* Rosc on ammonium hydroxide-induced model in Swiss albino mice is shown in Table 1. The results obtained indicate that both ethanol 250 and 500 mg/kg extract had significant anti-tussive activity in Swiss albino mice, when compared with reference standards ($P < 0.001$). The ethanolic 250 and 500 mg/kg extract treated groups of *A. calamus* Rosc reduced cough 15.6 ± 0.9 and15.0 ± 0.9, respectively.

DISCUSSION[11]

We investigate the anti-tussive effect of ethanolic and aqueous extract of *A. calamus* Rosc rhizome. The most commonly used animal model for anti-tussive activity and ammonium hydroxide-induced cough in mice. Cough is a common symptom and one of the main signs of respiratory tract diseases. Cough is a protective reflex mechanism which removes foreign material and secretions from the bronchi and bronchioles of the airways. It can be in various situations inappropriately stimulated; for example, by inflammation in the respiratory tract or neoplasia. In this study, the ethanol extract of *A. calamus* (250 and 500 mg/kg) showed a significant reduction of cough at different time interval and aqueous extract (250 and 500 mg/kg) showed significant action but compared to ethanolic extract decrease the reduction of cough.

CONCLUSION

Cough is a crucial protective mechanism of the respiratory system, but it can also be a symptom of various underlying diseases. It is the fifth most common reason for visits to ambulatory care units in the United States, accounting for 30 million visits yearly. The adverse effects of cough on the quality of life of patients with pulmonary diseases cannot be overemphasized. In addition, cough can be a side effect of certain medications, with ACE inhibitors being a common example.

To improve the evaluation of drugs at the preclinical stage, randomized crossover designs have been suggested to remove between-subject variability, a significant source of experimental error. Such designs may offer improved precision in detecting treatment effects, ultimately leading to better drug development[3,6,7,8]

In the present study, the anti-tussive efficacy of *A. calamus* rhizomes was investigated using the

Table 1: Ammonium hydroxide induced cough

S. No.	Drug	Dose	Frequency (min)				
			0	30	60	90	120
1.	Control	–	81.5±0.8	83.3±0.4	83.5±0.5	82.3±0.4	81.0±0.4
2.	Standard (dextromethorphan)	10 mg/kg	82.6±0.8	68.6**±0.4	52.5**±0.4	28.0**±0.7	15.6**±1.2
3.	*A. calamus* Aq.	250 mg/kg	83.3±0.8	73.8**±1.1	64.8**±1.0	55.5**±0.6	46.0**±0.5
4.	*A. calamus* Aq.	500 mg/kg	83.8±0.6	74.0**±1.1	63.3**±1.5	52.3**±1.7	40.8**±1.7
5.	*A. calamus* Eth.	250 mg/kg	81.5±0.8	66.5**±1.7	52.5**±1.3	39.5**±1.8	27.3**±3.6
6.	*A. calamus* Eth.	500 mg/kg	80.6±0.42	59.6±0.6	43.0**±1.46	29.3**±1.5	17.33**±1.4

Values are mean±SEM. n=10, **P<0.01, *P<0.05. *A. calamus*: *Acorus calamus*

ammonium hydroxide-induced cough model. The ethanolic extract of *A. calamus* (500 mg/kg) demonstrated excellent anti-tussive activity and significantly reduced the number of coughs when compared to the control groups. These findings suggest that *A. calamus* could be a potential candidate for the development of new anti-tussive agents.

It is important to note that further research is necessary to confirm these findings and establish the safety and efficacy of *A. calamus* as an anti-tussive agent. In addition, the mechanism of action of *A. calamus* in reducing cough needs to be investigated further.

Overall, the study underscores the importance of developing effective anti-tussive agents to improve the quality of life of patients with pulmonary diseases and highlights the potential of using natural products as sources for developing new drugs. The use of randomized crossover designs in preclinical studies could also lead to the development of more precise and effective therapies. Further research is necessary to fully elucidate the therapeutic potential of *A. calamus* and other natural products for the treatment of cough.[10,12]

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