Abstract

Ethanol extract of rhizomes of *Curcuma aromatica* (Zingiberaceae) was investigated for its antitussive effect on sulfur dioxide induced cough model in mice. The extract exhibited significant antitussive activity in a dose dependant manner. The activity was compared with the prototype antitussive agent codeine phosphate. The ethanolic extract at the dose of 100mg, 200mg and 400mg/kg body weight, po, showed 68%, 74% and 79% of inhibition of cough with respect to control group.

Introduction

*Curcuma aromatica* is an erect perennial herb found throughout India; also cultivated in some parts of West Bengal and Kerala. Rhizomes are tuberous, large, yellow, orange, red and aromatic inside, used externally in combination with astringents to bruises and sprains and considered tonic and carminative. They are also used in skin eruptions and infections and to improve complexions and in cough, hiccup, bronchitis, leucoderma and diseases of the blood (Ayurveda). An intraperitonial injection daily for eight days containing *C. aromatica* as one of the constituents to an adult mice with a hepatoma H22 ascites tumor inhibits (87%) cancer. The oil has shown important antimicrobial activity against *S. aureus*, *S. paratyphi*, *E. carotovora*, *P. solanacearum*, *P. lilicinum*, *A. niger* and *R. nodosus*. The fresh rhizomes contain the sesquiterpenes l-curcumene, á and b-pinene, 1,8-cineole, isofuranogermacrene, borneol, isoborneol, tetramethylpyrazine, germacrone, curdione, curcumol,
zedoarondiol, curcumenone, neocuprindinone, curcumin, demothoxycurcumin. The powdered rhizome is taken either alone or in combination with other species of Curcuma against intestinal worms by tribals of Meghalaya\(^3\).

**Materials And Methods**

**Preparation of the extract:**

Rhizomes of *Curcuma aromatica* were collected from the local market of Shimoga and were authenticated. Dried rhizomes were powdered, extracted with 70% ethanol in a Soxhlet, concentrated and dried under vaccuum. Qualitative chemical test of the extract showed the presence of flavonoids, terpenes and carbohydrates.

**Animals used**

Swiss Albino mice of either sex weighing between 20-30g, provided with standard diet (Hindustan Liver Ltd.) and water *ad-libitum*, maintained under standard laboratory condition were used for the study. Ethical clearance for conducting the experiment was obtained from Institutional Ethical Committee.

**Acute toxicity studies**

Accute toxicity studies of the ethanolic extract was carried out in mice by Stair case method\(^4\). No toxicity was observed upto the dose of 4.0g/kg body weight. The extract (suspended in a 0.25% gum acacia) was tested at three dose levels i.e. lower dose of 100mg, moderate dose of 200mg and the highest dose of 400mg/kg body weight.

**Screening for Antitussive (Cough suppresant) activity**

The method described by Miyagoshi *et al*\(^5\), also quoted by Pulok K Mukharjee\(^6\) was adopted to evaluate antitussive activity. The animals were divided into seven groups of five each.

I group served as a control group received vehicle only (0.25% gum acacia solution)

II, III and IV groups received test drug (alcoholic extract of *Curcuma aromatica*) at the dose of 100mg, 200mg and 400mg respectively.

V, VI and VII groups received 10mg, 20mg and 40mg of Codeine phosphate respectively.

The experimental model is shown schematically\(^6\) (Fig. 1), where A is a three-necked flask containing aqueous saturated Sodium hydrogen sulfite solution. By opening the stopcock of a burette B, sulfuric acid is introduced to generate Sulfur dioxide gas. The chemical reaction occurring in flask A is, \(2\text{NaHSO}_3 + \text{H}_2\text{SO}_3 \rightarrow 2\text{SO}_2 + \text{Na}_2\text{SO}_4 + 2\text{H}_2\text{O}\)

Sulfur dioxide gas (SO\(_2\)) is filled previously in A and C gas reservoir and opening the cocks c and b, pressure in the gas reservoir C was elevated which was recorded by water monometer D. Then the stopcock b is closed and then opened slightly till pressure in D...
reaches 75mm in water when the cock d was closed. These procedures are operated in a draft.

Initially the cough responses of all the groups are observed (zero minutes) by placing the animals in the desiccator E. The cocks c, f and e are opened in order and when the pressure in D becomes zero (0 mm) of H₂O, all the cocks are closed immediately. A certain amount, around 2 to 3ml of sulfur dioxide gas is introduced into the desiccator. After a minute of introducing the gas, the animal is taken out of the desiccator and frequency of cough is observed for five minutes in an un-ended filter funnel with a stethoscope at the tip in which mouse is confined. In the same fashion the frequency of coughs are observed for all the animals of all the groups at zero (0) minute, before drug administration and 30, 60 and 90 minutes after drug administration.

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Fig -1. Schematic representation of the apparatus used for the evaluation of Antitussive (cough suppressant) activity by sulfur dioxide induced cough model⁶
Statistical Analysis

The results are expressed as ± SEM. Student’s t-test was adopted to evaluate the value of significance and p values less than 0.01 was considered as significant.

Table 1
Effect of ethanolic extract of Curcuma aromatica on sulfur dioxide induced cough in mice

| Treatment | Frequency of cough (mean ± SEM) |
|-----------|---------------------------------|
|           | 0 min | 30 min. (% Inhibition) | 60 min. (% Inhibition) | 90 min. (% Inhibition) |
| Control   | 48.08±0.02 | 46.02±0.04 | 47.88±1.20 | 47.08±0.6 |
| 10mg Cp   | 47.26±0.02 | 20.04±0.02 (57%) | 18.0±0.01* (62%) | 15.08±0.04* (68%) |
| 20mg Cp   | 47.02±0.02 | 16.0±0.20* (65%) | 14±0.04* (70%) | 12.08±1.20* (74%) |
| 40mg Cp   | 48.02±0.02 | 12.08±0.04* (73%) | 10.02±1.22* (78%) | 6.22±0.01* (87%) |
| 100mg C.a | 48.08±0.02 | 30.08±0.02 (35%) | 20.08±0.06 (57%) | 15.08±1.02* (68%) |
| 200mg C.a | 48.06±0.02 | 22.07±1.08 (52%) | 15.08±1.22* (68%) | 12.08±0.46* (74%) |
| 400mg C.a | 47.18±0.02 | 16.08±0.08* (65%) | 12.02±0.04* (74%) | 10.08±0.22* (79%) |

Frequency of cough was counted for 5 min. after SO₂ gas challenge
*P<0.01 shows statistical significance compared to control, n=5
Cp—Codeine phosphate, C.a—Ethanolic extract of Curcuma aromatica rhizomes

Results And Discussion

The effect of ethanolic extract of Curcuma aromatica rhizomes on Sulfur dioxide induced cough in mice is shown in Table 1. The extract as well as the standard drug has shown the activity in dose dependant manner and the % of inhibition of cough was more significant after 90 minutes of drug administration. It has been observed that the extract has produced 68%, 74% and 79% inhibition at the dose levels of 100mg, 200mg and 400mg/kg respectively after 90 minutes of drug administration. It is evident from the data that the highest dose of 400mg was found to be more effective. Sulfur dioxide produces cough by upper airway irritation and cough produced by this chemical stimulation is more comparable to that in humans. Many of the currently available cough suppressants like Codeine, Ephedrine, Bromhexine, Guaifenesin etc, produces significant side effects such as constipation, respiratory depression, drowsiness and
addiction which makes their use unsatisfactory. Hence, currently there is need to develop safe and effective cough suppressants. The present study has provided an experimental evidence for protection against cough by *Curcuma aromatica* rhizomes, which justifies its traditional use in the treatment of cough.

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