ANTI-TUSSIVE ACTIVITY OF *OLDENLANDIA UMBELLATA* ON COUGH REFLEX INDUCED BY SULFUR DIOXIDE IN MICE.

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Summary

The benzene, petroleum ether and ethanol extracts of leaves of *Oldenlandia umbellata* were investigated for its anti-tussive effect on a cough model induced by sulfur dioxide gas in mice. The ethanol extract at doses of 250 and 500 mg/ kg showed maximum inhibition of cough reflex at 3 hours after drug administration and the anti-tussive activity was comparable to that of codeine phosphate, a standard anti-tussive agent.

Key words: *Oldenlandia umbellata*; sulfur dioxide; anti-tussive activity; cough; codeine phosphate

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Introduction

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-suppressant agents. The anti-tussive agents are used mainly to suppress dry and painful coughs. It should be mentioned that the use of this group of drugs suppresses only the symptom without influencing the underlying condition.

Therefore, we should prevent administration of such drugs in cough associated with chronic bronchitis, which is associated with harmful sputum thickening and retention of the same. In these cases, agents with expectorant activity such as secretolytics and mucolytics are preferable and can suppress cough by other mechanisms. The most frequently used anti-tussive drugs in clinical conditions are from a group of narcotic analgesics. Their anti-tussive action is very effective at doses below those required for pain relief. Their disadvantage is a high rate of unwanted effects, like depression of the respiratory center, decreased secretion in the bronchioles and inhibition of ciliary activity. Their administration can lead to increased sputum viscosity, decreased expectoration, hypotension and even constipation. All of these side-effects lead us to look for other substances, especially non-narcotics, which might prevent the pathological cough [1]. As most of the drugs from plants which have become important in modern medicine had an origin either from traditional systems of medicine or folklore, we made an attempt to explore the anti- tussive activity of *Oldenlandia umbellata* (OU).

OU of the family Rubiaceae is commonly found in parts of India, Burma, Srilanka, Cambodia and Indonesia [2]. It was once extensively cultivated along the Coromandel Coast (eastern India). OU is an annual herb with small irregular flowers, throughout the regions of different plains of India. It is known as chay root, Shaya, Indian madder, Impural, Impuraver in Tamil [3]. Hedyotis umbellata is the synonym of the herb. The roots and leaves of OU are well known for its medicinal activity and are used in the treatment of asthma, bronchitis and bronchial catarrh [4]. The decoction of leaves is used for gastric lavage of poisonous bites. Two year old plant root bark is the source of chay root dye. Chay root dye is used with a mordant to impart a red colour to fabrics such as calico, wool and silk [5]. The aerial parts of the plant is claimed to possess antibacterial activity. It inhibits tumour growth, modulates immune activity, stimulates reticulo endothelial system, phagocytosis and augment macrophage oxidative burst [6]. The plant is well known in siddha for its styptic property. Decoction of the root has febrifuge property [7].

Material and Methods

Plant material

Fresh leaves of OU (Rubiaceae) were collected from the Tirumala hills of Tirupati, Andhra Pradesh, identified and authenticated by Dr. K.Madhavachetty, Department of Botany, Sri Venkateswara University, Tirupati. A voucher specimen of the plant was deposited in the Institute's herbarium for further reference.

Preparation of extract

The leaf material was cleaned, shade dried, coarsely powdered and extracted with benzene, petroleum ether and ethanol using soxhlet apparatus. The benzene, petroleum ether and ethanol extracts were stored in a desiccator and used for the experiment after suspension in aqueous Tween 80 solution (2% v/v).

Animals

White albino mice of either sex (wistar strain) weighing between 30-40g each obtained from central animal house of the Institute were used for this experiment. The animals were housed in standard metal cages (five animals in each cage) and provided with food and water *ad libitum*. The animals were used for the experiment after an acclimatization period of one week before the experimental sessions. Animals were acclimatized to the laboratory conditions prior to experimentation and were given a standard laboratory diet (Hindustan lever Ltd, Mumbai) and water *ad libitum*. Food was withdrawn 12 hrs before and during the experimental period. Experimental protocol was approved by the Institutional animal ethical committee. Animal ethical norms were strictly followed during all experimental procedures and conducted according to the Indian National Science Academy guidelines for the use and care of experimental animals.

Anti-tussive activity

Anti-tussive effect against sulphur dioxide (SO_2) induced cough was evaluated using method described by [8-11]. The experimental model is shown in Figure 1 [12,13] where A is a 500 ml three-necked flask which contains aqueous saturated sodium hydrogen sulphite solution. By opening the stop-cock of a burette (B), the concentrated sulphuric acid was introduced to generate sulphur-dioxide gas. 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 SO_2 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.

The animals were divided into five groups, each containing ten mice. Group 1 animals served as control and received 2% v/v Tween 80 solution alone; group 2 received 10 mg/kg dose of codeine phosphate; group 3, 4 and 5 received 250, 500 mg/kg of benzene, petroleum ether and ethanol extract of OU respectively. The extract and codeine phosphate were suspended separately in 2% v/v aqueous Tween 80 solution.

Initially the cough response of all groups of animals was observed (0 minute) by placing the animals individually in desiccator E. Cocks c, f and e were opened successively and when the pressure in D became 0 (zero) mm H_2O, all the cocks were closed immediately. Certain volume of SO_2 gas (5 ml, which was fixed throughout the experiment) was introduced into the desiccator in this way. One minute after introduction of gas, the animal was taken out of the desiccator and the frequency of cough was observed for 5 minutes in an unended filter funnel with a stethoscope at the tip in which the mouse was confined. In this fashion the frequency of coughs was observed for all the animal groups at 1, 2 and 3 hr intervals after drug administration.

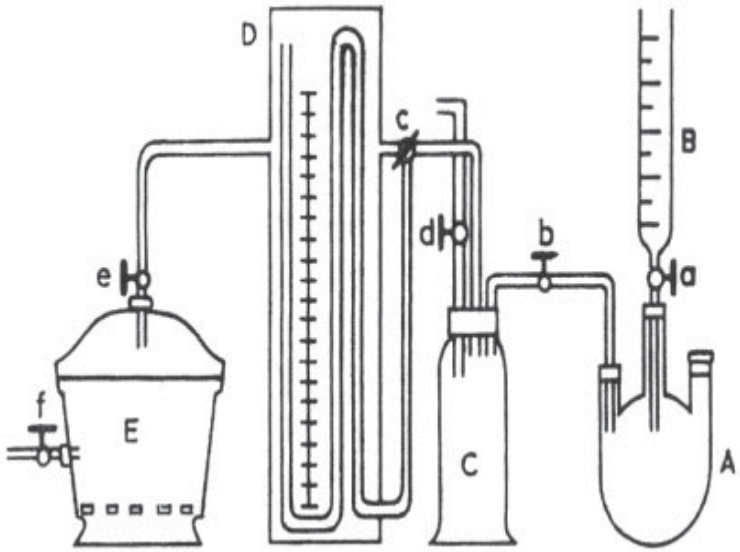

Fig.1. Apparatus for sulphur dioxide gas production [12].

A: Saturated $NaHSO_3$ solution in 500ml flask, B: Conc. H_2SO_4 in burette, C: Gas cylinder, D: Water manometer, E: Desiccator

Statistical Analysis

The experimental results are expressed as mean ± standard error of mean (SEM). Significance was evaluated using the Student's 't'-test. p-values <0.001 Vs control imply significance of the pharmacological effects in the experiment.

Results

The effect of OU extract on sulfur dioxide induced cough in the experimental animals is presented in Table 1. It was found that the frequency of cough of the control group remains more or less constant, i.e. it varied from 70±1.60 to 74±1.63 coughs/min. But a dose dependent inhibition of cough was observed with groups of animals treated with OU and the results were also comparable with that produced by codeine phosphate, a prototype antitussive agent. Benzene, petroleum ether and ethanol extract of leaves of OU at increasing doses, significantly and progressively inhibited the number of coughs after oral administration and the latency of the first cough was increased in the animals treated. The benzene (BEOU), petroleum ether (PEOU) and ethanol extract (EEOU) of leaves of OU at a dose of 250, 500 mg/kg relatively decreased the number of coughs (Table 1). The codeine phosphate at a dose of 10 mg/kg showed an inhibition of 64.86 %. Benzene extract at a dose of 250 and 500 mg/kg produced 29.73% and 36.49% inhibition; Petroleum ether at a dose of 250 and 500 mg/kg produced 28.38% and 41.89% inhibition; Ethanol extract at a dose of 250 and 500 mg/kg produced 31.35% and 60.81% inhibition respectively at the end of 3 hours.

Table 1.

Effects of OU on cough induced by SO_2 gas in mice

Treatment	Dose (mg/kg)	Frequency of Cough (mean ± SEM)		
		1 hr	2 hr	3 hr
Control	2% v/v Tween 80	70±1.60	72±1.73	74±1.63
Codeine phosphate	10	26±1.26* (62.86 %)	25±1.60* (65.18 %)	26±1.90* (64.86 %)
BEOU	250	58±1.25** (17.14 %)	56±1.46** (22.22 %)	52±1.65** (29.73 %)
	500	48±1.77* (31.43 %)	46±1.83* (36.11 %)	47±1.90* (36.49 %)
PEOU	250	52±1.78** (25.71 %)	55±1.81** (23.61 %)	53±1.67** (28.38 %)
	500	42±1.53* (40.00 %)	44±1.73* (38.89 %)	43±1.65* (41.83 %)
EEOU	250	40±1.75* (42.86 %)	38±1.65* (49.22 %)	36±1.75* (31.35 %)
	500	30±1.23* (57.14 %)	28±1.60* (61.11 %)	29±1.36* (60.81 %)

The frequency of cough was counted after SO_2 challenge. Values are mean ± S.E.M., n = 10; * $p < 0.001$, **$p < 0.01$ Vs control group, student's t-test.

Discussion

Cough 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 require suppression by antitussive drugs. In animals, coughing has been elicited by mechanical [14] or chemical irritation [15] and by electrical stimulation [16] of tracheal mucosa or by nerve stimulation [17]. Of all these methods, chemical or mechanical stimulation are more similar to the physiological event and also the experimental models generally used in man.

At present cough is treated by narcotic anti-tussive drugs such as codeine's groups. Unwanted and often life complicating side-effects of the above drugs cause attenuation of respiratory center, decrease the secretion and increase the viscosity and elasticity of sputum, decrease of expectoration, dependence (mainly in children) and bronchoconstriction [18]. The aims of current research are to find new substances or mechanisms, which could influence cough reflex without these side-effects or to decrease their occurrence as much as possible.

The present animal studies have shown ethanol extract of the leaves of OU to be an antitussive agent. It was significantly active in suppressing the SO_2-induced cough in mice. OU has no abuse potential whereas codeine is narcotic in nature and hence has high-abuse potential. These results corroborate the folklore claims on the effectiveness of the plant in managing 'tough' cough conditions. OU extract may prove useful and an effective antitussive agent in humans. Other pharmacological effects correlated with the antitussive activity, the general pharmacology and the toxicology of OU extract needs to be studied further.

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