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Evaluation of Antitussive Activity of *fruits of Terminalia chebula Retz.* on Cough Reflex induced by different Cough induced models in Mice

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Abstract

Cough is a natural reflex expulsive defense mechanism of the body, for clearing excess secretions, mucous, inhaled irritants, toxins or foreign substance in the respiratory tract. It is the most common symptom of respiratory disease. When cough becomes serious, opioids are effective, but they have side effects like sedation, constipation, some addiction liability and also compromise the respiratory function. Therefore, there is a need to have effective anti-tussive agent which do not have respiratory suppressant activity. The present study was carried out to evaluate anti-tussive activity of acetate and methanolic extract of fruit of *Terminalia.chebula* in ammonium hydroxide and Sulphur dioxide induced cough models in mice.

Key-Words: *Terminalia.chebula*, Cough, Antitussive activity

Introduction

Plant and animal materials have been used successfully for the treatment of human diseases since ancient times. Every country in the world has enlisted various indigenous herbal remedies according to the diseases and human requirements. Ayurveda is an original holistic system of diagnosis and treatment involving nutrition, hygiene and rejuvenation, developed and perfected in India over 5000 years ago (1). Ayurveda (Ayur-life, Veda-knowledge), is the knowledge of healthy living and is not merely confined to the treatment of illness. Ayurvedic medicines are largely based on herbal and herbomineral preparations and have specific diagnostic and therapeutic principles (2).

Cough is the most common respiratory symptom that has been experienced by every human. It is an essential protective and defensive act whose action secures the removal of mucus, noxious substances, and infections from the larynx, trachea, and larger bronchi. On the other hand, a number of patients have nonproductive cough, which is not associated with mucus clearance and may have a different stimulation. It may be the first overt sign of disease of airways or lungs and may significantly contribute to the spread of airborne infections and, in some instances, may result in severe functional and structural damage to the organism.

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The primary action of currently available cough suppressants (opiates, dextromethorphan, etc.) is on the central cough pathway. The significant side effects of these agents such as constipation, respiratory depression, dependence, drowsiness, and death from this action limit their use in human(3)

The causes of cough are obviously diverse but the common link between them all is the activation of subsets of airway sensory nerves. Diseases of the respiratory system can lead to activation of sensory nerves at the level of the airway lumen following the release of inflammatory mediators, increased mucus secretion or damage to the airway epithelium. Disorders of other organs that have neurons carried in the vagus (i.e. the oesophagus or the heart) probably interact with airway neurons in higher neuronal centres to elicit the cough reflex (4)

Terminalia chebula (*T. chebula*) is a flowering evergreen tree of the family Combretaceae. It has several common names such as black myrobalan, ink tree, or chebulic myrobalan (English), haritaki (Sanskrit and Bengali), harad (Hindi), harada (Marathi and Gujrati) Karkchettu (Telgu) and Kadukkaya (Tamil). In Tibet, *T. chebula* is called as the "King of Medicine"(5). It well known as 'haritaki' since it carries away all diseases or it is sacred to God Siva (Hara). Haritaki has several interesting synonyms like 'pathya', since it removes obstructions from the pathways and channels in the body; 'abhaya', since it

gives fearlessness; 'amrta', means an ambrosia; 'divya', means a divine herb; 'medhya', means a nerve tonic; 'pranada', means life saving; 'jivaniya', means a vitalizing herb; 'vayahstha', means one that promotes longevity and maintains youth; 'rasayana phala', means a rejuvenating fruit etc(6). *T. chebula* is routinely used as traditional medicine by tribals of Tamil Nadu to cure several ailments such as fever, cough, diarrhea, gastroenteritis, skin diseases, candidiasis, urinary tract infection and wound infections.(7). Phytochemical investigations of *Terminalia Chebula* have been reported on presence of tannins, carbohydrates, glycosides, phenols, alkaloids, terpenoids and flavonoids(8).

Material and Methods

Collection of plant

Fruits of *T.chebula* were collected from botanical garden, Indore (M.P) and identified and authenticated at Department of Botany, Govt. Agriculture College, Indore. A voucher specimen has been kept in the herbarium of our department for future references.

Drugs and chemicals

Fruits Of Terminalia Chebula, Petroleum Ether, Chloroform,Ethyl Acetate, Ethanol, H_2SO_4, Aq Sodium Hydrogen Sulphite, Ammonium hydroxide, Codine Phosphate, Dextromethaphan.

Preparation of extract

Fruits were purchased from local market of indore and identified and authenticated at Department of Botany, Govt. Agriculture College, Indore. A voucher specimen has been kept in the herbarium of our department for future references. shade dried and and powdered Fruits were subjected to successive solvent extraction using Petroleum ether, Chloroform, Ethyl acetate, Methanol as a solvent. All the four extract obtained were filtered, concentrated on water bath, dried in vacuum and stored in refrigerator for further experiment. Since main phytoconstituents flavonoids and alkaloids were found in ethyl acetate as well as in methanolic extract thus these two extract were taken for the further studies.

Experimental animals

For both the model of antitussive activity Swiss albino mice of either sex (20-30g) were used in the study. The animals were housed in polypropylene cages under standard conditions (12 h light; 12 h dark cycle; 25± 5oC; 35-60% humidity). They were fed with standard pellet diet (Pranav Agro Ltd, Dehradun) and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC/PCP/2014/49).

Pharmacological study

Sulphur Dioxide induced cough

Swiss albino mice were divided into four groups, each group containing six mice. The control group was treated with distilled water orally, and the positive control was treated with Codiene Phosphate. The remaining groups were treated with the ethyl acetate and methanol extract at doses of 500 mg/Kg body weight respectively.

Sulfur dioxide gas induced cough reflex in mice

The experimental model is shown in Figure 1 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.

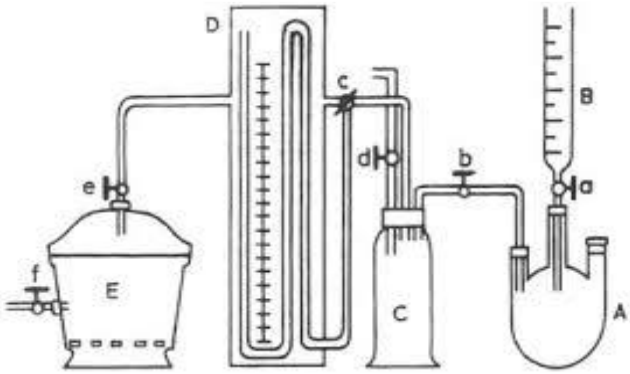

Figure 1

A: Saturated NaHSO3 solution in 500ml flask, **B**: Conc. H2SO4 in burette, **C**: Gas Cylinder, **D**: Water manometer, **E:** Dessicator and a, b, c, d, e, f are stop cocks.

The chemical reaction which occurred in flask A is as follows:

2 NaHSO3 + H2SO4 → 2 SO2 + Na2SO4+ 2 H2O

Flask A and gas cylinder C were filled with sulphur dioxide (SO2) 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 H2O, when stop-cock d was closed. The procedures were conducted in a draught. Cough response of all the groups are observed (0 minute) 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 H2O, all the cocks are closed immediately. A certain amount, 5ml 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 five minutes in an un-ended filter funnel with a stethoscope at the tip in which mice is confined. In the same fashion the frequency of cough are observed for all the animal groups after every 30 minutes for 2 hrs. (9).

The percentage frequency of cough reflex was calculated by the formula

% frequency of cough reflex= (1 – T / C) X 100

Where T= Cough reflex in tested drug treated in mice

C= Cough reflex in control group treated mice.

Ammonium hydroxide induced Cough

Swiss albino mice were divided into four groups, each group containing six mice. The control group was treated with distilled water orally, and the positive control was treated with dextramethorphan. The remaining groups were treated with the ethyl acetate and methanolic extract at doses of 500 mg/Kg body weight respectively. Anti-tussive activity was investigated on a classical mouse cough model induced by ammonia liquor. Each mouse was placed in a 300 ml special glass chamber and exposed to 40µl 25% NH4OH. The cough frequency produced during 2 min exposure period was counted. The cough frequency and latent period of cough were also recorded (10).

The percentage frequency of cough reflex was calculated by the formula

% Frequency of Cough Reflex= (1 – T / C) X 100

Where T= Cough reflex in tested drug treated in mice;

C= Cough reflex in control group treated mice.

Results and Discussion

The aim of the present study was to investigate the anti-tussive activity of ethyl acetate and ethanol fraction of fruits *Terminalia chebula* in experimental animal model. Anti-tussive agents or cough suppressants are used mainly to suppress dry and painful cough. They act to reduce the urge to cough. The larynx and extrapulmonary airways are richly supplied with non myelinated C- fibres and rapidly adapting receptors having myelinated Aδ- fibres. These are involved in the cough mechanism (11) . Vagal afferent nerve provide inputs to brainstem nuclei, primarily the nucleus of the solitary tract (nTS) that receive inputs from airway cough evoking afferents and generate cough reflex in body . Centrally acting antitussives such as codeine and dextromethorphan act within the central nervous system (CNS) at the level of the brain stem by depolarization or a dulling of the vagus nerve, the nerve leading from the brain stem and serving the chest area. Peripheral antitussive drugs act outside the CNS to inhibit cough by suppressing the responsiveness of one or more vagal sensory receptors that produce cough (12).

Antitussive animal models could be designed by mechanical stimulus, electrical stimulus, and chemical stimulus. In this experiment, chemicals like ammonium liquor and sulfur dioxide were used to induce cough. These models are widely used animal models for evaluating antitussive activity of a traditionally used drug. 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.(13)

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. Anti-tussive agents are used mainly to suppress dryand painful cough. Cough suppressants act to reduce the urge to cough. Nonmyelinated C-fibres and rapidly adapting receptors, which have myelinated Aδ-fibres, appear to be involved in cough. These putative cough receptors have myelinated afferents and are found mainly in the larynx and the extrapulmonary airways (18). Vagal afferent nerve provide inputs to brainstem nuclei, primarily the nucleus of the solitary tract (nTS) that receive inputs from airway cough evoking afferents and generate cough reflex in body. Centrally acting antitussives such as codeine phsophate and dextromethorphane act within the central nervous system (CNS) at the level of the brain stem by depolarization or a dulling of the vagus nerve, the nerves leading from the brain stem and serving the chest area. Peripheral antitussive drugs act outside the CNS to inhibit cough by suppressing the responsiveness of one or more vagal sensory receptors that produce cough (19, 20).

The *in vivo* antitussive activity of the ethyl acetate and methanolic extract of fruit of *T.chebula* was investigated for its effect on a cough model induced by sulphur dioxide gas in mice and found to have significant anti-tussive activity when compared with control and the standard drug Codiene phosphate. The ethyl acetate and methanolic extract of *T.chebula* plant was orally administered at the dose levels of 500 mg/kg b.w. showed maximum inhibition of cough by 82% and 81% respectively .The standard anti-tussive drug Codiene phosphate (10mg/kg b.w.) showed maximum inhibition of cough by 84%. It was found that both

extract of *T.chebula* showed anti-tussive activity and obtained percentage inhibition of cough reflex is approximately comparable as standard drug (Table 2 and 4).

Ammonium hydroxide well-described inducers of bronchoconstriction in individuals and are chemically related and, therefore, may share a common mechanism of action. Acute exposure of ammonium hydroxide causes dryness of nose and throat and a measureable increase in resistance to bronchial air flow.

In this model, ethyl acetate and methanolic extract of fruit of *T.chebula* orally administered at the dose of 500mg/kg b.w. showed maximum inhibition of cough by 82% and 81% respectively. The standard anti-tussive drug Dextromethorphan (10mg/kg b.w.) showed maximum inhibition of cough by 84% (Table 1 and 3).

Conclusion

To conclude, our study indicated that the ethyl acetate and methanolic extract of fruit of *T.chebula* demonstrated significant antitussive activity and obtained percentage inhibition of cough reflex is approximately comparable as standard drug. These effects are the important evidence for the traditional use of fruit of *T.chebula* in the treatment of cough and respiratory disorders.

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Table 1: Effect of ethyl acetate and methanolic extract of fruit of *T. chebula* on cough frequency in Ammonium hydroxide gas induced cough mice

TREATMENT(n=6)	Dose(mg/kg)	COUGH FREQUENCY IN MINUTES				
		0 min	30 min	60 mmin	90 min	120 min
Control	-	74.16±2.92	76.83 ±2.48	75.5±0.83	74.33±3.07	74.67±3.01
Dextromethaphan	10	76.33± 1.21	34.83±2.63**	22.33±1.86**	14.5±2.42**	11.5±1.04**
Ethyl acetate extarc	500	73±1.41	55.83±2.56**	42.5±2.25**	33.66±1.96**	13.33±2.42**
Methanol extract	500	74±1.41	62.67±3.14**	45.5±1.04**	34.17±3.06**	14.16±1.47**

Values are mean ± SEM, n= No. of animals in each group. ** $p < 0.05$ Significance versus control.

Table 2: Effect of ethyl acetate and methanolic extract of fruit of *T.chebula* on cough frequency in Sulphur dioxide gas induced cough mice

TREATMENT	DOSE(mg/kg)	COUGH FREQUENCY IN MINUTES				
		0 MIN	30 MIN	60 MIN	90 MIN	120 MIN
CONTROL		77.38±1.58	78.13±1.57	76.88±	75.63±1.44	76.25±1.51
Codiene Phospahte	10	76.38±1.55	31.75±1.31**	18.25±1.24**	13.88±1.30**	11.5±1.51**
Ethyl acetate extract	500	74.22±1.23	53.25±1.28**	44.71±1.04**	32.26±1.11**	13.42±1.36**
Methanol extarct	500	75.13±1.29	62.26±1.24**	49.21±1.47**	34.28±1.21**	14.47±1.26**

Values are mean ± SEM, n= No. of animals in each group. ** $p < 0.05$ Significance versus control.

Table 3: Effect of ethyl acetate and methanolic extract of fruit of *T.chebula* on % Inhibition in Ammonium Hydroxide gas induced cough mice

Treatment	Dose (mg/kg)	% INHIBITION OF COUGH REFLEX			
		30 MIN	60 MIN	90 MIN	120 MIN
Dextromethaphane	10	54%	70%	80%	84%
Ethyl acetate extarct	500	27%	43%	54%	82%
Methanol extract	500	18%	39%	54%	81%

Table 4: Effect of ethyl acetate and methanolic extract of fruit of *T. chebula* on % Inhibition in Sulphur dioxide gas induced cough mice

Treatment	Dose (mg/kg)	% INHIBITION OF COUGH REFLEX			
		30 MIN	60 MIN	90 MIN	120 MIN
Codiene Phospahte	10	59%	76%	81%	84%
Ethyl acetate extract	500	31%	41%	57%	82%
Methanol extract	500	20%	35%	54%	80%

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