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Anti-Tussive Activity of Ethanolic Leaf Extract of *Cymbopogon citratus* against Citric Acid Induced Cough in Guinea Pigs

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Abstract: Cough is a reflex of pulmonary irritant receptor, found in the epithelium of the respiratory tract, which are sensitive to both chemical and mechanical stimuli. Cough is a useful physiological mechanism that serves to clear the respiratory passages of foreign material and excess secretions and should not be suppressed indiscriminately. Anti-tussive drugs are the most reliving class for cough suppressants. Codeine is an effective antitussive, but it has some undesirable side effects like constipation, drowsiness, respiratory depression, dependence on chronic use etc., so it's necessary to seek a safe alternative from herbal source. Cymbopogon citratus belonging to the family Gramineae is an herb worldwide known as lemongrass. Anti-tussive activity of ethanolic leaf extract of Cymbopogon citrates (100 & 200mg/kg) was studied by inducing cough by citric acid in guinea pigs. Number of bouts in 5 minutes and percentage cough inhibition were measured. Codeine Phosphate was used as reference control. Both the doses of Cymbopogon citrates were significantly reduced the bouts in dose dependent manner and the percentage cough inhibition was increased which comparable as that of the reference control codeine phosphate. From the result it was concluded that the ethanolic leaf extract of Cymbopogon citrates exhibited anti-tussive activity against citric acid induced cough in guinea pigs.

Keywords: Cymbopogon citrates, Anti-tussive, Codeine Phosphate and Cough

INTRODUCTION

Cough is a protective reflex, its purpose being expulsion of respiratory secretions or foreign particles from air passage. It occurs due to stimulation of mechano or chemoreceptors in throat, respiratory passage or stretch receptors in the lungs. Acute cough symptoms commonly result from infection (upper respiratory tract infection (URTI) or common cold) or environmental exposure to smoke and/or allergens. Anti-tussive drugs are the most relieving class for cough suppressants. They are the drugs act in the CNS to raise the threshold of cough centre or act peripherally in respiratory tract to reduce the tussal impulses or both these actions. Trials of over-the-counter remedies, such as cough syrups and cough suppressants, have also failed to produce consistent, objective benefits [1]. Herbal medicines are part of a wide range of treatments such as phytotherapies, hydrotherapies, and Traditional Medicine, few of which are applied in conventional medicine.

Many synthetic drugs because of unwanted but unavoidable side effects have poor patient compliance. Therefore herbal treatment is being preferred over conventional treatments. Much attention and so scope is drawn towards herbal remedy of many respiratory disorders.

Cymbopogon citratus belonging to the family Gramineae is an herb worldwide known as lemongrass. The prefix 'lemon' owes to its typical lemon like odor, which is mainly due to the presence of citral, a cyclic monoterpene. Cymbopogon citratus a fast growing, perennial aromatic grass is native to South India and Sri Lanka, now widely cultivated in the tropical areas of America and Asia. Freshly cut and partially dried leaves are used medicinally and are the source of the essential oil. The plant is used extensively in Ayurvedic medicine.

Lemongrass is a folk remedy for coughs, elephantiasis, flu, gingivitis, headache, leprosy, malaria, ophthalmic, pneumonia and vascular disorders. Studies have shown that the lemon grass has antibacterial and antifungal properties. Mixed with pepper, it's a home therapy for menstrual troubles and nausea. The lemon grass is a good cleanser that helps to detoxify the liver, pancreas, kidney, bladder and the digestive tract. It cuts

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down uric acid, cholesterol, excess fats and other toxins in the body while stimulating digestion, blood circulation, and lactation; it also alleviates indigestion and gastroenteritis. It is said that lemon grass also helps improve the skin by reducing acne and pimples and acts as a muscle and tissue toner. Also, it can reduce blood pressure [2].

The essential oil consists of, mainly, citral a volatile oil with strong lemon fragrance. Citral is a mixture of two stereoisomeric monoterpene aldehydes; the Trans isomer geranial (40-62%) dominates over the cis isomer neral (25-38%) and is used in manufacture of perfumes, colored soaps and synthesis of Vitamin A [3]. Few biological activities like anti-microbial [4], antifungal [5], anti-protozoan [6], anti-oxidant [7], antidiarrhoeal [8], anti-mutagenic [9], analgesic & antianti-malarial inflammatory [10, 11], hepatoprotective activity [13] etc, of ethnobotanical claim of Cymbopogon citrates were documented. Current study was undertaken to evaluate the cough suppressant activity of ethanolic leaf extract of Cymbopogon citrates in mice.

MATERIALS AND METHODS Plant material

The leaves of Cymbopogon citrates were collected in the month of November 2016 from the outskirts of Pondicherry. It was identified and authenticated as Cymbopogon citrates by Scientist 'F' Botanical survey of India, Southern Regional Centre, Tamilnadu Agriculture University, and Coimbatore. The voucher specimen (BSI/SRC/12/42/2016-17/Sci/0553) has been deposited in department for further references.

Preparation of Extract

The fresh leaves of Cymbopogon citrates were washed, dried and cut into small pieces. The leaves were shade dried and subjected to steam distillation. The distillate collected was subjected to extraction with ethanol. The resulted ethanolic extract was dried, and stored in airtight container, protected from light.

Animals

Dunkon - Hartely Guinea pigs weighing between 350–400 g were used for the study. The animals were obtained from animal house of Sri

Lakshmi Narayana Institute of Medical Sciences, Pondicherry, India. On arrival the animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of $24 \pm 2^{\circ}$ C and relative humidity of 30–70 %. A 12:12 light: dark cycle was followed. All animals were allowed free access to water and fed with standard commercial pelleted rat chaw (Hindustan Lever Ltd, Mumbai). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics (932/a/06/CPCSEA) were Committee and accordance with the guidelines of the IAEC.

Evaluation of Anti-Tussive activity

Cough was induced by exposure to the aerosol of citric acid (7.5% w/v) introduced through a small opening at the side of the chamber using a nebulizer for 5 minutes. The frequency of cough during following 5 minutes was recorded. The animals showing 15±5 bouts of cough were selected for the study.

The day before the test they were placed individually in histamine chamber for 5 minutes for acclimatization. The selected animals were randomly assigned to 4 groups with six animals in each group. After an hour of rest the first group of animals was administered normal saline, second group received codeine phosphate (0.25gm/kg), and the third and fourth, group animals received the ethanolic leaf extract of Cymbopogon citratus at a dose of 100 & 200mg/kg respectively. All the test drugs were administered orally using gastric intubation tubes.

An hour after drug administration, they were subjected to citric acid aerosol exposure and the number of bouts in 5min after test drug administration was recorded. The percentage inhibition of cough was calculated.

STATISTICAL ANALYSIS

The values were expressed as mean \pm SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnet's 't' – test using graph pad version I. P values <0.05 were considered significant.

RESULTS

Table-1: Effect of Ethanolic Leaf Extract of Cymbopogon citratus on Citric Acid induced Cough in Guinea Pigs

Drug Treatment	Number of Bouts in 5 minutes	Percentage Inhibition
Control	18.88±0.63	-
Normal Saline		
Codeine Phosphate (250mg/kg, p.o)	2.48±0.17***	86.88
Cymbopogon citratus (100mg/kg, p.o)	6.54±0.43**	65.36
Cymbopogon citratus (200mg/kg, p.o)	2.75±0.16***	85.43

Values are in mean ± SEM (n=10), *P<0.05, **P<0.01, ***P<0.001 Vs Control

The anti-tussive activity of ethanolic leaf extract of Cymbopogon citrates on Cirtic acid induced Cough in guinea pigs were shown on table 1. Two dose levels (100 & 200mg/kg) of ethanolic leaf extract of Cymbopogon citrates were studied against Citric acid induced cough in guinea pigs. After test drug administration, the number of bouts (cough) was observed for 5 minutes. The control group, treated with normal saline and the number of bouts were 18.88±0.63. The reference control codeine phosphate, significantly (P<0.001) reduced the bouts to 2.48±0.17 and the percentage inhibition was 86.88%. The number of bouts observed with 100 & 200mg/kg of Cymbopogon citrates after citric acid administration was 6.54 ± 0.43 and 2.75 ± 0.16 respectively. The ethanolic leaf extract of Cymbopogon citrates at 100mg/kg significantly reduced (P<0.01) the cough as compared to control and 200mg/kg also significantly (P<0.001) reduced the cough as compared to control groups. The percentage inhibition of 100 & 200mg/kg of Cymbopogon citrates were found to be 65.36% and 85.43%. The anti-tussive effect produced by the ethanolic leaf extract of Cymbopogon citrates at 200mg/kg was equipotent as that of the reference control codeine phosphate.

CONCLUSION

The present study validate the traditional claims of ethanolic leaf extract of Cymbopogon citrates in the treatment of cough against Citric acid induced cough in guinea pigs. Further studies using receptor models may confirm the exact mechanism of action of Cymbopogon citrates.

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