



## PRE-CLINICAL INVESTIGATION OF ANTI-TUSSIVE ACTIVITY OF *HIBISCUS ROSA-SINENSIS* ON GUINEA PIG

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### ABSTRACT

Ayurveda suggests that decoction of leaves of *Hibiscus rosa-sinensis* could be beneficial to reduce cough. Codeine and other synthetic antitussive agents shows number of side-effects and thus to find better and safer alternate *Hibiscus rosa-sinensis* was evaluated for its antitussive activity. Dried powder of flower part of *Hibiscus rosa-sinensis* was extracted with methanol and the practical yield of methanolic extract was found to be 1% W/W. Anti-tussive effect of methanolic extract of *Hibiscus rosa-sinensis* was studied in Histamine chamber using citric acid (7.5%W/V) induced cough model, in which the methanolic extract of *Hibiscus rosa-sinensis* significantly decreased the number of coughing. From this we concluded that methanolic extract of *Hibiscus rosa-sinensis* possess antitussive activity.

**Keywords:** Anti-tussive, Cough, Codeine, *Hibiscus rosa-sinensis*, Citric acid

### INTRODUCTION

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<sup>1</sup>. 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<sup>1,2</sup>. Anti-tussive drugs are the most relieving class for cough suppressants. They are the drugs that 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<sup>3</sup>. This class has aim to control the cough rather than to eliminate. Anti-tussives should be used only in dry unproductive cough<sup>3</sup>. Opioids, nonopioids, antihistaminic and bronchodilators are different class of anti-tussives<sup>1,3,4,5</sup>.

Codeine is the widely used anti-tussive agent from opioid class. It is more selective for cough centres and treated as standard anti-tussive. Codeine exerts effects through opioid receptors present in neurons in the CNS and peripheral tissues. Opioid receptors are of three types identified as  $\mu$ ,  $\kappa$  and  $\delta$ <sup>3</sup>. These receptors are distributed throughout the brain, spinal cord and peripheral tissues. Mu receptors are primarily found in brainstem and medial thalamus. Kappa receptors are primarily found in limbic system, brainstem and spinalcords. Delta receptors are generally found in limbic and spinal cord area<sup>3,6</sup>. Even though the codeine is having narcotic effect so it is having abuses and drug dependence<sup>2,3</sup>. Constipation is also seen as a chief drawback of codeine<sup>1,2,3</sup>. At higher doses the drowsiness and respiratory depression can occur<sup>1,2,3</sup>. It is contraindicated in asthma and in patients who have diminished respiratory reserve<sup>2,3</sup>.

Looking at the dire need to find better and safer herbal alternate of codeine and other synthetic antitussive agents, this experiment was done to prove anti-tussive effect of the *Hibiscus rosa-sinensis*.

These are the dried petals of *Hibiscus rosa-sinensis* belongs to family Malvaceae. Commonly it is known as Shoeflower [Jamaica] or Jasud [Gujarati]<sup>7</sup>.

The leaves were alternate, simple and ovate to lanceolate, often with a toothed or lobed margin. The flowers are large, conspicuous, trumpet-shaped, with five or more petals,

ranging from white to pink, red, orange, purple or yellow, and from 4–18cm broad<sup>8,10</sup>.

Pharmacological actions of *Hibiscus rosa-sinensis* includes Pharmacological Actions: color<sup>6,8,9,10</sup>, bortifacient<sup>9</sup>, analgesic<sup>9</sup>, antidiarrhoic<sup>9</sup>, antiestrogenic<sup>9</sup>, antifungal<sup>9</sup>, anti-infectious<sup>9</sup>; anti-inflammatory<sup>9</sup>; Antipyretic<sup>9</sup>; astringent<sup>9</sup>, CNS depressant<sup>7</sup>, constipating<sup>7</sup>, Contraceptive<sup>9</sup>; demulcent<sup>9</sup>, dentifrice<sup>10</sup>, diuretic<sup>10</sup>, expectorant<sup>9</sup>, hemostat<sup>9</sup>; hypoglycemic<sup>9</sup>, hypotensive<sup>7</sup>; hypothermic<sup>7</sup>; insect attractant<sup>7</sup>; promotes hair growth and ; purgative<sup>7</sup>; refrigerant<sup>9</sup>; relaxes spasm<sup>7</sup>; soothes irritated tissue<sup>9</sup>.

### MATERIALS AND METHOD

#### Collection and extraction:

The herb of *Hibiscus* was collected from local region in Rajkot district of Gujarat. Morphological & microscopy of plant was authenticated by head of pharmacognosy, department of School of Pharmacy, RK University. The flowers were separated and dried between 55 ° to 60° C and then pulverized to very fine powder. The powder was subjected for Soxhlet apparatus using methanol as an extractor to prepare methanolic extract. The % yield of extract was found to be 1% W/W.

#### Selection of animals:

*Caviaporcellus* (Guinea pig) of weighing around 400-550gm were used for the study. The animals were procured from Animal House, Department of Pharmacology, School of Pharmacy, RK University Rajkot, India. The animals were placed at random and divided to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±20°C and relative humidity of 30 – 70 %. A light and dark cycle was followed. All animals were fed on standard balance diet and provided with water ad libitum. Experiments were carried out between 09:00 and 14:00 hrs. All the experimental procedures and protocols used in the study had been reviewed and approved by the Institutional Animal Ethical Committee (IAEC) and care of laboratory animals had been taken as per the guidelines of Committee for the purpose of control and supervision of experiments on animals (CPCSEA), Govt. of India (Registration No.1131/ac/07/CPCSEA.)

**Experimental design:** 4, 5, 11, 12, 13, 14

The study includes the anti-tussive effect of Methanolic extract of *Hibiscus rosa-sinensis*.

Procedure:

Model: Citric acid induced cough model (Histamine chamber):

The 18 animals (guinea pig) divided into 3 groups containing 6 animals each.

Disease control: 0.5ml 0.9 % saline solution, p.o.

Standard: codeine: 5 mg/kg, p.o.

Test: Methanolic extract of *Hibiscus rosa-sinensis*: 200 mg/kg, p.o.

After 10 minutes un-anesthetized, unrestrained animals were placed individually in a transparent Perspex Histamine-chamber, dimensions 30 X 20 X 20 cm and exposed to a nebulized aqueous solution of 7.5 % W/V citric acid for

seven min. The aerosol was produced by an air flow of 8liter/min through a Wright nebulizer. The output of nebulizer was 0.6±0.04 mlsolution/min. The same nebulizer was used throughout the experiments. During the last five min of the exposure, a trained observer continuously watched the animal and counted the numbers of coughs. Coughs could easily be distinguished from sneeze, since there is a clear difference in sound as well as in behavior of the animals.<sup>4,5,11,12,13,14</sup>

**Statistical analysis:**

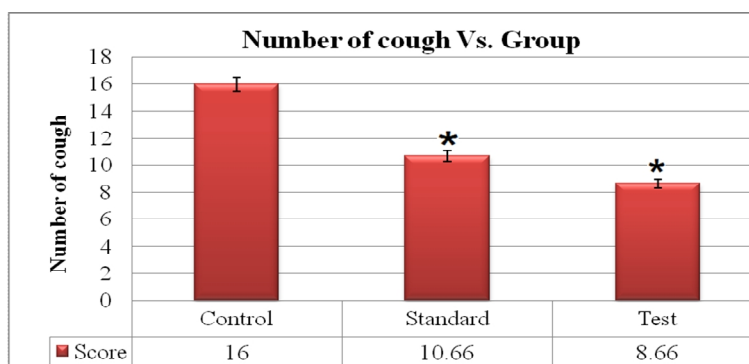
Data were expressed as mean±SEM. Comparison of baseline data with the number of coughs obtained in the presence of plant extracts and codeine were made using ANOVA followed by Tukey's test. Statistical significance was accepted at p ≤ 0.05.

Group	Disease Control	Standard	Test
Number of cough	16 ± 0.52	10.66 ± 0.42*	8.66 ± 0.33*

n=6, data were presented as SEM ± Mean

Disease control received 0.5 ml 0.9 % saline solution (vehicle), p.o., Standard received 5 mg/kg, p.o.

Test received Methanolic extract of *Hibiscus rosa-sinensis* (MEHR) 200 mg/kg, p.o., \*indicate statistical difference level, p ≤ 0.001



**Figure 1: Effects of codeine and methanolic extract of *Hibiscus rosa-sinensis* in citric acid induced coughing model.**

**RESULT:**

Exposure to citric acid in guinea pig produced coughing reflex. Codeine and methanolic extract of *Hibiscus rosa-sinensis* significantly reduced number of cough compared to disease control group, which suggests potential of antitussive activity of both drugs. (Table 1)

**DISCUSSION:**

The result of our study shows methanolic extract of flower of *Hibiscus rosa-sinensis* can significantly reduce number of coughing. The methanolic extract of *Hibiscus rosa-sinensis* contains flavanoids (hibiscitin), phenolic content as well as terpenoid compounds like β – sitosterol, caemphesterol, etc, which are probably responsible for the actions. Ganatra TH et al. has reported CNS-depressant activity of *Hibiscus rosa-sinensis* thus probably *Hibiscus rosa-sinensis* may act centrally and suppress the cough formation centrally. Further investigation is required on isolation, purification and characterization of chemical constitute from *Hibiscus rosa-sinensis*, which is responsible for antitussive activity of *Hibiscus rosa-sinensis* flower – petals.

**CONCLUSION:**

The methanolic extract of *Hibiscus rosa-sinensis* flower can decrease the number of coughing so it possesses anti-tussive activity and it could be better and safer herbal alternate of codeine.

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