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## A experimental study of *Bharangiguda Avaleha* prepared by two different processes

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### Abstract

All the time less theories of *Ayurveda* is availed to the patient only by means of *Bhaishajya Kalpana*. *Avaleha Kalpana* is the formulation, which is efficacious as well as preferred by the patients and thence, in the present study, two different samples of *Bharangiguda Avaleha* was prepared following two different processes as mentioned in *Chakradatta 12/25-30* and Iatro Chemistry of *Ayurveda* by *Bhagvandas* based on *Ayurveda Saukhya of Todaranda* citing *Acharya Gopura Rakshita* at 1/301, Pg. No. 70

- एक द्वित्रि शृतं कृत्वा दापयेद् गुणवृध्यये।।

In the present study test formulations, *Bharangiguda Avaleha-I*, *Bharangiguda Avaleha-II* and *Bharangi Churna* were tested for their comparative therapeutic efficacy in experimental models representing different aspect of the disease *Tamaka Shwasa* to provide scientific basis to their therapeutic application, Appropriate experimental models were planned with Immunomodulatory activity, Anti-inflammatory activity, Analgesic activity, Anti-tussive activity, Anti- histaminic activity, Effect on Bronchial smooth muscles.

**Keywords:** *Bharangiguda Avaleha-I & II*, Experimental Study - Immunomodulatory activity, Anti-inflammatory activity, Analgesic activity, Anti-tussive activity, Anti- histaminic activity, Effect on Bronchial smooth muscles

### Introduction

"*Anekavidha Kalpana*" of *Bhaishaja* (drug) the five basic *Kalpanas* has their own importance. This five basic *Kalpanas* are –

पञ्चविधं कषाय कल्पनमिति तद्यथा स्वरसः कल्कः, शृतः, शीतः, फाण्टः कषाय इति। [1]

Since these basic *Kalpanas* had several drawbacks such as short shelf life, taste, palatability etc., several *Upakalpanas* came into existence on the basis of "*Panchavidha Kalpana*" for e.g. *Avaleha Kalpana*, *Sandhana Kalpana*, *Sneha Kalpana* etc.

Among the above *Kalpanas* study of "*Avaleha Kalpana*" has been selected to evaluate the efficacy of these of "*Bahukalpam*" and to explore the hidden pharmaceutical properties. Hence, in the present study, two different samples of *Bharangiguda Avaleha* was prepared following two different processes as mentioned in *Chakradatta*<sup>2</sup> and Iatro Chemistry of *Ayurveda* by *Bhagvandas* based on *Ayurveda Saukhya of Todaranda* citing *Acharya Gopura Rakshita*<sup>[3]</sup>

- एक द्वित्रि शृतं कृत्वा दापयेद् गुणवृध्यये।।

Here these two processes are different in sense of preparation of *Kwatha*. In method of *Chakradatta*, *Kwatha* has been prepared only once and whereas in method of *Todaranda*, *Kwatha* has been prepared twice with objectives to increase therapeutic efficacy.

Throughout the course of development of the *Ayurvedic* science, animal experimental studies have been extensively used by the ancient learned scholars of *Ayurveda* for a wide variety of purposes such as – testing of meals served to the royal family by feeding the prepared food to crows/ peacocks or dogs to check for toxic contents/ poisoning before serving to them.

तत्र चतुर्विधो भूतग्रामः संस्वेदगजरायुजाण्डजोद्भिज्जसंज्ञः।

तत्र पुरुषः प्रधानं, तस्योपकरणमन्यत्।<sup>4</sup>

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The essence of this *Shloka* is that man occupies a supreme position among all the living creatures. Hence before administering drug to him it is desirable to experiment on other animals.

Screening of different type of source material for the biological activity viz. investigative and explorative studies in the lower animals establishing the pharmacodynamics actions of the drugs provide the necessary data helping in better understanding and thereby better therapeutic use of the drug. Moreover these are mandatory in order to convince the global population about the hitherto unexplored therapeutic utility of a drug as well as its safety for human use.

Good evidence of efficacy exists for certain herbal medicines but evaluation is inadequate says a report from WHO and the details can be seen as below <sup>[5]</sup>.

**Efficacy of certain Herbal Drugs:** Percentage of randomized clinical trials (RCTs) showing benefit of herbal medicines (based on 50 RCTs with 10 herbal medicines for 18 therapeutic indications)

18% - Same benefits as placebo

34% - More benefits than placebo

48% - Benefits reported – unlikely due to design or analytic flaws.

Optimal use and expanded credibility of the herbal drugs/compounds will therefore depend on developing an evidence base for safety and efficacy. This means consolidating the existing national and international studies and supporting new research to fill the evidence gaps perceived to be necessary to be filled to accept *Ayurveda* as a global system of medicine.

It is sometimes impossible to produce same etiopathological events as they occur in human being on *Dosha-Dushya* parlance in the animals, but we can prepare some pathological conditions like inflammation, ulcer etc. for the experimental model and test the drugs for their efficacy in relieving them.

In the present study test formulations, *Bharangiguda Avaleha-I*, *Bharangiguda Avaleha-II* and *Bharangi Churna* were tested for their comparative therapeutic efficacy in experimental models representing different aspect of the disease *Tamaka Shwasa* to provide scientific basis to their therapeutic application, Appropriate experimental models were planned with following aims and objectives:

#### Aims and Objectives

1. To compare the efficacy of test drugs i.e. *Bharangiguda Avaleha-I*, *Bharangiguda Avaleha - II* and *Bharangi Churna*.
2. To observe the effect of these formulations on the experimental models resembling the pathogenesis of *Tamaka Shwasa*.
3. To obtain data on the probable mode of action of these formulations.

#### Materials and Methods

##### Drugs

1. Raw drugs required for test preparations were procured from Dept. of Pharmacy, Gujarat Ayurved University, Jamnagar.
2. Both the test samples – *Bharangiguda Avaleha-I* and *Bharangiguda Avaleha-II* were prepared in the Department of *Rasashastra* and *Bhaishajya Kalpana* including Drug Research, I.P.G.T. & R. A., Jamnagar and *Bharangi Churna* was procured from Dept. of Pharmacy, Gujarat Ayurved University, Jamnagar.

#### Animals

Albino rats of Charles Foster strain and Swiss albino mice of either sex were selected from the animal house attached to the Pharmacology Laboratory of I.P.G.T. & R.A., Jamnagar. Animals were maintained on Pranava Agro's, Amrut brand, rat pellet feed and tap water gives *ad libitum* and exposed to natural day and night cycle. The experiments were carried out in accordance with the directions of the Institutional Animal Ethical committee.

#### Experimental Protocol

The animals were grouped at random, irrespective of sex into four groups. The first group was kept as control and tap water was administered to the animals in this group. The second, third and fourth group were treated with the test drugs viz. *Bharangiguda Avaleha-I*, *Bharangiguda Avaleha-II*, and *Bharangi Churna* respectively for the required period as per the individual experimental protocol.

#### Dose

Dose for experimental study was calculated by extrapolating the therapeutic dose to rat dose on the basis of body surface area ratio (Conversion factor – 0.018 for rats and 0.0026 for mice) by referring to the table of Paget and Barne's., Calculated this way the rat dose of *Bharangiguda Avaleha I & II* comes to 2.20 g kg<sup>-1</sup> body weight and *Bharangi Churna* comes to 0.55 g kg<sup>-1</sup> body weight. The mice dose of *Bharangiguda Avaleha I & II* comes to 3.12 g kg<sup>-1</sup> bodyweight and *Bharangi Churna* comes to 0.78 g kg<sup>-1</sup> body weight.

#### For Albino Rats

The dose of *Bharangiguda Avaleha - I & II* were fixed as 2.20 g Kg<sup>-1</sup> body weight.

The dose of *Bharangi Churna* was fixed as 0.55 g Kg<sup>-1</sup> body weight.

#### For mice

The dose of *Bharangiguda Avaleha I & II* were fixed as 3.12 g Kg<sup>-1</sup> body weight.

The dose of *Bharangi Churna* was fixed as 0.78 g Kg<sup>-1</sup> body weight.

#### Preparation Method of Test Drugs Solution for Animals

*Bharangiguda Avaleha-I & II* were made into fine suspension and diluted to suitable concentration to administer in the volume of 0.5 ml/100 g. body weight.

*Bharangi Churna* was made into fine suspension with Gum acacia (5%) and diluted to suitable concentration to administer in the volume of 0.5-ml/100 g. body weight.

The animals of control group received plain tap water.

#### Route of Drug Administration

The drug solutions were administered with the help of gastric catheter of suitable size sleeved on to a syringe nozzle.

#### Statistical Analysis

Student's t test for unpaired data has been used for analyzing the data generated during the study.

#### Immunomodulation Activity

Important causative factors of *Shwasa* (Asthma) according to *Ayurveda* as well as modern science are exposure to some external environmental factor i.e. *Raja*, *Dhooma*, *Anil* (*Vayu*) *Sevena* etc. (Pollen grains, dust particles etc.). Body has a

mechanism to maintain its homeostatic state during exposure to these factors it is known as “*Vyadhikshamatva*” and this can be equated to immune system or immune mechanism in the body.

In the present study to assess the comparative efficacy of test drugs, their effect was observed on antibody formulation against sheep red blood cells (SRBC).

#### a) Effect on Humoral Antibody Formation

##### Animals

For this experiment Charles Foster strain albino rats of either sex weighing between 160-300 g were selected and divided into four groups.

##### Methodology

The drugs were administered for 10 consecutive days, on 3<sup>rd</sup> day. 25% SRBC solution was injected intraperitoneally in the dose of 0.5ml/100 g of body weight. This SRBC solution was prepared from the sheep blood collected from the city slaughterhouse in a sterilized bottle containing Alsever's solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% sodium chloride). SRBC was thoroughly washed with sterilized normal saline by centrifuging and stored in Alsever's solution in a refrigerator till experimentation. On the 10<sup>th</sup> day of drug administration rats were sacrificed by cervical dislocation and blood was collected in separate test tubes. Blood from the same animal (Sheep) was used for both sensitization and to determine antibody titre. From the collected blood, serum was separated and incubating in a serological water bath for 30 minutes at 55°C to inactivate the complement in it. Serial two fold dilutions of the serum in sterile saline solution was made in the volume of 0.1 ml of micro-titre plate. 0.1 ml of thrice saline washed 2% SRBC was added to each well of the tray. The trays were covered and placed in refrigerator overnight. Antibody titre (heamagglutination titre) was noted next day. Titre was converted to log<sub>2</sub> values for easy comparison. Spleen, Thymus and Lymph nodes were dissected out from the animals and their weight was also recorded. Tissues were transferred to 10% formaldehyde solution for fixation and later on processed for histological studies.

#### b) Effect of Test Formulations on Cell Mediated Immunity

Cell mediated immunity – the second arm of the acquired or adaptive immunity primarily consists of T Lymphocytes (helper –T and cytotoxic –T cells). CD<sub>4</sub> lymphocytes help macrophages effect delayed hypersensitivity with the help of its two subpopulations – TH-1 cells and TH-2 cells. Delayed type hypersensitivity reactions are produced particularly against intracellular antigens. A deficiency of cell-mediated immunity manifests itself as a marked susceptibility to infection. Also, certain T cells can suppress antibody production. Failure of such regulation may result in unrestrained antibody production to self-antigens, which can cause autoimmune diseases, which in this instance may lead to asthma. There may not be a specific population of T cells that mediates suppression. There is evidence that in some situations CD<sub>8</sub> cells can produce suppression, but inhibitory lymphokines produced by CD<sub>4</sub> cells also can play this role.

Thus, with the evident role of the Cell-mediated immunity in the manifestation of asthma, the test drug were evaluated to assess their effect on cell mediated immunity against the solution prepared with Triple Antigen with Alum precipitates– mediated immunological edema. Immunological inflammation was produced in rats by injection of triple

antigen with alum precipitates in the following proportion into sub plantar tissue of rat hind paw.

Triple Antigen 1 ml Normal saline (0.9%) 4 ml Potash Alum (10%) 1 ml pH of the above solution was maintained between 5.6-6.8 by using 10% sodium carbonate.

##### Animals

The rats of either sex weighing between 170g to 270g were grouped into four groups each consisting of six rats.

##### Methodology

Initially the rats were sensitized by injecting the above said solution sub-cutaneously in the nape of the neck in a dose of 0.5ml/100 g body wt. The test drug administration began on the day of sensitization and continued for the next seven days. On 7<sup>th</sup> day, 1 hour after administration of the test drug, the rats were injected with 0.1 ml of the above said solution beneath plantar aponeurosis in the left hind paw. The paw volume was measured before, 24 hours and 48 hour after injecting this solution. The paw volume was measured with the help of a plethysmograph. Percentage increase in paw volume after injecting the above said solution in comparison to initial value was noted. Values from control group were compared to the values from test drug administered groups.

##### Anti Inflammatory Activity

Airway inflammation is one of the important features observed in Asthma; hence the test drugs were evaluated for anti-inflammatory activity.

#### a) Carrageenin Induced Paw Oedema in Rats.<sup>6</sup>

To screen the anti-inflammatory activity of all three trial drugs against carrageenin induced hind paw oedema.

##### Animals

24 rats of either sex weighing between 190-320 g were selected and divided into four groups.

##### Methodology

Drug was administered once for seven days. Initial paw volume of (Left) hind paw was taken by using a plethysmograph following Bhatt *et al* (1977) procedure.

##### Plethysmograph

The plethysmograph utilized consists of 10 ml glass vessel (2.5 ml x 65 mm) fixed to 2 ml syringe through pressure tubing. About 4 ml of mercury filled in the syringe and the mercury level was adjusted to zero mark on the micropipette. The space between the zero mark on the micropipette and fixed mark on the glass vessel was filled up with water with few drops of teepol. The initial level of fluid was adjusted and set at zero. The paw was immersed in water exactly up to the tibio-tarsal articulation. The increased level of water in the glass vessel was readjusted to the prefixed mark by releasing the pressure of the connected syringe. The level where water and mercury interface is seen in the micropipette was recorded as paw volume.

The drugs were administered daily for seven consecutive days. On the 7<sup>th</sup> day, one hour after administration of last dose, paw oedema was induced by injecting 0.1 ml of 1% carrageenin suspension in normal saline into plantar aponeurosis of left hind paw. The left hind paw volume was recorded after three hours after oedema induction by plethysmograph employing same procedure. The rats were

administered 2 ml/100 g. body weight of tap water to ensure uniform hydration and minimize variation in oedema formation.

By comparing to initial paw volume, increase in oedema was calculated as % increased in paw volume.

#### b) Formaldehyde Induced Paw Oedema in Rats <sup>[7]</sup>

To evaluate the comparative effect of the entire test formulations i.e. *Bharangiguda Avaleha-I*, *Bharangiguda Avaleha-II* and *Bharangi Churna* on formaldehyde induced paw oedema.

#### Animal

24 rats were selected with a body weight in the range of 160-220 g and divided into four groups, 6 in each group irrespective of weight and sex.

#### Methodology

The drugs were administered daily for seven consecutive days. On 7<sup>th</sup> day, Initial paw volume of each rat was noted with the help of plethysmograph. On the same day one hour after administration of drug, 0.1 ml of 3% formaldehyde solution was injected into the plantar aponeurosis of left hind paw. After 90 minute, after 24 hrs. and 48 hrs. paw volume was again recorded with plethysmograph. The percentage increases in paw volume of treated group as well as control group were compared to determine presence of anti-inflammatory effect in the test drugs.

#### Analgesic Effect

##### Formaldehyde Induced Paw Licking <sup>[8]</sup>

#### Animals

24 rats were selected with a body weight in the range of 160-220 g and divided into four groups, 6 in each group irrespective of weight and sex.

#### Methodology

The pain induced in rats by the injected 3% Formaldehyde solution was quantified immediately after the injection by noting the frequency of paw licking episode at the time intervals of 0-5 min., 5-10 min., 10-15 min., 15-20 min. and 20-30 min and onset of paw licking episode was also noted.

The initial phase, which is 0 – 10 min. represent the neurogenic pain and the second phase, which is observed between 10 - 30 min., represents inflammatory pain.

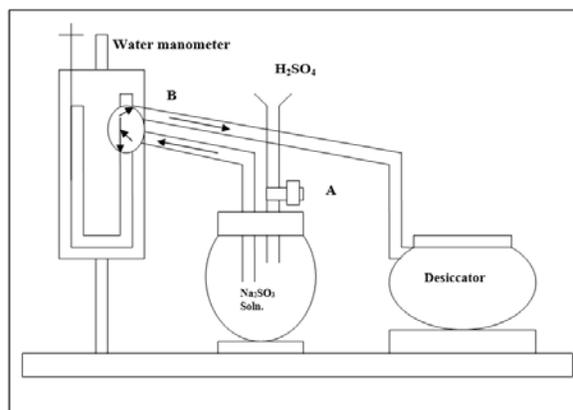
#### Anti – Tussive Activity

Cough can be a symptom of upper or lower respiratory tract infection, or may be a consequence of a non-infectious condition such as asthma, exposure to cigarette smoke, or aspiration of a foreign body. Whatever it is, when it occurs as a symptom with asthma or as a precursor of asthma, it can be very distressing and providing relief from the perturbing cough especially dry cough forms the part of the management of the disease. Thus, an effective drug combating the disease asthma would be appreciated more if it possesses anti-tussive property along with mitigating asthma. Hence, evaluation of anti-tussive effect of the test drugs was planned – using a cough model in which cough is induced by sulphur di-oxide gas in mice <sup>[9]</sup>

#### Animals

For this experiment mice of either sex weighing between 20-30 g were selected and divided into four groups.

#### Schematic Sketch of the Apparatus Used for Anti-tussive Effect



#### Methodology

A 500ml three-necked flask containing aqueous saturated sodium hydrogen sulfite ( $\text{Na}_2\text{SO}_3$ ) solution is taken. Into this bottle, concentrated Sulphuric acid ( $\text{H}_2\text{SO}_4$ ) is introduced drop by drop, the inflow being controlled by the cork –A as shown in the figure, to generate sulfur dioxide gas.  $\text{SO}_2$  is filled previously in the column of water manometer by opening the three-way cork-B such that the  $\text{SO}_2$  can enter the water manometer but without any exit way until the pressure generated reads 75 mm of water as recorded by the water manometer. Then the three-way cork-B is rotated in such a way that the volume of  $\text{SO}_2$  collected in the water manometer escapes into the desiccator (as shown in the sketch) and not into the flask containing sodium hydrogen sulfite solution. These procedures are operated in a drift. The mouse to be tested is placed in 1L desiccators and covered with the lid. A certain amount of  $\text{SO}_2$  is introduced to the desiccator by this procedure. The mice, after exposure to  $\text{SO}_2$  for one minute in the desiccators, were taken out of the desiccator and confined in an up-turned filter funnel. The free end of the funnel is attached to a stethoscope, by the help of which the cough reflex of the mice was heard and the number of cough episodes in 5 minutes was enumerated.

#### Antihistaminic Activity

##### Effect of Test Formulations on the Guinea Pig Ileum (*in vitro*)

Bronchial hyper-responsiveness and inflammatory reaction within the bronchial wall are the important pathological events observed in asthma. These two phenomena are due to release of mast cell mediators such as histamine, prostaglandin and leukotrienes. Because of this reason the test drugs were assessed for anti-histaminic property in isolated guinea pig ileum preparation.

#### Procedure

This experiment was set-up following standard procedure. A healthy male guinea pig was sacrificed by stunning and severing of neck blood vessels. Abdomen was opened by a mid line incision, ileum was identified; 3-4 cm of it was excised out and placed in petridish containing, oxygenated tyrode solution ( $\text{NaCl}$  137,  $\text{KCl}$  2.7,  $\text{CaCl}_2$  1:8,  $\text{MgCl}_2$  0.1,  $\text{NaHCO}_3$  11.9,  $\text{NaH}_2\text{PO}_4$  0.4 and Glucose 5.55 mM per litre). After placing suitable ligatures the tissue was setup in an isolated organ bath containing tyrode solution, which was oxygenated through, continued passage of  $\text{O}_2$ . The tissue was

allowed to rest for 30 minutes before eliciting responses to drugs. During resting period the tyrode solution in the organ bath was changed once in every 10 minutes. The tissue response was recorded through frontal writing level system on a smoked drum attached to kymograph (magnification 1 : 7 and preload of 500 mg). Initially the dose response was recorded with standard spasmogens i.e. to select a dose producing sub maximal response. Recording tissue response to test drugs followed this and its effect on the response elicited with histamine.

**Effect of Test Drug on Bronchial Smooth Muscles**

Trachea was removed from a freshly sacrificed guinea pig, spiral from it were cut longitudinally along the middle dorsal surface and through a series of transverse cuts made successively from alternate sides in such a manner that they overlap one another but do not transect the preparation completely. The spiral was mounted in an organ bath after

preserving it in refrigerator for 24 hours. The physiological salt solution used was modified Kreb’s solution, which contained the following ingredients :

NaCl	-	118.0 mM
KCl	-	4.7 mM
CaCl <sub>2</sub>	-	2.5 mM
MgSO <sub>4</sub> , 7H <sub>2</sub> O	-	1.2 mM
NaHCO <sub>3</sub>	-	2.5 mM
KH <sub>2</sub> PO <sub>4</sub>	-	1.2 mM
Glucose	-	5.55 mM

The tissue responses to drug *per se* and its modifying effect on the histamine induced control response were noted with the help of a Kymographic set up. The responses were recorded with the help of a side ways writing level (1:15) magnification with a 1 g weight as pre-load.

**Observations and Results  
Immunomodulation Activity**

**Table 1:** Effect of Test Drugs on Antibody Formation against SRBC in Rats

Group	Dose	Haemagglutination titre log <sub>2</sub>	% Change
Control	5 ml Kg <sup>-1</sup>	6.12 ± 0.85	-
<i>Bharangiguda Avaleha-I</i>	2.20 g Kg <sup>-1</sup>	5.19 ± 0.43	15.19↓
<i>Bharangiguda Avaleha-II</i>	2.20 g Kg <sup>-1</sup>	5.42 ± 0.60	11.44 ↓
<i>Bharangi Churna</i>	0.55 g Kg <sup>-1</sup>	6.12 ± 0.60	00.00

Data : Mean ± SEM                      ↓ = Decrease

The data pertaining to the effect of test drugs on antibody formation against SRBC after 10 days are included in Table-

1. None of the three test drugs could suppress anti-body formation against SRBC to significant extent. (Table-1)

**Table 2:** Effect of Test Drugs on Weigh of Spleen after 10 Days of Drug administration in SRBC Sensitized Rats

Group	Dose	Absolute Wt. (mg)	% Change	Relative Wt. mg/100gbody wt	% Change
Control	5 ml Kg <sup>-1</sup>	471.50 ± 34.28	-	179.2 ± 22.04	-
<i>Bharangiguda Avaleha-I</i>	2.20g Kg <sup>-1</sup>	454.50 ± 36.85	03.60↓	200.61 ± 14.08	11.93↑
<i>Bharangiguda Avaleha-II</i>	2.20g Kg <sup>-1</sup>	536.33 ± 41.03	13.75↑	207.21 ± 23.54	15.62↑
<i>Bharangi Churna</i>	0.55g Kg <sup>-1</sup>	529.00 ± 42.53	12.19↑	251.93 ± 24.74	40.57↑

Data : Mean ± SEM                      ↓ = Decrease                      ↑ = Increase

The above table contains data related to effect of test drugs on spleen weight in SRBC pre-sensitized rats. The test drugs did not affect spleen weight to significant extent in SRBC pre-

sensitized rats. The apparent increase and decrease observed were found to be statistically non-significant. (Table-2)

**Table 3:** Effect of Test Drugs on Weigh of Thymus after 10 Days of Drug administration in SRBC Sensitized Rats

Group	Dose	Absolute Wt. (mg)	% Change	Relative Wt. mg/ 100g body wt.	%Change
Control	5 ml Kg <sup>-1</sup>	700.83 ± 52.23	-	267.30 ± 35.01	-
<i>Bharangiguda Avaleha - I</i>	2.20g Kg <sup>-1</sup>	734.00 ± 35.14	04.73↑	329.96 ± 28.99	23.44↑
<i>Bharangiguda Avaleha - II</i>	2.20g Kg <sup>-1</sup>	744.17 ± 61.06	06.18↑	283.92 ± 23.86	06.22↑
<i>Bharangi Churna</i>	0.55g Kg <sup>-1</sup>	708.50 ± 44.14	01.09↑	342.05 ± 42.53	27.96↑

Data : Mean ± SEM                      ↑ = Increase

The above table contains data related to effect of test drugs on thymus weight in SRBC pre-sensitized rats. An apparent weak to moderate increase in thymus weight was observed in test drug administered groups in comparison to control group. However this increase was found to be statistically non-significant. (Table-3)

**Histology of Spleen, Thymus and Lymph Node**

**Spleen:** The spleen is known, as the largest lymphoid organ inside the body. It is included among the ductless glands. Besides it should be considered as functionally WBC forming and RBC destroying organ. It is covered by a serous

membrane or peritoneum except at hilum. A capsule of fibrous tissues containing numerous elastic fibers and some smooth muscles are found beneath it. On the surface, at hilum a deep indentation occurs which marks the entrance and exit of the splenic vessels. The frame network of this organ is distensible and capable of considerable change in volume (almost double the normal size) due to the abundance of the elastic tissue, some smooth muscle and the arrangement of fibrous connective tissue in wavy bundles. The space between the connective tissue septa is filled with soft sponge like tissue known as the splenic pulp. It fills up all the space between the connective tissue trabaculae to form a compact



**Table 6:** Effect of Test Drugs on Formaldehyde Induced Paw Oedema in Rats

Group	Dose	% Increase in paw volume after Formaldehyde injection					
		90 min.	% Change	24 hrs	% Change	48 hrs	% Change
Control	5 ml Kg <sup>-1</sup>	51.91±4.14	-	59.66±7.02	-	36.35±6.84	-
<i>Bharangiguda Avaleha-I</i>	2.20g Kg <sup>-1</sup>	13.14±3.13***	74.69↓	9.62±1.46***	83.87□	7.21±1.22**	80.16↓
<i>Bharangiguda Avaleha-II</i>	2.20g Kg <sup>-1</sup>	33.95±5.42*	34.59↓	22.06±4.57**	63.02□	14.01±3.02*	61.46↓
<i>Bharangi Churna</i>	0.55g Kg <sup>-1</sup>	27.42±4.84**	47.18↓	45.81±8.68	23.21□	19.75±3.07	45.67↓

Data : Mean ± SEM ↓ = Decrease \* P<0.05 \*\* P<0.01 \*\*\* P<0.001

From the data shown in Table-6 it could be seen that, test drug administration leads to 74.69%, 34.59% and 47.18% reduction in the formaldehyde induced hind paw oedema in the *Bharangiguda Avaleha-I*, *Bharangiguda Avaleha-II* and *Bharangi Churna* groups respectively, at 90 minutes after pedal injection of formaldehyde. The reduction is statistically highly significant in the groups treated with *Bharangiguda Avaleha-I* and *Bharangi Churna* and just significant in group treated with *Bharangiguda Avaleha-II*.

At 24 hours after pedal injection of formaldehyde, in *Bharangiguda Avaleha-I* and II administered groups a statistically highly significant decrease in paw edema was observed when compared to the control group. The decrease

was 83.87% in the former and 63.62% in the latter. The paw volume was not significantly affected by *Bharangi Churna*. In this group 23.21% decrease in paw volume was observed.

At 48 hours, apparent reduction of 80.16%, 61.64% and 45.67% in the paw oedema volume was observed in the groups administered with *Bharangiguda Avaleha-I* and *Bharangiguda Avaleha-II* and *Bharangi Churna* respectively. The reduction is statistically highly significant in the group treated with *Bharangiguda Avaleha-I*, just significant I group treated with *Bharangiguda Avaleha-II* and just failed to reach significant level in group treated with *Bharangi Churna*.

**Analgesic effect**

**Table 7:** Effect of Test Drugs on Formaldehyde Induced Paw Licking in Rats

Group	Dose	Onset of paw licking (in sec.)	Frequency of paw licking			
			First phase (0-10 min.)	% Change	Second Phase (10-30min.)	% Change
Control	5 ml Kg <sup>-1</sup>	34.50 ± 6.13	12.00 ± 1.34	-	23.67 ± 2.51	-
<i>Bharangiguda Avaleha - I</i>	2.20g Kg <sup>-1</sup>	33.17 ± 4.73	10.17 ± 1.60	15.25↓	21.33 ± 3.52	09.88↓
<i>Bharangiguda Avaleha - II</i>	2.20g Kg <sup>-1</sup>	11.00 ± 1.32	14.50 ± 1.71	20.83↑	16.67 ± 3.31	29.57↓
<i>Bharangi Churna</i>	0.55g Kg <sup>-1</sup>	30.83 ± 6.38	7.50 ± 2.40	37.50↓	13.3 ± 3.66*	43.68↓

\*P<0.05 Data : Mean ± SEM ↓ = Decrease ↑ = Increase

The data related to the effect of test drugs on duration of formaldehyde induced paw licking response are shown in Table-7. It could be observed from the depicted data in the phase I response marginal to moderate decrease in paw licking episodes was observed in *Bharangiguda Avaleha-I* and *Bharangi Churna* administered groups. In *Bharangiguda Avaleha-II* a moderate increase was observed. However none of the observed changes were found to be statistically significant in comparison to control group. Further a statistically non-significant shortening of latency of onset of paw licking was observed. During the second phase of the response decrease of weak to moderate magnitude was

observed in all the test drug administered groups in comparison to the control group. The decrease was 9.88%, 29.57% and 43.68% in *Bharangiguda Avaleha-I*, *Bharangiguda Avaleha II* and *Bharangi Churna* administered groups respectively. Only the decrease observed in *Bharangi Churna* administered group was found to be statistically significant. (Table-7)

**Anti-tussive Activity**

**Anti – Tussive Activity of Test Drugs on Chronic Administration (7 Consecutive Days) In Mice**

**Table 8:** Effects of Test Drugs on SO<sub>2</sub> Induced Cough Reflex after 7 days Drug Administration

Group	Dose	No. of cough episodes per 5 min.	% Change
Control	5 ml Kg <sup>-1</sup>	84.17 ± 3.58	-
<i>Bharangiguda Avaleha-I</i>	2.20g Kg <sup>-1</sup>	49.83 ± 1.35***	40.79↓
<i>Bharangiguda Avaleha-II</i>	2.20g Kg <sup>-1</sup>	64.83 ± 2.55**	22.98↓
<i>Bharangi Churna</i>	0.55g Kg <sup>-1</sup>	63.17 ± 3.38**	24.95↓

\*\* P<0.01 \*\*\* P<0.001 Data : Mean ± SEM ↓ = Decrease

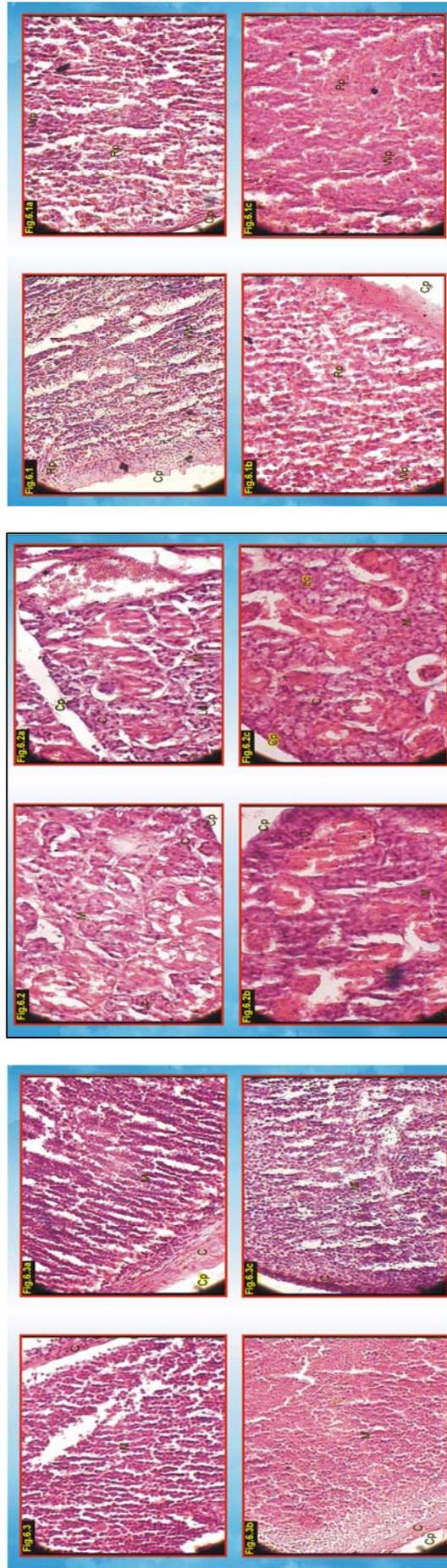
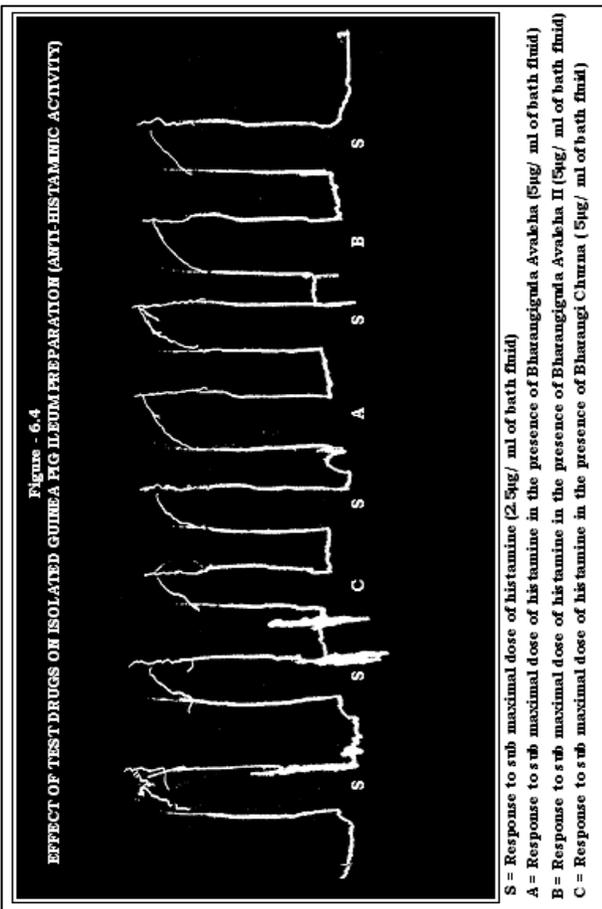
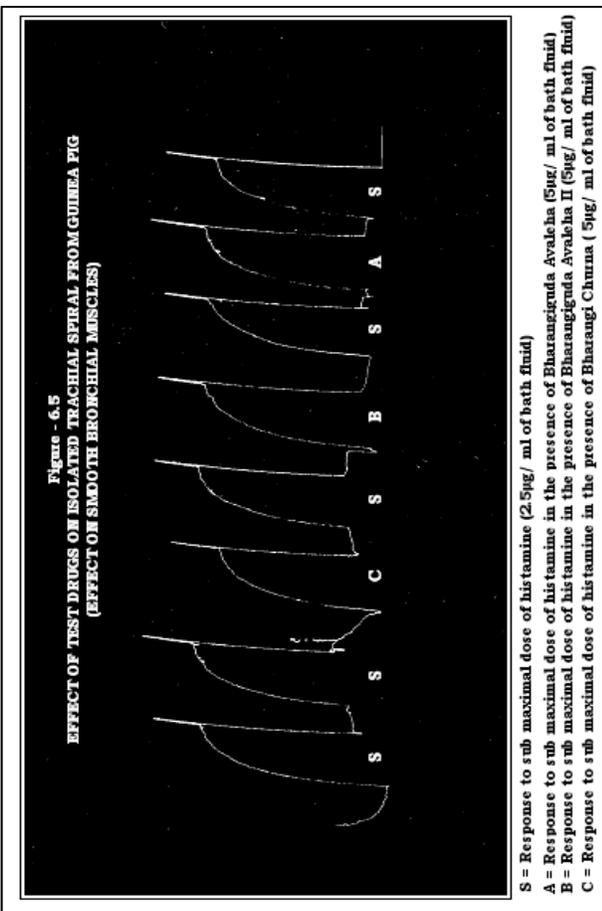
The data pertaining to the effect of test drugs on SO<sub>2</sub> induced cough episodes in mice 7 days after drug administration are included in Table-8. The three test drugs viz. *Bharangiguda Avaleha-I*, *Bharangiguda Avaleha-II* and *Bharangi Churna* reduced cough reflex by 40.79%, 22.98% and 24.95% respectively. The observed effect was found to be statistically highly significant in comparison to control group. (Table-8)

**Evaluation for Anti-histaminic effect**

At dose level (5 µg/ml of both fluid) none of the three test drugs could affect histamine induced contractions. The Kymographic recording is presented in figure-6.4.

**Effect on Bronchial Smooth Muscle - (In Vitro):**

None of the three test drugs *per se* could produce any effect and also they did not modify histamine induced contractions. The Kymographic recording can be seen in figure-6.5.



**Discussion**

**Pharmacological Study**

- It is a well-known fact there are many factors and pathogenic pathways, which are involved in the manifestation of disease condition. For proper therapeutic intervention it is important to know the factors that are modulated by the drug and the stages, which are crucial for the suppression of the pathogenesis of particular disease in focus. However, it is not always possible or feasible to obtain precise information on these aspects. Animal experimentation would be helpful in obtaining useful information on some of the putative mechanisms of action.
- The main patho-physiological features of bronchial Asthma are the occurrence of recurrent bouts of bronchospasm. The bouts may last for few minutes to few hours. Sometimes there may be basal level of bronchospasm even during in between period of bouts. The bronchoconstriction is due to the hypersensitiveness of bronchial musculature leading to its hyperactivity. This results in reduction in the diameter of the air way and oedema in the cellular layers covering the bronchial wall. There are number of allergic factors which act to precipitate a hypersensitivity reaction in majority of the cases. In cases where the exact nature of the causative factors is not known viral infections of the upper respiratory tract can be one of the main cause of bronchial hyperactivity. In the first type of hyper-reaction immune reactions of Type-I (local anaphylactic reaction) is involved. During first exposure to a probably airborne allergen, the person becomes sensitized i.e. it initiates series of mechanisms involved in the formation of IgE type of anti bodies which are also reagenic anti bodies which gets attached to mast cell especially of air way. When the airway is again exposed to the antigen after sensitization antigen and antibody reaction occurs on the surface of mast cell membrane. This leads to mast cell degranulation resulting in massive release of locally active autacoids like histamine, leukotrienes, and platelet activating factors, bradykinin and prostaglandins. These mediators diffuse throughout the airway wall causing airway muscle contraction and vascular leakage. Elaboration and mediation of second set of mediators like Granulocyte Macrophage Stimulating Factors (GM-CSF), interleukins 4, 5, 9 and 13 follow this, mediated by

thymus dependent lymphocytes. The result would be sustained broncho-constriction, cellular infiltration of mucosa followed by erosion leading to exposure of sensory nerve endings <sup>[10]</sup> This further heightens the hyper-responsiveness of the airway.

- It is widely known for some time that bronchial hyper-reactivity is linked to inflammation of the airway mucosa. However the exact mechanisms involved in this are yet to be elucidated in an unequivocal manner. Eosinophils are assumed to play important role. Presently available evidence rule out the possibility of Eosinophil related mechanism as the sole mechanism of the airway hyper-reactivity. It is likely that mediators activated neural and humoral pathways are also involved.<sup>11</sup> Involvement of non-adrenergic and non-cholinergic neural pathway is being activity investigated. The present thinking is that bronchospasm occurs as a result of interaction of multiple factors. The mediators released cause broncho-constriction and hyper-reaction occurs as a result of activation of neuronal and humoral pathways.
- From the above - summarised patho-physiology of the disease it is clear that for effective treatment, it is necessary to administer a drug with multiple mechanism of action. Important therapeutic approaches would be-
  1. Reduction in the antibody formation
  2. Prevention of mast cell degranulation
  3. Antagonising the effect of mediators released from the mast cells.
  4. Antagonism of cholinergic stimulation induced bronchoconstriction (anti-cholinergic effect)
  5. Direct relaxation of airway smooth muscle.
  6. Reduction in the bronchial hyper-responsiveness.
- Based on the review of the above patho-physiological factors and availability of facilities the present study was undertaken on three test preparations viz. *Bharangiguda Avaleha-I*, *Bharangiguda Avaleha-II* and *Bharangi Churna* to evaluate them for Immunomodulatory, Cell Mediated Immunity, Anti-Inflammatory, Analgesic, Anti-tussive, Anti-Histaminic activities and effect on bronchial smooth muscle *in vitro* conditions.
- To facilitate overall consideration of the results while arriving at a conclusion the results have been presented as a consolidated statement in Table- 9.

**Table 9:** Consolidated Statement of the Results Obtained During Pharmacological Evaluation

Sr. No.	Parameters	Group A	Group B	Group C
I.	<b>Immunomodulation Activity</b>			
	i) Effect on Humoral Antibody formation	NS↓	NS↓	NE
	a) Spleen Weight - Absolute wt. - Relative wt.	NS↓ NS↑	NS↑ NS↑	NS↑ NS↑
	b) Thymus Weight - Absolute wt. - Relative wt.	NS↑ NS↑	NS↑ NS↑	NS↑ NS↑
	<b>ii) Effect on Cell Mediated Immunity</b>			
	a) After 24 hours	NS↑	NS↑	NS↑
	b) After 48 hours	NS↓	NS↓	NS↓
	II.	<b>Anti-Inflammatory Activity</b>		
i) Carrageenin Induced Paw Oedema		S↓	NS↓	HS↓
<b>ii) Formaldehyde Induced Paw Oedema</b>				
a) After 90 minutes		HS↓	S↓	MS↓
b) After 24 hours		HS↓	MS↓	NS↓
c) After 48 hours		MS↓	S↓	NS↓
III.	<b>Analgesic Activity</b>			

i) Formaldehyde Induced Paw Licking				
	a) Neurogenic Phase	NS↓	NS↑	NS↓
	b) Inflammatory Phase	NS↓	NS↓	S↓
IV.	Anti-tussive Activity	HS↓	MS↓	MS↓
V.	Anti-histaminic Activity	NE	NE	NE
VI.	Effect on Bronchial Smooth Muscles	NE	NE	NE

**Group A:** *Bharangiguda Avaleha-I* **Group B:** *Bharangiguda Avaleha-II*

**Group C:** *Bharangi Churna*

NE - No effect

HS - Highly significant

MS - Moderately significant

S – Significant

NS – Non-significant

↑ - Increase; ↓ - Decrease

### ➤ Effect on Humoral Antibody formation

- A potent immunosuppressant action is expected of a drug combating asthma. Hemagglutinating antibody (HA) titer is a primary method for studying the humoral response. 10 days after the drug schedule, the test samples failed to influence antibody formation against SRBC in a significant manner. The spleen and thymus weight were not affected to significant extent. The result indicates that the test drugs do not influence antibody formation. (Table-1, 2 & 3)

### Effect on Cell Mediated Immunity

- Cell mediated immunity, the second arm of the acquired immunity is responsible for delayed type hypersensitivity and certain T cells suppress antibody production. The test samples were evaluated to assess their effect on cell-mediated immunity against an experimental model, which is supposed to represent CMI. It involved injection of the suspension of Triple Antigen – Alum precipitated combination (Alum adjuvant), in normal saline– to produce immunological edema. This immunological oedema represents cell-mediated immunity. The results obtained show that no significant CMI suppression effect in all the three tests drugs. However it has to be pointed out that in *Bharangiguda Avaleha-I* administered group a moderate 35% decrease was observed in paw oedema measured at 48 hrs. after the injection of the sensitizing agent. This indicates moderate suppression of CMI. Thus in addition to anti-inflammatory activity this formulation is likely to contribute to clinical effectiveness through CMI modulation. (Table-4)
- The exact mechanism through which immunological oedema suppression occurs remains to be elicited. In antibody formation the antigen which enters the body is processed by antigen presenting cells (APCs) like macrophage, dendritic cells, langerhans cells etc. and is presented to T-lymphocytes which gets activated and secrete cytokines like inter leukin-2 (IL-2), IL-1, IL-6, interferon, Granulocyte, Macrophage Colony Stimulating Factor (GM-CSF). CMI is amplified by  $\gamma$ -interferon by enhancing the process of antigen processing by macrophages. Macrophage migration inhibition factor inhibits movements of macrophages from the affected site. Interleukin (IL-2) acts on the activated T-lymphocyte and helps in their clonal expansion. It also activates cytotoxic lymphocytes and B-lymphocytes. T-lymphocytes modulates the adherence, locomotion and activation of eosinophils leading to accumulation at the site of immune reaction. Activated eosinophils further add to the tissue injury.

### Anti-inflammatory activity

- The data obtained during the study clearly show presence of significant anti-inflammatory activity in all the three formulations in both the test models. They differ only in the extent of the activity produced. In Carrageenin paw oedema test *Bharangi Churna* produced most potent anti-inflammatory activity, *Bharangiguda Avaleha-I* produced moderate but statistically significant suppression while *Bharangiguda Avaleha-II* produced moderate but statistically non-significant suppression. In the formaldehyde paw oedema test significant oedema suppression was observed in all the three test preparations. The most potent being *Bharangiguda Avaleha-I*, followed by *Bharangiguda Avaleha- II* and moderate suppression was observed with *Bharangi Churna*. This clearly shows that the test formulations have inflammatory suppression effect by inhibiting the fluid exudates and proliferative phases of inflammation. Carrageenin paw oedema represents acute inflammation with predominant involvement of fluid exudation in the initial phases followed by cell emigration. Formaldehyde oedema represent proliferative phase of the inflammation with prominent role for the fibroblasts. The exact mechanism of the observed anti-inflammatory activity remains to be elucidated. However it can be suggested that the anti-inflammatory activity may be due to inhibition in the formation of phlogistic mediators at the site of inflammation or modulation of their effect on vessel walls. The effect on proliferative phase may involve decrease in the activity of the fibroblasts through decrease in the formation of the relevant growth factors. However it is not known whether the results observed in these models can be extrapolated to the airway inflammation observed in asthma in which different factors may be involved. (Table-5 & 6)
- Sensory neuropeptides like substance p are considered to play an important role in neurogenic inflammation [12] Substance p leakage in guinea-pig airways causes increased vascular permeability. It would be interesting to assess the test preparations for activity against substance P induced inflammatory response to ascertain whether it has specific inhibitory effect against neurogenic inflammation or not.

### Analgesic Activity

- The Phase-I of the formaldehyde induced algogenic effect is supposed to represent neurogenic pain involving release of neuropeptides. The Phase-II of formaldehyde induced analgesia represents inflammatory pain.
- *Bharangiguda Avaleha-I* produced only weak effect on Phase II algogenic effect; *Bharangiguda Avaleha-II* produced moderate but statistically non-significant decrease whereas *Bharangi Churna* produced significant

decrease. The basis for this decrease may be the presence of anti-inflammatory activity. However there was no exact correlation in the activity profile observed in experimental inflammation and experimental allergenic response indicating involvement of additional factors. (Table-7)

#### Anti-tussive activity

- Cough is one of the commonest presenting symptoms in Asthma and is one among the symptom triad of asthma. Coughing may be initiated either voluntarily or reflexively. Any disorder resulting in inflammation, constriction, infiltration or compression of airways can be associated with cough. Sulfiting agents, such as potassium metabisulfite, potassium and sodium bisulfite, sodium sulfite and sulfur dioxide, which are widely used in the food and pharmaceutical industries as sanitizing and preserving agents, also can produce acute airway obstruction (especially in sensitive individuals) (E.R. McFadden, Jr, 2001) with cough as a presenting symptom. Hence, evaluation of anti-tussive effect of the test drugs was carried out – using a cough model in which cough is induced by sulphur di-oxide gas in mice<sup>[8]</sup> at 7 days after drug administration. Statistically highly significant reduction in the number of cough reflex was observed in all the 3 test samples. The observed results clearly indicate all the three test preparation have powerful anti-tussive activity. This activity becomes apparent on chronic dose administration. Cough is a protective reflex mechanism, which helps in the removal of foreign material and airway secretions from both bronchi and bronchioles. However in conditions involving airway inflammation and neoplasia of the respiratory tract it is inappropriately stimulated. In such situations it is required to administer drugs to suppress the inappropriate and troublesome cough. Clinically effective anti-tussive agents produce their effect by suppressing the cough center present in the brain stem. In asthma the cell factors released from the activated eosinophils act on the epithelium lining of the airway leading to its destruction. This exposes the underlying nerve endings, which get easily irritated leading to bouts of cough. The nerve endings are excited by neuropeptides through an action on the  $\mu$ - opioid receptors. In the anti-tussive effect observed with the preparations the following mechanism may be operative. The drugs may be suppressing the cough center in the brain stem.
- In the model employed for induction of cough in the present study the cough is caused by the irritation of the airway epithelium. Thus it is likely to be due to non-immunological mechanism- probably through suppression of cough center. Based on the results obtained it can be suggested that all the three preparations are good anti-tussives, they can be employed for suppressing cough due to different causes. (Table-8)

#### Anti-histaminic Activity

- Since histamine is one of the mediators released by the mast cells. It was thought useful to ascertain whether the test preparations produce anti-histaminic effect or not.
- Studies on isolated guinea pig ileum preparation showed, that, none of the test preparations possess anti-histaminic activity. At dose level (5  $\mu$ g/ml of both fluid) all the three

test drugs did not affect histamine-induced contractions. This indicates that anti-histaminic activity does not contribute to the therapeutic utility of the preparations. (Fig.-6.4)

#### Effect on bronchial musculature

- All the three test preparations were evaluated for their effect on bronchial smooth muscle contraction. None of them possess any effect *per se* and they also did not modify the responses to histamine. This again rules out bronchodilator activity as one of the mechanisms of action of the test drugs. (Fig.-6.5)
- The above two tests indicate that the test drugs have no anti-histaminic and bronchodilator activities. The observed therapeutic efficacy do not depend on these properties. However it may produce tissue desensitization, whether this mechanism is operative *in vivo* remains to be ascertained.
- Review of literature<sup>13</sup> pertaining to *Clerodendron serratum* (*Bharangi*), the main plant around which the formulations used in the present study have been built, indicate the presence among other effects anti-allergic, anti-histaminic, bronchoconstrictor activity. In the present these effects were not apparent to significant effect. The reason may be that the active principles responsible for the above reported activity may not be present in significant quantity in the present formulations. Even if they are present their effect might have been opposed by active principles possessing opposite effect. The idea behind selection of *Bharangiguda Avaleha* was to ascertain whether addition of *Guda* (Jaggery) enhances the biological activity of the preparation or not. For it has been clearly mentioned in the classics that addition of *Guda* (Jaggery) enhances the palatability of the preparation, it helps in the preservation of activity of the ingredients and also potentiate the therapeutic properties.
- If we make an attempt to determine which of the three test samples is likely to produce best therapeutic effect we need to consider the results obtained and their importance in the therapeutic utility. The main underlying and initiating cause is the inappropriate immune reaction. Thus the drug with best immunosuppressant activity should be the ideal choice. Analysis of the results indicates that the formulations do not differ with respect to their effect on anti-body formation. In CMI test only *Bharangiguda Avaleha-I* produced moderate but statistically non-significant effect. Thus it can be suggested that for *Bharangiguda Avaleha-I* ingredients provide better immunomodulation (mainly CMI related) in comparison to *Bharangi Churna* alone. In contrast to this for anti-inflammatory activity best results were observed in *Bharangi Churna* followed by *Bharangiguda Avaleha-I* and *Bharangiguda Avaleha-II* had only a weak activity. In anti-tussive activity all the three samples produced very good effect though the best effect was observed in *Bharangiguda Avaleha-I*. Thus based on the comparative activity profile it can be suggested that in the experimental study *Bharangiguda Avaleha-I* has better profile in comparison to other two formulations.
- Critical analysis of the above profile shows that *Bharangiguda Avaleha-I* seems to be the ideal preparation followed by *Bharangiguda Avaleha-II* and *Bharangi Churna*. However it is to be mentioned that all

the three preparations are likely to be therapeutically active only the magnitude of relief obtained may vary at a given dose level.

### Conclusion

- The test samples failed to influence antibody formation against SRBC in a significant manner. The spleen and thymus weight were not affected to significant extent. The results obtained show that no significant CMI suppression effect in all the three tests drugs.
- The histo-pathological sections of the internal organs revealed that decrease in cellularity in the spleen was noted in *Bharangi Churna* administered group, whereas no changes were seen in the other two samples administered groups.
- Decrease in cellularity in histo-pathological slides of the thymus was observed in the *Bharangiguda Avaleha-I & II*, whereas did not show any significant change in the cyto-architecture of the thymus in *Bharangi Churna* administered group.
- Decrease in cellularity in the lymph node was noted in *Bharangiguda Avaleha-II* administered group, whereas no changes were observed in the other two test formulations administered groups.
- A statistically significant reduction in Carrageenin induced paw oedema was noted in *Bharangiguda Avaleha-I* administered group. Statistically highly reduction was observed in *Bharangi Churna* treated group, while *Bharangiguda Avaleha-II* produced moderate anti-inflammatory activity but statistically non-significant suppression.
- In the Formaldehyde paw oedema test significant oedema suppression was observed in all the three test preparations. The most potent being *Bharangiguda Avaleha-I*, followed by *Bharangiguda Avaleha- II* and moderate suppression was observed with *Bharangi Churna*.
- A statistically highly significant reduction in episodes of cough was recorded in anti-tussive activity in the entire three formulations administered group.
- On the basis of results obtained of the experimental models of pharmacological study, *Bharangiguda Avaleha-I* is more effective than *Bharangiguda Avaleha-II* and *Bharangi Churna* in comparison to control group (tap water).

### Reference

1. Cha. Su. 4/7
2. Chakradatta 12/25-30
3. Iatro Chemistry of Ayurveda by Bhagvandas based on Ayurveda Saukhya of Todarananda citing Acharya Gopura Rakshita at 1/301, 70
4. Su. Su. 1/30
5. Wealth of India
6. Method of winter, 1962.
7. Procedure of Bwownlee. (1950) as described by Nataraja, 1985.
8. Method as described Bitter. 2000.
9. Tokagi's method, Ref: PM –Miyagoshi, M, 1996, 275-278
10. Chaudhari SK. Quintessence of medical pharmacology New Central Book Agency (P) Calcutta. 1997, 385-398
11. Boushey HA. in: Basic and clinical pharmacology (Ka-Zung, B.G. Ed.) 2001, 353.

12. Barnes PJ, Fanchung K, page C.P. Inflammatory mediators and Asthma. Pharmacological Review, 1988; 40:49-72.
13. Database on Medicinal Plants Used in Ayurveda, CCRAS- New Delhi. I, 73.