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A pharmacological study of Bharangiguda Avaleha and Bharangyadi Arishta with special reference to Shwasa

Dr. Alpesh T Jarsania and Dr. Nipa A Jarsania

Abstract
The living body is a biological apparatus of spectacular capabilities, particularly in the context of drug-cell interaction. When any drug is applied through it's therapeutic route either locally or systemically it interacts with its target cell to produce the desired action. The discipline of pharmacology attempts to elucidate the intricate mechanism that underlies the cause and effect of this interaction.
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 two different formulations, Bharangiguda Avaleha from the reference Chakradutta was chosen for the present study and to comprehend the relative efficacy of Arishta Kalpana, the drug Bharangi with the usable part as Twaka [Cha. Su. 25/48(1)] and with the support of the presence of the pharmaceutical preparation "Bharangisura" (Su Utt. 61/38-40) the ingredients of Bharangiguda Avaleha were suitably processed to prepare the Bharangyadi Arishta were tested for their comparative therapeutic efficacy in experimental models representing different aspect of the disease Shwasa to provide scientific basis to their therapeutic application, appropriate experimental models were planned with Immunomodulatory activity, Anti-inflammatory activity, Analgesic activity, Anti- histaminic activity, Effect on Bronchial smooth muscles.

Keywords: Bharangiguda Avaleha, Bharangyadi Arishta, Experimental Study - Immunomodulatory activity, Anti-inflammatory activity, Analgesic activity, Anti- histaminic activity, Effect on Bronchial smooth muscles.

Introduction
An urge soothe the sufferings, is as old as the urge for the secured life. The oriental thinking found the path to mitigate the sufferings - be it physical, mental or spiritual in the form of science of life, i.e. Ayurveda. The pondering mind of our Acharyas, considering the multitude nature of the herbal resources and multifactorial nature of the disease - envisaged multiple Kalpanas – i.e. to suit the person, disease, Kala or the Satmya in overall.
Ayurveda can be concised and understood in "Trisutra" i.e. Hetu, Linga and Aushadha from which Aushadha means Bhaishaja -

> भैषजं नाम तद्यदुपकरणायोपकल्पते भिषजो...। [1]

The importance of Bhaishaja is mentioned under the heading of "Chikitsa Chatuspada" in which properties of dravyas should be as follows:

> बहुकल्पं बहुगुणं सम्पन्नं योग्यमौषधम्..[2]

Among, "Anekavidha Kalpana" of Bhaishaja (drug) the five basic Kalpanas have their own importance. This five basic Kalpanas are –

> पञ्चविधं कषाय कल्पनमिति तद्यथा –
> स्वरसः कल्कः, श्रृतः, शीतः, फाण्टः कषाय इति।। [3]

These basic Kalpanas are mentioned in Charaka Samhita and the reason behind their origin can be seen in the following reference -

सातत्यात्स्वाद्वभावाद्या पथ्यं द्वेष्यत्वमागतम्।।
कल्पनाविधिभिस्तैस्तैः प्रियत्वं गमयेत पुनः।।[4]

Since these basic Kalpanas had several drawbacks such as short shelf life, taste, palatability etc., several Upakalpanas came into existence on the basis of ''पञ्चविध कल्पना'' for e.g. Avaleha Kalpana, Sandhana Kalpana, Sneha Kalpana etc.
Among the above Kalpanas study of **"**Avaleha Kalpana" and "Arishta Kalpana" has been selected to evaluate the efficacy of these of ''बहुकल्पम`"" and to explore the hidden pharmaceutical properties.
Moreover because of their relative longer shelf life and their utility in curative, preventive and promotive nature, their role in the field of health is distinctly evident.
Hence, one of the Avaleha Kalpana, Bharangiguda Avaleha from the Ref. Chakradutta[5] was chosen for the present study. To comprehend the relative efficacy of Arishta Kalpana, the drug Bharangi with the usable part as Twaka[6] and with the support of the presence of the pharmaceutical preparation "Bharangisura"[7] the ingredients of Bharangiguda Avaleha were suitably processed to prepare the Bharangyadi Arishta. Arishta was prepared by using yeast as fermenting initiator and as per general method of Sandhana Kalpana.
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 पुरूषः प्रधानं, तस्योपकरणमन्यत्।।[8]

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.
The living body is a biological apparatus of spectacular capabilities, particularly in the context of drug-cell interaction. When any drug is applied through it's therapeutic route either locally or systemically it interacts with its target cell to produce the desired action. The discipline of pharmacology attempts to elucidate the intricate mechanism that underlies the cause and effect of this interaction.
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 two different formulations, Bharangiguda Avaleha and Bharangyadi Arishta were tested for their comparative therapeutic efficacy in experimental models representing different aspect of the disease 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 formulations i.e. Bharangiguda Avaleha and Bharangyadi Arishta.
2. To observe the effect of these formulations on the experimental models resembling the pathogenesis of Shwasa.
3. To obtain data on the probable mode of action of both formulations.

Materials and Methods

Drugs

1. Raw drugs required for both preparations were procured from Dept. of Pharmacy, Gujarat Ayurved University, Jamnagar.
2. Both the drugs Bharangiguda Avaleha and Bharangyadi Arishta were prepared in Rasashastra and Bhaishajya Kalpana Department Including Drug Research, I.P.G.T. & R. A., Jamnagar.

Animals

Albino rats of Charles Foster strain 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 Navachakan oil mills, Amrut brand, rat pellet feed and tap water gives *ad libitum* and was exposed to natural day and night cycle.

Experimental Protocol

The animals were grouped at random, irrespective of sex into three groups. The first and second groups were treated with test formulation i.e. Bharangiguda Avaleha and Bharangyadi Arishta respectively and third group was kept as control and tap water was administered to the animals in this group.

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). Calculated this way the rat dose of Avaleha comes to 2.25 g kg^{-1} and was rounded of to 2.5 g kg^{-1} dose.

For Albino Rats

- The dose of Bharangiguda Avaleha was fixed as 2.5 g Kg^{-1}
- The dose of Bharangyadi Arishta was fixed as 3.6 ml Kg^{-1}
- Both the preparations contained equal proportion of ingredients at the above dose level.

Route of drug administration

Bharangiguda Avaleha was made into fine suspension and diluted to suitable concentration to administer in the volume of 0.5 ml/100 g. body weight. Bharangyadi Arishta was administered as it is. The animals of control group received plain tap water. The drug solutions were administered with the help of gastric catheter.
It was administered through an oral catheter of suitable size.

Immunomodulation Activity

Important causative factors of Shwasa (Asthma) according to Ayurveda as well as modern science is exposure to some external environmental factor i.e. Raja, Dhooma, Anil (Vayu) seven 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 210-270 g were selected and divided into three groups. Group A received drug Bharangiguda Avaleha, Group B rats were treated with Bharangyadi Arishta and the tap water was administered to third group, which served as the control.

Methodology

The drugs were administered for 10 consecutive days, on 3rd 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 strelised 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 10th 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 inactivated complement in it. Serial two fold dilutions of the serum in sterile saline solution were 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 (heamaglutination titre) was noted next day. Titre was converted to $\log_2$ 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

Unlike antibody mediated immune response which is mediated through the formation of antibody by the plasma cells, in cell mediated immunity T-lymphocytes directly react with antigen to cause its destruction. CMI is also mediated by the release of lymphokines. Antibodies and complements are not involved in this reaction. The phenomenon is responsible for the rejection of foreign cells (Tissue transplantation is one such reaction).

The test drug was evaluated to assess their effect on cell mediated immunity against triple antigen mediated immunological oedema.

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 using 10% sodium carbonate. The rats of either sex weighing between 130 to 300 g were grouped into three group each consisting of six rats. The Group A received drug Bharangiguda Avaleha, Group-B rats were treated with Bharangyadi Arishta and the tap water was administered to third group which served as the control. Initially the rats were sensitized by injecting the triple antigen with alum precipitates subcutaneously in the nape of the neck in a dose of 0.5 ml/100g body wt. The test drug administration began on the day of sensitization and continued for the next five days. On 5th day, 1 hour after administration of the test drug, the rats were injected with 0.1 ml triple antigen with alum precipitates beneath plantar aponeurosis in the left hind paw. The paw volume was measured before, 24 hours and 48 hour after injecting this alum adjuvant. The paw volume was measured with the help of a plethysmograph. Percentage increase in paw volume after alum adjuvant injection in comparison to initial value was noted. Values from control group were compared to the values from test drug administered group.

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) The model employed was carrageenin induced paw oedema

Ref. - Method of winter et al (1962) was adopted to screen the anti-inflammatory activity of both the trial drugs against carrageenin induced hind paw oedema.

Animals

24 rats of either sex weighing between 140-220 g were selected and divided into four groups. For Group-A, Bharangiguda Avaleha was administered and Group-B animals were treated with Bharangyadi Arishta. Control group was treated with tap water.

Methodology

Drug was administered once for five days. Initial paw volume of (Lt) 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 five consecutive days. On the 5th 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

To test the comparative effect of both test formulations Bharangiguda Avaleha and Bharangyadi Arishta on formaldehyde induced paw oedema, procedure of Bwownlee (1950) as described by Nataraja (1985) was employed.

18 rats were selected with a body weight in the range of 140-290 g and divided into three groups, 6 in each group irrespective of weight and sex. Group-A was administered with Bharangiguda Avaleha, to group B rats Bharangyadi Arishta was administered and group C rats received tap water to serve as control.

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 3 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

Ref. : Effect of test drugs on formaldehyde paw liking test was carried out as described Bitter et al (2000).

Animals

18 Rats of either sex weighing between 140-290 g were selected and divided into 3 groups of six each. Group-A was treated orally with Bharangiguda Avaleha, Group-B was administered Bharangyadi Arishta while tap water was given to the rats in the Group-C which served as control.

Methodology

The pain induced in rats by injected 3% Formaldehyde solution was quantified immediately after the injection frequency of paw licking episode were noted 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 peaks around 5 min. represent the neurogenic pain and the second phase, which is observed between 15-20 min., represent inflammatory pain (Bitter *et al* 2000).

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 cms of it was excised out and placed in petridish containing, oxygenated tyrode solution (NaCl 137, KCl 2.7, $CaCl_2$ 1:8, $MgCl_2$ 0.1, $NaHCO_3$ 11.9, NaH_2PO_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 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 it's 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
$CaCl_2$	-	2.5 mM
$MgSO_4$, $7H_2O$	-	1.2 mM
$NaHCO_3$	-	2.5 mM
KH_2PO_4	-	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

I. Immunomodulation Activity

Table 1: Effect of test drugs on antibody formation against SRBC in rats

Group	Dose	Haemaggulatination titre log 2 Mean ± SEM	% change
Control	5 ml Kg^{-1}	6.01 ± 0.15	-
Bharangiguda Avaleha	2.5 g Kg^{-1}	6.65 ± 0.56	10.65 □
Bharangyadi Arishta	3.6 ml Kg^{-1}	6.47 ± 0.34	7.65 □

(a) Effect on Antibody formation

The data pertaining to the effect of test drugs on antibody formation against SRBC are included in Table 1. Both the Bharangi preparations failed to influence antibody formation against SRBC in a significant manner (Table 1).

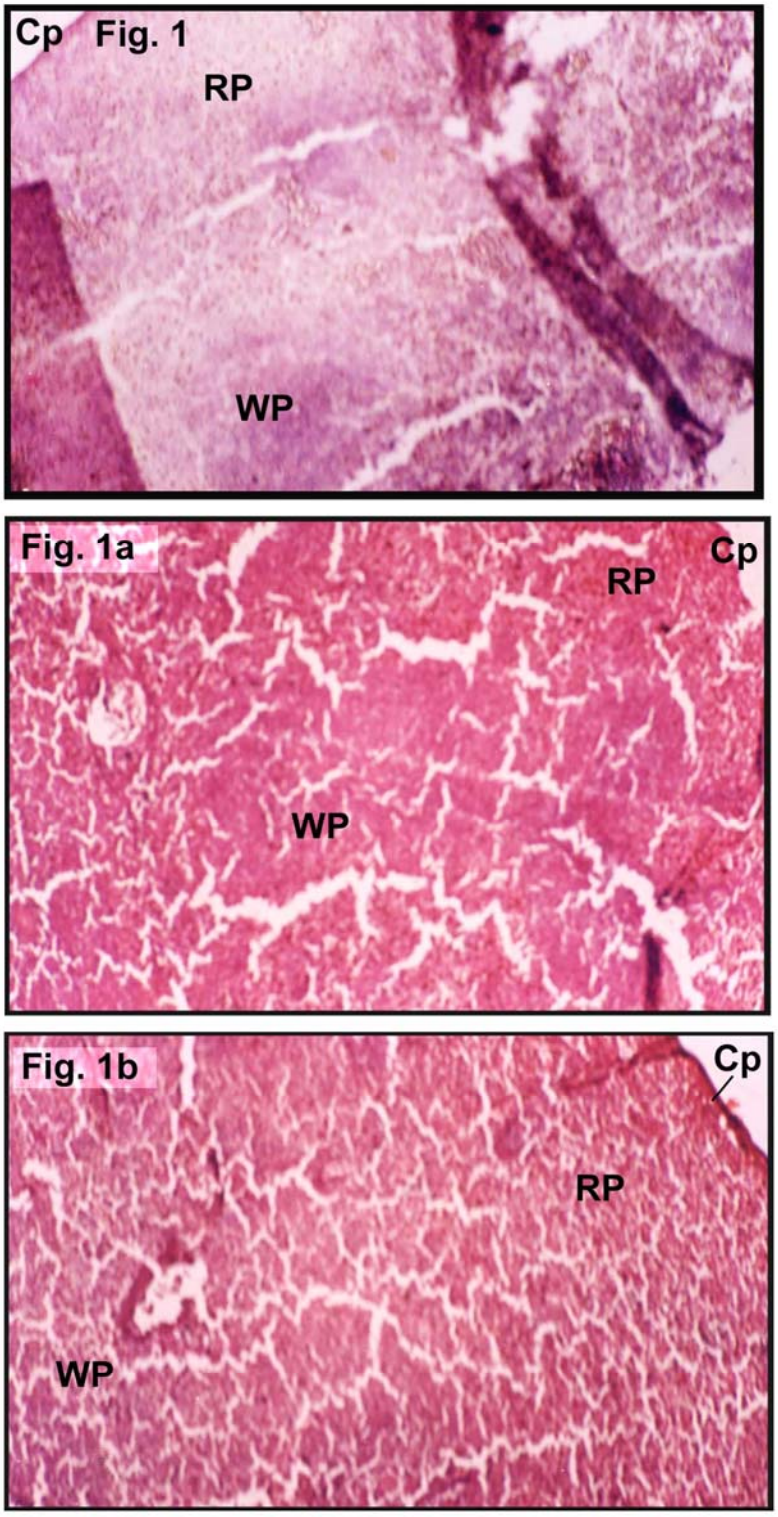

Fig. 1, 1a & 1b : Photomicrographs of Spleen sections.
1-Control Group, 1a Bharangiguda Avaleha treated group and 1b Bharangyadi Arishta Group,
Cp. : Capsule, WP : White pulp, RP : Red pulp (1 x 100-magnification)
Note : Increased WP proportion in Bharangiguda Avaleha (1a) group in comparison to control

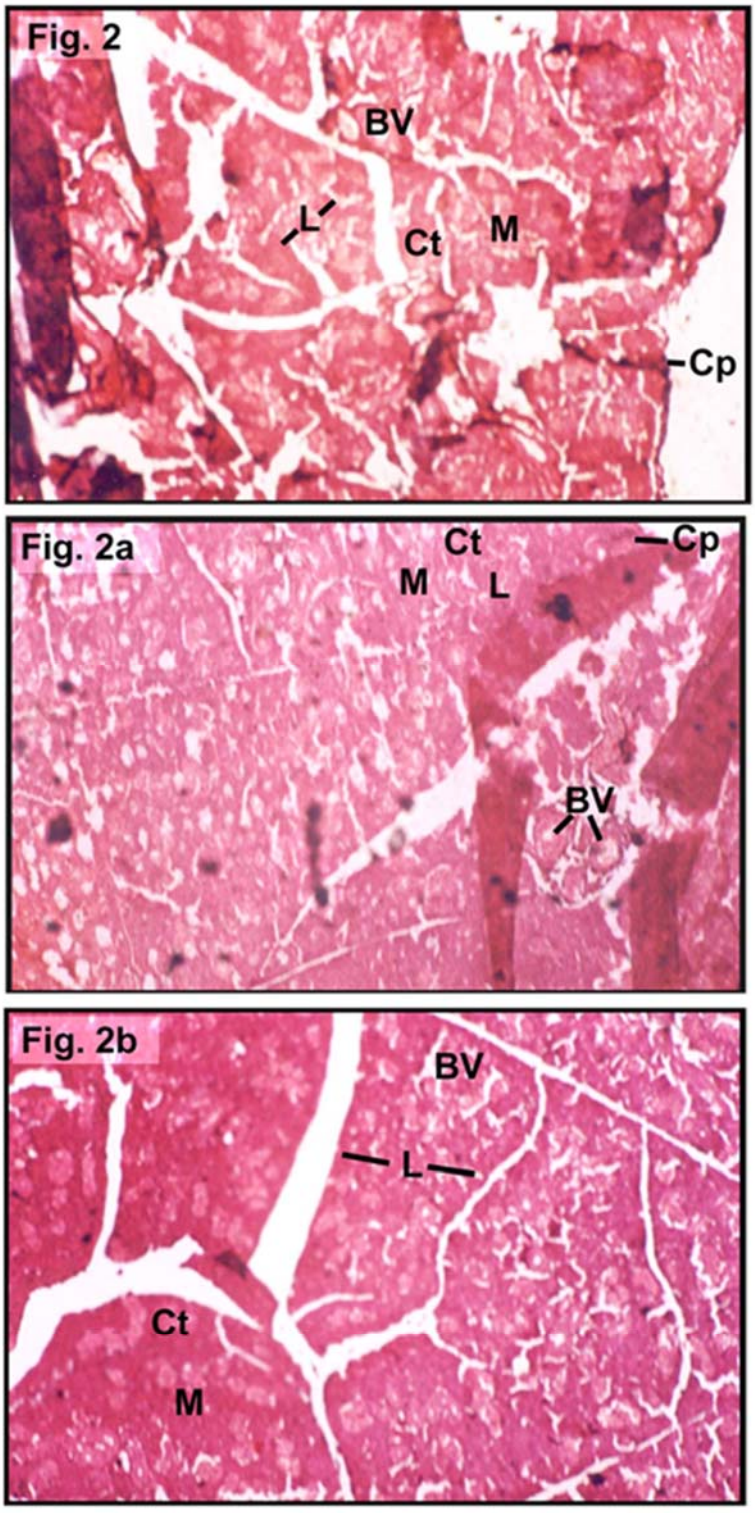

Fig. 2, 2a and 2b : Photomicrographs of thymic sections, 2-control, 2a-Bharangiguda Avaleha treated group, 2b Bharangyadi Arishta treated group Cp-Capsule, Ct-Cortex, M-Medula, BV-Blood Vessels, L-Lobule (1 x 100 Magnification) Note : Decreased cellularity in Bharangiguda Avaleha treated Group.

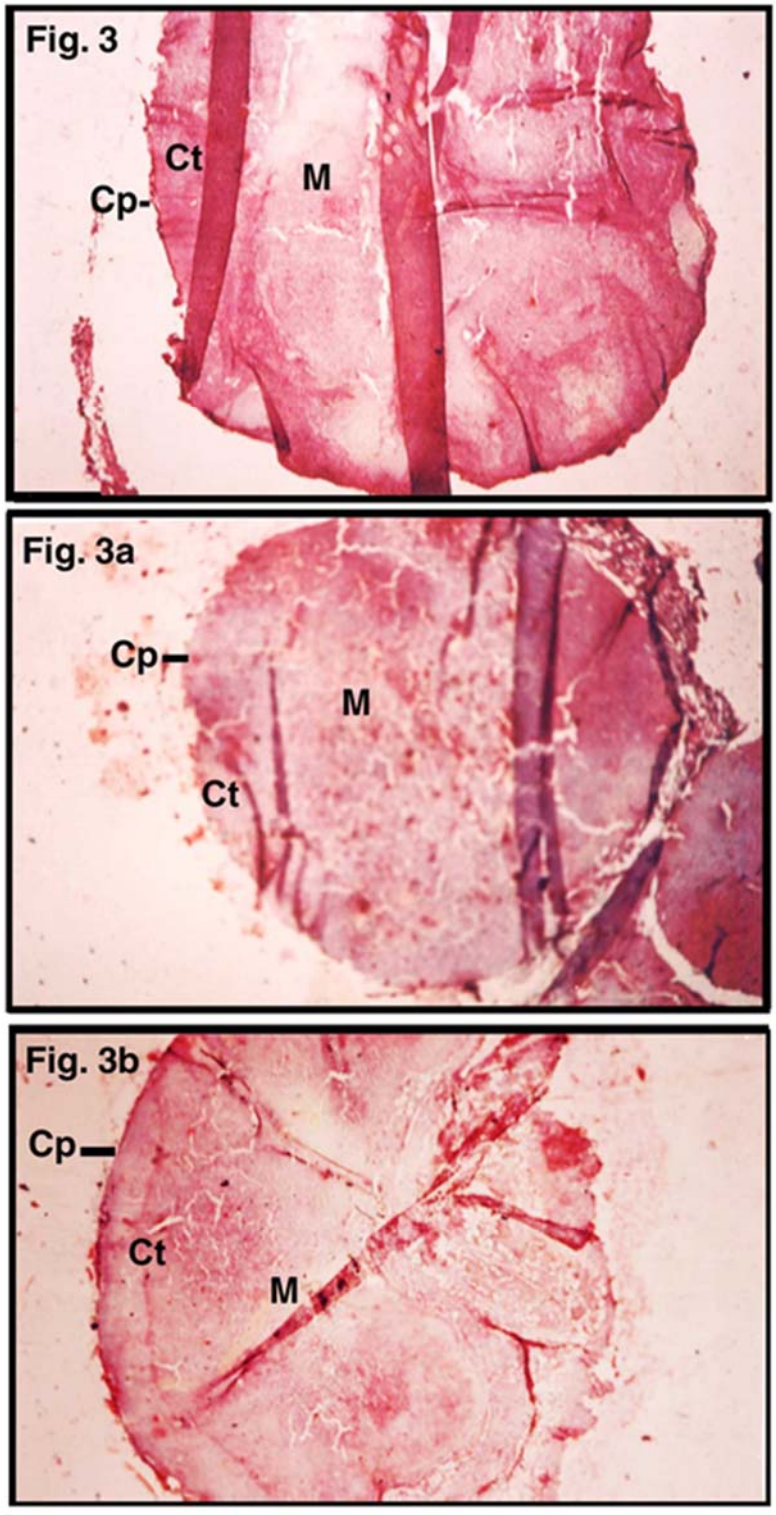

Fig. 3, 3a & 3b : Photomicrographs of Lymphnode Sections, 3-Control, 3a-Bharangiguda Avaleha treated Group, 3b-Bharangyadi Arishta treated Group Cp-Capsule, Ct-Cortex, M-Medulla, (1 x 100 - Magnification) Note : No difference in the Cytoarchitecture in test drug administered group

Table 2: Effect of test drugs on spleen, thymus weight in SRBC sensitized Rats

Group	Dose	Spleen mg/100g body wt. Mean ± SEM	% change	Thymus mg/100g body wt. Mean ± SEM	% change
Control	5 ml Kg^{-1}	0.70 ± 0.12	-	0.80 ± 0.08	-
Bharangiguda Avaleha	2.5 g Kg^{-1}	0.65 ± 0.05	7.14 ↓	0.66 ± 0.03	17.5 ↓
Bharangyadi Arishta	3.6 ml Kg^{-1}	0.76 ± 0.04	8.57 ↑	0.73 ± 0.06	8.75 ↓

(b) Effect on spleen and thymus weight

Table-2 contains data related to effect of test drugs on spleen and thymus weight in SRBC sensitized rat. The test drugs did not affect spleen and thymus weight significantly in SRBC sensitized rat. The apparent increase and decrease observed were found to be statistically non-significant (Table 2).

(c) Histology of Spleen, thymus and lymph node

(i) Spleen

Microscopic examination of section of spleen obtained from different groups was carried out. Increase in the proportion of white pulp was observed in Bharangiguda Avaleha administered group in comparison to control group. In Bharangyadi Arishta administered group no significant change in the cytoarchitecture of spleen could be observed. Photograph of representative section can be seen in figures- 1, 1a and 1b.

(ii) Thymus

Microscopic examination of thymus section obtained from different groups showed decrease in the cellularity in Bharangyadi Arishta administered group. In Bharangiguda Avaleha administered group no significant change in thymus could be observed. Photographs of representative section can be seen in figures- 2, 2a and 2b.

(iii) Lymph nodes

Scanning of microtome sections of lymph node obtained from different groups did not show any significant change in the lymph node in the test drug administered group in comparison to control group. Photomicrographs of representative sections can be found in figures 3, 3a and 3b.

Table 3: Effect of test drugs on alum adjuvant induced immunological paw oedema in pre-sensitized Rat.

Group	Dose	% Increased in paw volume after alum adjuvant injection			
		24 hours Mean ± SEM	% change	48 hours Mean ± SEM	% change
Control	5 ml Kg^{-1}	66.77 ± 23.47	-	44.59 ± 14.87	-
Bharangiguda Avaleha	2.5 g Kg^{-1}	67.96 ± 8.03	1.78 ↑	41.26 ± 7.96	7.47 ↓
Bharangyadi Arishta	3.6 ml Kg^{-1}	33.90 ± 2.53	49.23 ↓	35.98 ± 7.70	19.31 ↓

(d) Effect on cell-mediated immunity

The data on the effect of test formulation on alum adjuvant induced immunological oedema have been presented in table 3. In Bharangiguda Avaleha administered group oedema formulation of 24 hours after pedal injection of alum adjuvant was not affected significantly in comparison to control group. At 48 hours slight decrease of 7.47% in oedema formation was observed in comparison to control group, which was not statistically significant. In Bharangyadi Arishta administered group on apparent 49.23% decrease in oedema in comparison to control group was observed at 24 hours after pedal injection of alum adjuvant. However due to variation in the data this decrease did not reach statistically significant level. At 48 hour 19.31% but statistically non-signification decrease in oedema formation was observed in comparison to control group (Table 3).

II. Anti-inflammatory activity

Table 4: Effect of test drugs on Carrageenin induced Paw oedema in Rats

Group	Dose	% Increased in paw volume after 3 hour after Carrageenin injection Mean ± SEM	% change
Control	5 ml Kg^{-1}	123.11 ± 23.80	-
Bharangiguda Avaleha	2.5 g Kg^{-1}	102.80 ± 21.23	17.00 ↓
Bharangyadi Arishta	3.6 ml Kg^{-1}	77.70 ± 31.72	36.88 ↓
Standard Indomethacin	5 mg Kg^{-1}	58.50 ± 9.09	52.48 ↓

The data pertaining to the effect of test drugs on Carrageenin induced hind paw oedema in rats are presented in table-4. An apparent 17% decrease in oedema formation was observed in Bharangiguda Avaleha administered group in comparison to control group. In Bharangyadi Arishta administered group the decrease was 36.88%. However, the observed decrease was found to be statistically non-significant (Table 4).

Table 5: Effect of test drugs on Formaldehyde induced Paw oedema in Rats

Group	Dose	% Increased in paw volume after 3 hour after Formaldehyde injection Mean ± SEM	% change
Control	5 ml Kg^{-1}	67.17 ± 11.03	-
Bharangiguda Avaleha	2.5 g Kg^{-1}	50.79 ± 8.21	24.38 ↓
Bharangyadi Arishta	3.6 ml Kg^{-1}	60.86 ± 4.44	9.39 ↓

The data pertaining to the effect of test drugs on Formaldehyde induced hind paw oedema in rats are presented in table-5. An apparent 24.38% decrease in oedema formation was observed in Bharangiguda Avaleha administered group in comparison to control group. In Bharangyadi Arishta administered group only marginal decrease of 9.39% was observed. However, the observed decrease was found to be statistically non-significant (Table 5).

III. Analgesic effect

Table 6: Effect of test drugs on Formaldehyde induced Paw licking in Rats

Group	Dose	Onset of paw liking (in sec.)	Frequency of paw liking			
			First phase (0-20 Min.) Mean ± SEM	% change	Second Phase (20-30 Min) Mean ± SEM	% change
Control	5 ml Kg^{-1}	85 ± 22.17	6.33 ± 1.36	-	7.5 ± 3.17	-
Bharangiguda Avaleha	2.5 g Kg^{-1}	38.33 ± 6.01	8.5 ± 1.48	34.28 ↑	10.33 ± 2.74	37.73 ↑
Bharangyadi Arishta	3.6 ml Kg^{-1}	28.66 ± 2.30	12.00 ± 1.93 *	89.57 ↑	19.5 ± 4.20 *	160 ↑

* $P < 0.05$

The data related to the effect of test drugs on duration of formaldehyde induced paw licking response are shown in table-6. If could be observed from the depicted data that there is an apparent increase in the frequency of paw licking during the phases in both the drugs. The increase was 34.28% and 37.73% respectively in phase-I and phase-II in Bharangiguda Avaleha administered group. However the increase was found to be statistically non-significant. In Bharangyadi Arishta group the increase was 89.57% during phase-I and 160% during phase-II. The increase was found to be statistically significant (Table 6).

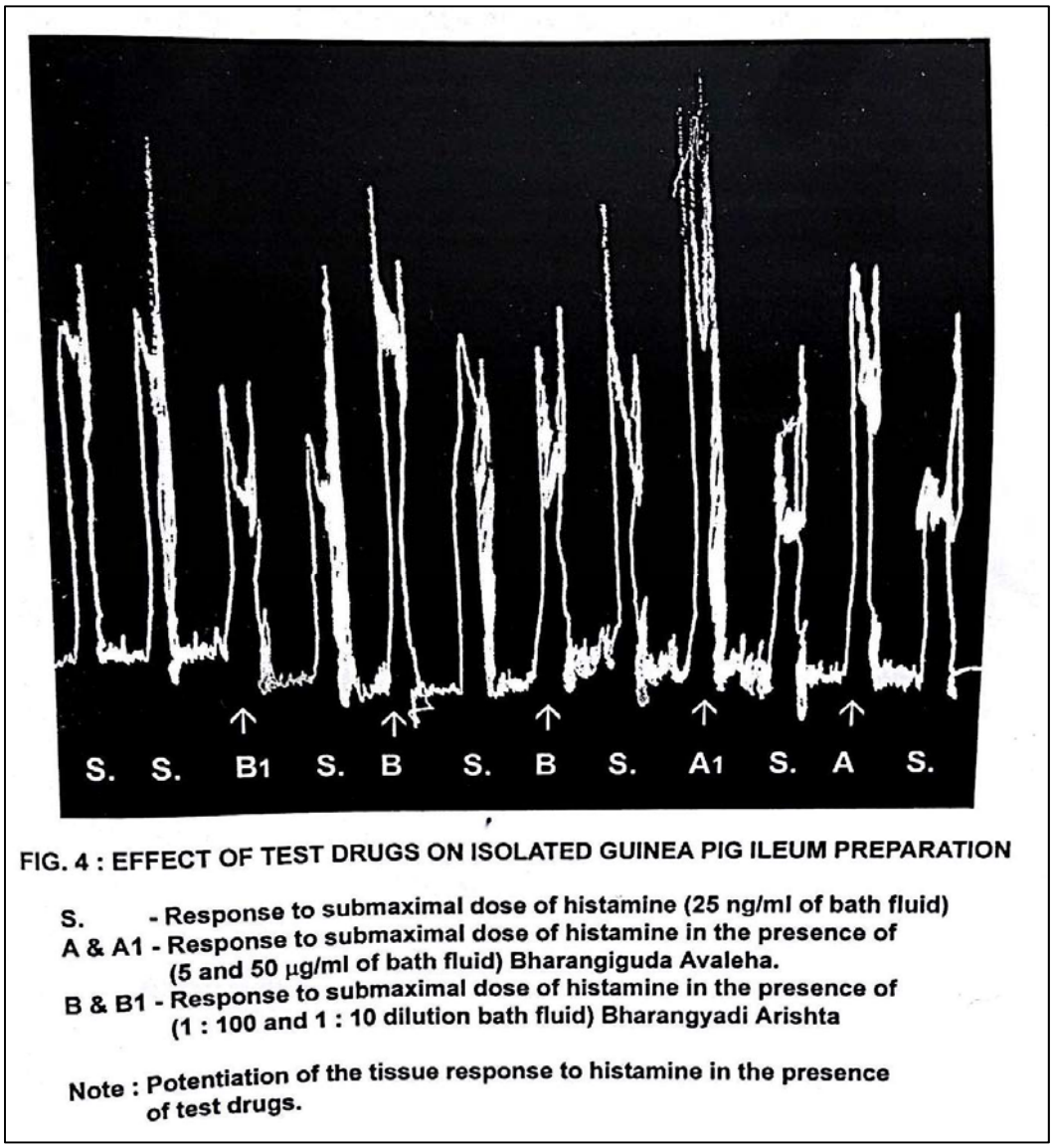

FIG. 4 : EFFECT OF TEST DRUGS ON ISOLATED GUINEA PIG ILEUM PREPARATION

S. - Response to submaximal dose of histamine (25 ng/ml of bath fluid)
A & A1 - Response to submaximal dose of histamine in the presence of (5 and 50 μg/ml of bath fluid) Bharangiguda Avaleha.
B & B1 - Response to submaximal dose of histamine in the presence of (1 : 100 and 1 : 10 dilution bath fluid) Bharangyadi Arishta

Note : Potentiation of the tissue response to histamine in the presence of test drugs.

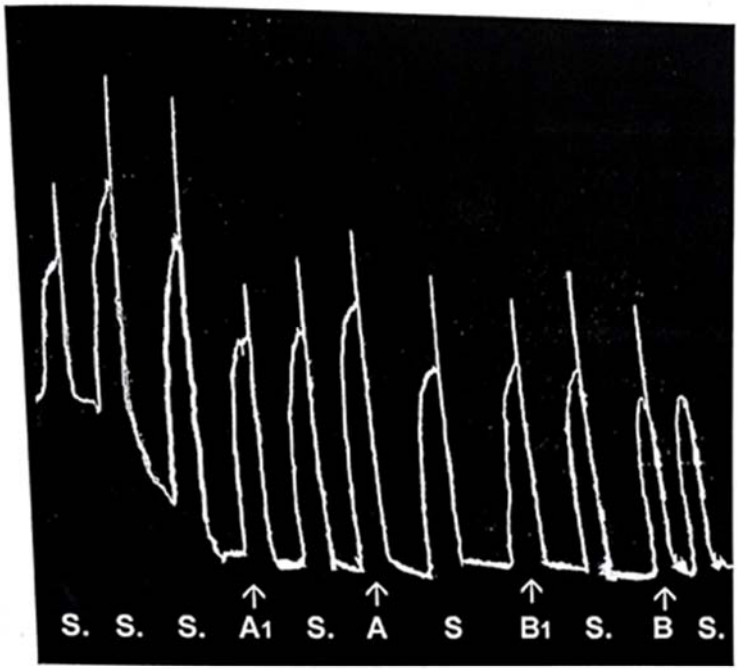

FIG. 5 : EFFECT OF TEST DRUGS ON ISOLATED TRACHEAL SPIRAL FROM GUINEA PIG.

S. - Response to submaximal dose of histamine (2.5 μg/ml of bath fluid)
A & A1 - Response to submaximal dose of histamine in the presence of Bharangiguda Avaleha (5 and 50 μg/ml of bath fluid).
B & B1 - Response to submaximal dose of histamine in the presence of Bharangyadi Arishta (1 : 100 and 1 : 10 dilution bath fluid).

Note : Test drugs did not modify the tissue response to histamine .

IV. Evaluation for Anti-histaminic effect

At lower dose level (5 g/ml of both fluid) both the test drugs did not affect histamine induced contractions. At higher dose level (5 g/ml of both fluid) a moderate potantiation of the contraction was observed with both the drugs. The Kymographic recording is presented in figure-4.

V. Effect on Bronchial Smooth Muscle - (*in vitro*)

Both the test drugs neither produced any effect per se and they modified the Sub-maximal dose histamine induced contractions. The Kymograghic recording can be seen fig-5.

Discussion

The prescription of a general practitioner would contain a powder compound, one or two types of tablets and necessarily a liquid medicine-such as any Asava or Arishta. This pattern might incorporate an Avaleha preparation depending on the age, palatability, disease condition and acceptance by the patient. This reflects the immense popularity of the Avaleha and Sandhana Kalpana. But surprisingly least number of explorative works has been carried out in these two fields. Moreover only a single work has been done to adjudge the relative efficacy of the Avaleha and Sandhana Kalpana. But this study is afresh in itself that all the ingredients were kept common in both forms of pharmaceutical procedures and was subjected to critical analysis. The disease Shwasa is being very rampant in the young work force in today's society, its association being gradually increasing with increased westernized life-style, and is higher prevalence in around Jamnagar. All these points were considered positively before choosing the disease Shwasa.

A scan through the ancient literature revealed seven references of Bharangiguda from various classics such as Vrunda Madhava, Chakradatta, Vangasen, Gadanigraha, Bhava Prakasha, Yoga Chintamani, Bhaishajya Ratnavali. This repetition might denote the popularity of the compound, which in turn reflects its efficiency in counteracting the disease. Keeping this view in mind, this formulation was chosen for the critical exploration.

Twak being enumerated as one among nine Asava Yonis, and a reference from Sushruta Uttartantra points out of the usage of Bharangi twak in the preparation of Sura i.e. Sandhana Kalpana – helped as to modify the Bharangiguda Avaleha i.e. Avaleha Kalpana with all its ingredients into Bharangyadi Arishta i.e. Sandhana Kalpana.

Thus the same ingredients processed through two different pharmaceutical procedures viz. Avaleha and Sandhana procedures enabled us to compare the role of the pharmaceutical procedure (Kalpana) in enhancing /modifying therapeutic efficacy.

Table 7: Activity Profile of Bharangiguda Avaleha and Bharangyadi arishta

No.	Test / Parameters	Bharangiguda Avaleha	Bharangyadi Arishta
1	Immunomodulation activity i) Effect on Humoral antibody formation a. Spleen weight b. Thymus weight	↑ ↓ ↓	↑ ↑ ↑
	ii) Effect on cell mediated immunity a) After 24 hrs. b) After 48 hrs.	↑ ↓	↓ ↓
2	Anti-inflammation activity		
	i) Carrageenin induced paw oedema	↓	↓
	ii) Formaldehyde induced paw oedema	↓	↓
3	Analgesic activity - Formaldehyde induced paw licking	↑	↑S
4	Anti-histaminic activity	Pro-histamine	Pro-histamine
5	Effect on bronchial smooth muscle	-	-

- No effect
↑SSignificant Increase ↓S Significant Decrease
↑ Non Significant Increase ↓ Non Significant Decrease

A consolidated account of the activity profile of both the test preparations is provided in Table 7.

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 allergenic 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 become sensitised i.e. it induces mechanism of 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 sensitisation 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, leukotrines, 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 bronchoconstriction, cellular infiltration of mucosa followed by erosion leading to exposure of sensory nerve endings[9]. 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 [10] 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-

i) Reduction in the antibody formation
ii) Prevention of mast cell degranulation
iii) Antagonising the effect of mediators released from the mast cells.
iv) Antagonism of cholinergic stimulation induced bronchoconstriction (anti-cholinergic effect)
v) Direct relaxation of airway smooth muscle.
vi) 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 two preparations-Bharangiguda Avaleha and Bharangyadi Arishta to evaluate them for Immunomodulatory, Cell Mediated Immunity, Anti-Inflammatory, Analgesic, Anti-Histaminic activities and effect on bronchial smooth muscle *in vitro* conditions. The discussion will be centred on the results obtained from the studies carried out evaluate for the following mechanism of action.

Immunomodulation

A) Humoral Antibody formation

The test drug was evaluated for its effect on antibody formation against SRBC in rats. At the dose level studied the drug did not modify antibody formation to a significant extent. The spleen and the thymus weights were not affected to significant extent. The results indicate that the test drug do

not influence anti-body formation. Though increase in white pulp proportion was seen in spleen, treated with Bharangiguda Avaleha group and decrease in the cellularity of thymus in Bharangyadi Arishta administered group it was not linked to the anti-body formation against SRBC. The obtained results clearly indicate that the preparations do not have modulatory effect on anti-body formation hence anti-body formation suppression is not the mechanism of action for whatever therapeutic efficacy observed with the preparation at the dose level studied.

B) Cell Mediated Immunity

The test drugs were studied for their effect on triple antigen-alum precipitates combination (alum adjuvant) induced immunological oedema in rats. This immunological oedema represents cell-mediated immunity. The results obtained show that the drug exhibit a weak to moderate CMI suppression effect which in case of Bharangiguda Avaleha is delayed in its onset. This indicates that the CMI suppression may contribute at least partially to the therapeutic efficacy of the preparations at the dose level studied.

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, dentritic 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 □-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 β-lymphocytes. T-lymphocytes modulate the adherence, locomotion and activation of eosinophils leading to accumulation at the site of immune reaction. Activated eosinophils further add to the tissue injury. It is possible that the test drugs may be interfering in one or more of the above mechanisms albeit in a modest way to produce weak to moderate CMI suppression.

Anti-inflammatory Activity

Neither of the preparations studied could produce anti-inflammatory activity in the experimental model employed viz. carrrageenin and formaldehyde induced paw oedema. In fact pro-inflammatory effect was observed in both the preparation. This clearly rules out anti-inflammatory activity as one of the mechanisms of anti-asthmatic activity of the preparations at the dose level studied.

Sensory neuropeptides like substance p are considered to play an important role in neurogenic inflammation [11] 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. Evidence available from this study does not support this contention. The phase algogenic response due to formaldehyde injection to the paw is supposed to be neurogenic in nature in which neuropeptide like substance P are supposed to be involved the test drugs failed to modulate this response indicating that they may not modulate neurogenic pathways and neurotransmitters involved in neurogenic algogenic and inflammatory reaction.

Analgesic Activity

The Phase-I is reported to represent neurogenic pain involving release of neuropeptides. The Phase-II of formaldehyde induced analgesia represents inflammatory pain.

There was an apparent increase in the frequency of paw licking during both the phases in both the drugs; however, in Bharangyadi Arishta group, the increase was found to be statistically significant, during both the phases. This rules out presence of analgesic effect in the test preparations. This and the results of the anti-inflammatory study clearly indicate that anti-inflammatory activity is not present in the test preparations and it is not a contributing factor to the clinical efficacy at the dose level studied.

Anti-Histaminic Activity

Since histamine is one of the mediators released by the mast cells. It was though useful to ascertain whether the test preparations produce anti-histaminic effect or not.

Studies on isolated Guinea pig ileum preparation showed, that, neither of the preparations possess anti-histaminic activity. At lower dose level (5 μg/ml of both fluid) both the test drugs did not affect histamine-induced contractions. At higher dose level (50 μg/ml of both fluid) a moderate potentiation of the histamine contraction was observed with both the drugs. This indicates that anti-histaminic activity does not contribute to the therapeutic utility of the preparations.

Bronchodilator activity

As already mentioned the test drug was evaluated for bronchodilator activity in isolated tracheal spiral preparation of Guinea pig. The results obtained did not indicate presence of bronchodilator activity per se and also it did not affect the contraction induced by spasmogen (histamine). This again rules out bronchodilator activity as one of the mechanisms of action of the test drugs.

Review of literature [12] 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 enhances the biological activity of the preparation or not. For it has been clearly mentioned in the classics that addition of guda enhances the palatability of the preparation, it helps in the preservation of activity of the ingredients and also potentiate the therapeutic properties. The results obtained in this study did not show much difference between the two formulations. The exact reason for not observing any difference is not clear and would require consideration of all the related aspects.

Thus it can be suggested from the overall analysis of the data generated during the present study that moderate suppression of the cell mediated immunity can be the main contributing factor to whatever beneficial effect observed with the test preparations. There is no qualitative or quantitative difference between the preparations with respect to the expression of biological activity. It would be interesting to prepare the test drugs in other formulation forms and evaluate for the above mechanism of actions.

Conclusion

The test formulations - Bharangiguda and Bharangyadi Arishta were subjected to evaluation of -

1. Immunomodulatory Activity
2. Anti-inflammatory Activity
3. Analgesic Activity
4. Anti-histaminic Activity
5. Effect on Bronchial smooth muscles

On the basis of results obtained it can be suggested that moderate suppression of the cell-mediated immunity can be the main contributing factor to whatever beneficial effect observed with the test preparations**.** There is no qualitative or quantitative difference between the preparations with respect to the expression of biological activity. It would be interesting to prepare the test drugs in other formulation forms and evaluate for the above mechanism of actions.

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