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An Experimental Study on a Shwashara (Anti Asthmatic) Ayurvedic Formulation as a Whole & Its Individual Herbs for Treating Cigarette Smoke Induced Asthma in Mice Model

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Abstract: ***A quantitative research method allows us to see the effects of changes in the variables of interest by keeping other relevant variables under control. Experimental studies involve the random assignment of participants into different groups (e. g. experimental, control) in order to determine the causal effect of a certain condition (independent variable) on a certain outcome (dependent variable). The experimental study on the current topic has been done to conduct the following – a) Acute Toxicity Test of the formulation and herbs b) Cigarette Smoke Induced Asthma in Mice Model c) Dissection, Hematological study and Tissue Collection for Histopathology of Mice. Experimental studies are considered the gold standard in studies because researchers are able to determine causal effects with more confidence than when using any other research method. This study provides the scientific data for the proper establishment of efficacy and potency of*** **the shwashara formulation (bharangi+sunthi+pippali) [1] and also of its individual herbs i. e. bharangi, sunthi and pippali and demonstration of their anti - asthmatic activity in cigarette smoke induced asthma in mice.**

Keywords: Experimental study, Acute Toxicity Test, Cigarette Smoke Exposed Mice Model, Histopathology, Hematology, Bharangi + Sunthi + Pippali, Asthma

1. Introduction

Medicinal plants are playing very active role in traditional medicines for the treatment of various ailments. Here we are demonstrating the efficacy of *bharangi+sunthi+pippali* as a whole formulation and individually in treating tamak shwas (bronchial asthma). For this purpose we have done acute toxicity study of the formulation and its individual herbs for dose fixation, hematological study and histopathological study of lung tissue of the smoke induced asthmatic mice after treatment. In the present study, the formulation consists of Bharangi (*Clerodendrum indicum Linn.)*, Sunthi (*Zingiber officinale Rosc.)*, and Pippali (*Piper longum Linn*), is described in 'Yogaratnakar' for the treatment of shwas disorders[1]. The constituent herbs of the research drug are reported for pharmacological activities which are beneficial in treatment of tamak shwas (BronchialAsthma). Bharangi (*Clerodendrum indicum Linn.)* has anti - histamine activity[2]. The roots have antispasmodic, anti - inflammatory activity[3]. Sunthi (*Zingiber officinale Rosc.)* shows marked anti - inflammatory activity in rats which is comparable to prednisolone[4]. Acetone extract of rhizomes of *Z. officinale* is attributed with the antioxidant property[5]. The milk extract of Pippali (*Piper longum Linn.)* reportedly increases the rate of respiratory flow[6]. Significant effect in controlling the frequency and severity of the asthmatic attack was observed with *P. longum* powder in a clinical study[7]. Currently, approximately one - fourth of asthma patients are smokers[12]. Herbal preparations have been cited as the third most popular complementary treatment modality[8]. So the main goal of this present study is to evaluate the effect of bharangadi yoga and its individual herbs in treating cigarette smoke induced tamak shwas (bronchial asthma) on mice.

2. Materials and Methods

2.1 Instruments for experimental study:

1) **For acute toxicity study:**
- Weighing machine
- Feeding gavage

2) **For exposing mice to cigarette smoke [11]:**
- Acrylic transparent sheets
- Vacuum pump
- Oxygen pump
- Transparent Pipes
- Regulator
- Temperature indicator
- Cigarette holder and cigarettes.

Acute Toxicity Studies:
Acute toxicity studies are conducted to determine the short - term adverse effects of the formulation when administered in a single dose or in multiple doses during a period of 24 hours in mice. Acute toxicity studies provide information on:
1) The potential for acute toxicity in humans,
2) An estimate of safe acute doses for humans,
3) The potential target organs of toxicity,
4) Time course of drug - induced clinical observations,
5) The appropriate dosage for multiple - dose toxicity studies, and
6) Species differences in toxicity[9]

Figure: Feeding mouse with gavage

The Acute Toxicity study of the research drugs were done, following the OECD guidelines to find out the toxic effect and LD - 50 of the drug.

- **Animals** - Healthy, young 8 - 12 weeks old mice weighing not more than 25gm were selected for the study. The animals were divided into 4 groups having 2 animals in each group.
- **Dose and Dosage** - The research formulation was administered to the animals orally in the different dosages - 300mg/kg, 500mg/kg, 1000mg/kg, 1500mg/kg and 2000mg/kg body weight.
- **Route of Administration** - Oral by using animal gavages feeding needle.
- **Method** - The animals were fasted overnight (maximum 4h) ad - libitum, followed by oral administration of a single dose of the research drug to each animal as per their bodyweight. Food was with held for further 2 - 4 hours after the administration of research drug.1^0
- **Observation** - Symptoms such as increased motor activity, decreased motor activity, salivation, lacrimation, convulsion and other toxic effect were observed at an interval of 1 h, 2 h, 4 h, 8 h, 12 h and 24 h. Animals were observed up to 14 days after administration of the research drug.

Exposing mice for cigarette smoke:

Preparation of animals:

Female Swiss Albino Mice of 20 - 25gms to be taken after weighing properly. The animals were housed in polypropylene cages and maintained at 24 ± 2ºC under 12h light dark cycle and fed adlibitum with standard pellet diet and were led to have free access to water. Permission for the study was obtained from the Institutional Ethical Committee of IPGAE&R, Kolkata.

Procedure of whole body cigarette smoke exposure of mice:

According to the protocol, mice to be placed in the whole body cigarette smoke exposure chamber[11]. This chamber has been maintained by good oxygen and air ventilation supply so that smoke didn't stick in this chamber. Here Mice have been kept for maximum 10 mins during this study daily for 6 weeks. 'Paris' Branded filter rolled cigarette selected where one cigarette contain Virginia type Tar: 13 mg and contain nicotine 1.1mg. Total cigarette in each packet was 20 cigarettes and total number of packets of cigarette used for this study was approx.20. During the period of this study every mouse got normal feeding and water, rest properly at institutional animal house. The physiological and behavioral changes were observed and recorded.

Figure: Cigarette smoke exposure chamber

Table representing the number of cigarettes used every week, along with the smoke exposure time and duration for establishment of asthmatic mice model

No. of weeks	No. of cigarettes	Exposure time with duration
1st	2	10mins daily for 7 days
2nd	4	10mins daily for 7 days
3rd	6	10mins daily for 7 days
4th	8	10mins daily for 7 days
5th	10	10mins daily for 7 days
6th	12	10mins daily for 7 days
7th	No smoke exposure was done & mice were kept for observation	

Procedure to evaluate research drug efficacy in Asthmatic Model

- After smoke exposure for 6 weeks and all mice got rest for 7days. Dosage of the research drugs were selected after acute toxicity study.
- In this time period the physiological and behavioral changes were recorded for each mouse properly.
- Grouping of the animals: mice were divided into 6 groups and 6 animal were in each group

Dissection & Collection of blood & tissue for further study

After the experiment the mice were euthanized and dissected. After collection of blood by cardiac puncture from all groups sent for haematological study and lung was collected from all the 6 groups of mice for histopathological study

Table representing the experimental groups & treatment received with dosage and duration:

No. of Group	Name of the group	Treatment received	Time
G I	Positive Control	Deriphyllin p. o. (10mg/kg bd. wt.)	For 14 days
G II	Negative Control	Distilled water	For 14 days
GIII	RD1 (Bharangi)	Bharangi 600 mg/kg b. w.	For 14 days
GIV	RD2 (Sunthi)	Sunthi 600 mg/ kg b. w.	For 14 days
G V	RD3 (Pippali)	Pippali 600 mg/ kg b. w.	For 14 days
G VI	RD4 (Formulation)	Formulation 600 mg/ kg b. w	For 14 days

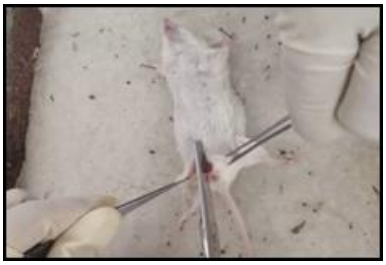


Figure: Mouse dissection

Figure: Internal structure of mouse

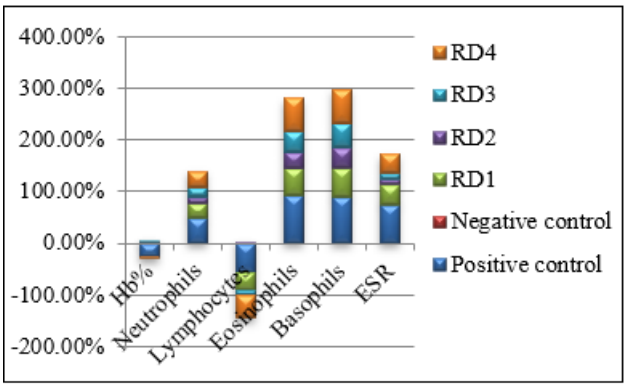


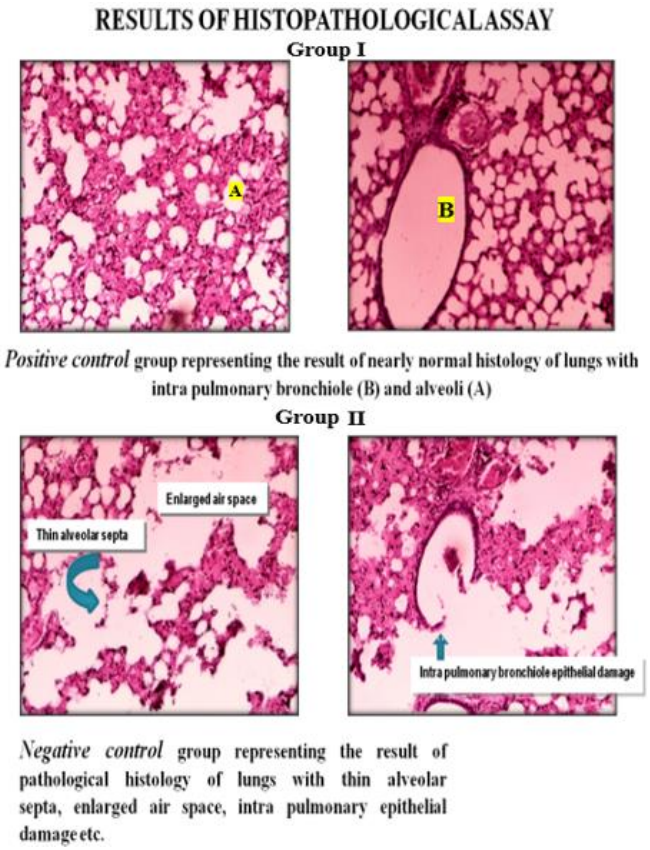
Figure: Comparative results of blood parameters of mice in different treated groups

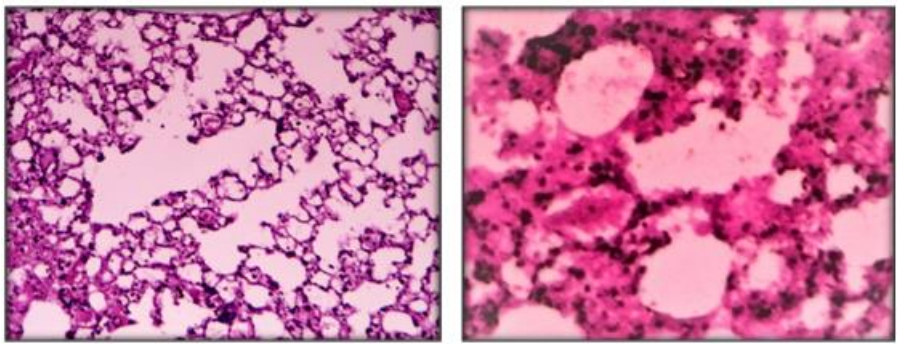
***Note: Further the individual herbs as well as the formulation will be addressed as the following-*
RD1- Bharangi; RD2- Sunthi; RD3- Pippali; RD4- the formulation i.e. Bharangi+Sunthi+Piappali
****RD- Research Drug;***G - Group*

Observations and Results:

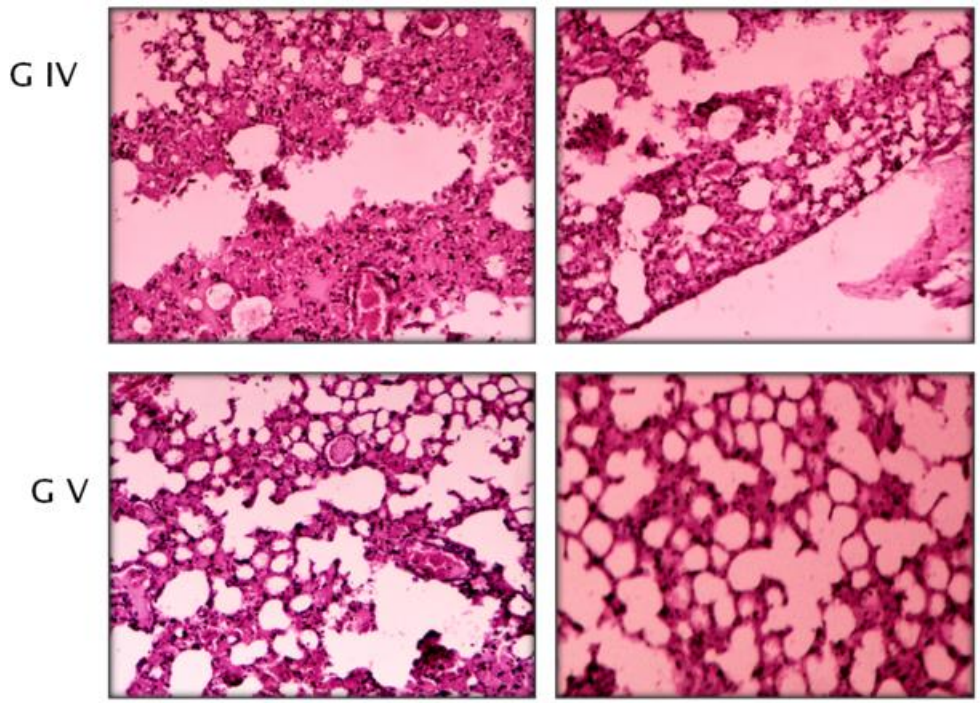
- **Physiological & behavioral changes observed in cigarette smoke induced asthmatic mice after receiving treatments:** After thorough observation of the physiological and behavioral changes of mice which includes body weight, food and water intake, body hair, gait, movement, eye, ear, lacrimation, salivation etc. for 14 days of giving the herbs and the formulation, it can be concluded that RD4 (Group VI) and RD1 (Group III) had shown better results where RD4 - the formulation was more efficacious. The rest drugs i. e. RD2 (Group IV) and RD3 (Group V) have also shown somewhat good results but were not satisfactory. However, RD3 has shown better results than RD2

- **Results of Hematological study:**
  After assessing overall result in relation to the blood parameters, found that the formulation (RD4) are very close to the blood parameters of positive control group then RD1, then RD3 and then RD2. Which indicates the formulation RD4 has more potency to reduce the symptoms of bronchial asthma in cigarette smoke exposed asthmatic mice.

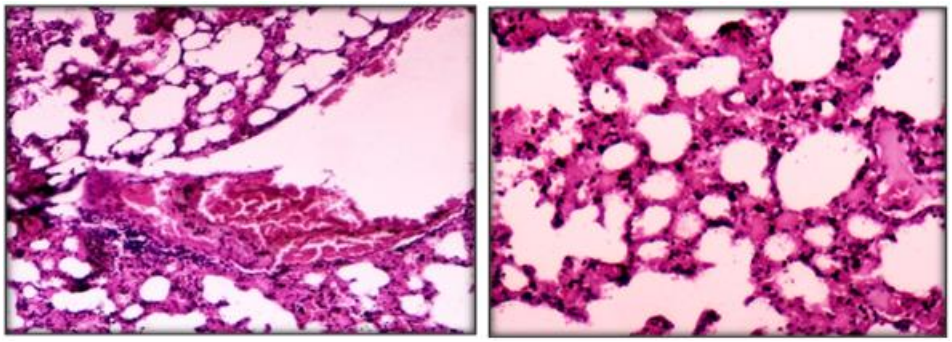




Group III representing the result of after treatment histology of mice lungs with RDI- minimal alveolar enlargement

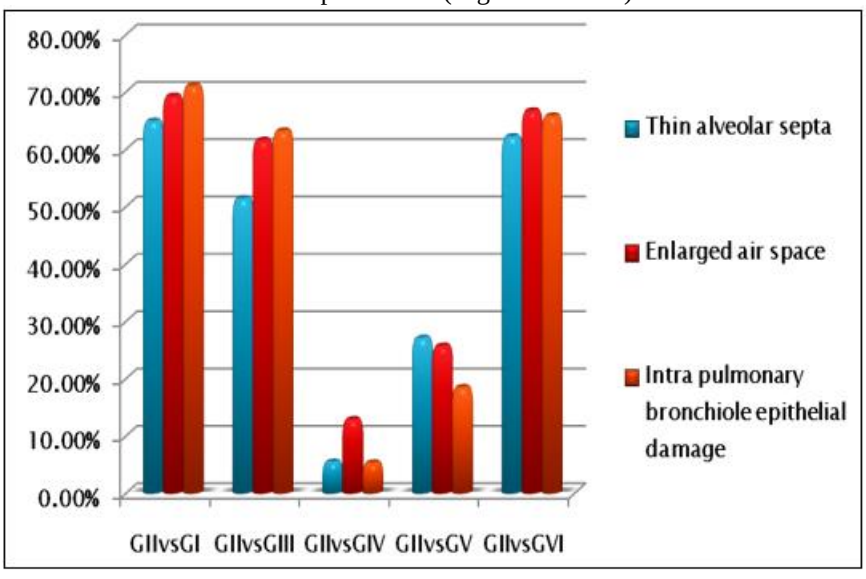


Group IV and Group V representing the result of after treatment histology of mice lungs with RD2 and RD3- Moderate alveolar enlargement. Though comparatively group V shows better result than group IV



Group VI representing the result of after treatment histology of mice lungs with RD4- Alveolar enlargement is more minimal than group III, absence of peribronchial inflammation

Pairwise comparison of percentage inhabitation in histopathological findings of mice lung tissue within various treated groups with respect to GII (negative control)



Impression:
The result of histopathological slide study of mice lungs under microscope in various treated groups showing maximum recovery in GI (standard modern drug) then GVI (RD4) > GIII (RDI)> GV (RD3)> GIV (RD2). Where GII is negative control

3. Discussion

The acute toxicity study of the formulation and its individual herbs on swiss albino mice showed neither any toxic symptoms or adverse behavioral changes nor any mortality up to the maximum dose 2000 mg/kg body weight signifying no acute toxicity of the research formulation and the herbs. Although a few motor and sensory behavioral changes were observed in the first few hours it later subsided after 4 to 5hours and showed no further toxic symptoms up to next 24 hours and then up to 14 days, which indicates there was no LD - 50 up to the dose of 2000 mg/kg/ body wt. The dose 600mg/kg body weight was selected for further experimental study and also clinical study on the basis of behavior and other physiological sign and symptoms of all animals.

For Cigarette smoke induced asthma in mice model, Whole body cigarette smoke exposure chamber was made to conduct the preclinical study. Albino Mice were selected to develop asthma.

Haematological study of mice blood in each treated groups comparison to positive control group & Histo-Pathological slide study of mice lung in each treated groups in comparison to negative control group: Showed maximum recovery in GI (Standard modern drug) then GVI(RD4) > GIII(RD1) > GV(RD3) > GIV(RD2).Where GII is negative control and GI is positive control group. The histopathological study was done following 3 parameters- Condition of alveolar septa, size of the air space, intra pulmonary bronchiole epithelial changes. On the basis of these parameters, arbitrary scoring methods of the histopathological study of lung tissue in experimental mice of each treated group by random inspection of 10 fields of each slide under microscope, revealed the more satisfactory result of RD4 (formulation i.e. Bharangi+Sunthi+Pippali) then RD1 (Bharangi), then RD3 (Pippali) then RD2 (Sunthi).

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