

**PREPARATION AND ASSESSMENT OF ANTI-ASTHMATIC
ACTIVITY OF DIFFERENT EXTRACT OF IPOMOEA AQUATICA,
CINNAMOMUM ZEYLANICUM, AND PIPER LONGUM
CONTAINING POLYHERBAL FORMULATION**

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ABSTRACT

Objective: To evaluate the antiasthmatic activity of developed polyherbal formulation of *Ipomoea aquatica*, *Cinnamomum zeylanicum*, and *Piper longum*. **Methods:** Water or chloroform extracts of different plants were prepared. A polyherbal formulation was developed and subjected for antiasthmatic activity. **Results:** Hydroalcoholic extract of *S. aromaticum* exhibited a prominent anxiolytic effect. **Conclusions:** The present study shows that polyherbal formulation with water base synergistically improved the level of GSH in both BAL fluid and lung tissue homogenates.

KEYWORDS: Asthma, *Ipomoea aquatica*, *Cinnamomum zeylanicum*, *Piper longum*, Polyherbal Capsule, Antiasthmatic activity.

1. INTRODUCTION

Nearly 7–10% of the world population suffers from bronchial asthma. Asthma is a common airways disease. It belongs to chronic inflammatory disease. Airflow obstruction and bronchospasm are the main characteristics of Asthma. Wheezing, coughing and shortness of breath are some of the common symptoms. The cause of asthma can be genetic and environmental. It can be diagnosed by the pattern of symptoms, response to therapy, and by spirometry. The occurrence of asthma has increased significantly since the 1970s. Asthma

can be atopic (extrinsic) and non-atopic (Intrinsic). There are a vast range of drugs available; the relief offered by them is mainly emblematic and temporary. At the same time these drugs also produce aftereffects. This force an ambitious need to identify effective and safe remedies to treat bronchial asthma (Govindan *et al.*, 1999). The current approved contemporary medicine or allopathy has constantly developed over the years by scientific and observational efforts of scientists. However, the basis of its development remains rooted in traditional medicine and therapies (Rana, 2008). About 80% of the world population, primarily in developing countries for primary health care are using herbal medicines (Kamboj, 2000; Chopra *et al.*, 1956). By the assessment of the current status of health care system in adequacies of synthetic drugs is likely to be more evident in the coming years. It has been reported that there has been a startling increase in number of diseases and disorders caused by synthetic drugs prompting a switch over to traditional herbal medicine (Ghule & Patil, 2001). Ayurveda is a traditional Indian Medicinal System practiced for thousands of years. Considerable research on pharmacognosy, chemistry, pharmacology and clinical therapeutics has been carried out on ayurvedic medicinal plants. The polyherbal formulations described in Ayurveda have been the basis of treatment of various human diseases. Selection of scientific and systematic approach for the biological evaluation of herbal formulations based on their use in the traditional systems of medicine forms the basis for an ideal approach in the development of new drugs from plants.

In the present study herbal capsule have been developed as polyherbal formulations from the selected plants based on mechanism of action, useful in the bronchial asthma i.e. *Ipomoea aquatica* leaf (bronchodilatation, anti-inflammatory and antioxidant), *Cinnamomum zeylanicum* bark (anti-inflammatory) and fruit of *Piper longum* Linn (immunomodulatory & bioavailability enhancing activity). These plants contain several phytochemicals, which may simultaneously target multiple components of asthma and may bring about benefits through a synergistic or additive action.

2. MATERIALS AND METHODS

2.1 Collection of plant material

The *Cinnamomum zeylanicum* (dried bark), *Piper longum* (dried stem) were procured from the AHS Enterprises, Gwalior, Madhya Pradesh and certificate of authentication was provided by them. The plant of *Ipomoea aquatica* was collected in the month of the

September 2008 from the village Gormi and it was authenticated from CDRI Lucknow (U.P.). (Voucher specimen no.-J-31).

2.2 Preparation of extract

Different plant parts were washed with distilled water to remove dirt and soil, and shade dried. The dried materials were powdered and passed through a 10-mesh sieve. The coarsely powdered materials of each plant was defatted with petroleum ether (60-800). Fifty grams of each of the air-dried and coarsely powdered plant material was extracted with 200 ml each of chloroform and water using a soxhlet apparatus. The extracts were filtered and concentrated by distilling off the solvents and evaporated to dryness using water bath to get crude extract.

2.3 Acute toxicity study

The acute toxicity of plant extract was evaluated in mice (25-35 g) using the OECD Guideline. The water extracts were reconstituted in water and the chloroform extracts are reconstituted in DMSO. Two doses of the extracts 1600 mg/kg and 3200 mg/kg were administered to the rat. For each dose and extract a group of four mice were used. Food and water were withheld two hours prior to and two hours post oral administration. The animals were weighed daily, and food and water consumption were recorded. They were also observed for general behaviour and mortality for 14 days. After 14 days the mice were anaesthetised with ether and blood was withdrawn from orbital plexus for various hematological and biochemical analysis. They were sacrificed by cervical dislocation and vital organs were removed, weighed and preserved.

2.4 Evaluation of Antiasthmatic activity

Male Hartley guinea pigs weighing 500 to 700 g were used to carry out in-vivo antiasthmatic activity. The bioavailability studies were carried on Adult albino rats (Wistar) of either sex weighing 250 ± 20 g. The animals were maintained in ordinary animal cages in a constant 12-h light/dark cycle. Food and water were available *ad libitum*. All protocols were in accordance with guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA) in India CPCSEA [Ref: SRCOP/IAEC/15/09] & CPCSEA [Ref: SRCOP/IAEC/16/09].

Table No. 1: Experimental study design and treatments (Anti asthmatic studies)

S.No.	Group	Treatment
1	Normal Control	No treatment
2	Standard Control	No treatment
3	Group 1	Standard drug
4	Group 2	Saline
5	Group 3	IA-W
5	Group 4	IA-C
7	Group 5	CZ-W
8	Group 6	CZ-C
9	Group 7	PL-W
10	Group 8	PL-C
11	Group 9	IA+CZ+PL-W
12	Group 10	IA+CZ+PL-C

Where **W**; Water extract: **C**; Chloroform extract: **IA**; *Ipomoea aquatica*: **CZ**; *Cinnamomum zeylanicum*: **PL**; *Piper longum*: **IA+CZ+PL**; *Ipomoea aquatica*, *Cinnamomum zeylanicum*, and *Piper longum*.

3. RESULTS

3.1 Evaluation of Respiratory Hyperreactivity: Final challenge with ovalbumin aerosol (0.5% in normal saline) was performed on seventh day of treatment, 30 minutes after the last dose. The guinea pigs were placed in the histamine chamber and challenged with an aerosol of ovalbumine (5mg/ml in water) for 30 min. Airway abnormalities of the animals were visibly monitored by two trained observers who were blinded to the group assignment of the animals for the changes in the respiratory activity of the animals subjected to different treatments. Evaluation of latency time (min) for the appearance of first respiratory abnormalities assessed as the time between onset of aerosolization and the first stroke of cough and the frequency of cough till 10 min after first cough stroke was measured according to the method of Gupta et al., 1968.

3.2 Statistical Analysis

All the values were expressed as Mean \pm SEM and were statistically analyzed using one way ANOVA followed by Tukey's multiple comparison tests. The $p < 0.05$ was considered to be statistically significant.

3.3 Acute toxicity test

Two doses of the extracts 1600 mg/kg and 3200 mg/kg were administered to the rat. No mortality and no behavioral changes were observed on these doses.

3.4 Evaluation of Antiasthmatic Activity

3.4.1 Effect against antigen induced airway hyper responsiveness

Challenge with 0.5% OVA aerosol developed prominent airway abnormalities in the form of severe cough strokes with the signs of dyspnoea that took place 4.75 ± 0.86 min after the onset of aerosolization in PBS treated sensitized guinea pigs (SC) with the cough frequency of 21.17 ± 2.48 cough strokes in 10 min. whereas unsensitized guinea pigs (NC) showed no substantial abnormalities and dyspnoea apart from sporadic cough strokes arising approximately 22.76 ± 2.72 min after onset of aerosolization.

Group-1 did not show any relief with severe cough strokes that took place 4.97 ± 0.86 min with the cough frequency of 21.35 ± 3.37 . There is increased cough latency in Group2 and Group3 treated animals (15.56 ± 0.98 min and 22.29 ± 1.99 min respectively) however no clear cut sign of dyspnoea was observed in both cases. Animals treated with Group4 and Group5 resist the airway hyper reactivity for 12.49 ± 2.12 and 15.56 ± 1.39 min with the cough frequency of 15.49 ± 1.22 and 11.79 ± 1.28 respectively. Group-6 pre-treated animals showed no significant elevation in cough latency (5.59 ± 1.83 min) but Group-7 animals could resist the onset of cough stroke for 13.20 ± 1.28 min with severe dyspnoea symptoms at both the doses. Group-8 showed marked increase in latency period of 21.89 ± 2.11 min with marked decrease in cough frequency (9.89 ± 1.76). Group-9 showed increased latency period of 25.79 ± 3.11 min with marked decrease in cough frequency (5.11 ± 1.57) and half of the population didn't even show any abnormalities with any signs of dyspnoea. Group-10 showed increased latency period of 18.98 ± 3.11 min with decrease in cough frequency (13.11 ± 1.57).

3.4.2 Assessment of Airway Inflammation

Exposures to antigen induced a significant cellular infiltration into BAL fluid recovered from sensitized animals (SC) than normal control (NC) group ($12.6 \times 10^3 \pm 1.49$ cells/ml versus $21.33 \times 10^3 \pm 3.81$ cells/ml). Using this model, the effect of chronic administration of the extracts alone and in combination was studied. Eosinophilic infiltration into lungs and BAL fluid has been considered the hallmark of inflammation. The count of eosinophils in the BAL fluid of sensitized guinea pigs was markedly enhanced ($2.42 \times 10^3 \pm 0.31$ cells/ml) denoting the severe inflammation when compared to normal control ($0.46 \times 10^3 \pm 0.02$ cells/ml).

In Group-9 Chronic administration of combination of extract significantly inhibited the cellular infiltration ($12.57 \times 10^3 \pm 3.22$ cells/ml) and prominent decrease in eosinophilia ($0.502 \times 10^3 \pm 0.09$ cells/ml) compared to Group-3 ($14.98 \times 10^3 \pm 1.55$ cells/ml and $0.93 \times 10^3 \pm 0.04$ cells/ml). Group-6 did not show any significant effect on cellular infiltration but notable effect was observed within Group-7 on both total leukocyte and eosinophilic count ($16.35 \times 10^3 \pm 1.44$ cells/ml and $1.54 \times 10^3 \pm 0.25$ cells/ml). Group-4 and Group-5 were found to possess moderate anti-inflammatory activity as it reduced the eosinophilic infiltration that was comparable to Group-2 and Group-3 ($2.42 \times 10^3 \pm 0.08$ cells/ml and $1.50 \times 10^3 \pm 0.76$ cells/ml respectively). Unlike the effect on Group-9, Group-8 failed to show any synergistic or additive effect in reducing the total leukocyte and eosinophil count ($14.00 \times 10^3 \pm 1.10$ cells/ml and $0.98 \times 10^3 \pm 0.03$ cells/ml respectively). Group-1 did not show any significant effect on cellular infiltration on both total leukocyte and eosinophilic count.

4. DISCUSSION

The protective effect of the extracts of these crude drugs alone and in combination was studied on respiratory hyper reactivity, airway eosinophilic accumulation and bronchial inflammation induced by an exposure to OVA aerosol in asthmatic guinea pigs. Guinea pig is the most commonly used small animal species in preclinical studies for evaluation of antiasthmatic activity of drugs. The intraperitoneal injection of 20 µg OVA and 100 mg Al(OH)₃ twice in a gap of seven days caused allergic reactions in male Hartley guinea pigs and the success rate of sensitization method was 90.66% (68 of 75 sensitized guinea pigs showed allergic reactions to inhaled antigen). Exposure to inhaled antigen developed pulmonary asthma-like response in antigen induced sensitized guinea pigs which is in connection with increased eosinophilic accumulation in the bronchial tissue and BAL fluid associated with chronic airway inflammation. Methanolic and chloroform extracts of *Ipomoea aquatica* was evaluated and turned to be a potent bronchodilator accompanied with notable antioxidant and anti-inflammatory activity. In present study we found that asthma-like response in antigen sensitized guinea pigs was inhibited synergistically by seven days treatment of IA+CZ+PL-W. The synergism may be because of *Ipomoea aquatica* extract (aqueous) induced inhibition of release of histamine and other bronchoconstrictors from target tissue; *Piper longum* (aqueous extract) enhanced smooth muscle relaxation due to bronchial airway clearance. The airway hyper responsiveness to the inhaled antigen was almost abolished with the pre-treatment of IA+CZ+PL-W. *Piper longum* (chloroform extract) on the other hand was not at all comparable with *Ipomoea aquatica* extract when cough

latency time and frequency of cough strokes were measured by behavioral symptoms. The combination of chloroform extracts of all three drugs (IA+CZ+PL-Cl) was also studied but there was no additive or synergistic effect found with this combination. *Cinnamomum zeylanicum* extract alone found to possess effect on antigen induced hyper responsiveness but was not comparable to *Ipomoea aquatic* extract. *Cinnamomum zeylanicum* extract may prevent hyper responsiveness due to its potent antiinflammatory activity that reduced the cough frequency and improved the latency time.

Eosinophilic infiltration is known to be a hallmark of bronchial asthma and several studies had reported a significant eosinophilia in BAL fluid of asthmatic patients. The significant correlation between the concentration of major basic protein and the number of eosinophils in BAL fluid postulates the role of eosinophil-derived mediators in the development of bronchial hyperreactivity (Wardlaw et al 1988).

5. REFERENCES

1. Chang RK, Guo X, Burnside B, Couch R., (2000) Fast-dissolving tablets. *Pharm Technol*; 24:52-8.
2. Chopra RN, Nayar SL, Chopra IC. In: Glossary of Indian medicinal plants, Council of Scientific and Industrial Research, New Delhi, 1956; 1: 197.
3. Ernst E. Complementary therapies for asthma: what patients use. *J Asthma*, 1998; 35: 667-671.
4. Greenberger PA. Therapy in management of rhinitis asthma complex. *Allergy Asthma Proc*, 2003; 24: 403-407.
5. Huntley A, Ernst E. Herbal medicines for asthma: a systemic review. *Thorax*, 2000; 55: 925-929.
6. Kamboj VP. Herbal medicine. *Cur Sci*, 2000; 78(1): 35-39.
7. Kemper KL, Lester MR. Alternative asthma therapies: an evidence-based review. *Contemp Pediatr*, 1999; 16: 162-195.
8. Lyengar MA, jambaiah KM, Rao GM. Studies on an antiasthma kada: A Proprietary Herbal Combination Part-I Clinical Study. *Indian Drug* 1994; 31(5): 183-186
9. MMWR. (1996) Asthma mortality and hospitalizations among children and young adults: United States. *MMWR* 45:350.
10. Nadeem M, Dandiya PC, Pasha KV, Imran M, Balani DK, Vohora SB. Hepatoprotective activity of some herbal formulations available in India. *Indian Drugs*, 1996; 33: 390-396.

11. Peter J.B., Robert A.M., George R.B., editors. Principles of pharmacology: Basic concepts and clinical applications. New York; Thomson Publishing Company: 1995.
12. Sears, M. R. Descriptive epidemiology of asthma. *Lancet* 1997; 350: 1-4.
13. Wardlaw, A.J. Molecular basis for selective eosinophil trafficking in asthma: a multistep paradigm. *J. Allergy. Clin. Immunol.* 1999; 104: 917-26.