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# Evaluation of antiasthmatic activity of a polyherbal formulation containing four plant extracts.

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**ABSTRACT**

**Objective:** We evaluated the antiasthmatic potential of a polyherbal formulation using several experimental models.
**Materials & Methods:** Adult Wistar albino rats were used for mast cell stabilization protocol. Acetylcholine and histamine-induced bronchospasm were conducted on guinea pigs.
**Results:** The results of the acetylcholine and histamine-induced bronchospasm paradigms demonstrate bronchospasmolytic activity of polyherbal formulation (300 mg/kg, p.o.). The spasmolytic effect was characterized by prolongation of onset of bronchospasm and reduction of asphyxia as compared to control group ($P < 0.01$). In the mast cell stabilization paradigm, compound 48/80 treatment produced 76% of mast cell degranulation. Polyherbal formulation (1, 10, and 100 µg/ml) showed dose-dependent significant reduction in mast cell degranulation ($P < 0.01$) as compared to the compound 48/80-treated animals. The probable mechanism for the antiasthmatic action of the polyherbal formulation could be antihistaminic, anticholinergic and mast cell membrane stabilization. The $LD_{50}$ of polyherbal formulation was 2262.7 mg/kg, p.o.
**Conclusion:** The results of our study, for the first time, show that the polyherbal formulation possesses antihistaminic, anticholinergic and mast cell stabilizing properties and therefore can be a candidate for the antiasthmatic treatment. Future research should focus on the molecular mechanism of responsible constituent for antiasthmatic action.

**Keywords:** Antiasthmatic, Antihistaminic, Anticholinergic, Mast cell stabilization, Polyherbal formulation.

## 1. INTRODUCTION

Bronchial asthma is an inflammatory disorder of the airways characterized by various airway obstruction, airway inflammation and bronchial hyper responsiveness (Djukanovic *et al*., 1990) and is a global health problem that results from a complex interplay between genetic and environmental factors (Phillip, 2003). Nearly 7–10% of the world population suffers from bronchial asthma. Among several respiratory diseases affecting man, bronchial asthma is the most common disabling syndrome. Despite the availability of a wide range of drugs, the relief offered by them is mainly symptomatic and short lived. Moreover, these drugs produce side effects. Therefore, there is a dire need to identify effective and safe remedies to treat bronchial asthma (Govindan *et al*., 1999). The current accepted modern medicine or allopathy has gradually 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).

Herbal medicines are being used by nearly about 80% of the world population, primarily in developing countries for primary health care (Kamboj, 2000). Assessing the current status of health care system in adequacies of synthetic drugs is likely to be more glaring in the coming years. It has been reported that there has been an alarming increase in number of diseases and disorders caused by synthetic drugs prompting a switch over to traditional herbal medicine (Ghule

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& 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 light of above background, the present study aimed at evaluation of a polyherbal formulation for the possible antiasthmatic action using experimental animals.

## 2. MATERIALS AND METHODS

### Collection of plant materials

The leaves of *Solanum xanthocarpum*, *Murraya koenigii*, *Aegle marmelos* and *Caesalpinia bonduc* were collected from the out field of Junagadh city, Gujarat, India in February-March 2007. The plant materials were identified and authenticated by Dr. P. Parmar, Botanical Survey of India, Jodhpur, Rajasthan, India. Voucher specimens (SU/DPS/Herb/19-22) of the collected plant samples were deposited in the Department of Pharmaceutical Sciences, Saurashtra University, Rajkot, Gujarat, India for future reference.

### Drugs and chemicals

Compound 48/80, disodium chromoglycate (DSCG), histamine dihydrochloride, acetylcholine chloride, atropine sulfate, mepyramine meleate, and toluidine blue were purchased from Sigma (St. Louis, MO, USA). Histamine solution was freshly prepared in normal saline (NaCl, 8.5 g/l). All the other chemicals were of analytical grade.

### Extraction

Leaves of different plants 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 (500 g) of each plant was defatted with petroleum ether ($60\text{-}80^0$) and then extracted separately with ethanol (95%, v/v) in a Soxhlet apparatus. The extracts were filtered and concentrated by distilling off the solvents and evaporated to dryness using water bath to get crude ethanol extract.

### Experimental animals

Antihistaminic and anticholinergic studies were conducted on guinea pigs (350-500 g) of either sex. For mast cell stabilization paradigm, adult Wistar albino rats weighing 140–160 g of either sex were used. All the experimental animals were fed on commercial pellet diet (Amrut, Pranav Agro Industries Ltd, India). They were group housed under standard conditions of temperature ($22 \pm 2^0$C), relative humidity ($60 \pm 5\%$) and 12:12 light/dark cycle, where lights on at 0700 and off at 1900 h). They were divided in groups of ten animals each. The saline fed group served as control and one group was treated with a standard drug. Before experimentation, the animals were kept on fast for 24 h but water was given *ad libitum*. During experiments, animals were also observed for any alteration in their general behavior.

All the experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC), N. R. Vekaria Institute of Pharmacy and Research Centre, Junagadh, Gujarat, India (approval number-NRVCP/IAEC/08-2k7/01). All the experiments and the care of the laboratory animals were according to current ethical guidelines by the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India, New Delhi.

### Preparation of the test extracts

The polyherbal formulation was suspended in 1% SCMC in distilled water and administered orally or intraperitoneally. The control animals were given an equivalent volume of SCMC vehicle. The standard group received various standard anti-asthmatic drugs.

### Phamacological screening (Antiasthmatic paradigms)

#### *Histamine-induced bronchospasm in guinea pigs (Armitage et al., 1961)*

The guinea pigs fasted for 24 h were exposed to an atomised fine mist of 2% histamine dihydrochloride aerosol (dissolved in normal saline) using nebulizer at a pressure of 300 mm Hg in the histamine chamber (24 x 14 x 24 cm, made of perplex glass). Guinea pigs exposed to histamine aerosol showed progressive signs of difficulty in breathing leading to convulsions, asphyxia and death. The time until signs of convulsion appeared is called pre-convulsion time (PCD). By observation experience was gained so that the preconvulsion time can be judged accurately. As soon as PCD commenced, animals were removed from the chamber and placed in fresh air to recover. In the present experiments the criterion used was time for onset of dysponea and percent protection was calculated. Those animals which developed typical histamine asthma within 3 min were selected out three days prior to the experiment and were given habituation practice to restrain them in the histamine chamber. They were divided in groups of ten animals each. Mepyramine (8 mg/kg) and polyherbal formulation (300 mg/kg) were administered intraperitonially 30 min prior to exposure. Animals, which did not develop typical asthma within 6 minutes were taken as protected.

#### *Acetylcholine-induced bronchospasm in guinea pigs (Kumar & Ramu, 2002*)

Procedure was similar to the histamine aerosol study except that animals were exposed to aerosol of 0.5% acetylcholine in another set of animals (n = 10). Atropine sulphate (2 mg/kg) was used as a standard drug.

***Mast cell degranulation by compound 48/80***

This was carried out as per the method described by Kaley and Weiner (1971) with little modification. Male albino rats were sacrificed by cervical dislocation. The animals were immediately injected with 15 ml of pre-warmed (37°C) buffered salt solution (NaCl 137 mM; KCl 2.7 mM; $MgCl_2$; 1 mM; $CaCl_2$ 0.5 mM; $NaH_2PO_4$ 0.4 mM; Glucose 5.6 mM; HEPES 10 mM) into the peritoneal cavity, and massaged gently in this region for 90 s, to facilitate cell recovery. A midline incision was made and the peritoneum was exposed. The pale fluid was aspirated using a blunted plastic Pasteur pipette, and collected in a plastic centrifuge tube. The fluid was then centrifuged at 1000 rpm for 5 min, and the supernatant discarded to reveal a pale cell pellet. The cell pellets were re-suspended in fresh buffer and re-centrifuged. Aliquots of the cell suspension were incubated with the test compounds or disodium cromoglycate, before challenge with compound 48/80. The aliquots were carefully spread over glass slides and the mast cells were stained with 1% toluidine blue and counterstained with 0.1% light green. The slides were dried in air and the mast cells counted from randomly selected high power objective fields (X450). The effect of polyherbal formulation on mast cells was studied by incubating the mast cells for 10 min with the above formulation in a concentration of 1, 10 or 100 µg/ml. In another set of experiments the mast cells which were pre-incubated with polyherbal formulations were exposed to the mast cell degranulator, compound 48:80 (10 µg/ml) and the incubation continued for a further 10 min. Then, the mast cells were carefully spread over glass slides. The percent degranulation of the mast cells was calculated. Disodium cromoglycate (DSCG) (20 µg/ml) was included in one of the study group for comparison.

**Table 1.** Effect of polyherbal formulation on mast cell degranulation[a]

| Pre-treatment (µg/ml) | Degranulation after treatment (%) | |
|---|---|---|
| | Vehicle | Compound 48/80 (10 µg/ml) |
| Vehicle | 33.4 ± 0.93 | 75.8 ± 1.36 |
| DSCG (20) | 12.8 ± 3.07** | 20 ± 1.79$^{\#\#}$ |
| Polyherbal formulation (1) | 27.4 ± 2.16$^{ns}$ | 57.4 ± 2.32$^{\#\#}$ |
| Polyherbal formulation (10) | 25.8 ± 2.18$^{ns}$ | 45.8 ± 2.31$^{\#\#}$ |
| Polyherbal formulation (100) | 17.6 ± 1.94** | 30.4 ± 0.81$^{\#\#}$ |

[a]Each value represents the mean ± S.E.M. of five observations; [ns]Not significant, *$P$ < 0.05, **$P$ < 0.01 vs. control (vehicle treated), one-way ANOVA followed by Dunnett's $t$-test; $^{\#}P$ < 0.05, $^{\#\#}P$ < 0.01 vs. control (Compound 48/80 treated), one-way ANOVA followed by Dunnett's $t$-test.

**Acute toxicity test (Determination of $LD_{50}$)**

The acute toxicity test ($LD_{50}$) was determined according to the procedure described by Lorke (1983). Albino mice (20–25 g) of either sex were used. This method involved an initial dose finding procedure, in which the animals were divided into three groups of three animals per group. Doses of 10, 100 and 1000 mg/kg were administered intraperitoneally (i.p.), one dose for each group. The treated animals were monitored for 24 h for mortality and general behavior.

From the result of the above step, four different doses of 200, 400, 800 and 1600 mg/kg were chosen and administered i.p. respectively to four groups of one mouse per group. The treated animals were again monitored for 24 h. The $LD_{50}$ was then calculated as the geometric mean of the lowest dose showing death and the highest dose showing no death.

**Preparation of polyherbal formulations**

The polyherbal formulations was composed of ethanolic extracts of *Solanum xanthocarpum* (50 mg), *Aegle marmelos* (50 mg), *Caesalpinia bonduc* (100 mg), and *Murraya koenigii* (100 mg).

**Table 2.** Effect of polyherbal formulation on histamine-aerosol in guinea pigs[a]

| Treatment | Dose (mg/kg, i.p.) | Protection (%) |
|---|---|---|
| Control | saline, 1.0 ml/kg | 0 |
| Mepyramine | 8 | 90*** |
| Polyherbal formulation | 300 | 80** |

[a]n=10 in each group; **$P$ < 0.01, ***$P$ < 0.001 vs. control; ($\chi^2$ test with Yate's correction)

## 3. RESULTS AND DISCUSSION

The results of the present study revealed mast cell stabilization, antihistaminic and anticholinergic actions of polyherbal formulation.

In the mast cell stabilization paradigm, 33% of mast cells were degranulated in the control group. Addition of DSCG (20 µg/ml), and the polyherbal formulation (100 µg/ml) reduced the percentage of mast cell degranulation ($P$ < 0.01) compared to the control group. Compound 48/80 (10 µg/ml) produced about 76% degranulation of mast cells. Pretreatment with DSCG (20 µg/ml) and the polyherbal formulation (1, 10, and 100 µg/ml) significantly reduced ($P$ < 0.01) degranulation of mast cells as compared to compound 48/80-tretaed control group (Table 1). The protection given by them at higher concentrations was comparable to that of DSCG, a potent mast cell stabilizing agent. These results suggest

that polyherbal formulation protects mast cells from compound 48/80-evoked degranulation.

In the histamine aerosol study, the control animals showed convulsion during the first 3 min of the experiment. Prior treatment of polyherbal formulation (300 mg/kg, i.p.) protected the animals (Table 2) to a significant extent ($P < 0.01$) from the development of asphyxia produced by histamine aerosol confirming that it has antihistaminic activity. The role of histamine in asthma is well established (Nelson, 2003). The close resemblance of pulmonary responses to histamine challenge in both guinea pigs and humans, as well as the anaphylactic sensitization made this species the model of choice. In the present study, guinea pigs were used because of the extreme sensitivity of their airways to the primary mediators of bronchoconstriction, including histamine and leukotrienes, and their ability to be sensitized to foreign proteins. Although there are various model of asthma, guinea pig airways react to histamine, acetylcholine, leukotrienes, and other bronchoconstrictors in a manner similar to that seen in humans (Agrawal *et al*., 1991). Another similarity between the guinea pig model and asthmatic patients is that enhanced bronchoconstriction occurs in both species following sensitization, in response to β-adrenergic antagonists (Matsumoto *et al*., 1994). Thus, the guinea pig model resembles the human allergic pathology in several aspects, especially in terms of mediator release. Histamine antagonists can be conveniently recognized and assayed by their ability to protect guinea pigs against lethal effects of histamine-induced bronchospasm (Broadbent & Bain, 1964). Mepyramine, a standard anti-histaminic drug, (8 mg/kg, i.p.) significantly protected 90% of animals from asphyxia ($P < 0.001$).

**Table 3.** Disease Severity Score of Azithromycin Vs Placebo Group

| Treatment | Dose (mg/kg, i.p.) | Preconvulsion time (sec) | Protection (%) |
|---|---|---|---|
| Control | saline, 1.0 ml/kg | 128 ± 2.72 | - |
| Atropine | 2 | 460 ± 4.33 | 72.17** |
| Polyherbal formulation | 300 | 285 ± 3.70 | 55.08** |

[a]Values are mean ± S.E.M. (n = 10); **$P < 0.01$ vs. control; Dunnett's *t*-test after one-way ANOVA.

Acetylcholine-aerosol provoked bronchoconstriction in all animals of control group. The polyherbal formulation (300 mg/kg, i.p.) significantly protected animals ($P < 0.01$) from acetylcholine-induced bronchoconstriction. Atropine sulphet (a standard anti-muscarinic drug, 2 mg/kg, i.p.) significantly prolonged pre-convulsion time to 460 ± 4.33 sec ($P < 0.01$) and protected animals from asphyxia (Table 3). The $LD_{50}$ of the polyherbal formulation was calculated to be 2262.7 mg/kg, i.p.

In the early stage of asthma, release of inflammatory mediators like histamine, acetylcholine, leukotrienes, and prostaglandins are triggered by exposure to allergens, irritants, cold air or exercise (Bosquet *et al*., 2000). Some of these mediators directly cause acute bronchoconstriction. Spasmolytic drugs like β-adrenergic agonists, xanthine derivatives and anticholinergic drugs are used as quick relief medications in such acute asthmatic attacks (Horwitz & Busse, 1995). In the present study, we have used histamine and acetylcholine as spasmogens in the form of aerosols to cause immediate bronchoconstriction in guinea pigs. Mepyramine (8 mg/kg) and atropine sulphate (2 mg/kg) were used as reference standard against histamine and acetylcholine induced bronchospasm respectively (Shah & Parmar, 2003). The bronchodilatory effect of polyherbal formulation was found comparable to the protection offered by both the reference standard drug mepyramine and atropine sulphate.

## 4. CONCLUSION

In conclusion, the results of present investigation suggest that, polyherbal formulation have significant bronchodilatory activity against histamine and acetylcholine. However, further studies are suggested to establish molecular mechanism and also to isolate and characterize the active principles responsible for the action.

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