

## RESEARCH ARTICLE

## IN-VITRO EVALUATION OF IMMUNOMODULATORY EFFECT OF POLYHERBAL COMPOUND - *BHARANGYADI*

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### ABSTRACT

*Bharangyadi* compound is a polyherbal drug use in Ayurveda system of medicine for the management of allergic disorders such as allergic rhinitis, allergic asthma etc. The present study was carried out to evaluate the immunomodulatory activity of ethanolic extract of polyherbal compound "*Bharangyadi*" on swiss albino mice. Cyclophosphamide induced immunosuppression model was used to assess the activity of drug. Cyclophosphamide was given in the dose of 30mg/kg body weight through i.p route. 500mg/kg body weight of *Bharangyadi* polyherbal drug was given through oral route. The extent of protection against immunosuppression caused by Cyclophosphamide was evaluated after 14 days of drug administration, by estimating hematological parameter and neutrophil adhesion test. Ethanolic extracts of *Bharangyadi* Compound showed pronounced immunoprotective activity by increasing the depleted levels of total WBC count and RBC, % Hb, and % neutrophils adhesion. The extract was found to be an effective immunomodulatory agent.

**Keywords:** *Bharangyadi* Compound, ethanolic extract, Immunomodulatory activity, Cyclophosphamide.

### INTRODUCTION:

A substance that alters the immune response by augmenting or reducing the ability of the immune system to produce antibodies or sensitized cells that recognize and react with the antigen that initiated their production is known as immunomodulator<sup>1</sup>. Some immunomodulators are naturally present in the body, and certain of these are available in pharmacologic preparations. Immunomodulators and their role in the pathogenesis of various allergic diseases are the new emerging area of research. New information regarding the molecular mechanisms of allergic disorders has led to a variety of novel therapeutic approaches including use of herbal immunomodulators. Strategies to inhibit inflammatory pathways are many and include targeting the cell of origin of inflammatory cytokines and mediators, targeting the released mediator or cytokine, or inhibiting the effects of the released cytokine or mediator by blocking the mediated effects on the target cell. All of these approaches have been undertaken with various molecules but the most attracting approach is to modulate the immune response so as to inhibit the initial inflammatory process. *Bharangi* (*Clerodendrum serratum*), *Sati* (*Hedychium spicatum*) and *Pushkarmoola* (*Inula racemosa*) are indigenous Ayurvedic drugs use in the treatment of *Svasa roga* (dyspnea).

In Ayurvedic system of medicine mainly polyherbal compounds are used for the treatment of Asthma. *Bharangi* (*Clerodendrum serratum*) is found to have anti-inflammatory<sup>2,3</sup>, antihistaminic, antiallergic, antioxidant and hepatoprotective properties<sup>4</sup>. In Ayurvedic system of medicine, it is mainly used in respiratory tract diseases<sup>5</sup>. *Sati* (*Hedychium spicatum*) is found to possess hypotensive, hypoglycaemic, anti-inflammatory, vasodilator, antispasmodic, tranquilizer, anti-bacterial, anti-fungal, CNS-depressant, hypothermic, spasmolytic & analgesic effects<sup>6,7,8</sup>. *Pushkarmoola* (*Inula racemosa*) has been found

prove beneficial for cardiovascular system, angina and dyspnoea<sup>9,10</sup>. *Bharangyadi* is a mixture of *Clerodendrum serratum*, *Hedychium spicatum* and *Inula racemosa*.

Therefore this study was planned to assess the ethanolic extract of polyherbal compound (*Bharangyadi*) for immunomodulatory activity to search probable mode of its action in allergic diseases.

The hydroalcoholic extract of drug was chosen for the trial, extracted by hot percolation using soxhlet apparatus. The hydroethanolic extract was chosen as most of the secondary metabolites are soluble in water & alcohol; moreover the drug was planned to be given through nasal route in aresol form, the only media suitable for human nasal delivery of drug is water and alcohol act as preservative. As the drug was used for the treatment of Acute Asthma given via Nebulization mechaine in aresol form the drug should be in liquid form. Cyclophosphamide acts on both cyclic and intermitotic cells, resulting in general depletion of immunocompetent cells. Cyclophosphamide (CP) is an alkylating agent widely used in anti-neoplastic therapy<sup>11</sup>. It is effective against a variety of cancers such as lymphoma, myeloma and chronic lymphocytic leukemia<sup>12,13</sup>. CP induced immunosuppression is reported to prompt various types of infection<sup>14,15</sup>.

### MATERIALS AND METHODS

#### Plant material:

The plants *Bharangi* (*Clerodendrum serratum*), *Sati* (*Hedychium spicatum*) and *Pushkarmoola* (*Inula racemosa*) were collected from local market of Varanasi. The identification of the drugs was done by Prof.A.K. Singh, Department of Dravyaguna, S.S.U., Varanasi ( Identification number DG/ AKS / 604).

Table 1: Content of "Bharangyadi Compound"

Name of the Drug	Botonical name	Part Uesd	Approx. quantity in 100ml of extract
<b>Bharangi</b>	Clerodendrum serratum	Moola ( Root)	20mg
<b>Sati</b>	Hedychium spicatum	Moola (Root)	20mg
<b>Pushkarmoola</b>	Inula racemosa	Moola (Root)	20mg

### Extraction of the plant material and sample preparation:

Hydroalcoholic Extraction ( Distilled water: Ethanol = 2:1) of drug was carried out by hot percolation method through soxhlet apparatus. Thereafter extract was dried using rotary evaporator and dried extract was put to the process of standradization. The percentage yield was noted.

### Drugs and Chemicals

Cyclophosphamide (Sigma, life science) was used as standard immunosuppressant. All the other reagents and chemicals used in studies were of analytical grade.

### Animals:

Eight week-old healthy, laboratory bred, Swiss albino mice of either sex (20-25g) were maintained under standard laboratory conditions such as temperature 22–25°C, 12 hour light/dark cycle and provided with water and pellet food ad libitum. The experiments were conducted as per the guidelines of CPCSEA (Committee for the purpose of control and supervision of experiments on animals, India), Ministry of Social Justice and Empowerment, Government of India. **The research protocol was approved by Institutional Research committee of Institute of Medical Sciences, BHU- India.**

### Immunomodulatory Activity

The ethanolic extract of Polyherbal compound was subjected to evaluation of Immunomodulatory Activity using Cyclophosphamide induced immunosuppression model and neutrophil adhesion test.

### Preparation of sample

The ethanolic extract of the plant were dissolved in distilled water and administered orally at a dose of 500 mg/kg b/w with the help of feeding cannula.

### Preparation of Cyclophosphamide<sup>16</sup>

The Cyclophosphamide was suspended in 0.5% Carboxy methyl cellulose solution in distilled water. The solution was administered intraperitoneally at a dose of 30 mg/kg b/w.

### Cyclophosphamide induced immunosuppression

The animals were divided into the 3 groups containing 6 animals in each group. Group1 (Control group) received Carboxy Methyl Cellulose (CMC) for 14 days and group 2 (Challenge group) received CMC for 10days, on 11th, 12th and 13th day Cyclophosphamide intraperitoneally at a dose of 30mg/kg b/w. Groups 3 (Test group) received ethanolic extract of the drug at a dose of 500mg/kg body weight orally for 14 days. On days 11,12 and 13th day Cyclophosphamide solution was given intraperitoneally at a dose of 30mg/kg b/w one hr after the administration of

the extract. This experimental study was a part of clinical study in which the anti-asthmatic effect of the compound given via nasal route through nebulization was evaluated, hence the acute toxicity study and Multiple Ascending Dose Determination Study had been conducted before experimental study. Acute toxicity study of the compound showed that the drug is innocuous and very safe for therapeutic use with infinite LD<sub>50</sub> value. The dose determination study had showed that drug has maximum therapeutic effect between the range of 500mg- 1g. Thus 500mg dose was selected to evaluate the immunomodulator effect of the drug.

### Hematological Test<sup>17-19</sup>

At the end of the treatment, mice were light anaesthetized by using di-ethyl ether. The blood was collected from the retro-orbital plexus using heparinised capillary tubes and Hematological tests were carried out. The WBC count was done by Turke's method <sup>[17]</sup> RBC by Hayem's method <sup>[18]</sup>, and haemoglobin by Sahli's method <sup>[19]</sup>. The results are shown in Fig 1-4.

### Neutrophil adhesion test<sup>20</sup>

Total leukocyte counts (TLC) and differential leukocyte counts (DLC) were analyzed by fixing blood smears and staining with Field stain I and II- Leishman's stain. After initial counts, blood samples were incubated with 80mg/ml of nylon fibers for 15 min at 37°C. The incubated blood samples were again analyzed for TLC and DLC. The product of TLC and % neutrophil gives neutrophil index (NI) of blood sample.

Percent neutrophil adhesion was calculated as shown below

$$\text{Neutrophil adhesion (\%)} = \text{NI u} - \text{NI t} \times 100$$

NI u

Where

NI u = Neutrophil index of untreated blood sample.

NI t = Neutrophil index of treated blood sample.

### Statistical Analysis

The data were expressed as the mean  $\pm$  standard deviation of the means (SEM) and statistical analysis was carried out employing student's t-test and one-way analysis of variance (ANOVA) followed by Dunnett's multiple 't' comparison test.

### RESULTS

#### Effectiveness of *Bharangyadi* compound against drug-induced immunosuppression

Administration of Cyclophosphamide (30 mg/kg, i.p) produced a significant decrease in the Total Leukocyte Count from  $6.2 \pm 0.081$  to  $2.98 \pm 0.214$ , RBC count from

5.02 $\pm$ 0.116 to 3.02 $\pm$ 0.152, and % hemoglobin from 15.49 $\pm$ 0.081 to 9.32 $\pm$ 0.153 (P<0.01). This was found to be consistent with earlier studies which state that Cyclophosphamide induces immune dysfunction through reactive intermediate-induced damage to the cells of the immune system. Evaluation of effect of hydroethanolic extract of *Bharangyadi* Compound on Cyclophosphamide induced immunosuppression indicated good protection by increasing all the hematological parameters. WBC count, RBC count, and % hemoglobin values observed were better than untreated control groups (Fig. 1-4).

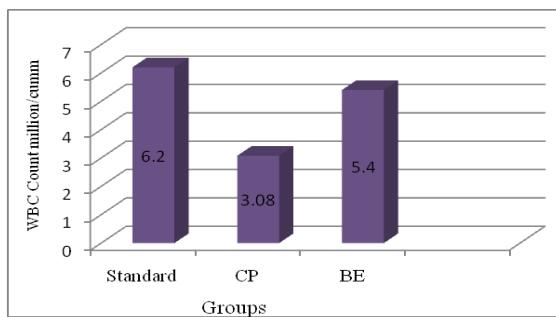


Figure 1: Showing Effect of *Shirishadi* compound on cyclophosphamide induced depletion in WBC counts

All values are mean $\pm$ SEM, n=6.

\*\*P<0.001 when compared with control group and, \*\*\*P<0.001, when compared with Cyclophosphamide treated group (Students t test).  
bP<0.01, when compared with Cyclophosphamide treated group (One way ANOVA).

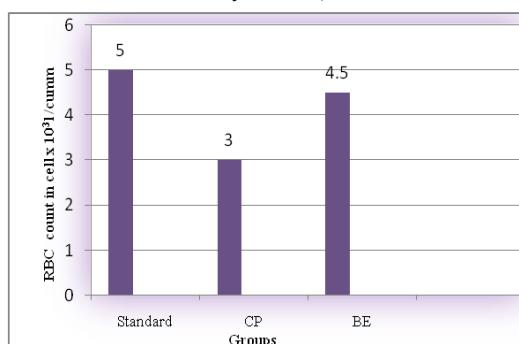


Figure 2: Effect of ethanolic extract of *Bharangyadi* Compound on cyclophosphamide induced depletion in RBCs counts

All values are mean $\pm$ SEM, n=6.

\*\*\*P<0.001 when compared with control group and, \*\*\*P<0.001, when compared with Cyclophosphamide treated group (Students t test).  
bP<0.01, when compared with Cyclophosphamide treated group (One way ANOVA).

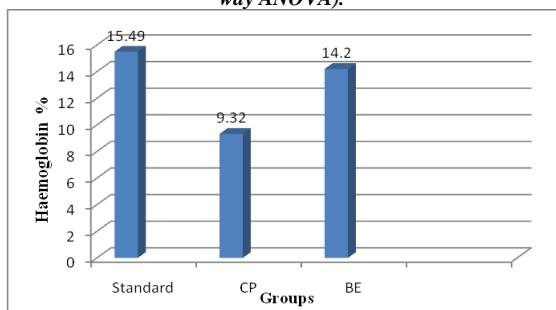


Figure 3: Effect of ethanolic extract of *Bharangyadi* Compound on Haemoglobin estimation.

All values are mean $\pm$ SEM, n=6.

\*\*\*P<0.001 when compared with control group and, \*\*\*P<0.001, when compared with Cyclophosphamide treated group (Students t test).  
bP<0.01, when compared with Cyclophosphamide treated group (One way ANOVA).

#### Neutrophil adhesion test

The % neutrophil adhesion in control group animals was, 25.76 $\pm$ 1.585, in CP treated group was 14.44 $\pm$ 1.08, in *Bharangyadi* hydroethanolic treated group it was 22.25 $\pm$ 1.90 (Fig 4). The results of neutrophil adhesion test indicating that there was significant (P<0.001) increase in neutrophil adhesion after administration of *Bharangyadi* ethanolic extract.

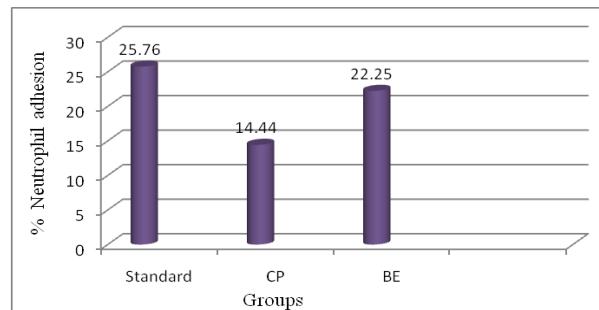


Figure 4: Effect of ethanol extract of *Bharangyadi* Compound on neutrophil adhesion test on Cyclophosphamide treated mice.

All values are mean $\pm$ SEM, n=6.

\*\*P<0.01 when compared with control group, \*\*\*P<0.001 when compared with Cyclophosphamide treated group. (Students t test).  
b. P<0.01 when compared with Cyclophosphamide treated group (one way ANOVA).

#### DISCUSSION & CONCLUSION:

An immunomodulator is any substance that helps to regulate the immune system. This "regulation" is a normalisation process, so that an immunomodulator helps to optimise immune response. Use of herbs for improving the overall resistance of body against common infections and pathogens has been a guiding principle of Ayurveda. The basic mechanisms by which the herbs defend the body against infection have two probable ways 1. by destroying the pathogens and 2. by enhancing the immune system of the body. Mechanism of drug action is essential to understand for its proper application. The dynamic and complex nature of immune system can be better understood after immune challenge thus using cyclophosphamide induced immune suppressive mice model is most reliable method for evaluation of immunomodulation effect. The study affirms that ethanolic extracts of *Bharangyadi* Compounds are effective immunomodulatory agent. The effectiveness of extract-treated animals in overcoming the side effects of cyclophosphamide induced immunosuppression provides evidence for balancing and adaptogenic effectiveness of extracts. The extracts potentiated the non-specific immune response. This may be attributed to different phytoconstituents. Increase in percent neutrophil is attributed to marginalization of phagocytic cells i.e. improved defensive response under normal circumstances. Evaluation of effect of hydroethanolic extract *Bharangyadi* Compounds on Cyclophosphamide induced immunosuppression indicated good protection by increasing all the hematological parameters. WBC count, RBC count, and % hemoglobin values observed were better than untreated control groups. Administration of Cyclophosphamide produced a significant decrease in the Total Leukocyte Count from 6.2 $\pm$ 0.081 to 2.98 $\pm$ 0.214,

RBC count from  $5.02\pm0.116$  to  $3.02\pm0.152$ , and % hemoglobin from  $15.49\pm0.081$  to  $9.32\pm0.153$  ( $P<0.01$ ). Groups treated with *Bharangyadi* extract showed WBC count =  $5.4\pm1.26$ , RBC count=  $4.5\pm0.67$ , Hbgm% =  $14.2\pm0.74$  ( $p<0.001$ ) and Neutrophil adhesion  $22.25\pm1.90$ .

Thus with the result of this preliminary study it can be conclude that the *Bharangyadi* compound holds promise for being used as an immunostimulating agents.

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## CONCLUSION

The ethanolic extracts of polyherbal compounds have protected the animal against Cyclophosphamide induced immunosuppression indicating its profound immunostimulatory activity.