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Pharmacological Study

Immunomodulatory effect of ethanolic extract of *Shirishadi* compound

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Abstract

Immunomodulators are substances that helps to regulate the immune system. The basic mechanisms by which the herbs defend the body against infection have two probable ways- one by destroying pathogens and other by enhancing the body immunity. *Shirishadi* compound is a polyherbal drug used in Ayurvedic 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 "*Shirishadi*" on Swiss albino mice. Cyclophosphamide (CP) induced immunosuppression model was used to assess the activity of drug. CP was given in the dose of 30 mg/kg body weight through i.p route. 500 mg/kg body weight of *Shirishadi* polyherbal drug was given through oral route. The extent of protection against immunosuppression caused by CP was evaluated after 14 days of drug administration, by estimating hematological parameters and neutrophil adhesion test. Ethanolic extracts of *Shirishadi* 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.

Key words: *Shirishadi* compound, cyclophosphamide, immunomodulatory activity

Introduction

In recent years, there has been growing interest in the field of herbal medicines research and search for promising potential compounds for investigating the immunomodulatory compounds from natural products. The immune system is designed to protect the host from invading pathogens and to eliminate disease. Plants are the essential and integral part in Complementary and Alternative Medicine (CAM) and this is due to their ability to develop formation of secondary metabolites like proteins, flavonoids, alkaloids, steroids and phenolic substances, which are in turn used to restore health and heal many diseases. Herbal drugs are believed to enhance the natural resistance of the body against infection and their immunomodulatory activities have been reported in numerous plants.[1]

The concept of immunomodulation relates to nonspecific activation of the function and efficiency of macrophages, granulocytes, complement, natural killer cells and lymphocytes and also to the production of various effector molecules generated by activated cells. It is expected that theses nonspecific effects give protection against different pathogens including bacteria, viruses, fungi etc., and constitute an alternative to conventional chemotherapy.

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The Polyherbal preparation used in present study contains *Shirisha* (*Albezia lebbeck (L.)* Benth, leguminosae), *Nagarmotha* (*Cyperus rotundus* Linn., Cyperaceae), and *Kantakari* (*Solanum surattense*, Mimosaceae). *Albezia lebbeck* reported to have anti-inflammatory, anti-anaphylactic effect, prevent mast cell degranulation and protect the sensitized guinea pig from antigen induced anoxic convulsion. [2-4] Previous researches shown that it is effective against bronchospasm induced by histamine acid phosphate and shown to exert di-sodium –cromoglycolate like action on mast cells.[5]

Kantakari (*Solanum surattense*) is one of the members of the *Dashmula* (ten roots) of the Ayurveda. A decoction of the root is given with the addition of long pepper and honey, in cough and catarrh, and with rock salt and asafoetida in spasmodic cough.[6] Plant has been investigated for much of responses and as well a pilot study on the clinical efficacy of *Solanum surattense* as a dried whole plant shown significant improvement in some respiratory diseases like bronchial asthma.[7] *Cyperus rotundus* is also found to have

anti-inflammatory, anti-pyretic and analgesic activities along with antioxidant activity.[8-10]

Cyclophosphamide (CP) acts on both cyclic and intermitotic cells, resulting in general depletion of immunecompetent cells. CP is an alkylating agent widely used in anti-neoplastic therapy.[11] It is effective against a variety of cancers such as lymphoma, myeloma, and chronic lymphocytic leukemia.[12,13] CP induced immunosuppression is reported to prompt various types of infection.[14,15]

The present study is aimed at investigating the immunomodulatory potency of the ethanol extract of *Shirishadi* Compound using Cyclophosphamide induced immunosuppression model by evaluating the effect of the extract on various hematological parameters and Neutrophil adhesion test in Swiss albino mice.[16]

Materials and Methods

Plant material

The plants *Albizia lebbeck* (L.) *Benth* (*Shirisha*), *Cyprus rotundus* (*Mustaka*) and *Solanum surattense* (*Kantakari*) were collected from local market of Varanasi. The identification of the drugs was done by Department of Dravyaguna, S.S.U., Varanasi (Identification number DG/AKS/604).

Contents of *Shirishadi* compound are tabulated in Table 1

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 the extract was dried using rotary evaporator and dried extract was put to the process of standardization. The percentage yield was noted.

Drugs and Chemicals

CP (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 (20-25 g) were maintained under standard laboratory conditions such as temperature 22-25°C, 12 h light/dark cycle and provided with water and pellet food *ad libitum*. The experiments were conducted as per the guidelines of 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 for evaluation of immunomodulatory activity using CP induced immunosuppression model and neutrophil adhesion test.[16]

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

The CP was suspended in 0.5% Carboxy methyl cellulose (CMC) 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 1 (Control group) received CMC for 14 days and group 2 (Challenge group) received CMC for 10 days, on 11th, 12th and 13th day CP intraperitonially at a dose of 30 mg/kg b/w. Groups 3 (Test group) received ethanolic extract of the drug at a dose of 500 mg/kg body weight orally for 14 days. On days 11, 12 and 13th day CP solution was given intraperitonially at a dose of 30 mg/kg b/w one hour after the administration of the extract.[17] This experimental study was a part of a 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_{50} value. The dose determination study had showed that drug has the maximum therapeutic effect between the range of 500 mg^{-1} g. Thus, 500mg dose was selected to evaluate the immunomodulator effect of the drug.

Hematological test

At the end of the treatment, mice were anesthetized by using di-ethyl ether. The blood was collected from the retro-orbital plexus using heparinized capillary tubes and hematological tests were carried out. The WBC count was done by Turke's method,[18] RBC by Hayem's method,[19] and haemoglobin by Sahli's method.[20] The results are shown in [Figures 1-4].

Neutrophil adhesion test

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 80 mg/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.[21]

Percent neutrophil adhesion was calculated as shown below

Table 1: Contents of *Shirishadi* compound

Name of the drug	Botonical name	Part uesd	Approx. quantity in 100 ml of extract (mg)
Shirisha	*Albezzia lebbeck (L.) Benth.*	*Twaka* (Bark)	20
Nagarmotha	*Cyprus rotundus (L.)*	*Kanda* (Rhizome)	20
Kantkari	*Solanum surattense*	*Panchanga* (whole plant)	20

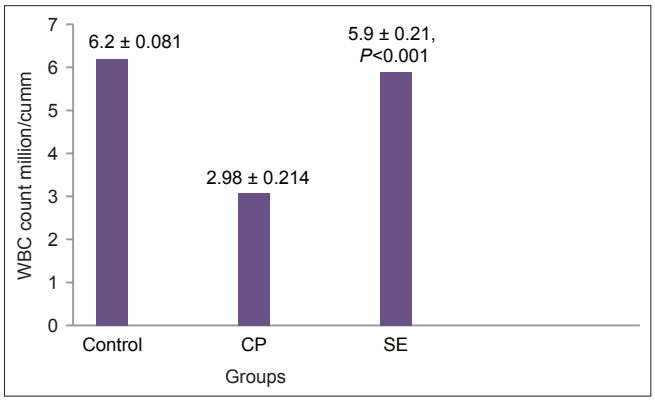

Figure 1: Effect of hydroethanolic extract of *Shirishadi* (SE) on WBC Count in CP treated mice. All values are mean ± SEM, *n* = 6. ****P* < 0.001 when compared with control group and, ###*P* < 0.001, when compared with cyclophosphamide (CP) treated group (Students *t* test). b*P* < 0.01, when compared with CP treated group (one way ANOVA)

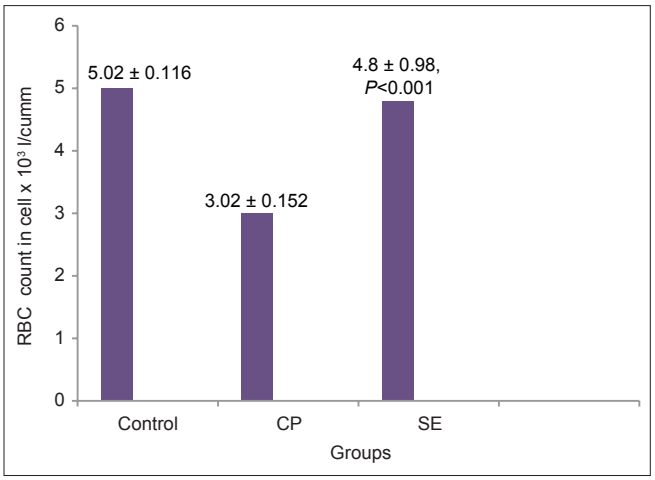

Figure 2: Effect of hydroethanolic extract of *Shirishadi* compound on RBC count in CP treated mice. All values are mean ± SEM, *n* = 6. ****P* < 0.001 when compared with control group and, ###*P* < 0.001, when compared with CP treated group (Students *t* test). b*P* < 0.01, when compared with CP treated group (one way ANOVA)

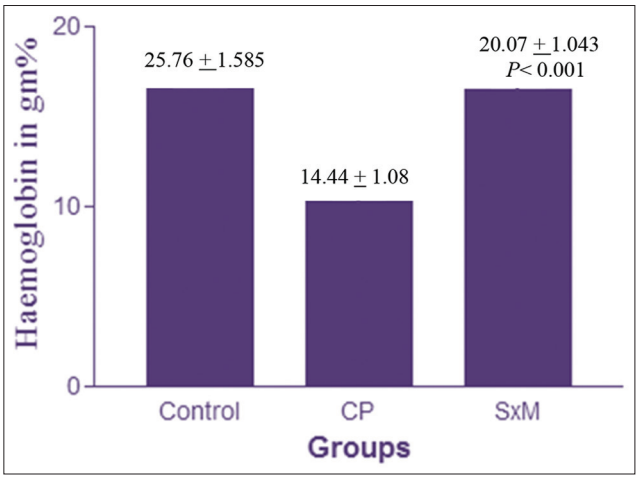

Figure 3: Effect of hydroethanolic extract of *Shirishadi* compound on Haemoglobin estimation in CP treated mice. All values are mean ± SEM, *n* = 6. ****P* < 0.001 when compared with control group and, ###*P* < 0.001, when compared with CP treated group (Students *t* test). b*P* < 0.01, when compared with CP treated group (one way ANOVA)

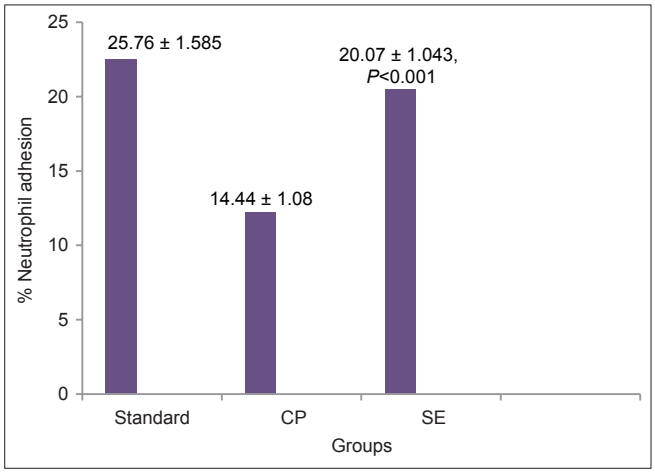

Figure 4: Effect of hydroethanolic extract of *Shirishadi* compound on neutrophil adhesion test in CP treated mice. All values are mean ± SEM, *n* = 6. ***P* < 0.01 when compared with control group, ###*P* < 0.001 when compared with CP treated group (Students t' test). b*P* < 0.01 when compared with CP treated group (one way ANOVA)

Neutrophil adhesion (%) = NI u – NI t × 100

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 ± standard error 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 against drug-induced immunosuppression

Administration of CP (30 mg/kg, i.p) produced a significant decrease in the Total Leukocyte Count from 6.2 ± 0.081 to 2.98 ± 0.214, RBC count from 5.02 ± 0.116 to 3.02 ± 0.152, and % hemoglobin from 15.49 ± 0.081 to 9.32 ± 0.153 ($P < 0.01$). This was found to be consistent with earlier studies which state that CP induces immune dysfunction through reactive intermediate-induced damage to the cells of the immune system.[18] Evaluation of effect of ethanolic extract of *Shirishadi* Compound on CP induced immunosuppression indicated good protection by increasing all the haematological parameters. WBC count, RBC count, and % hemoglobin values observed were better than untreated control groups [Figures 1-3].

Neutrophil adhesion test

This test is an indicative of the marginalization of phagocytic cells in the blood vessels, i.e. an indication

of immunostimulation. The % neutrophil adhesion in control group animals was, 25.76 ± 1.585, in CP treated group it was 14.44 ± 1.08, in drug treated group it was 20.07 ± 1.043 [Figure 4. The results of neutrophil adhesion test indicate that there was significant ($P < 0.001$) increase in neutrophil adhesion after administration of ethanol extract.

Discussion

Shirishadi compound is a polyherbal preparation consisting of *Shirisha* (*Albezzia lebbeck*), *Nagarmotha* (Cyprus *rotundus*) and *Kantakari* (*Solanum surattense*) prescribed in Ayurveda for several several diseases and its constituents have been investigated for different pharmacological properties. [1-11] Use of herbs for improving the overall resistance of body against common infections and pathogens has been a guiding principle of Ayurveda. The compound has been used and reported in many such formulations. However, there is no systematic study of its immunomodulatory activity. Hence in the present study, the immunomodulatory activity of ethanol extract of this polyherbal preparation was investigated.

The dynamic and complex nature of immune system can be better understood after immune challenge thus cyclophosphamide induced immune suppressive mice model is most reliable method for evaluation of immunomodulation effect. Haematological parameter such as Total WBC, RBC, Haemoglobin and neutrophil constitutes the key components of the immune system. A rise or fall in the number of these cells affects the health/immune constitution of the body as they are known to recognize the foreign antigens and mount an immune response.[15] Hence these parameters were chosen to study the immunomodulatory activity of the ethanol extract of *Shirishadi* compound.

The study affirms that ethanolic extract of the *Shirishadi* Compound is an effective immunomodulatory agent. The effectiveness of extract-treated animals in overcoming the side-effects of CP induced immunosuppression provides evidence for balancing and adaptogenic effectiveness of extract. The extract potentiated the non-specific immune response. This may be attributed to different phytoconstituents. Increase in percentage of neutrophil is attributed to marginalization of phagocytic cells i.e. improved defensive response under normal circumstances. Thus, with the result of this preliminary study it can be concluded that the *Shirishadi* compound holds the promise for being used as an immunostimulating agent.

Conclusion

The ethanolic extract of *Shirishadi* compound has protected the mice against CP induced immunosuppression indicating its profound immunostimulatory activity.

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हिन्दी सारांश

शिरीषादि कम्पाउण्ड के एल्कोहलीय अर्क का इम्युनोमोडुलेट्री प्रभाव

कजारिया दिव्या, त्रिपाठी जे. एस., तिवारी एस. के., पंड्या बी. एल.

शरीर की प्रतिरक्षा प्रणाली को नियमित करने वाले पदार्थों को इम्युनोमोडुलेट्री कहते है । यहां नियमित करने का अर्थ प्रतिरक्षा प्रणाली को सम्यक बनाये रखना हैं, अर्थात जिन पदार्थो के द्वारा इस प्रणाली को साम्यावस्था में बनाये रखते हुयें सुचारू रूप से कार्यकारी बनाया जाता है उन्हें इम्युनोमोडुलेट्री (व्यधिशक्ति नियंत्रक) कहते है । पादप औषधियों द्वारा संक्रमण से शरीर की रक्षा मुख्यतः दो प्रकार से होती है – १. संक्रमण कारक जीवाणुओं का नाश करके २. शारीर की प्रतिरक्षा प्रणाली को मजबूत बनाकर । शिरीषादि यौगिक का प्रयोग मुख्यतः अनूर्जता जनित व्याधियो के नाश हेतु होता है यथा allergic rhinitis, allergic asthma इत्यादि । प्रस्तुत शोध कार्य में शिरीषादि यौगिक के इम्युनोमोडुलेट्री गुण का परीक्षण किया गया । इस शोध कार्य के अन्तर्गत शिरीषादि यौगिक के अल्कोहलीय अर्क (Ethanolic extract) का प्रयोग एक विशिष्ट जाति के चूहों (wistar albino rats) पर किया गया है । इस शोध कार्य में Cyclophosphamide द्वारा शरीरस्थ प्रतिरक्षा प्रणाली में होने वाले क्षय तथा औषध द्वारा इस क्षय की प्रतिपूर्ति को मानक के रूप में प्रयोग किया गया हैं । इस प्रयोजनार्थ CP की 30mg/kg शारिरिक भार की मात्रा तथा शिरीषादि यौगिक की 500 mg/kg शारीरिक भार की मात्रा का प्रयोग किया गया हैं । शारीरिक प्रतिरक्षा प्रणाली में औषध द्वारा होने वाली वृद्धि का मुल्यांकन १४ दिनों पश्चात् विभिन्न रक्त परीक्षणों एवं Neutrophil adhesion परीक्षा द्वारा किया गया है । इस शोधकार्य में पाया गया कि शिरीषादि यौगिक के द्वारा प्रतिरक्षा प्रणाली में महत्वपूर्ण वृद्धि गयी । शिरीषादि यौगिक के प्रयोग से WBC, RBC, Hb% तथा Neutrophil इत्यादि में महत्वपूर्ण वृद्धि पायी हुई । इस शोध–कार्य से ये स्पष्ट होता है कि इम्युनोमोडुलेट्री यौगिक एक सशक्त एवं प्रभावी औषधि है ।