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## Effect of Herbal Preparations on Cellular Immunity in Rats.

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### ABSTRACT

The present study was planned to evaluate the effect of Sitopaladi churna & a herbal preparation on cell mediated immunity: by haematological parameters & by phagocytic activity of polymorpho nuclear (PMN) cells in rats. Effect of Sitopaladi churna (1000 mg/ kg), Decoction of herbs (10 ml/ kg) containing Embelia ribes seeds, Cymbopogon citratus (lemon grass), Zingiber officinale (ginger), Ocimum Sanctum (tulsi) on cell mediated immunity was evaluated by haematological parameters and by studying phagocytic activity by PMN cells. Both & herbal preparations shown increased cellular immunity as compared to control. Both herbal preparations shown comparable results with Septilin syrup (marketed by Himalaya) which was used as positive control ( $P > 0.05$ ). The study demonstrates that herbal preparations used in this study trigger cell mediated immunity.

**Keywords:** Cell mediated immunity, Herbal preparations, Haematological parameters, Sitopaladi churna, Phagocytic activity

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## INTRODUCTION

There are two types of immune system that is innate and adaptive immune system. If a pathogen reaches the barriers, the innate immune system provides immediate, but nonspecific responses. The cells of the immune system are special types of leukocytes called lymphocytes. B cells and T cells are the major types of lymphocytes and are derived from pluripotential hematopoietic stem cells in the bone marrow. B cells are involved in the humoral immune response, whereas T cells are involved in cell-mediated immune responses. The B cell antigen specific receptor is an antibody molecule on the B cell surface and recognizes whole pathogens without any need for antigen processing. Macrophages play a significant role in the regulation of Immunological reactions through various functions including phagocytic elimination of foreign or denatured substances and secretion of cytokines and reactive oxygen species<sup>1</sup>.

Compounds that are capable of interacting with the immune system to up regulate or down regulate specific aspects of the host response can be classified as immune modulators. Those compounds which appear to stimulate the human immuneresponse are being sought for the treatment of cancer, immunodeficiency diseases, or conditions associated with immunosuppression. They are used for combinational therapy with antibiotics; and as adjuvants for vaccines<sup>2</sup>. Whereas immune suppressants are used reduce the immune response of the body against antigen, and are used mainly in organ transplants.

Plant extracts are among the attractive sources of new drugs and have been shown to induce promising immunostimulatory effects<sup>2</sup>. Plant and plant products are being used as a source of medicine since long. Non – toxic herbal preparations are used to improve the general health by stimulating body's immunity. Herbal preparation may be effective and safer than conventional medicines. Herbal immunomodulating agents might be able to provide an alternative to costly immunotherapeutic agents<sup>3</sup>.

Sitopaladi churna is a recipe of traditional Ayurvedic pharmacopoeia well known and effective in relieving coughs associated with various respiratory disorders. One of its ingredient , the herb pipli (alcoholic extract) has been reported to have immunomodulatory activity<sup>4 - 6</sup>. Ocimum Sanctum , one of the ingredient of decoction of herbs has been reported to have immunomodulatory activity<sup>7-9</sup>.

Sitopaladi churna & decoction of herbs are claimed to have immunostimulant activity, but not yet proved by animal/ clinical studies. Therefore this study was planned.

Daswani et al, have studied immunomodulatory activity of septilin. So Septilin syrup has been

used as positive control. The current study aimed at exploring effect of these herbal preparations on the cellular immunity in rats.

## MATERIAL AND METHODS:

Experimental protocol was approved by Institutional Animal Ethical Committee (IAEC). Sprague Dawley rats weighing 200-250gms housed in polypropylene cages were used. They were fed pellet diet and water *ad-libitum*. The rats were maintained under standard conditions of temperature ( $25^{\circ}\text{C} \pm 5^{\circ}\text{C}$ ) and relative humidity ( $55 \pm 10\%$ ) & 12 hours night & day cycle. Rats of either sex were used.

### Study treatment :

- 1. Sitopaladi Churna:** manufactured by Shree Baidyanath Ayurved Bhavan pvt. Ltd. was purchased from market. It is a mixture of powders of *Bombusa arundinacia* (Ext), *Cinnamomum zeylanicum*, *Elettaria cardamomum* (Fruit), *Piper longum* (Fruit), Sugar candy.
- 2. Decoction of herbs:**

### Preparation of decoction of herbs:

The herbs *Embelia ribes* seeds, *Cymbopogon citratus* (lemon grass), *Zingiber officinale* (ginger), *Ocimum Sanctum* leaves were purchased from local market. To prepare decoction, 100 ml of purified water was taken, heated to boiling, then ingredients of Table 1 were added. Boiling was continued till the quantity of water became half. It was allowed to cool & filtered. The decoction of herbs was freshly prepared daily.

**Table 1: Composition of decoction of herbs**

Ingredients	Quantity
<i>Embelia ribes</i> seeds	10 nos
<i>Cymbopogon citratus</i> (lemon grass)	2 leaves
<i>Zingiber officinale</i> (ginger)	1/2"
<i>Ocimum Sanctum</i> leaves	10 leaves

**Septilin syrup: manufactured by Himalaya was purchased by market.**

### Experimental Design:

Animals were divided into four groups, having eight rats in each groups.

Group I: Vehicle for Control

Group II: Septilin Syrup (Dose 2 ml/ kg) (Positive control).

Group III: Sitopaladi churna (Dose 1000 mg/ kg).

Group IV: Decoction of herbs (Dose 10 ml/ kg).

All the four groups received the respective vehicle / test drug daily for 28 days by oral route of drug administration.

**Blood collection from animals:**

On 29<sup>th</sup> day animals were anaesthetized for blood sampling. Blood samples were collected by retro –orbital puncture using capillary tubes.

**Haematological analysis**

The fresh whole blood samples were used for the estimation of total leucocyte counts by using Neubauer's counting chamber & differential leucocyte counts were done by fixing blood smears & staining with Leishman's stain.

**In vitro phagocytic activity of polymorphonuclear cells:**

The fresh whole blood drops (4-5) were collected on a clean, dry glass slide and placed in a moist chamber to permit adherence of PMN cells, after which the clot was gently removed without disturbing the adherent PMN cells. This layer of PMNs was covered with a suspension of *Candida. albicans* (yeast cells) (106 candida/mL) and incubated for 1 h. The slide was then stained with Giemsa stain and the effect of herbal preparations on phagocytic activity was expressed as the percentage of cells showing phagocytosis and phagocytic index as the average number of *Candida* per PMN<sup>10</sup>.

**Statistical analysis:**

All the results were expressed as Mean± Standard deviation (SD). Data were analyzed using one-way Analysis of Variance (ANOVA) followed by Tukey-Kramer multiple comparison test.  $p < 0.05$  were considered as statistically significant.

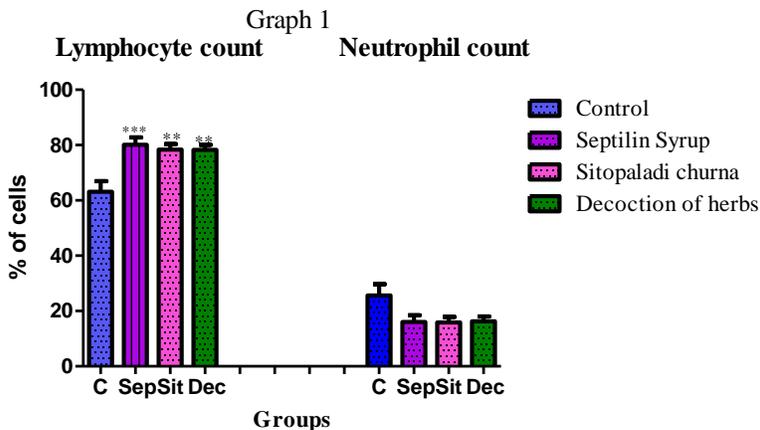
**RESULTS AND DISCUSSION:**

Bodyweights before and after drug treatment did not differ significantly from the control group ( $p > 0.05$ ) (Table 2).

**Table 2: Effect of herbal preparations on weight gain (n= 8 per group)**

	Control	Septilin syrup	Sitopaladi churna	Decoction of herbs
Weight gain in gm	28.5± 13.82	30.63±22.35	33.88±29.43	32.75±21.80

There was significant increase in lymphocyte count in Decoction of herbs treated group & Sitopaladi churna treated group ( $p < 0.01$ ) when compared to control. Increase in lymphocyte count of both the groups was comparable with Septilin syrup treated group ( $p > 0.05$ ) Table 3, Graph 1. Neutrophil count was not significantly different from the control group ( $p > 0.05$ ) Table 3, Graph 1.



**Graph 1: Effect of study treatments on lymphocyte and neutrophil count.**

\*\*\*Control vehicle Septilin Syrup P<0.001

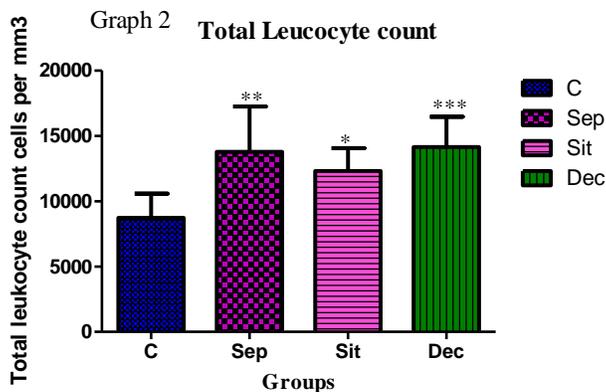
\*\*Control vehicle Sitopaladi churna P<0.01

\*\*Control vehicle Decoction of herbs P<0.01

Total leukocyte count increased in both Decoction of herbs treated group(p<0.001) & Sitopaladi churna treated group (p< 0.05) when compared to control. Increase in total leukocyte count of both the groups was comparable with Septilin syrup treated group (p> 0.05)Table 3, Graph 2.

**Table 3: Effect of herbal preparations on haemogram (n=8 per group)**

	Control	Septilin Syrup	Sitopaladi churna	Decoction of herbs
Total leukocyte count cells per mm <sup>3</sup>	8700 ±1900	14000±3500	12000±1800	14000±2300
Lymphocyte count (%)	63 ± 11	80 ± 7.5	78 ± 5.7	78 ± 5.4
Lymphocyte count(Absolute)	5600 ±1800	11000 ±3200	9700±2000	11000±2100
Neutrophil count (%)	26 ± 11	16 ± 6.9	16 ± 5.6	16 ± 5.1
Neutrophil count (Absolute)	2200±930	2100±880	1900±630	2300±870



**Graph 2: Effect of study treatments on leucocyte count.**

\*\*Control vehicle Septilin Syrup P<0.01

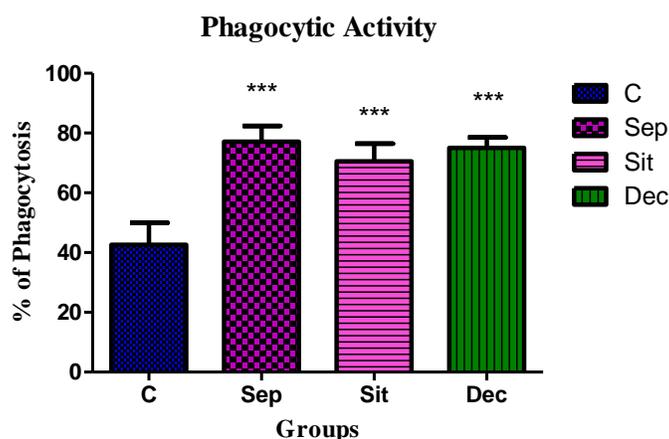
\*Control vehicle Sitopaladichurna P<0.05

\*\*\*Control vehicle Decoction of herbs P<0.001

There was increase in phagocytic activity in Decoction of herbs treated group as well as Sitopaladi churna treated group (p< 0.001) when compared to control & were comparable with Septilin syrup treated group. (Table 4, Graph 3)

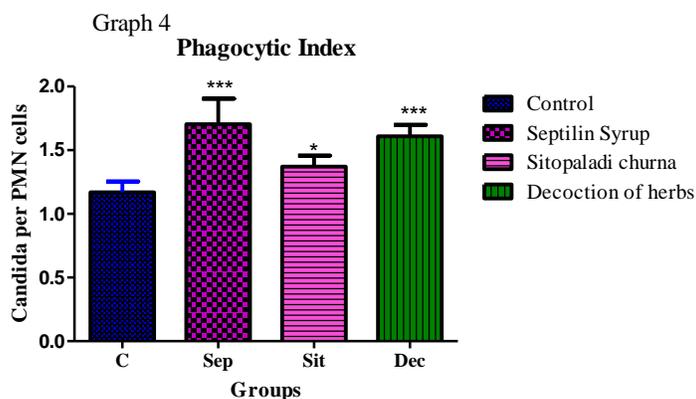
**Table 4: Effect of herbal preparations on Phagocytic activity (n= 8 per group)**

	Control	Septilin Syrup	Sitopaladi churna	Decoction of herbs
PMN showing phagocytosis	43 ± 7.4	77 ± 5.2	71 ± 5.9	75 ± 3.5
Phagocytic index	1.2 ± 0.08	1.7 ± 0.20	1.4 ± 0.08	1.6 ± 0.09



**Graph 3: Effect of study treatments on phagocytic activity.**

Phagocytic index was increased in Decoction of herbs treated group (p<0.001) when compared to control & was comparable with Septilin syrup treated group. There was increase in phagocytic index in Sitopaladi churna treated group (p< 0.05) when compared to control. But the phagocytic index of Sitopaladi churna treated group was less than Septilin syrup & Decoction of herbs treated groups. (Table 4, Graph 4)



**Graph 4: Effect of study treatments on phagocytic index.**

\*\*\*Control vehicle Septilin Syrup  $P < 0.001$

Control vehicle Sitopaladichurna  $P < 0.05$

\*\*\*Control vehicle Decoction of herbs  $P < 0.001$

Immunomodulation is a procedure which can alter the immune system of an organism by interfering with its functions. If it results in an enhancement of immune reactions it is named as an immunostimulative drug which primarily implies stimulation of specific and non specific system, i.e. granulocytes, macrophages, complement, certain T & B-lymphocytes and different effect or substances. Immuno-suppression implies mainly to reduce resistance against infections, stress and may occur on account of environmental or chemotherapeutic factor<sup>11, 12</sup>.

The present study shows increase in lymphocyte count (T and B) and total leucocyte count in both Sitopaladi churna & Decoction of herbs treated groups. Both the study treatments showed comparable results with Septilin treated group marketed by Himalaya as a immunostimulator.

The role of phagocytosis is the removal of microorganisms and foreign bodies, dead or injured cells. The increase in the phagocytic activity reflects the enhancement of the phagocytic function of mononuclear macrophage and nonspecific immunity. Phagocytosis by macrophages is important against the smaller parasites and its effectiveness is markedly enhanced by the opsonisation of parasites with antibodies and complementing C3b, leading to a more rapid clearance of parasites from the blood. The present study shows increase in phagocytic activity in both Sitopaladi churna & Decoction of herbs treated groups. The results shown comparable results with Septilin treated group marketed by Himalaya as a immunostimulator. However, the effect of Decoction of herbs & Septilin syrup on phagocytic index is better than that of Sitopaladi churna treated group.

The significant increase in the levels of lymphocytes, total leucocyte count, phagocytic activity confirms the cell mediated immunity of Sitopaladi churna & Decoction of herbs.

#### CONCLUSION:

These herbal preparations have shown promising results in this model. They are also being studied in another model i.e. *E. coli* induced sepsis. Further nitric oxide (NO) has been shown to exert immunomodulatory effects including effects on immune cell adherence & function, cellular proliferation & cytokine production<sup>13</sup>. We further propose to study role of NO in immunomodulatory activity of these herbal preparations. Considering the results of these studies, these herbal preparations may be studied in clinical set up.

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