Original article:

Study of immunomodulatory effect of sitopaladi churna in mice

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Abstract:

Title: To study immunomodulatory effect of sitopaladi churna in mice.

OBJECTIVES:

1. To evaluate immunomodulatory activity of sitopaladi churna by determining host resistance against E.Coli induced sepsis in mice
2. To elucidate probable mechanism of action of sitopaladi churna.

METHODS: Immunomodulatory activity of sitopaladi churna was evaluated by determining host resistance against E.coli induced sepsis. The mechanism of action was studied in mice which survived from sepsis, with the help of histopathology of spleen, thymus along with evaluation of bone marrow cells.

RESULTS: The sitopaladi churna has increased the survival in sepsis induced mice. It has increased cellularity in PALS, marginal zone & follicles of spleen as well as cortex, medulla of thymus. The test drug also increased myeloid & lymphoid cells in bone marrow. All the results were statistically significant as compared with control & were comparable with septilin syrup group which was used as standard treatment.

CONCLUSION: Pretreatment with sitopaladi churna before induction of sepsis have shown prominent increase in the survival rate due to its immunostimulant activity.

Keywords: sitopaladi churna, sepsis, spleen, thymus, bone marrow

Introduction:

Sepsis is the leading cause of death in critically ill patients in the developed world. The overall mortality rate in patients of sepsis is 25–30% and mortality in patients with abdominal sepsis can be as high as 60%. Although several bacteria have been identified as causative organisms in peritonitis, Escherichia coli remains one of the most common pathogens in intraperitoneal infections as reported by Rosemarijn Renckens et al [1]. Richard S. Hotchkiss et al[2] have done three independent autopsy studies in adult, paediatric, and neonatal patients who died of sepsis.

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In these studies they observed that there was profound depletion of T and B lymphocytes in these patients. Their studies in both primate models and humans with sepsis have shown that apoptosis is the pathogenic mechanism responsible for the death of lymphocytes. Extensive apoptosis of lymphocytes and dendritic cells is most likely an important factor contributing to the immunosuppression that is a hallmark of patients with sepsis. It is also reported that sepsis causes multiple organ failure & increases apoptosis of T cells in thymus as well as in spleen and bone-marrow.

Peritonitis is a life-threatening situation; the host's capacity to control the bacterial load within the peritoneum may decide the survival of host. Peritoneal infection models in the mouse using Escherichia coli involve large numbers of bacteria to initiate the infection[3]. Therefore in this study Escherichia coli were used to induce sepsis in mice.

Sitopaladi churna is a recipe of traditional ayurvedic pharmacopoeia. It is well known to be effective in relieving cough associated with various respiratory disorders[4].

Septilin is a marketed immunostimulant preparation. Sharma S.B et al have recommended use of septilin for immunosuppressed high risk patients[5]. Many scientists, have studied immunomodulatory activity of septilin in animals[5-7]. Therefore in this study septilin syrup has been used as standard drug.

This study evaluated immunomodulatory activity of sitopaladi churna in sepsis model & its mechanism of action. The parameters studied were % survival, evaluation of lymphoid & myeloid cells in bone marrow, histopathology of spleen, thymus to check immunostimulant activity of the study treatments.

Materials and Methods:

Experimental protocol was approved by Institutional Animal Ethical Committee (IAEC).

Swiss Albino mice weighing 20-25 g housed in polypropylene cages were used. They were fed pellet diet and water ad-libitum. They were maintained under standard conditions of temperature (25 ^{o}C ±5^{o}C) and relative humidity (55±10%) along with 12 hours night & day cycle. Animals of either sex were used.

Study Treatments:

1. Sitopaladi churna:

Manufactured by Shree Baidyanath Ayurved Bhavan Pvt. Ltd was purchased from market.

2. Septilin syrup:

Manufactured by The Himalaya Drug Company was purchased from market.

Experimental Design:

Animals were divided into three groups, with thirty mice in each group.

Group I: Vehicle for Control (0.5 % Sodium carboxy methyl cellulose in distilled water)[8]

Group II: Septilin Syrup (Dose 2 ml/ kg) (Standard therapy).

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

All the three groups received the respective study treatments daily for 28 days by oral route. On 29th day, abdominal sepsis was induced in the test mice by challenging them, intraperitoneally, with 3 x 10^{8}

CFU /ml of E. coli suspension. The test mice were observed for 7 days. The alive & dead mice were noted down, to calculate the % survival from sepsis. On the 8th day of survival, the protected mice were sacrificed. Spleen & thymus were removed and fixed in 10 % formalin. After processing, five micrometer sections were stained with H&E and analyzed by a pathologist who was blinded for groups. Cellular density was studied in compartments of spleen like : - a)periarterial lymphoidal sheath (PALS) b)marginal zone (MZ) and c)follicles. Cellular density was studied in compartments of thymus like : - a) cortex and b) medulla. Each parameter was graded on a scale from 0 to 4, as follows: 0 = normal, 1 = minimal, 2 = mild, 3 = moderate, 4 = marked. The total immunostimulant score was expressed as the sum of the scores for all parameters; the maximum values were 16 [9].

To evaluate the myeloid & lymphoid cells, slides were prepared from bone marrow of femur of the same mice. The slides were dried, fixed with methanol, stained with May Grunwald- Giemsa stain & the myeloid & lymphoid (granulocytic) cells were calculated [10].

Statistical analysis:

Data were analysed using the statistical software program Prism (GraphPad). The host resistance against E. coli induced abdominal sepsis results were analysed using the Chi-square test. The effect of test drugs on spleen & thymus results were analysed using kruskal wallis test followed by Dunns test for multiple comparison. The effect of test drugs on myeloid cells were analyzed using one-way Analysis of Variance (ANOVA) followed by Tukey-Kramer multiple comparison test. For all tests $p<0.05$ was considered as statistically significant.

Results:

Table I: Effect of test drugs on E. coli induced sepsis in mice (n = 30 per group)

Results expressed as % survival.

Treatment	Dead	Alive	% survival
Vehicle treated	25	05	16.60
Septilin syrup	20	10	33.00**
Sitopaladi churna	18	12	40.00**

**= comparison with vehicle treated group (P<0.01).

Statistically significant increase in survival was seen with test drug ie sitopaladi churna treated group when compared to control group & the increase was comparable with the septilin syrup treated group used as a standard therapy. **Table I, Fig. 1.**

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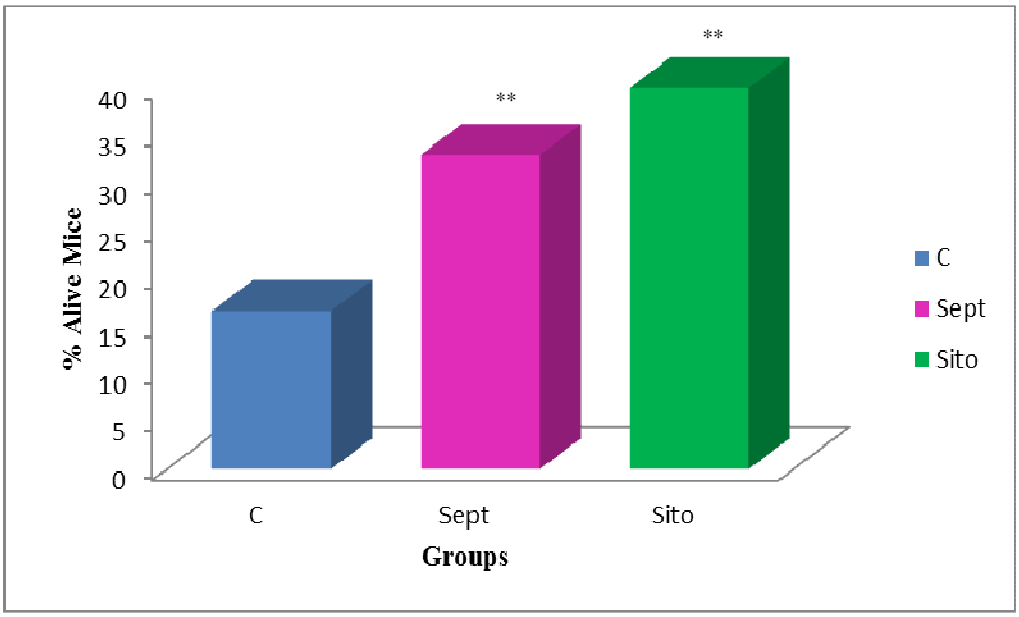
Fig. 1 % survival from sepsis



**= comparison with vehicle treated group (P<0.01)

Table II: Effect of test drugs on Periarterial lymphoidal sheath of spleen

Periarterial lymphoidal sheath (PALS) was screened for number of lymphocytes.

(n= 16 per group) Results expressed in median.

Groups	**n**	**Number of lymphocytes (scores)**
Vehicle treated	5	1.00
Septilin syrup	10	8.00 ***
Sitopaladi churna	12	6.50*

*** = Comparison with vehicle treated group (P< 0.001)

* = Comparison with vehicle treated group (P< 0.05)

The score of periarterial lymphoidal sheath of spleen in sitopaladi churna treated group was statistically significant as compared to vehicle treated group & the results were comparable with septilin syrup treated group used as a standard therapy. **Table II, Fig. 2**.

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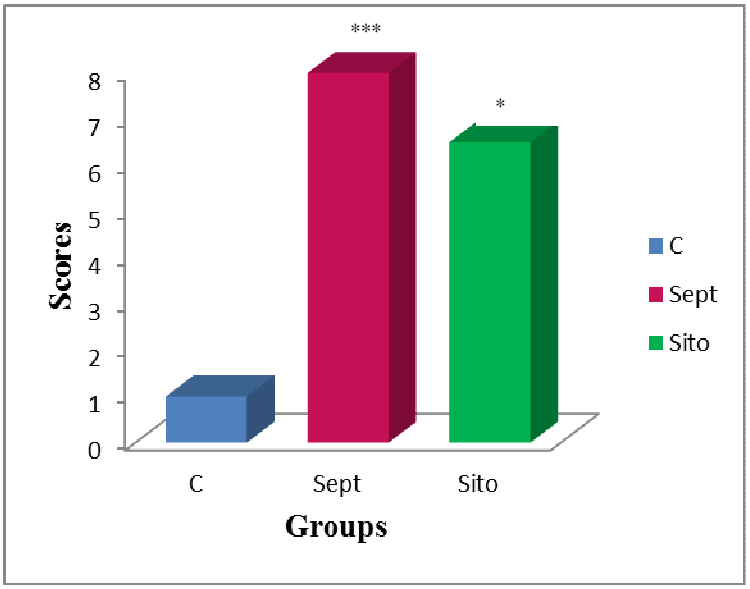
Fig. 2 Effect of drugs on periarterial lymphoidal sheath of spleen



*** = Comparison with vehicle treated group (P< 0.001)

* = Comparison with vehicle treated group (P< 0.05)

Table III: Effect of study treatments on marginal zone of spleen

Marginal zone was screened for number of lymphocytes.

Results are expressed in median.

Groups	**n**	**Number of lymphocytes (scores)**
Vehicle treated	5	1.00
Septilin syrup	10	4.50 **
Sitopaladi churna	12	4.00*

*= Comparison with vehicle treated group (P<0.05)

** = Comparison with vehicle treated group (P< 0.01)

Score of marginal zone of spleen was statistically significant with sitopaladi churna treated group as compared to control & was comparable with standard therapy ie septilin syrup treated group. **Table III, Fig. 3.**

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Table IV: Effect of study treatments on follicles of spleen

A follicle was screened for number of lymphocytes and germinal centres.

Results are expressed in median.

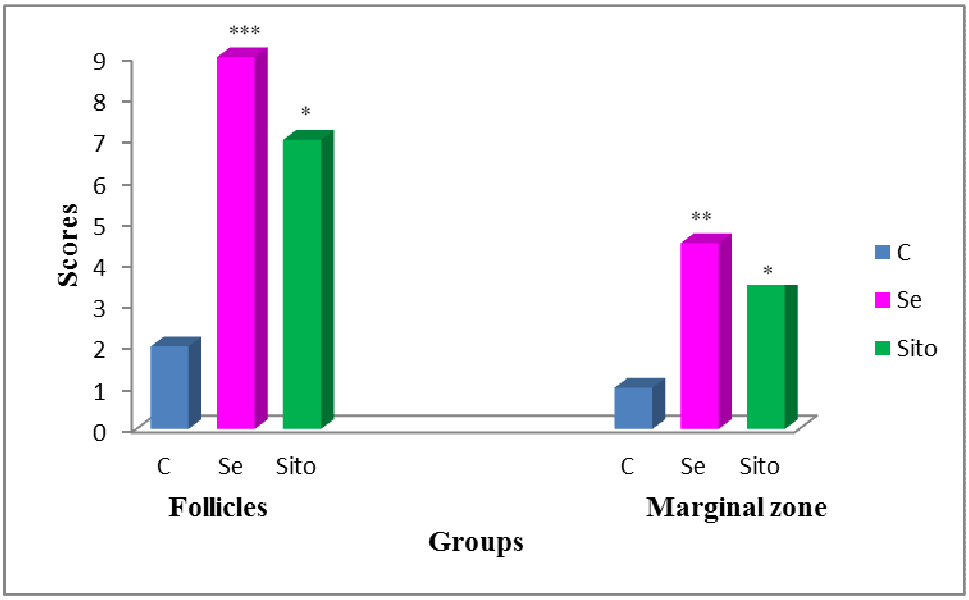
Groups	N	Number of lymphocytes & germinal centres (scores)
Vehicle treated	5	2.00
Septilin syrup	10	9.00 ***
Sitopaladi churna	12	7.00*

*= Comparison with vehicle treated group (P<0.05)

*** = Comparison with vehicle treated group (P< 0.001)

Score of follicles of spleen in sitopaladi churna treated mice was significantly higher as compared to vehicle treated group. However there was no significant difference between standard therapy i. e. septilin syrup treated group, they were comparable. **Table IV, Fig. 3.**

Fig. 3 Effect of study treatments on Follicles & marginal zone of spleen



*= Comparison with vehicle treated group (P<0.05)

** = Comparison with vehicle treated group (P< 0.01)

*** = Comparison with vehicle treated group (P< 0.001)

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Table V: Effect of study treatments on cortex of thymus

Thymus cortex was screened for number of lymphocytes.

Results are expressed in median.

Groups	n	Number of lymphocytes (scores)
Vehicle treated	5	1.0
Septilin syrup	10	4.0**
Sitopaladi churna	12	4.0*

** = Comparison with vehicle treated group (P< 0.01)

*= Comparison with vehicle treated group (P<0.05)

Sitopaladi churna treated group has shown significantly higher score in cortex of thymus in protected mice when compared to vehicle treated group. The results were comparable with septilin syrup treated group which was used as standard therapy. **Table V, Fig.4.**

Table VI: Effect of study treatments on medulla of thymus

Thymus medulla was screened for number of lymphocytes.

Results are expressed in median.

Groups	n	Number of lymphocytes (scores)
Vehicle treated	5	1.00
Septilin syrup	10	4.0**
Sitopaladi churna	12	4.0*

*= Comparison with vehicle treated group (P<0.05)

** = Comparison with vehicle treated group (P< 0.01)

The score with sitopaladi churna treated group is significantly more as compared to vehicle treated group. The results were comparable with septilin syrup treated group which was used as standard therapy. **Table VI, Fig. 4**.

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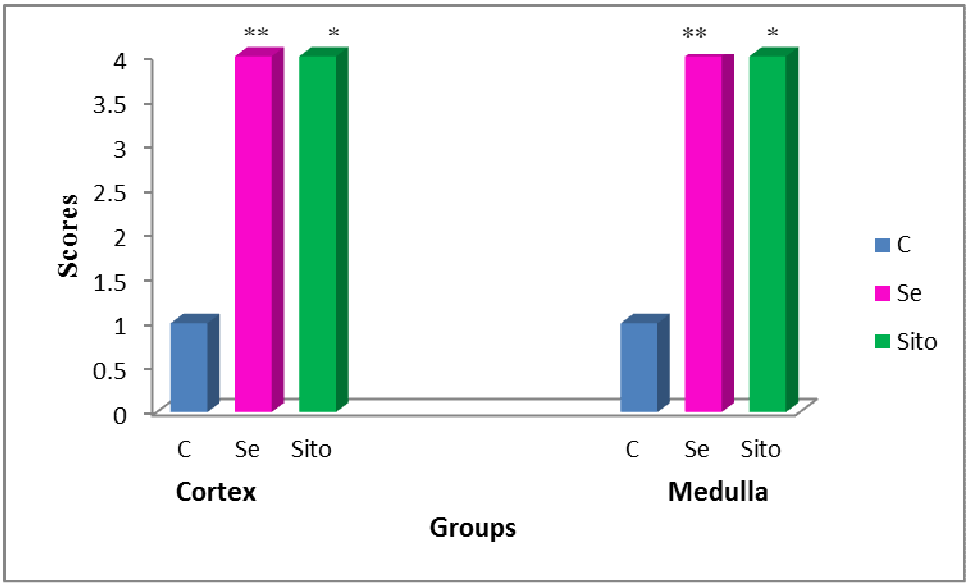
Fig. 4 Effect of study treatments on cortex & medulla of thymus



*= Comparison with vehicle treated group (P<0.05)

** = Comparison with vehicle treated group (P< 0.01)

Table VII: Effect of study treatments on myeloid & lymphoid cells

Results expressed in mean ± SD.

Groups	**n**	**% leucocytes**
Vehicle treated	5	70±4.6
Septilin syrup	10	72±5.8
Sitopaladi churna	12	80±5.3 ** $$

**= Comparison with vehicle treated group (P<0.01)

$$ = Comparison with septilin syrup treated group (P<0.01)

There was statistically significant increase in myeloid & lymphoid cells in sitopaladi churna treated group as compared to control group as well as septilin syrup treated group which was used as standard therapy. **Table VII Fig. 5**.

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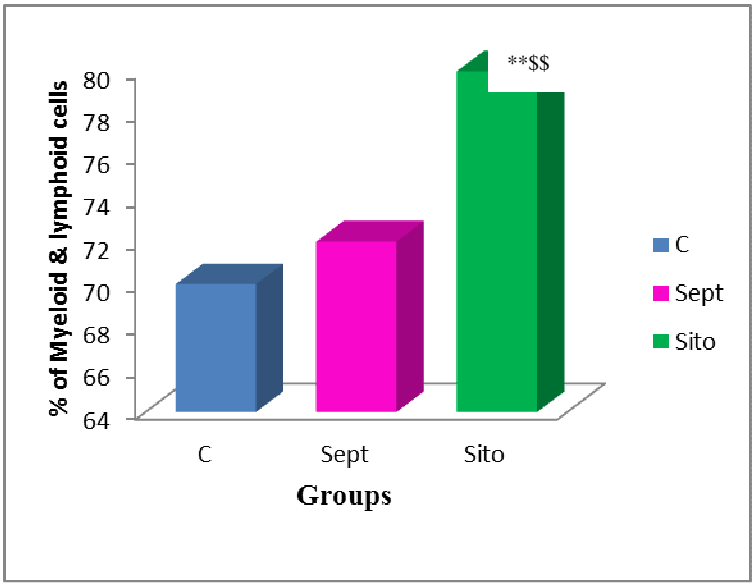
Fig. 5 Effect of study treatments on myeloid & lymphoid cells



** = Comparison with vehicle treated group (P< 0.01)

$$ = Comparison with septilin syrup treated group (P<0.01)

Discussion:

Sepsis is a complex clinical syndrome which results due to damaged host response to infection & consumes vast healthcare resources[11]. In spite of this, mortality is very high in patients with severe sepsis. There is need of new adjunctive therapies for treating sepsis. As per Kruger et al the new therapies will overcome the mortality problem if they possess immune-modulating properties [12].

Maronpot R et. al.[13] have reported that the assessment of immunomodulatory activity requires subjective & quantitative histological evaluation of various lymphoid organs. Haley P et. al.[14] state that each lymphoid organ has separate compartments which support specific immune functions, therefore each compartment should be evaluated individually for changes.

In this study, pretreatment with sitopaladi churna for 28 days showed increased % survival from sepsis. This effect of sitopaladi churna confirms that it has immunostimulant action. Further to elucidate the mechanism of action of sitopaladi churna, histopathology of spleen, thymus & evaluation of bone marrow cells were carried out in protected mice from sepsis. It may be noted that 'n' differ in different groups due to different % of survival in these groups.

Bone marrow is a primary lymphoid organ. All the cells of the immune system are derived initially from the bone marrow. The immunomodulators affect cell production in bone marrow. Immunostimulant drug

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treatment, stimulate the cell production in bone marrow. Myeloid & lymphoid (M &L) cells were evaluated for assessing the cellularity of bone marrow[10,15-16].

The present study shows significant increase in myeloid & lymphoid cells of bone marrow in sitopaladi churna treated group than control group as well as standard drug therapy i. e. septilin syrup. This effect of sitopaladi churna indicates stimulation of production of leucocytes in bone marrow. Therefore we suggest that this indicates enhanced specific as well as nonspecific immunity with sitopaladi churna.

Radhika et. al. have reported immunostimulant activity of Andrographis paniculata leaves as it increases blood lymphocyte count & splenic lymphocyte count[17]. Spleen is the largest secondary lymphoid organ & contains about 25 % of the body's lymphocytes which initiates immune responses to blood-borne antigens and thus can respond to systemic infections[18-20].

T lymphocytes are present mainly in PALS of spleen. B lymphocytes & few macrophages are present in marginal zone as well as in follicles of spleen[21]. The results of this study shows statistically significant stimulation in T as well as B cell areas of spleen. Thus it can be said that sitopaladi churna stimulates both, cell mediated & humoral immunity via T as well as B cell stimulation in spleen.

Thymus is an important organ of immune system & plays important role in maturation of lymphocytes. [22-24]. They provide cellular immunity against intracellular microorganisms e.g. bacteria, viruses. Gail Pearse et. al.[25]reported that the cortex of thymus represents mainly premature T lymphocytes & medulla of thymus mainly contains mature T lymphocytes & few B lymphocytes.

In the present study, results of thymus histopathology, indicates that sitopaladi churna treated group shows prominent immunostimulant activity which is the result of stimulation of cell mediated immunity.

In view of this study it can be suggested that, sitopaldi churna has stimulated both cell mediated as well as humoral mediated immune response. The immunostimulant action of sitopaldi churna has helped mice to fight with the bacterial load as well as to overcome immunosuppression caused due to sepsis & thus increased survival rate in sepsis induced mice.

Conclusion:

The results of present study suggest that sitopaladi churna has statistically significant immunostimulant activity which is comparable to the standard therapy i.e. septilin syrup. Therefore therapy with sitopaldi churna may represent a novel approach in the treatment of this highly lethal disorder, as it improves survival by prevention from apoptosis of lymphocytes in sepsis. Sitopaldi churna can also be evaluated for other immunocompromised conditions like AIDS & cancer chemotherapy. It can also be used prophylactically to prevent the occurrence of the infections as it has stimulated immune system under normal conditions.

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