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## Effect of Some Immunomodulating Agents on Nitric Oxide Levels

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### ABSTRACT

This study evaluated effect of atorvastatin, sitopaladi churna and a decoction of herbs on nitric oxide (NO) levels in rats. Nitric oxide levels in blood were estimated by Cortas &Wakid method. Atorvastatin (10 mg/ kg), 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) increased nitric oxide levels as compared to control ( $p < 0.05$ ). Atorvastatin, sitopaladi churna, decoction of herbs has shown comparable results with septilin syrup (marketed by The Himalaya Drug Company) which was used as positive control ( $p > 0.05$ ). The study demonstrates that atorvastatin, sitopaladi churna & decoction of herbs increased nitric oxide levels which may mediate their immunostimulant activity.

**Keywords:** nitric oxide, atorvastatin, sitopaladi churna, a decoction of herbs, immunostimulant.

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## INTRODUCTION

In mammalian cells, NO is produced along with L-citrulline by the enzymatic oxidation of L-arginine. Intensive investigations over the past two decades have demonstrated important role of enzymatically produced NO in diverse physiological processes, many of which are relevant to understanding the pathogenesis of infection. NO may contribute to the morbidity of infection by acting as a vasodilator, myocardial depressant, and cytotoxic mediator. On the other hand, cytoprotective, immunoregulatory, and antimicrobial properties of NO have a salutary and probably essential role in the infected host. In vitro studies of phagocytic cells and a variety of microbial targets have demonstrated cytokine-inducible microbiostatic or microbicidal activity which is L-arginine-dependent and inhibitable by competitive NO synthase inhibitor such as N G -monomethyl-L-arginine. NO-donor compounds have been shown to inhibit or kill microbes when administered in vitro. Although susceptibility is not universal, NO-related antimicrobial activity has been demonstrated against a remarkably broad range of pathogenic microorganisms including viruses, bacteria, fungi, and parasites [1].

NO is involved in the pathogenesis and control of infectious diseases, tumors, autoimmune processes and chronic degenerative diseases. Protective and toxic effects of NO are frequently seen in parallel [2]. Its widespread production [by three different NO synthase [NOS] and the fact that its activity is strongly influenced by its concentration, NO continues to surprise and perplex immunologists. Today, there is no simple, uniform picture of the function of NO in the immune system [3]. Wander Rogério Pavanelli et al recognized three types of global NO-mediated functions: [i] endothelium dependent relaxation [ii] neurotransmission. And [iii] cell-mediated immune response [4].

There are controversies about the role of nitric oxide. Is it protective or toxic? The mechanism of action of immunostimulants may involve NO pathway as demonstrated by Yoshiyuki Hattori. They showed that immunostimulants increase nitric oxide [NO] and tetrahydrobiopterin [BH4] synthesis in vascular smooth muscle cells[VSMC] by co-inducing expression of an isoform of NO synthase [iNOS] and GTP cyclohydrolase I [GTPCH] [5]. This study was therefore planned to explore the effect of some immunomodulatory agents on levels of nitric oxide in rats.

Atorvastatin, sitopaladi churna & a decoction of herbs have been studied for their immunostimulant effect in different animal models by the authors. Authors found that all these test drugs have an immunostimulant action. Daswani et al, have studied immunomodulatory activity of septilin. So Septilin syrup has been used as positive control [6].

## MATERIAL AND METHODS

Experimental protocol was approved by Institutional Animal Ethical Committee [IAEC]. Sprague Dawley rats weighing 200-250 gm 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. Atorvastatin calcium:

Atorvastatin calcium was received from Emcure Pharmaceutical Pvt. Ltd., Bhosari, Pune as a gift sample.

##### 2. 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.

##### 3. 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 volume reduced to half. It was allowed to cool & filtered.

<b>Ingredients</b>	<b>Quantity</b>
Embeliaribes seeds	10 nos
Cymbopogoncitratus(lemon grass)	2 leaves
Zingiberofficinale (ginger)	1/2"
Ocimum Sanctum leaves	10 leaves

The decoction of herbs was freshly prepared daily.

##### 4. Septilin syrup:

Manufactured by The Himalaya Drug Company was purchased by market.

#### EXPERIMENTAL DESIGN:

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

**Group I:** Vehicle for Control

**Group II:** Septilin Syrup [Dose 2 ml/ kg] [Positive control].

**Group III:** Atorvastatin calcium [Dose 10 mg/ kg].

**Group IV:** Sitopaladi churna [Dose 1000 mg/ kg].

**Group V:** Decoction of herbs [Dose 10 ml/ kg].

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

Atorvastatin calcium was suspended in water containing 0.5 % Sodium Carboxy methylcellulose [7].

#### 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.

#### Estimation of nitric oxide level:

Fresh blood samples were collected [about 2.5 ml] in plain bulbs. Serum was separated after 2 hours. The levels of nitric oxide were estimated by Cortas & Wakid method by using spectrophotometer at wavelength of 545 nm [8].

#### STATISTICAL ANALYSIS:

All the results were expressed as Mean  $\pm$  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

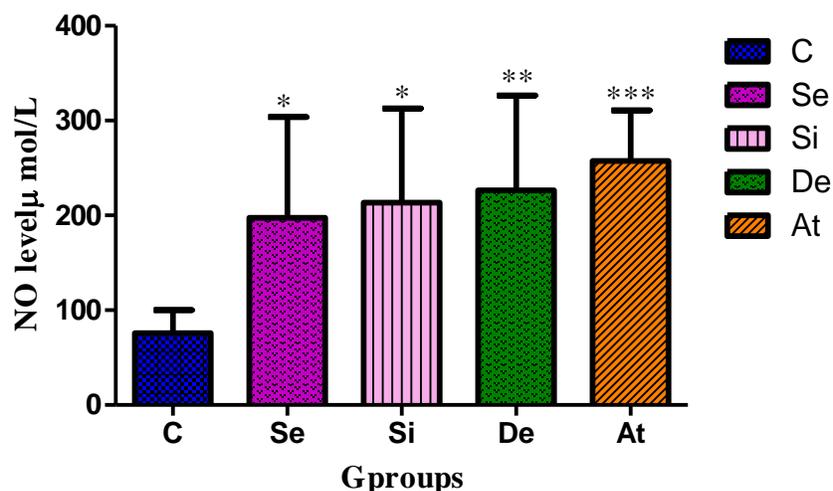
Body weights before and after drug treatment did not differ significantly from the control group [ $p > 0.05$ ] **Table 2**

<b>Table 2: Effect of study treatments on weight gain (n= 8 per group)</b>					
	Control	Septilin syrup	Sitopaladichurna	Decoction of herbs	Atorvastatin
Weight gain in gm	29 $\pm$ 14	31 $\pm$ 22	34 $\pm$ 29	33 $\pm$ 22	27 $\pm$ 17

There was significant increase in nitric oxide levels in atorvastatin, sitopaladi churna & decoction of herbs treated groups [ $p < 0.05$ ] when compared to control. Increase in nitric oxide levels in atorvastatin, sitopaladi churna & decoction of herbs treated groups was comparable with septilin syrup treated group [ $p > 0.05$ ] **Table 3, Graph 1**

<b>Table 3: Effect of study treatments on nitric oxide levels (n=8 per group)</b>					
	Control	Septilin Syrup	Sitopaladichurna	Decoction of herbs	Atorvastatin
NO level $\mu$ mol /L	76 $\pm$ 24	200 $\pm$ 110	210 $\pm$ 99	230 $\pm$ 100	260 $\pm$ 53

Graph 1: Effect of immunomodulating agents on nitric oxide levels.



\*Control vehicle Vs sepsilin syrup p<0.05  
 \*Control vehicle Vs sitopaladichurna p<0.05  
 \*\*Control vehicle Vs decoction of herbs p<0.01  
 \*\*\*Control vehicle Vs atorvastatin p<0.001

### DISCUSSION

Either iNOS [inducible nitric oxide synthase] or eNOS [endothelial nitric oxide synthase] have been found in macrophages, dendritic cells, and natural killer [NK] cells and in cell lines, clones, hybridomas and tumor cells of B or T cell origin. Whether primary T lymphocytes or B lymphocytes express any of the NOS isoforms remain questionable. The activity of NO is not restricted to the site of its production. As an uncharged gas, NO radicals are highly diffusible. Low-molecular weight S-nitrosothiols [such as S-nitrosoglutathione], S-nitrosylated proteins, and nitrosyl-metal complexes can function as long-distance NO vehicles, which liberate NO either spontaneously or after cleavage by ectoenzymes found on cells such as T and B lymphocytes.[3]

Paivi Ekman et al reported that, alpha interferon [IFN-α] brings about inducible nitric oxide synthase [iNOS] expression in normal human peripheral blood monocytes, whereas IFN-γ, together with lipopolysaccharide [LPS], has been known for years to be an efficient inducer of iNOS in rodent macrophages[9].

By Andrés Vazquez-Torres proved that Interferon γ appears to augment antibacterial activity predominantly by enhancing NO production, although a small iNOS-independent effect was also observed. [10].

In the immune system, NO mediates host protection through either directly, as a microbiostatic or microbicidal agent, or indirectly, modulating chemical modifications crucial for the biological activity of innate and acquired immunity cell lines [4].

One of the most prominent functions of NO in immune system is its participation in protective immunity against various intracellular pathogens including viruses, bacteria and protozoa. Furthermore, the killing activity of NO has also been showed effective in host defence against tumour cells and all antigens. NO production is rapidly triggered in cells of the innate immune system, after the parasite is detected and later by adaptive immune cells. A delicate, yet not completely understood, interplay exists between the components of the immune response and the concentration of NO [4].

The present study shows increase in nitric oxide levels in atorvastatin, sitopaladi churna, and decoction of herbs treated groups. The results are comparable with septilin treated group marketed by The Himalaya Drug Company as an immunostimulant. Here in this study septilin syrup was used as positive control because of previous report of Daswani et al in her study it has been shown that septilin syrup increased phagocytic activity as well as phagocytic index. Besides there are no standard immunostimulant drug except levamisole & BCG. The authors have studied immunostimulant action of atorvastatin, sitopaladi churna & decoction of herbs in different models. All these preparations have shown immunostimulant property as these preparations increased total leucocyte count, lymphocytes, phagocytic activity & phagocytic index.

Significant increase in the levels of nitric oxide in atorvastatin, sitopaladi churna, and decoction of herbs treated group confirms the role of nitric oxide in immunoregulation & these study treatments act as immunostimulants through NO pathway.

### CONCLUSION

This study has demonstrated immunostimulant effect of atorvastatin, sitopaladi churna, and decoction of herbs by elevating the levels of nitric oxide. The authors are also investigating immunomodulatory activity of atorvastatin, sitopaladi churna, decoction of herbs in E. coli induced sepsis model. It is proposed to study the immunostimulant effect of all study treatments by studying histopathological changes of spleen.

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