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## Effect of Atorvastatin on Cellular Immunity in Rats

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### ABSTRACT

This study evaluated cell mediated immunity of atorvastatin in rats. Cell mediated immunity was evaluated by haematological parameters and by studying phagocytic activity of PMN cells. The atorvastatin (10 mg/ kg) increased cellular immunity as compared to control ( $p < 0.01$ ). Atorvastatin has shown comparable results with septicin syrup (marketed by The Himalaya Drug Company) which was used as positive control ( $p > 0.05$ ). The study demonstrates that atorvastatin used in this study stimulates cell mediated immunity.

**Keywords:** cell mediated immunity, atorvastatin, haematological parameters, phagocytic activity

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## INTRODUCTION

Statins have a variety of properties that are independent of their lipid lowering ability. These anti-inflammatory, antioxidant, immunomodulatory, and antiapoptotic features have been collectively referred to as pleiotropic effects [1]. According to WulfPalinski et al, statins have immunosuppressive activity. They act by inhibiting the expression of class II major histocompatibility antigens (MHC-II) on human macrophages, endothelial cells, and smooth muscle cells (SMC) stimulated by interferon  $\gamma$  (IFN $\gamma$ ). [2]. As reported by Hsin-Yun Sun et al statins can reduce oxidative stress, suppress lymphocyte-endothelium interaction and T cell receptor expression, and modulate the adaptive immune system [3]. However, Ohn A. Chow et al considered alternative i.e. innate immunity cells for expressing mechanism of statins as an immunostimulator. They proved that statin therapy, *in vitro* & *in vivo* increases the ability of phagocytes to kill human pathogens [4]. As per Goronzy JJ et al statins increase the number of regulatory T cells (Tregs) *in vivo* by inducing the transcription factor forkhead box P32. The increase may be beneficial in stabilizing atherosclerotic plaque by reducing the effector T-cell response within the atheroma [5].

There are controversies about the immunomodulator role of atorvastatin. Is it an immunosuppressive or immunostimulant? This study was therefore planned to explore the effect of atorvastatin on cellular immunity in rats.

## 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. Septilin syrup:** Manufactured by The Himalaya Drug Company was purchased by market.

### Experimental Design

Animals were divided into three groups, with 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).

All the three 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 [6].

### 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) ( $10^6$  candida/mL) and incubated for 1 h. The slide was then stained with Giemsa stain and the effect of atorvastatin on phagocytic activity was expressed as the percentage of cells showing phagocytosis and phagocytic index as the average number of candida per PMN [7].

### 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 1).

Table 1: Effect of atorvastatin on weight gain (n= 8 per group)

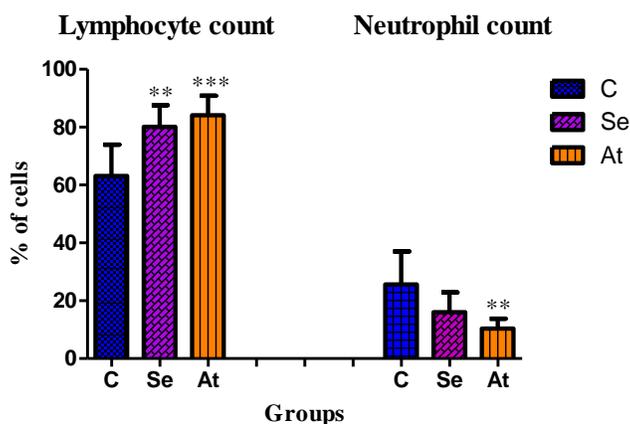
	Control	Septilin syrup	Atorvastatin
Weight gain in gm	29 $\pm$ 14	31 $\pm$ 22	27 $\pm$ 17

There was significant increase in Lymphocyte count in atorvastatin treated group ( $p < 0.001$ ) when compared to control. Increase in lymphocyte count in atorvastatin treated group was comparable with septilin syrup treated group ( $p > 0.05$ ) **Table 2, Graph 1**. Neutrophil count was significantly decreased in atorvastatin group ( $p < 0.01$ ) when compared to control group. The decrease in neutrophil count in atorvastatin treated group was comparable with septilin syrup treated group ( $p > 0.05$ ) **Table 2, Graph 1**.

**Table 2: Effect of atorvastatin on haemogram (n=8 per group)**

	Control	Septilin Syrup	Atorvastatin
Total leukocyte count cells per mm <sup>3</sup>	8700 ± 1900	14000 ± 3500	15000 ± 1900
Lymphocyte count (%)	63 ± 11	80 ± 7.5	84 ± 6.8
Lymphocyte count (absolute)	5600 ± 1800	11000 ± 3200	13000 ± 2100
Neutrophil (%)	26 ± 11	16 ± 6.9	10 ± 3.4
Neutrophil (absolute)	2200 ± 930	2100 ± 880	1600 ± 470

Graph 1

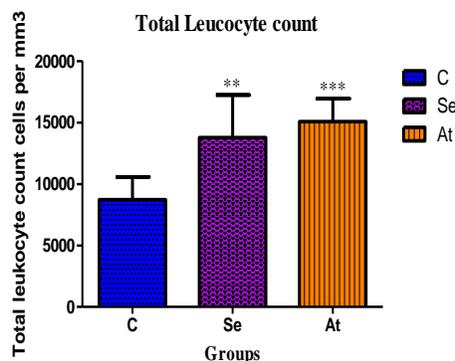


\*\*Control vehicle Vs Septilin Syrup  $p < 0.01$

\*\*\*Control vehicle Vs Atorvastatin  $p < 0.001$

\*\*Control vehicle Vs Atorvastatin  $p < 0.01$

Graph 2



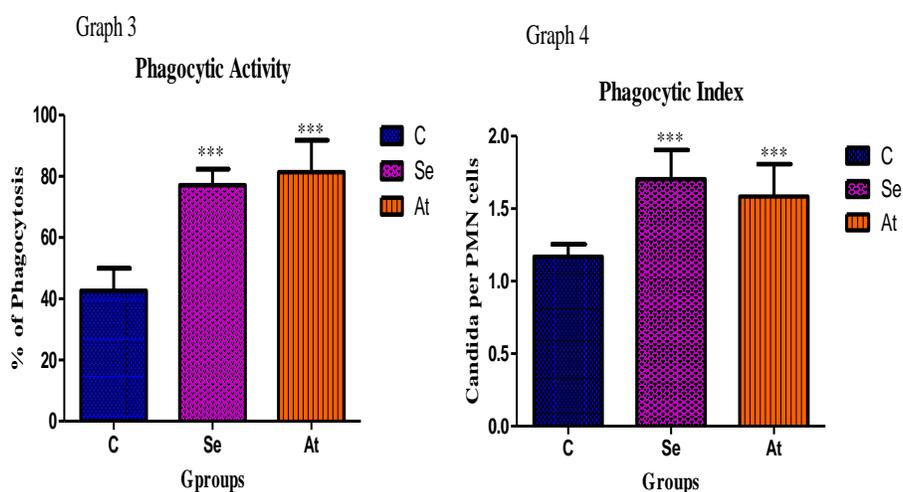
\*\*Control vehicle Vs Septilin syrup  $p < 0.01$

\*\*\*Control vehicle Vs Atorvastatin  $p < 0.001$

Total leukocyte count was increased in atorvastatin treated group ( $p < 0.001$ ) when compared to control. Increase in total leukocyte count was comparable with septilin syrup treated group ( $p > 0.05$ ) Table 2, Graph 2. There was increase in phagocytic activity & phagocytic index in atorvastatin treated group ( $p < 0.001$ ) when compared to control. The increase was comparable with septilin syrup treated group ( $p > 0.05$ ) (Table 3, Graph 3 & 4).

**Table 3: Effect of atorvastatin on Phagocytic activity (n= 8 per group)**

	Control	Septilin Syrup	Atorvastatin
PMN showing phagocytosis	43 ± 7.4	77 ± 5.2	81 ± 10
Phagocytic index	1.2 ± 0.08	1.7 ± 0.20	1.6 ± 0.22



\*\*\*Control vehicle VsSeptilin syrup  $p < 0.001$

\*\*\*Control vehicle Vs Atorvastatin  $p < 0.001$

## DISCUSSION

Immunomodulation 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 immunostimulant which primarily implies stimulation of specific and non specific immune system, i.e. granulocytes, macrophages, complement, certain T & B-lymphocytes and different effector substances. Immuno-suppression implies mainly to reduced resistance against infections, stress and may occur on account of environmental or chemotherapeutic factor. [8,9] The present study shows increase in lymphocyte count (T and B), total leucocyte count, phagocytic activity as well as phagocytic index in atorvastatin treated group. 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. Ohn A. Chow et al proved that prior opsonization of the bacteria

increased overall killing by statin-treated neutrophils, hinting that other innate antibacterial activities of the phagocytic cells could be statin responsive [4]. 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. [8]The significant increase in the levels of lymphocytes, total leucocyte count, phagocytic activity & phagocytic index confirms increased cell mediated immunity in atorvastatin treated group.

### CONCLUSION

Atorvastatin has shown promising results in this model. The authors are also investigating immunomodulatory activity of atorvastatin in E. coli induced sepsis. Further, nitric oxide (NO) has been shown to exert immunomodulatory effects on immune cell adherence & function, cellular proliferation & cytokine production. It has been reported that statins upregulate eNOS function. NO-donor compounds have been shown to inhibit or kill microbes when directly 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 [10]. We further propose to study role of NO in immunomodulatory activity of atorvastatin.

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