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# Pharmacological Investigation of Pitavastatin and Lovastatin on Inflammation – *In vitro*

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**Abstract**

**Aim** : Statins are the most commonly used medications for the treatment of hyperlipidemia. The recent literature report showed that statins may eventually leads to disregulation of immune response. The present study claimed that regular usage of statins accelerated inflammatory potential and increased risk for developing rheumatoid arthritis.

**Methods** : The role of statins on inflammation were evaluated using various *In vitro* studies such as COX inhibitory assay, LOX inhibitory assay, iNOS synthase level, estimation of myeloperoxidase and cellular nitrite level using LPS stimulated RAW 264.7 macrophage cell lines.

**Conclusion** : The result obtained from Inducible nitric oxide synthase level, Cyclooxygenase and Lipoxygenase inhibitory assay of both test drugs such as Pitavastatin and Lovastatin showed less percentage inhibition on inflammation at a Concentration range of 25- 100µg/ml. The test drugs triggered the release of various pro inflammatory mediators on RAW 264.7 cell lines by increasing the myeloperoxidase enzyme activity and concentration of cellular nitrite level. The present findings confirmed that statins exhibited little anti-inflammatory effect by accelerating the risk for affecting inflammation.

**Keywords :** Carrageenan, Hyperlipidemia, Lipoxygenase, Macrophage, Rheumatoid arthritis

## INTRODUCTION

Inflammation is a protective response to the immune system to cover the cells from different stimuli such as chemicals, radiation, pathogens [1]. The inflammatory reactions are initiated by accumation and recruitment of leucocytes [2]. The most important markers of inflammation is characterized as edema,pain,redness which causes the vascular and inflammatory response to infection and injury [3]**.** Statins (hydroxy methyl glutaryl coenzyme A reductase inhibitors) are widely accepted for the treatment of hyperlipidemia. In addition to these drugs have been shown to be effective in reducing the risk of cardiovascular morbidity and mortality in patients with hypertension, or type II diabetes. The recent studies have shown that statins have immunomodulatory property leads to dysregulation of autoimmune system [4]. Statins elicited its effect by releasing various inflammatory cytokines such as tumour necrosis factor, interleukin 1, interleukin 6 and reducing the level of nitric oxide formation through leucocyte - endothelial cell adhesion [5].The present study we select Pitavastatin and Lovastatin. for the pharmacological investigation on inflammation. Both Pitavastatin and Lovastatin are known for its powerful serum cholesterol lowering activity showed similar pharmacological profile but differ only in their chemical composition. These drugs exhibited its action by inhibiting HMG COA reductase the first commited enzyme of cholesterol biosynthesis [6].

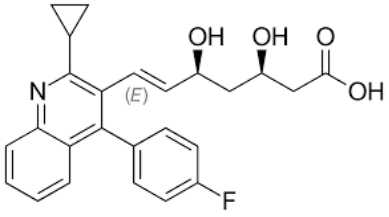


Figure 1 : Chemical structure of Pitavastatin

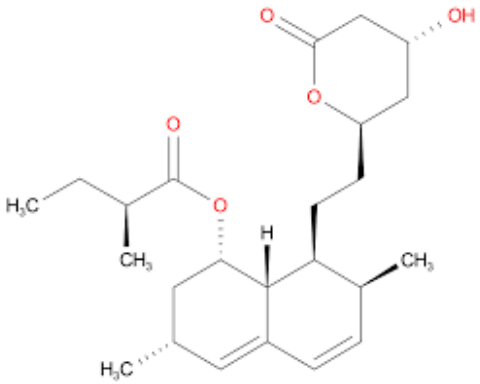


Figure 2 : Chemical structure of Lovastatin

## MATERIAL AND METHODS

### Reagents and Chemicals

The pure drugs Pitavastatin and Lovastatin were obtained from Yarrow Chem Products ( Mumbai, India). Tris hydrochloricacid buffer, Sodium linoleate, L- Arginine, Dithiothreitol, Guiacol and Tetrahydrpterin were collected from Sigma Aldrich Co Ltd ( Bangalore, India). Trichloroacetic acid, Manganese chloride, Sulphosalicylic acid, Sodium hydroxide, Hexadecyl trimethyl ammonium bromide were purchased from Nice Pharma ( Kochi, India). The reagents such as Glutathione and Arachidonic acid were obtained from chemit laboratories ( Hyderabad, India).

### Cell Culture

RAW 264.7 cells was initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecco's modified Eagles medium, DMEM ( Sigma aldrich, USA).The cell line was cultured in 25 $cm^2$ tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate (Merck, Germany) and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphoteracin

B (2.5µg/ml). Cultured cell lines were kept at 37ºC in a humidified 5% $CO_2$ incubator (NBS Eppendorf, Germany). The cells were grown to 60% confluency followed by activation with 1 µL lipopolysaccharide (LPS: 1µg/mL). LPS stimulated RAW cells were exposed with different concentration (25, 50, 100 µg/mL) of sample solution and diclofenac sodium, a standard anti-inflammatory drug in varying concentration corresponding to the sample was added and incubated for 24 hours. After incubation the anti-inflammatory assays were performed using the cell lysate.

**Cyclooxygenase Inhibitory Assay**

The reaction mixture were incubated at 25˚C. The process of reaction was activated by the addition of arachidonic acid and terminated by the insertion of trichloro aceticacid in hydrochloric acid. The tubes are boiled for 20 minutes followed by centrifuged the samples. After cooling the COX activity was measured at 632 [7].

**Lipoxygenase Inhibitory Assay**

The LOX activity was determined by adding following reagents such as Tris-HCl buffer and sodium lonoleate to cell lysate. The activity was measured at 234nm [ 8].

**Inducible Nitric Oxide Synthase level Estimation**

Initially cell lysate was homogenized with 2 ml of HEPES buffer. The assay system contained substrate 0.1 ml L-Arginine, 0.1 ml manganese chloride, 0.1 ml 30µg dithiothreitol (DTT), 0.1 ml NADPH, 0.1 ml tetrahydropterin, 0.1 ml oxygenated haemoglobin and 0.1 ml of sample. Absorbance was measured at 401nm [9].

**Estimation of Cellular Nitrite Level**

To 0.5ml of cell lysate, 0.1ml of sulphosalicylic acid was mixed and vortexed for 30 minutes. The samples were then centrifuged at 5,000 rpm for 15 minutes. To 200µL of protein free supernatant, 30µL of 10% NaOH ,300µL of Tris-HCl buffer and 530µL of Griess reagent was added and incubated in the dark for 10 to15 minutes. The absorbance was read at 540nm against a griess reagent blank. Sodium nitrite solution was used as the standard .The amount of nitrite present in the sample was estimated from the standard curves obtained [10].

**Myeloperoxidase Activity**

50mM potassium phosphate buffer and 0.57% hexadecyl trimethyl ammonium bromide (HTAB) was homogenized with cell lysate. Then the samples were centrifuged at 2000g for 30 minutes at 4˚C. Collected the supernant present in the sample for measuring MPO activity.Sample was activated by the addition of 50mM Phosphate buffer containing 1.67mg/ml guaiacol and 0.0005% Water.The change in absorbance at 460 nm was measured [11].

## RESULTS

The percentage inhibition of Cyclooxygenase inhibitory assay, Lipoxygenase inhibitory assay and Inducible nitric oxide synthase level estimation using different concentrations of Diclofenac sodium, Pitavastain and Lovastatin were perfomed on RAW 264.7 cell lines. The result obtained from this three studies showed that there was a rise in percentage inhibition in both test drugs and standard at 100µg/ml. The result obtained from estimation of cellular nitrite level on RAW 264.7 cell lines showed that there was a dose dependent decrease in the concentration of nitrite level, where as both the test drugs elicited rise in nitrite concentration ranges from 25 - 100µg/ml. Myeloperoxidase released by neutrophils binds to macrophages initiating a molecular cascade pathway resulting in the secretion of Interleukin -1, Interleukin -8 and Interferon α/β. The data showed that there was an increase in myeloperoxidase activity of Pitavastatin and Lovastatin due to decrease in the inhibition of enzyme activity.

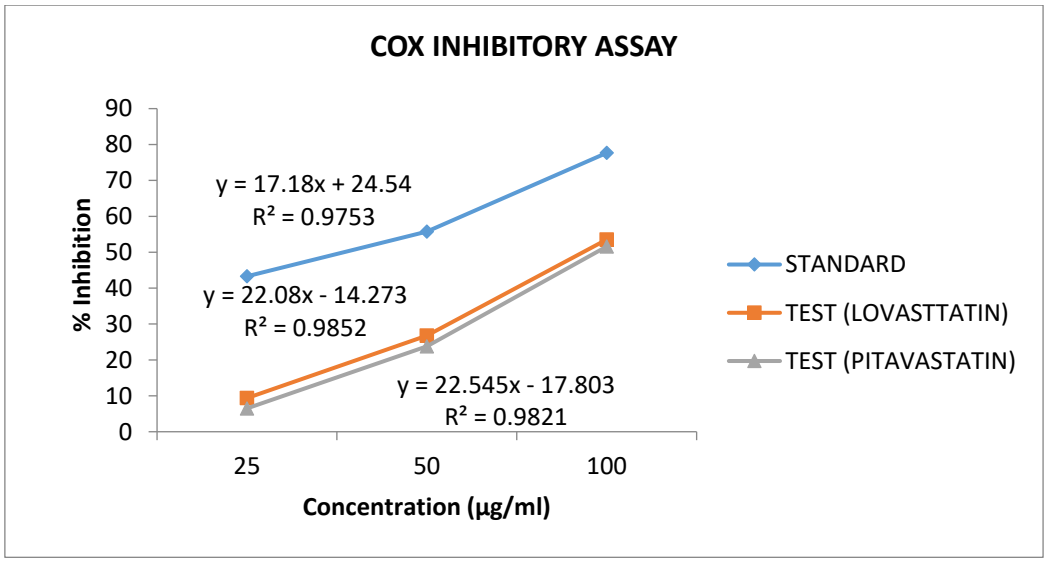


Figure 3 : Effect of Pitavastatin, Lovastatin & diclofenac on COX activity in RAW 264.7 cell lines.

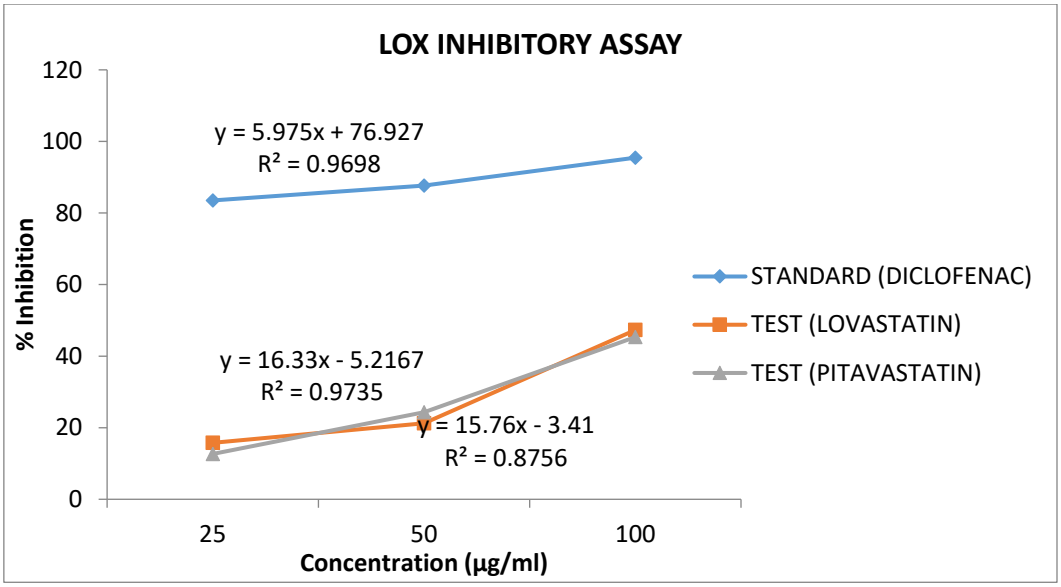


Figure 4 : Effect of Pitavastatin, Lovastatin & diclofenac on LOX activity in RAW 264.7 cell lines.

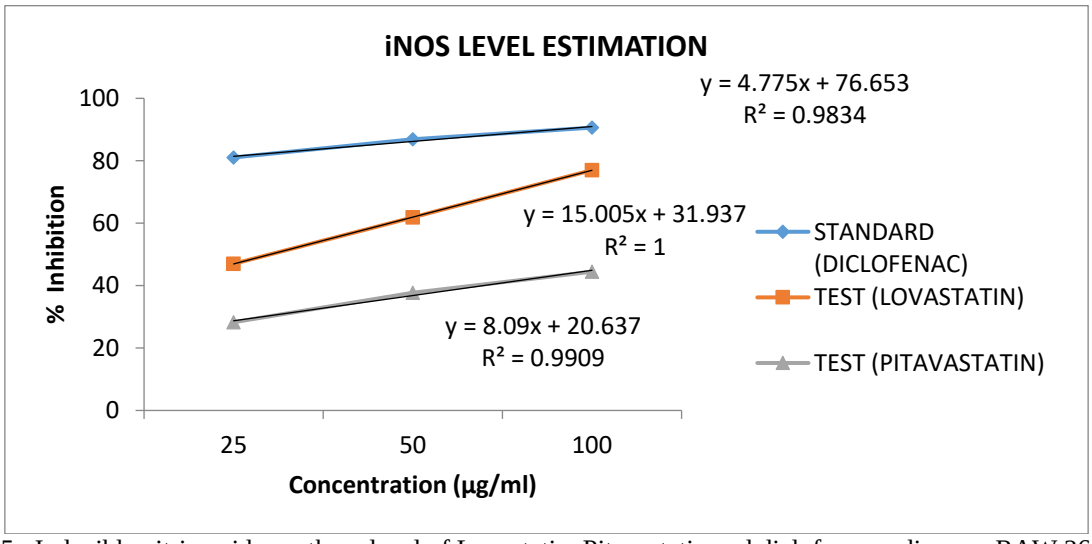


Figure 5 : Inducible nitric oxide synthase level of Lovastatin, Pitavastatin and diclofenac sodium on RAW 264.7 cell lines.

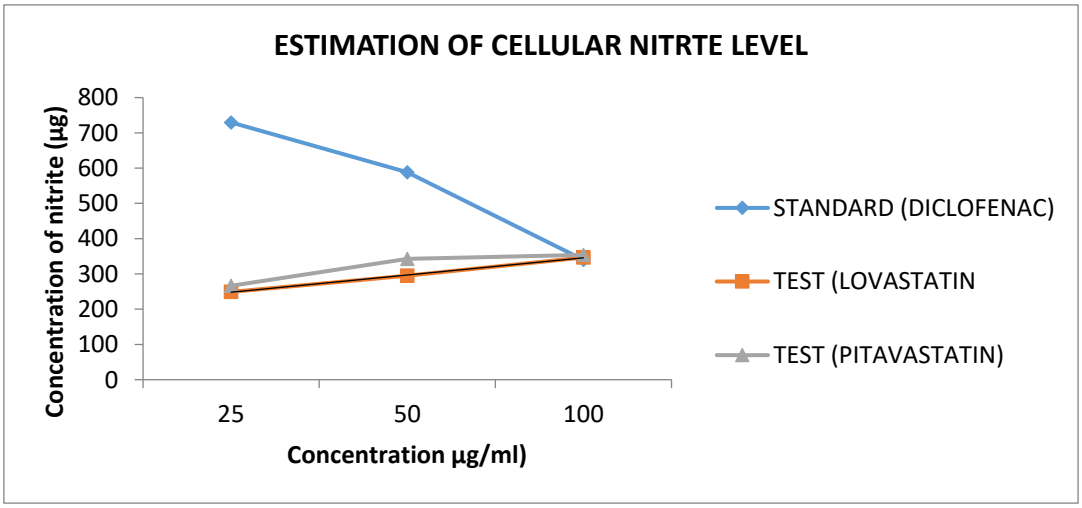


Figure 6 : Estimation of cellular nitrite level of Lovastatin, Pitavastatin and diclofenac sodium on RAW 264.7 cell lines.

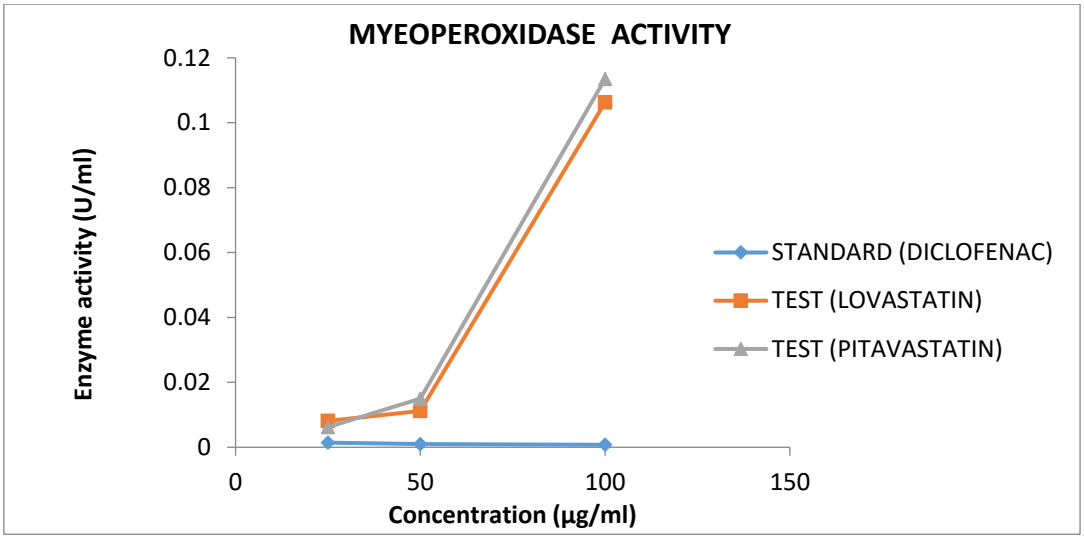


Figure 7 : Effect of myeloperoxidase activity of Lovastatin, Pitavastatin and d iclofenac Sodium on RAW 264.7 cell lines.

## DISCUSSION

The data obtained from the studies such as COX inhibitory assay, LOX inhibitory assay and inducible nitric oxide synthase level estimation assay showed that standard diclofenac sodium exhibited excellent anti inflammatory activity. This was further confirmed by the $IC_{50}$ values of these compounds. The study proposed that both pitavastatin and Lovastatin had little effect on inflammation. The decreased cellular nitrite level of diclofenac sodium proposed that capacity to inhibit nitric oxide synthase, thus the study elicited that diclofenac sodium had better antiinflamatory activity. The result indicated that decreased myeloperoxidase level of diclofenac sodium treated RAW 264.7 cell lines showed an excellent anti-inflammatory activity.

## CONCLUSION

The study was aimed at revealing the role of pitavastatin and lovastatin on inflammation. Result obtained from the *in vitro* inflammatory studies except Myeloperoxidase activity assay and estimation of cellular nitrite level showed that statins had little effect on onflammation when compared with standard drug. The rise in concentration of myeloperoxidase in RAW 264.7 cell lines triggered the release of pro inflammatory mediators through the activation of molecular cascaded pathway. The increased concentration of cellular nitrite level elicited that usage of statins may precipitate inflammation through the excess production of nitric oxide in cells.

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## REFERENCE

[1] Linlin chen, Huidan Deng, Hengmin Cui, Jing Fang, Zhicai Zao, Juliang Deng, Yungulum Li, Xung Way, Ling Zhao.Inflammatory response and inflammation - associated disease in organs. J.of Oncotarget; 2018,9(6), 7204- 7218.

[2] Leelaprakash G, Dass SM, Road B. Available online http://www.ijddr.in Covered in Official Product of Elsevier , The Netherlands © 2010 IJDDR INVITRO ANTI-INFLAMMATORY ACTIVITY OF METHANOL EXTRACT OF ENICOSTEMMA AXILLARE. 2011;3(3):189–96.

[3] Ferrero-Miliani L, Nielsen O, Andersen P, Girardin S. Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1β generation. Clin Exp Immunol. 2007; 147:227–235.

[4] Bijina B, Chellappan S, Krishna JG, Basheer SM, Elyas KK, Bahkali AH, et al. drug and as seafoof preservative Protease inhibitor from Moringa oleifera with potential for use as therapeutic drug and as seafood preservative. Saudi J Biol Sci [Internet].2011;18(3):273-81.Availablefrom:http://dx.doi.org/10.1016/j.sjbs.2011.04.002.

[5] Reddy VJS, Rao PGD, Lakshmi GR. A REVIEW ON ANTI-ARTHRITIC ACTIVITY OF SOME MEDICINAL PLANTS. 2014;5(4):2061–73.

[6] Dhobale S, Narad V, Gaikwad D. Estimation of Lovastatin in Pharmaceutical Formulationby Area under Curve Spectrophotometric Method. 2017;6(3):85–93.

[7] Chen Y, Shen S, Lee W, Hou W, Yang L, Lee TJF. Inhibition of Nitric Oxide Synthase Inhibitors and Lipopolysaccharide Induced Inducible NOS and Cyclooxygenase-2 Gene Expressions by Rutin , Quercetin , and Quercetin Pentaacetate in RAW 264 . 7 Macrophages. 2001;548.

[8] Choi YH, Lim H, Heo MY, Kwon DY, Kim HP. Korean Fermented Bean : 5- Lipoxygenase Inhibition. 2008;11(May 2007):539–43.

[9] Salvemini D, Wang Z, Bourdon DM, Stern MK, Currie MG, Manning PT. Evidence ofperoxynitrite involvement in the carrageenan-induced rat paw edema. 1996;303:217–20.

[10] Pankiewicz J, Wanat A, Anna P, Goła I. Determination of nitrite / nitrate in human biological material by the simple Griess reaction. 1998;274(x):177–88.

[11] Pulli B, Ali M, Forghani R, Schob S, Hsieh KLC, Wojtkiewicz G, et al. Measuring Myeloperoxidase Activity in Biological Samples. 2013;8(7):1–10.