CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
SJIF Impact Factor: 7.187

Available online at: http://www.iajps.com *Research Article*

PREDICTING ACCELERATED RHEUMATOID ARTHRITIC INCIDANCE OF STATINS BY *IN VITRO* TECHNIQUES

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Article Received: March 2022 **Accepted:** March 2022 **Published:** April 2022

Abstract:

Statins are the drugs used for the treatment of hyperlipidemia along with cholesterol lowering activity. In addition to cholesterol lowering activity these drugs may eventually leads to dysregulation of immune response. The primary objective of the present study focused on the induction of autoimmunity.The autoimmune response of statins is mediated by regulating leucocyte-endothelial cell adhesion, reducing nitric oxide production and promote the level of inflammatory cytokines such as tumour necrosis factor (TNF-α), interleukin 1 and interleukin 6. The dual effects of lipoprotein improvement and arthritic potential might be expected to confer an accelerated arthritic onset in patients with rheumatoid arthritis. The main study performed is denaturation of protein and inhibition of proteases assay. The result showed that pitavastatin and lovastatin administration during the active range of 62.5 – 500µg/ml indicated highest inhibitory profile on denaturation of proteins. The statins also exhibited maximum protease inhibitory activity during the percentage range of 12.28- 64.4% as compared to standard drug Diclofenac sodium. The recent evidence obtained from the study indicated both statins had little effect on accelerating rheumatoid arthritis. The study claimed that both Pitavastatin and lovastatin use promoted a significant role for developing rheumatoid arthritis.

Keywords: *Pitavastatin, Lovastatin, Protein denaturation, Protease inhibition, Autoimmunity*

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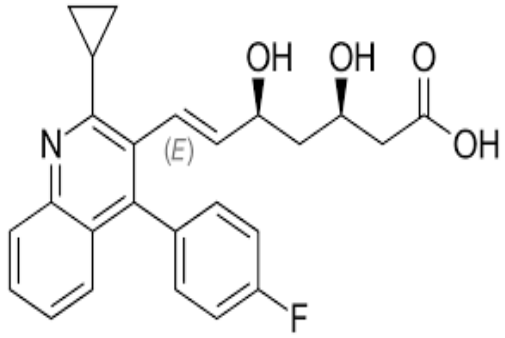
Please cite this article in press Jithin Mathew et al, ***Predicting Accelerated Rheumatoid Arthritis Incidance Of Statins By In Vitro Techniques.,*** *Indo Am. J. P. Sci, 2022; 09(04)*

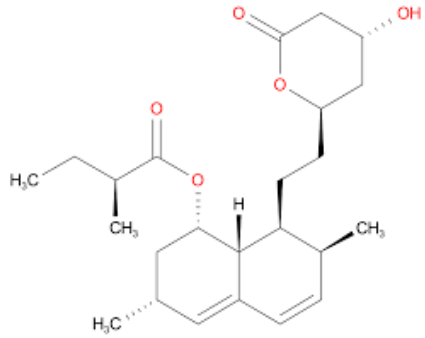
INTRODUCTION:

Rheumatoid arthritis (RA) is an inflammatory disease affects peripheral joints, damage of articular tissues and damage of joints. As the advancement of disease condition, it may created higher risk for bone damage and cartilage destruction [1,2,3]. Rheumatoid arthritis mainly affected joints later leads to death and arthralgic disability. It has been known for more than 50years that rheumatoid arthritis is associated with increased death rates compared with the common population which develops due to a complex interaction between traditional risk factors (dyslipidemia, Blood pressure) and those related to the inflammatory disease. Statins have lipid-lowering effects, and also the effect on arthritic potential that regulating leukocyte-endothelial cell adhesion, reducing nitric oxide production. It promoted the formation of various inflammatory mediators. The dual effects of lipoprotein improvement and arthritic potential might be expected to confer an accelerated arthritic onset in patients [4,5]. The immunomodulating effects promoted the formation of autoimmunity caused autoimmune disorders. The literature report suggested that continuous usage of statins resulting accelerated arthritic onset [6].

Pitavastatin[7,8] and Lovastatin [9,10,11] are the drugs falls under the category of Hydroxy methyl glutaryl-coenzyme(HMG COA) reductase inhibitor used for the management of hypercholestremia. The literature analysis proved that there was no method has been made to evaluate the off labeled use of both statins on arthritis. The present study focused to demonstrate whether statin use will increase the risk for developing rheumatoid arthritis.



Pitavastatin



Lovastatin

Figure 1: Chemical structure of Pitavastatin and Lovastatin.

MATERIALS AND METHODS:

Lovastatin and Pitavastatin were supplied by Yarrow chem Products Mumbai, India and were certified to contain 97% (w/w) and 98% (w/w) respectively on dry basis. Trypsin purchased from Biolaxi Corporation Limited Thane, India. 1N Hydrochloric acid, 70% per chloric acid and distilled water were procured from cimson scientific private limited kottayam, India. Bovine serum albumin fraction (AR), phosphate buffer and 20mM Tris Hydrochloric acid buffer were purchased from Sigma aldrich co limited, Bangalore, India. In addition, UV Visible spectrophotometer (SLII9 Systronics) CO_2 Incubator (NBS, Eppendorf, Germany) and Centrifuge (REMI RM12c).

Protein Denaturation Assay

Different concentrations of sample such as 62.5 µg/ml - 500µg/ml were prepared from stock solution. Both distilled water and bovine serum albumin present in test control. The test solution consists of 0.45ml of bovine serum albumin and different concentrations of sample (62.5, 125, 250 and 500 µg/ml). The reference compound was Diclofenac sodium. 1N Hydrochloric acid used for pH adjustment. Test compounds were incubated for a temperature of 37˚C for 20 minutes later the temperature increased up to 57˚C for 3 minutes. After cooling Phosphate buffer of 2.5ml added in each tube. Absorbance was measured using UV Visible spectrophotometer at 416nm [12, 13, 14].

Pharmacological evaluation of statins was determined by denaturation of protein. The maximum inhibitory activity of Pitavastatin and Lovastatin were found to be the range of 62.5 to 500 µg/ml. The statins showed more inflammatory potential than standard drug. Lovastatin possessed IC_{50}value of 48.92µg/ml and pitavastatin possessed IC_{50} value of 48.51µg/ml whereas diclofenac sodium possessed an IC_{50}

value of 16.47µg/ml.

Proteinase Inhibition Assay

Trypsin, Tris HCl buffer and test samples are present in reaction mixture. The mixture placed for incubation for 5 minutes at 37˚C. After the addition of casein to the mixture again it kept under incubation for 20 minutes. At the end add 70% per chloric acid and centrifuged at 3000 rpm for 10 minutes. The measurement of absorbance was done at 200nm [15,16].

RESULT AND DISCUSSION:

Pharmacological evaluation of statins was determined by denaturation of protein. The maximum inhibitory activity of Pitavastatin and Lovastatin were found to be the range of 62.5 to 500 µg/ml. The statins showed more inflammatory potential than standard drug. Lovastatin possessed IC_{50}value of 48.92µg/ml and pitavastatin possessed IC_{50} value of 48.51µg/ml whereas diclofenac sodium possessed an IC_{50} value of 16.47µg/ml.

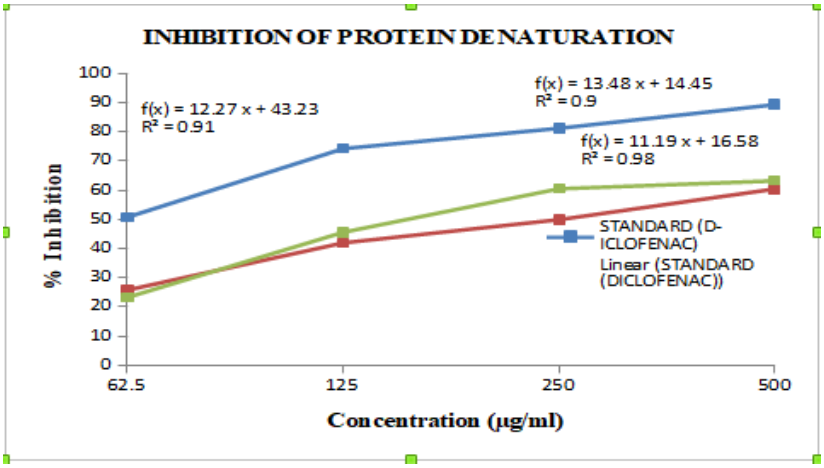


Figure 2: Effect of Percentage inhibition of pitavastatin, lovastatin and diclofenac on protein denaturation.

In the present study, *in vitro* arthritic effect of Pitavastatin and Lovastatin was evaluated by using antiprotease assay. The study showed Diclofenac sodium (reference drug) showed concentration dependent inhibition of antiprotease activity. It indicated maximum inhibition 90 at 500 µg/ml. Both Pitavastatin and Lovastatin exhibited anti-proteinase activity but not effective as Diclofenac sodium. This was further confirmed by IC_{50} values. IC_{50} value of diclofenac sodium, lovastatin and pitavastatin was found to be 17.28, 49.69, 50.46 µg/ml respectively. Proteinase has been implicated in inflammatory conditions. The study revealed that both test drugs exhibited a concentration dependent anti-proteinase activity. However, Diclofenac sodium was found to be more active.

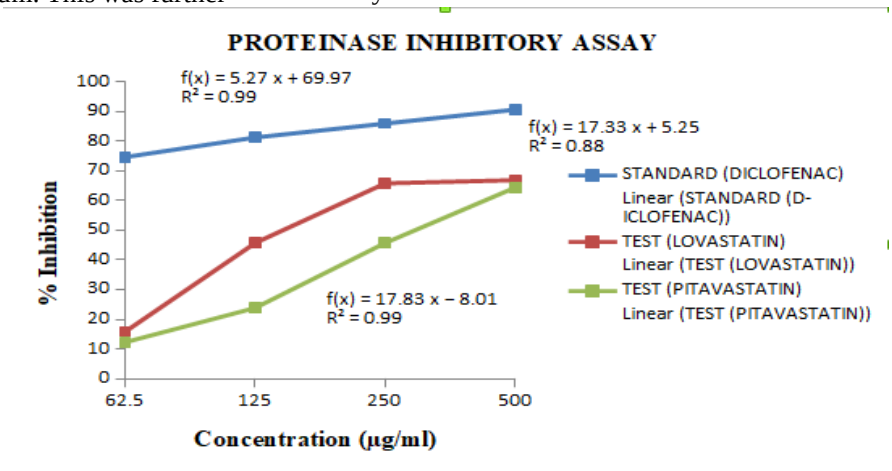


Figure. 3 : Effect of Percentage inhibition of pitavastatin, lovastatin and diclofenac on proteinase inhibitory assay.

CONCLUSION:

The *in vitro* arthritic activity of Pitavastatin and Lovastatin was studied using protein denaturation and proteinase inhibition assay. The present findings exhibited both of this test drugs elicited little effect on inflammation when compared to Diclofenac sodium. The study claimed that statin use was associated with increased risk of developing rheumatoid arthritis.

ACKNOWLEDGEMENT

The authors thank Yarrow Chem Products, Mumbai, for providing Pitavastatin and Lovastatin as samples for this work. They also thank Prof. Dr. Jyoti Harindran, Principal, Department of Pharmaceutical Sciences, Cheruvandoor, Kottayam.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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