Induction and Prognosis of Rheumatoid Arthritis: Biological Determination by Denaturation of Proteinase Technique and Antiproteinase Assay

In Vitro evaluation of Pitavastatin and .Lovastatin on Arthritis.

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Abstract: Statins are the drugs used for the treatment of hyperlipidemia along with 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 biological studies performed are denaturation of proteins 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. Statins also exhibited maximum protease inhibitory activity during the percentage range of 12.28- 64.4%. The recent evidence obtained from the study indicated both statins had little effect on Rheumatoid arthritis. The study claimed that both Pitavastatin and Lovastatin use promoted higher chance for developing Rheumatoid arthritis.

Index Terms: Pitavastatin, Lovastatin, Proteinase denaturation, Proteinase Inhibtion, Autoimmunity

I. INTRODUCTION

Rheumatoid arthritis (RA) is an inflammatory disease affects peripheral joints, damage of articular tissues and joints. As the advancement of disease condition, it may created higher risk for bone damage and cartilage destruction[1.2.3]. Rheumatoid arthritis mainly affects on 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. This 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, 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 the progression of autoimmune disorders. The literature report suggested that continuous usage of statins resulting accelerated arthritic onset [6.7]..

Lovastatin and Pitavastatin are the drugs falls under the category of HMG COA reductase inhibitor used for the management of hypercholesteremia[8,9].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 [10,11].

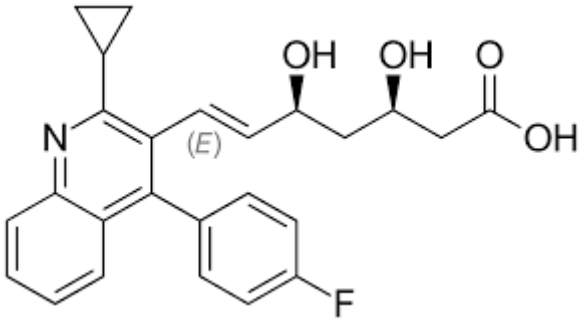

Figure 1 : Chemical structure of Pitavastatin

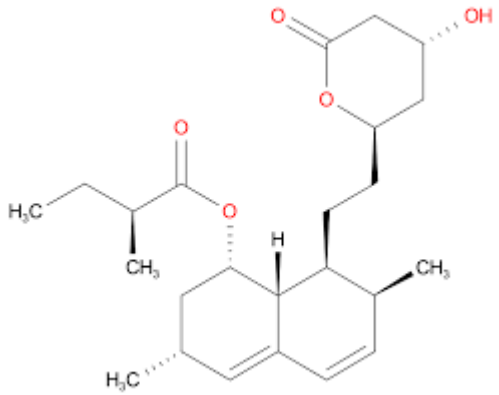

Figure 2 : Chemical structure of Lovastatin

II. 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 was 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 are 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 6.3. Test compounds were incubated for a temperature of 37˚C for 20 minutes later the temperature increased upto 57˚C for 3 minutes. After cooling Phosphate buffer of 2.5ml added in each tubes. Absorbance was measured using UV Visible spectrophotometer at 416nm [12, 13,14].

Proteinase Inhibition Assay

Trypsin, Tris HCl buffer and test sample 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% perchloric acid and centrifuged at 3000 rpm for 10 minutes. The measurement of absorbance was done at 200nm [15].

III. RESULTS AND DISCUSSION

Protein Denaturation Assay

Pharmacological evaluation of statins were determined by denaturation of proteins. The details are recorded in Table 1. 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 IC50 value of48.92µg/ml and Pitavastatin possessed IC_{50} value of 48.51µg/ml whereas IC50value of diclofenac sodium possessed an IC_{50}value of 16.47µg/ml.

Table 1: Effect of Pitavastatin, Lovastatin & diclofenac sodium on inhibition of protein denaturation method

Sample	Concentration (µg/ml)	OD of test at 416nm	OD of product control at 416nm	% Inhibition	IC_{50} Value
Control	_	0.0583±0.0001	_	_	-
Standard (Diclofenac)	62.5 125 250 500	0.227 ± 0.0001 0.0311 ± 0.0001 0.0553 ± 0.0004 0.0876 ± 0.0004	0.1983 ± 0.0001 0.0161 ± 0.0001 0.0443 ± 0.0001 0.0819 ± 0.0001	50.77 74.27 81.13 89.36	16.47
Test (Lovastatin)	62.5 125 250 500	0.1267 ± 0.0001 0.1764 ± 0.0001 0.1989 ± 0.0001 0.2011 ± 0.0001	0.0684 ± 0.0001 0.1308 ± 0.0004 0.1592 ± 0.0001 0.1700 ± 0.0004	25.8 41.98 49.94 60.43	48.92
Test (Pitavastatin)	62.5 125 250 500	0.1367 ± 0.0001 0.1832 ± 0.0001 0.1984 ± 0.0001 0.2122 ± 0.0004	0.0764 ± 0.0004 0.1404 ± 0.0001 0.1672 ± 0.0001 0.1832 ± 0.0004	23.26 45.60 60.51 63.22	48.51

(n=3), values were expressed as mean ± SD.

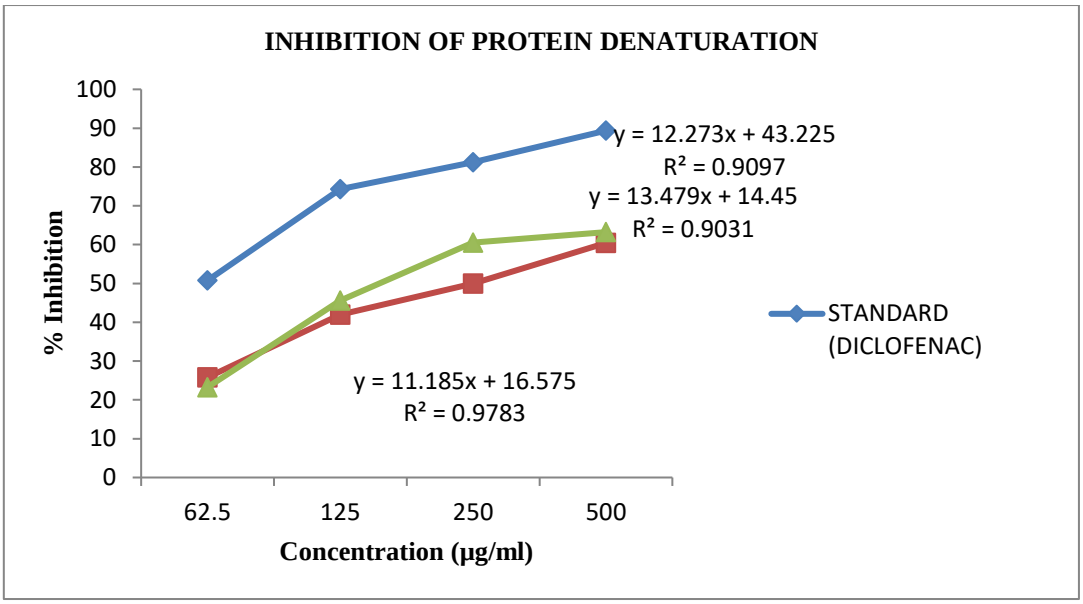

***Figure 3*: Percentage inhibition of Lovastatin, Pitavastatin and diclofenac sodium on protein denaturation assay.**

Proteinase Inhibition Assay

In vitro arthritic effect of Pitavastatin and Lovastatin was evaluated by using antiproteinase assay. The data are recorded in Table 2. The study showed diclofenac sodium (reference drug) showed concentration dependent inhibition of antiproteinase activity. It showed maximum inhibition 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 ant proteinase activity. However diclofenac sodium was found to be more active.

Table 2 : Effect of Pitavastatin, Lovastatin and Diclofenac on Proteinase Inhibition Assay.

Sample	Concentration (µg/ml)	OD of test at 200nm	OD of product control at 200nm	% Inhibition	IC_{50} Value
Control	_	0.9113 ± 0.0001	_	_	_
Standard (Diclofenac)	62.5 125 250 500	1.186 ± 0.0001 1.142 ± 0.0004 1.431 ± 0.0002 1.934 ± 0.0004	0.9558 ± 0.0004 0.9720 ± 0.0001 1.015 ± 0.0004 1.849 ± 0.0004	74.67 81.26 85.98 90.66	17.28
Test (Lovastatin)	62.5 125 250 500	0.1466 ± 0.0001 0.1732 ± 0.0001 0.2154 ± 0.0001 0.2217 ± 0.0001	0.0993 ± 0.0001 0.1427 ± 0.0002 0.1962 ± 0.0001 0.2031 ± 0.0004	15.83 45.73 65.83 66.90	49.69
Test (Pitavastatin)	62.5 125 250 500	0.1487 ± 0.0001 0.1896 ± 0.0001 0.2165 ± 0.0004 0.2218 ± 0.0004	0.0994 ± 0.0001 0.1468 ± 0.0001 0.1859 ± 0.0001 0.2018 ± 0.0001	12.28 23.84 45.73 64.41	50.46

(n=3), values were expressed as mean ± SD.

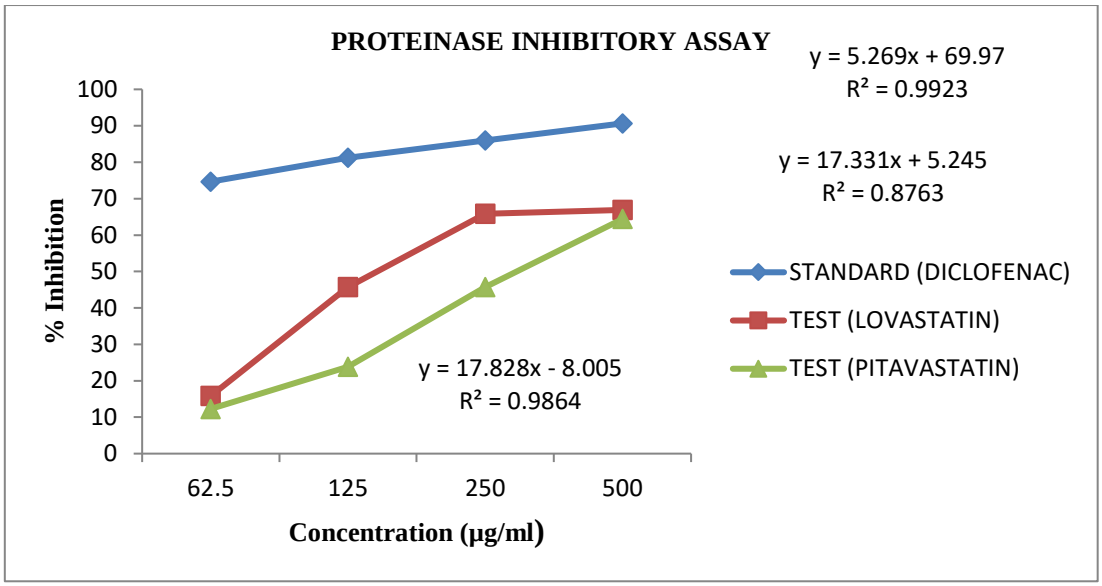

Figure 4: Percentage inhibition of Lovastatin, Pitavastatin and diclofenac sodium on proteinase inhibition assay.

IV. CONCLUSION

The study revealed that both Pitavastatin and Lovastatin had a strong influence on accelerating arthritic onset. The result obtained from proteinase denaturation assay elicited that the test drugs exhibited denaturation of proteins thus accelerated arthritic incidence, However the standard drug Diclofenac sodium exhibited remarkable inhibition of proteinase enzyme. The Antiproteinase activity showed that a concentration dependent proteinase inhibition. Both the studies claimed that statin administration accelerated arthritic incidence.

V. ACKNOWLEDGEMENT

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