ISSN 2583– 0260 (online) *ISSN 2394 -7837 (print)*

A STUDY ON ANTI-INFLAMMATORY EFFECT OF CHRYSIN

Jijymol, K. K.[1] and Saja, K.

Received: 24/5/2022 **Revised: 25/6/2022** **Accepted:28/6/2022**

Abstract
The present study indicated that chrysin significantly decreases the expression of MMP-9 and MMP-2 in foam cells. Our result suggests that chrysin has significant therapeutic potential for the management of inflammatory diseases, particularly in atherosclerosis.

Key words: Chrysin, matrix metalloproteinase, atherosclerosis, anti-inflammation, ELISA

Introduction

Atherosclerosis is an inflammatory disease, which involves multiple processes including endothelial dysfunction, inflammation, vascular proliferation and extracellular matrix (ECM) degradation (Ross 1999). Matrix metalloproteinases (MMPs) are a large family of extracellular matrix degrading endopeptidases, which play a major role in many physiological and inflammatory condition (Yadav etal., 2011) .MMPs produced by macrophages play crucial role in the development and progression of many vascular diseases particularly in atherosclerosis.

In the presence of OxLDL monocytes undergo differentiation and become macrophages. This macrophage uptake OxLDL and form lipid laiden foam cells, and produce various kinds of inflammatory cytokines and ECM degrading enzymes like MMPs. This MMPs determine the stability of atherosclerotic plaque, and increased production of MMPs leads to plaque rupture and thrombosis.

Chrysin is a flavonoid derived from passion flower, Indian trumpet flower, honey andpropolis (Wolfman C *et al.,*1994 , Rapta P et al.,1995 and Williams CA et al.,1997). It is reported to have anti-spasmodic, anti-microbial, anxiolytic, anti-oxidant and anti-inflammatory activities. (Dao TT, et al.,2004).As chrysin is reported to have anti-inflammatory property (Warda Mohamed Kaidama and Rajesh N. Gacche 2015) the objective of our present investigation was to see, whether anti- inflammatory effect of chrysin involves downregulation of MMPs in foam cell model system.

Materials and Methods

Materials

RAW 264.7 cell line is a macrophage like cell line derived from Balb/ c mice, is obtained from National Centre for cell Science (NCCS). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 2mM L- glutamine, penicillin and streptomycin. Cells were incubated at 37 0 C in 5% CO 2.

Methods

Isolation and chemical modification of LDL

Isolation and oxidation of LDL was done according to the procedure of Thio et al (Thao N K et al.,1999). Low density lipoprotein (LDL) was isolated from human plasma. LDL was precipitated by Heparin–MnCl 2 method. One portion of pellet was separated as native LDLand the remaining portion of pellet was subjected to oxidation. For oxidation incubate LDLwith CuSO 4 for 6 hours and dialysed against 1X PBS containing EDTA for 48 hours with regular buffer change. Degree of LDL oxidation was measured as the amount of thiobarbituric acid reactive substance produced.

Estimation of thiobarbituric acid reactive substance (TBARS)

Department of Biochemistry, University of Kerala, Thiruvananthapuram, Kerala, India
[1]Present Address: Post Graduate Department and Research Centre of Botany, Mahatma Gandhi College Thiruvananthapuram, Kerala, India, *email: jkuttappan507@gmail.com*

Thiobarbituric acid reactive substances were estimated by the method of Adriana et al. (Adriana et al.,2001).

Reagents

A. 0.1 M Tris - HCl buffer (pH= 7.5)

B. TBA – TCA – HCl Reagent

Oxidised LDL and native LDL was dissolved in 0.1 M Tris – HCL buffer and kept it for 5 minutes. TBA – TCA – HCl reagent was added to this and mixed thoroughly, kept in boiling water bath for 15 minutes. After cooling, the precipitate was removed by centrifugation at 1000 rpm at 10 minutes. Absorbance was read at 535 nm against the reagent blank.

Cell Viability Assay (MTT ASSAY)

The effect of chrysin on cell viability was determined with the 3- (4,5 Dimethylthiazol – 2-yl) – 2,5 diphenyltetrazolium bromide (MTT) assay (Slater et al., 1993). For MTT assay cells were seeded in 96 well plates. After 70% confluency, cells were treated with different concentrations of chrysin (10. 20, 30, 50, 80 and 100 µM) for 24 hours. 5mg/ml MTT solution was added and incubated for 4 hours at 37 0 C. Then MTT solvent (Isopropanol) was added and read absorbance at 570nm.

Enzyme linked immunosorbent assay (ELISA)

ELISA was done according to the procedure of Engvall and Perlman (Engvall and Perlman,1971). Different concentration of antigen is pre- coated on to the ELISA plate served as the antigen. Incubate the plate overnight at 37 0 C. Plates were washed with washing buffer (PBS –Tween 20) three times. Add blocking buffer in to the wells and incubate for 1 hour at room temperature. Again washed with PBS-Tween 20. After washing primary antibody is added to each well and incubate 37 0 C for an hour. Wash with PBS – Tween 20. Add secondary antibody (1: 1000) to the wells and incubate 37 0 C for an hour. Add substrate into each well and incubate in dark for 30 minutes. O-phenyelnediamine in citrate buffer with H 2 O 2 was used as substrate. Add HCL stop solution and absorbance is read at 450 nm using ELISA plate reader.

Statistical Analysis

Significance of the samples were determined by one-way ANOVA (and nonparametric) using Graph pad prism (Version 5.04).

Results and Discussion

Model System for the Study

RAW 264.7 murine macrophage cell line is treated with oxidized LDL for 48 hours, which provide the in vitro foam cell model system.

Oxidation of LDL -TBARS assay

LDL was isolated and oxidized with CuSo 4 as described in methodology. Production of thiobarbituric acid reactive substance indicate the degree of LDL oxidation. The result shows that the degree of oxidation in the OxLDL preparation is significantly higher than the native LDL.

Effect of chrysin on cell viability - MTT Assay

In order to study the effect of chrysin on the viability of cells, RAW 264.7 cells were maintained in culture in the presence of different concentrations (10, 20, 30, 50, 80 and 100 µM) of chrysin for 24 hours and treated with MTT. The amount of formazan crystals produced is directly proportional to the number of viable cells and the results are given in figure 3.The result indicates that, there was no significant change in viability of cells on treatment with chrysin at lower concentrations. The maximum concentration of chrysin (100 µM) treatment also showed more than 85% viability.

Effect of chrysin on the production of TNF-α by foam cells

In order to study the effect of chrysin on inflammation, the production of pro-inflammatory marker TNF-α by foam cells was studied. RAW 264.7 cells were maintained in culture in the

presence of OxLDL (50 µg/ml) and OxLDL+ chrysin (10,20,30 and 50 µM) for 48 hours. The medium was collected and production of TNF-α was measured by ELISA using specific antibody against TNF-α and the results are given in figure 1.

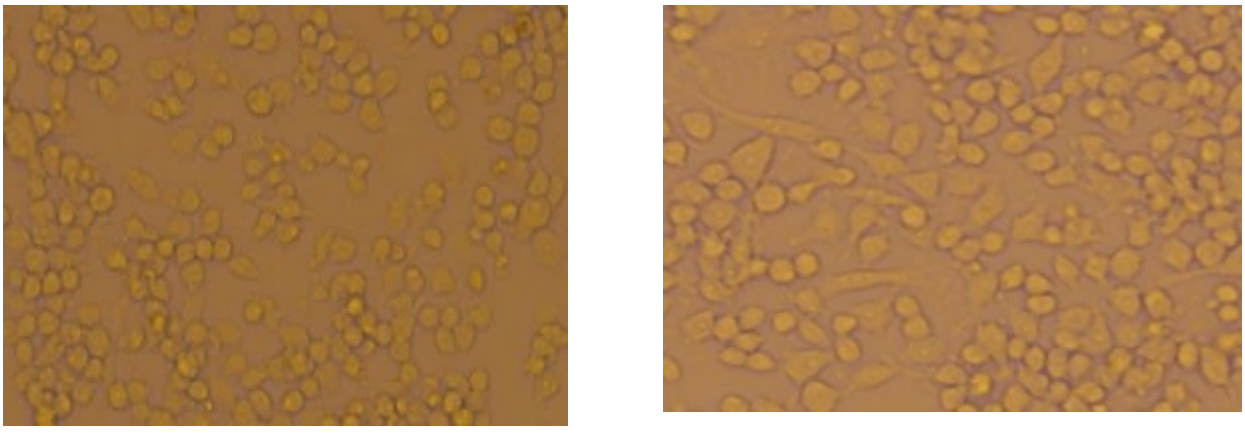
The result shows that, the production proinflammatory cytokine TNF-α was significantly upregulated in cells treated with OxLDL, and the effect was subsequently down regulated by chrysin in a concentration dependent manner indicating the anti-inflammatory effect of chrysin in foam cells.

High fat diet is a major risk factor for the development of Atherosclerosis. Atherosclerosis and its progression involve, vascular endothelial activation and dysfunction, foam cell formation and vascular smooth muscle cell migration and proliferation. Foam cells produce several inflammatory cytokines and ECM degrading enzyme like MMPs (Galis et al.,1995), these MMPs determine the stability of atherosclerotic plaque.

Here we used, RAW 264.7 macrophage cells treated with OxLDL as model system to mimic foam cell in vitro. In our system also there was significant upregulation in the production of MMP-9 and MMP-2 on treatment with OxLDL.

Since chrysin is reported to have anti-inflammatory effect, we studied the effect of chrysin on the expression and regulation of MMPs in foam cells. Cells were maintained in culture in the presence of OxLDL and different concentration of chrysin (10, 20, 30 and 50µM) for 48 hr. ELISA showed significant upregulation in the production of MMP -9 and MMP-2 in foam cells and the effect was reversed by chrysin in a concentration dependent manner. Recent studies reported that, chrysin inhibits foam cell formation through promoting cholesterol efflux from RAW 264.7 macrophages (Shuai Wang et al., 2015) and showed anti-atherogenic potential in Wistar rats (Anandhi et al., 2014). More over chrysin inhibited the expression of MMP-9, MMP-2 and MMP-7 in Gastric cancer cells (Yong Xia et al., 2015). Our results also indicated that chrysin downregulated both the MMP -9 and MMP- 2 expression (figures 2 and 3).

MMPs produced by foam cells play vital role in the development and progression of atherosclerosis, downregulation of MMPs by chrysin has great therapeutic potential. Further studies are required to understand the mechanism of action of chrysin on the expression and regulation of MMPs in foam cell model system.



A. Control cells **B.** Foam cell

Figure 1. RAW 264.7 cells were maintained in culture with OxLDL for 48 hours

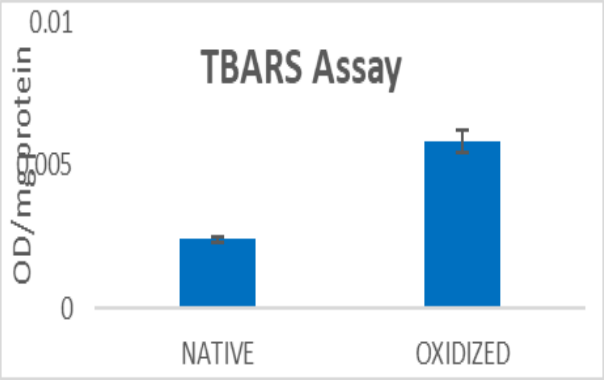


Figure 2. Oxidation of LDL TBARS assay

RAW 264.7 cells were maintained in culture in the presence of different concentrations of chrysin (10, 20, 30,50, 80 and 100 µM) for 24hours. Untreated cells served as control. The cells were treated with MTT and the viable cells produced formazan crystals. Result is the average of triplicate experiments ±SEM. Significant on comparison with control, *p<0.0001.

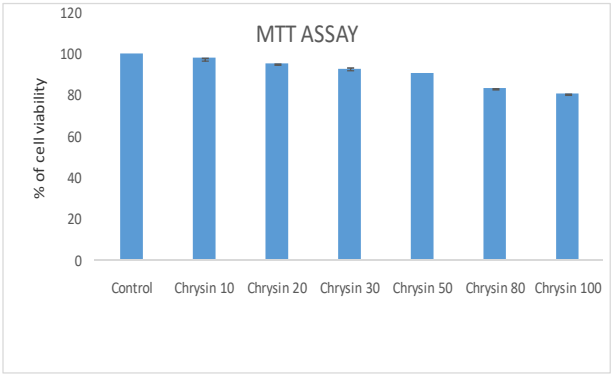


Figure 3. Effect of chrysin on the cell viability

Raw 264.7 cells were maintained in culture in the presence of OxLDL (50µg/ml) and OxLDL + chrysin in different concentration for 48 hrs. Untreated cells served as control. Medium was collected and protein equivalent volumes were used in ELISA, using specific antibodies against TNF-α. Results given are the average of triplicate experiments ±SEM. Significant on comparison with control and OxLDL control, OxLDL control and chrysin treated,*p<;0.0001.

References

Anandhi R, Thomas PA, Geraldine P. Evaluation of the antiatherogenic potential of chrysin in Wistar rats. Mol Cell Biochem (2014). 385:103–13.

Dao TT, Chi Y S , Haeil Park :- Synthesis and inhibitory activity against COX- 2-catalyzed prostaglandin production of chrysin derivatives. Bioorg. Med. Chem. Lett. (2004); 14: 1165-1167.

Galis Z. S., M. Muszynsk G. K, Sukhova, E. Simon-Morrissey, and P. Libby:-Enhanced expression of vascular matrix metalloproteinases induced in vitro by cytokines and in regions of human atherosclerotic lesions. Ann. N. Y. Acad. 1995. Sci. 748: 501–507.

Ross R :- Atherosclerosis--an inflammatory disease (1999), 340(2), 115-126.

Shuai Wang, Xue Zhang, Mingyue Liu, Hong Luan, Yubin Ji, Peng Guo ,Chongming Wu .Chrysin inhibits foam cell formation through promoting cholesterol efflux from RAW264.7 macrophages, Pharmaceutical Biology, (2015). 53:10, 1481-1487.

Wei Dou, Jingjing Zhang, Eryun Zhang, Aning Sun, Lili Ding, Guixin Chou, Zhengtao Wang and Sridhar Mani :- Journal of Pharmacology and Experimental Therapeutics June(2013), 345 (3) 473-482

Wolfman C, Viola H, Paladini A, Dajas F, Medina JH :- Possible anxiolytic effects of chrysin, a central benzodiazepine receptor ligand isolated from Passiflora coerulea. Pharmacol Biochem Behav (1994). 47:1–4

Yadav RK, Gupta SP, Sharma PK, Patil VM :-Recent advances in studies on hydroxamates as matrix metalloproteinase inhibitors: a review (2011).