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Protective Effects of Tricin Against Oxldl-Stimulated Inflammatory and Oxidative Stress Responses in RAW 264.7 Cells

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ABSTRACT

Oxidized low density lipoprotein (OxLDL) induced macrophage activation plays a major role in inflammatory and oxidative stress mediated disorders. The present study investigated the protective effect of tricin against OxLDL stimulated inflammatory and oxidative stress responses in RAW 264.7 cells. RAW 264.7 macrophages were pretreated with tricin (15 µM) or quercetin (25 µM) 1 h prior to OxLDL stimulation (50 µg/ml) and incubated for 24 h. DAPI staining revealed nuclear condensation and altered nuclear morphology in OxLDL treated cells, indicating cellular stress and inflammatory injury, whereas tricin treatment cells preserved nuclear integrity and reduced cellular damage. OxLDL stimulation significantly increased, total COX activity, PGE_2 levels MPO activity, MDA levels, and the pro-inflammatory cytokines TNF-α and IL-1β, while reducing SOD activity and anti- inflammatory cytokines IL-10 and TGF-β. Tricin treatment markedly suppressed total COX activity, PGE_2 production, MPO activity, lipid peroxidation, and inflammatory cytokine levels, while restoring antioxidant defence through increased SOD activity and anti-inflammatory cytokine levels. The findings of the present study demonstrated that tricin possesses significant anti-inflammatory and antioxidant activities against OxLDL-induced macrophage injury and may serve as a promising natural therapeutic agent for OxLDL associated inflammatory disorders.

KEYWORDS: Tricin; OxLDL; Inflammation; Oxidative stress; Cytokines; RAW 264.7 cells.

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I. INTRODUCTION

Inflammation is an important immune response that helps prevent infection and tissue damage. It plays a crucial role in maintaining tissue homeostasis through activation of several molecular and cellular signaling pathways. However, various inflammatory diseases and pathological conditions can arise as a result of prolonged or dysregulated inflammatory responses (Ahmed et al., 2011). The pathophysiology of chronic inflammatory disorders, such as atherosclerosis and cardiovascular diseases, is directly linked to oxidative stress as well as inflammation (Mittal et al., 2014). By secreting inflammatory cytokines, reactive oxygen species (ROS), and inflammatory mediators, macrophages contribute significantly to the initiation and development of inflammatory responses (Yu et al., 2013). Oxidized low density lipoprotein (OxLDL) is recognized as a major stimulator of macrophage activation, foam cell formation, and inflammatory injury during the progression of atherosclerosis (Poznyak et al., 2020; Mushenkova et al., 2021). Exposure of macrophages to OxLDL induces excessive production of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β), along with increased prostaglandin E2 (PGE_2) synthesis and cyclooxygenase (COX) mediated inflammatory responses (Li et al., 2021). OxLDL-mediated macrophage activation is also associated with excessive reactive oxygen species (ROS) generation, increased lipid peroxidation, elevated myeloperoxidase (MPO) activity, and depletion of endogenous antioxidant enzymes such as superoxide dismutase (SOD), thereby aggravating oxidative stress and inflammatory injury (Lara-Guzmán et al., 2018). Elevated malondialdehyde (MDA) levels serve as important biomarkers of lipid peroxidation and oxidative membrane damage, whereas elevated MPO activity reflects

inflammatory oxidative injury. In contrast reduced SOD activity contributes to impaired cellular antioxidant defence under OxLDL stimulated conditions (Lara-Guzmán et al., 2018).

Natural flavonoids have gained significant attention owing to their potent antioxidant and anti-inflammatory activities. Tricin, a naturally occurring flavone present in cereal grains and medicinal plants, is known to possess anti-inflammatory, antioxidant, and cytoprotective properties. Previous studies have demonstrated that tricin inhibits the production of inflammatory mediators and mitigates macrophage inflammatory responses through regulation of inflammatory signaling pathways (Li et al., 2021; Shalini et al., 2015). In addition, tricin has been reported to protect cells against oxidative stress-induced damage by scavenging reactive oxygen species and enhancing endogenous antioxidant defence systems. Moreover, tricin exhibits protective effects against inflammatory damage in various experimental inflammatory models, highlighting its potential therapeutic relevance in inflammation associated disorders (Shalini et al., 2015). Quercetin is a well-known flavonoid that has been extensively studied for its established antioxidant and anti-inflammatory properties. Previous reports have shown that quercetin effectively suppresses inflammatory cytokine production and alleviates oxidative stress in activated macrophages. Therefore, quercetin was employed as a comparative compound in the present study to evaluate the protective effects of tricin.

Recent investigations on flavonoid based interventions in OxLDL-stimulated macrophages have highlighted the importance of antioxidant phytochemicals in mitigating inflammatory injury and oxidative stress. Flavonoids have been demonstrated to suppress inflammatory cytokine production, reduce oxidative stress generation, inhibit foam cell formation, and improve macrophage dysfunction associated with OxLDL exposure (Li et al., 2022).

However, the protective role of tricin against OxLDL-induced inflammatory and oxidative stress responses in RAW 264.7 macrophages has not been fully elucidated. Therefore, the present study aimed to evaluate the anti-inflammatory and antioxidant effects of tricin in OxLDL stimulated RAW 264.7 macrophages by assessing nuclear morphological alterations using DAPI staining, oxidative stress markers, inflammatory enzyme activity, and inflammatory cytokines.

II. MATERIALS AND METHODS

A. Materials

RAW 264.7 macrophages were obtained from National Centre for Cell Science (NCCS, Pune, India). Dulbecco's modified Eagle's medium (DMEM) was brought from himedia. Methanol from merck, antibiotic-antimycotic solution and FBS from gibco, ELISA and culture plates were obtained from NUNC. All other reagents used were of analytical grade. Tricin was purchased from Sigma-Aldrich and quercetin from MP Biomedicals. OxLDL was isolated from human blood.

B. Cell Culture and Experimental Conditions

RAW 264.7 macrophages were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), penicillin-streptomycin antibiotics, and 1.5% sodium bicarbonate. A sterile cellulose acetate membrane filter with a pore size of 0.2 µm was used to filter the medium. Cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2 with media replacement every alternate day until approximately 70% confluency was attained.

C. Isolation and Oxidation of LDL

Blood was collected from healthy individuals and serum was separated by centrifugation. Very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) fractions were precipitated from the serum using phosphotungstic acid and $MgCl_2$ in the ratio of 1:3:5. The mixture was vortex mixed for 30 s, allowed to stand at room temperature for 10 min and centrifuged to collect the pellet.

The pellet was dissolved in 0.15 N NaCl to prepare LDL followed by dialysis against phosphate-buffered saline (PBS). Oxidation of LDL was induced by the addition of 10 µM $CuSO_4$ and incubated at 37°C for 12 h. The resulting oxidized LDL (OxLDL) preparation was then extensively dialyzed against PBS containing 0.3 mM Ethylenediaminetetraacetic acid (EDTA) for 2 days with periodic buffer changes. The degree of LDL oxidation was evaluated using the thiobarbituric acid reactive substances (TBARS) assay.

D. Experimental design

RAW 264.7 cell line were divided into four groups. Tricin and quercetin were induced 1hour prior to the induction of OxLDL

Group I - Control

Group II - OxLDL (50 µM)

Group III - OxLDL+ Tricin (15 µM)

Group IV - OxLDL+ Quercetin (25 µM)

LDL was isolated according to the method described by Havel et al. (1955), and oxidized LDL (OxLDL) was prepared following the method described by Nguyen-Khoa et al. (1999). The degree of LDL oxidation was determined using the thiobarbituric acid reactive substances (TBARS) assay.

The dose of tricin was selected based on a previous study by Shalini et al. (2012), while the dose of quercetin was selected with reference to an earlier report by Bhaskar et al. (2013).

RAW 264.7 macrophages were pretreated with tricin (15 µM) or quercetin (25 µM) for 1 h prior to stimulation with OxLDL (50 µg/ml). Cells were then incubated for 24 h, after

which culture supernatants and cell lysates were collected for further biochemical analyses.

1) DAPI Staining

DAPI staining was performed according to the method described by Kapuscinski (1995). RAW 264.7 macrophages were cultured on sterile coverslips in a 24-well plate and maintained in DMEM supplemented with 10% FBS at 37°C in a 5% CO_2 incubator. After 24 h, cells were treated with tricin and quercetin 1h prior to the induction of OxLDL. After treatment for 24 h cells were washed with PBS, fixed with 4% paraformaldehyde for 15 min, and permeabilized using 0.1% Triton X-100 for 5 min. Cells were then stained with DAPI solution (1 µg/mL) for 5–10 min in the dark and washed with PBS to remove excess stain. Nuclear morphological changes were observed under a fluorescence microscope using a DAPI filter.

2) Enzyme linked immunosorbent assay (ELISA)

Enzyme-linked immunosorbent assay (ELISA) was carried out according to the method described by Engvall and Perlmann (1971) for the quantification of inflammatory mediators using specific monoclonal antibodies. IL-1β, TNF-α, IL-10 and TGF-β levels were estimated in culture supernatants, whereas PGE_2 levels were analyzed in cell lysates using indirect ELISA.

3) Estimation of protein content

Protein content in the samples were estimated by the method of Lowry et al. (1951) using alkaline copper reagent and Folin - Ciocalteu reagent. The intensity of the developed colour was measured spectrophotometrically at 560 nm.

Calculation of total protein = O D of test × concentration of standard /O D of standard

4) Assay of Cyclooxygenase (COX) Activity

Cyclooxygenase (COX) activity was estimated by the thiobarbituric acid (TBA) method described by Shimizu et al. (1981). The reaction mixture consisted of 1 ml Tris-HCl buffer, 50 µl hemoglobin, and 50 µl enzyme sample. The reaction was initiated by by adding 100 µl arachidonic acid and incubated at 37°C for 20 min. The reaction was terminated by adding 10% trichloroacetic acid (TCA) in HCl, followed by the addition of 200 µl thiobarbituric acid (TBA). The mixture was then heated in a boiling water bath for 20 min, cooled, and centrifuged at 1000 rpm for 3 min. The absorbance of the supernatant was measured spectrophotometrically at 532 nm, and COX activity was determined accordingly.

5) Assay of Myeloperoxidase (MPO) Activity

Myeloperoxidase (MPO) activity was determined as an index of inflammatory response according to the method described by Bradley et al. (1982) with slight modifications After treatment, RAW 264.7 cells were washed twice with ice cold phosphate buffered saline (PBS) and harvested by scraping in 50 mM potassium phosphate buffer containing 0.5% hexadecyl trimethyl ammonium bromide (HTAB) (pH 6.0). The cell suspension was homogenized on ice and centrifuged at 20,000 g for 30 min at 4°C. The resulting supernatant was used for MPO activity assay. The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 6.0), 0.5 mM O-dianisidine dihydrochloride, 0.0005% H_2O_2, and the enzyme sample. The reaction was initiated by the addition of H_2O_2, and the change in absorbance was measured spectrophotometrically at 460 nm. MPO activity was normalized against total protein concentration and expressed as units/mg protein.

6) Assay of Malondialdehyde (MDA)

Malondialdehyde (MDA) level was determined by the method described by Ohkawa et al. (1979). Briefly, 0.2 ml enzyme sample was mixed with 0.2 ml of 8.1% sodium dodecyl sulfate (SDS), 1.5 ml acetic acid buffer (pH 3.5), and 1.5 ml of 0.8% thiobarbituric acid (TBA). The final reaction volume was adjusted to 4 ml with distilled water and heated in a boiling water bath at 95°C for 1 h. After cooling under running tap water, 1 ml distilled water and 5 ml butanol-pyridine reagent were added and mixed vigorously. The mixture was then centrifuged at 4000 rpm for 10 min, and the absorbance of the organic layer was measured at 532 nm against blank.

7) Assay of Superoxide Dismutase (SOD) Activity

Superoxide dismutase (SOD) activity was measured according to the method described by Kakkar et al. (1984). The assay mixture contained 1.2 ml sodium pyrophosphate buffer, 0.1 ml phenazine methosulfate, 0.3 ml nitroblue tetrazolium (NBT), 0.2 ml appropriately diluted enzyme sample, and distilled water to make a final volume of 3 ml. The reaction was initiated by the addition of 0.2 ml NADH and incubated at 30°C for 90 s. The reaction was stopped by adding 1 ml glacial acetic acid. The reaction mixture was vigorously mixed with 4 ml n-butanol and allowed to stand for 10 min, followed by centrifugation. The absorbance of the separated butanol layer was measured at 560 nm against butanol blank. One unit of SOD activity was defined as the amount of enzyme required to inhibit chromogen formation by 50% under the assay conditions and was expressed as units/mg protein.

E. Statistical analysis

Values are expressed as mean ± SEM of six values of each sample for in vitro experiments. The results were analyzed using a statistical program SPSS/PC+, Version 11.0 (SPSS Inc. Chicago, USA). Difference among the experimental groups was analyzed by one way analysis of variance (ANOVA), followed by Duncan's multiple range test for pair wise group comparisons. All results were considered statistically significant at $P \leq 0.05$ (Steel et al., 1997). Graphs were generated using Graph pad Prism 5.0 (San Diego, CA, USA).

III. RESULTS

A. Effect of Tricin on Nuclear Morphology by DAPI Staining in OxLDL-stimulated RAW 264.7 cells

DAPI staining was carried out to access nuclear morphological alterations in RAW 264.7 macrophages following OxLDL stimulation and treatment with tricin and quercetin. Control cells exhibited normal and uniformly stained nuclei, whereas OxLDL treated cells showed nuclear condensation and altered nuclear morphology, indicating cellular stress and inflammatory injury. Treatment with 15 µM tricin reduced nuclear condensation and preserved nuclear morphology compared with OxLDL treated cells. Similar effects were observed in 25 µM quercetin treated cells.

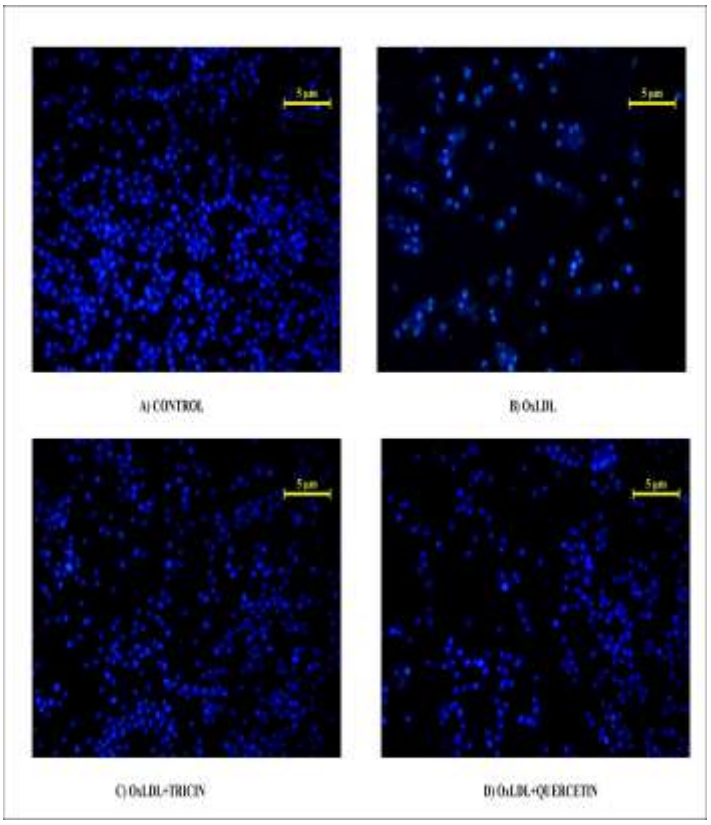

Figure 1: Effect of tricin on DAPI assay in RAW 264.7 cells observed under fluorescence microscopy (10X). A - untreated control. B - treated with oxidized low density lipoprotein (OxLDL, 50 µg/ml). C - received OxLDL + Tricin (15 µM). D - received OxLDL + Quercetin (25 µM). RAW 264.7 macrophages were pretreated with tricin (15 µM) or quercetin (25 µM) for 1 h prior to stimulation with OxLDL for 24 h. Tricin treated cells showed improved nuclear integrity with reduced cellular damage compared to the OxLDL treated cells. Similar findings have been shown in quercetin treated group.

B. Effect of Tricin on Total cox activity and PGE_2 in OxLDL-stimulated RAW 264.7 cells

OxLDL stimulation significantly increased total COX activity and PGE_2 levels in RAW 264.7 macrophages compared with the control group, indicating enhanced inflammatory mediator synthesis. Pretreatment with 15 µM tricin significantly attenuated total COX activity, which subsequently led to decreased PGE_2 production in OxLDL stimulated macrophages. Similarly, quercetin treatment also attenuated COX activity and reduced PGE_2 levels.

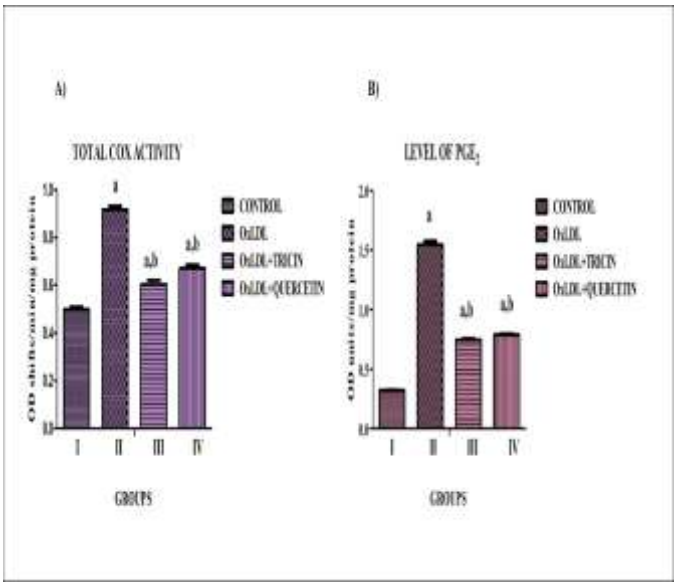

Figure 2: A) Effect of tricin on total COX activity determined by spectrophotometry. B) Effect of tricin on prostaglandin E_2 (PGE_2) levels by ELISA. RAW 264.7 macrophages were pretreated with tricin (15 µM) or quercetin (25 µM) for 1 h prior to stimulation with oxidized low density lipoprotein (OxLDL 50 µg/ml) for 24 h. Total COX activity and PGE2 levels were analyzed spectrophotometrically and by enzyme-linked immunosorbent assay (ELISA), respectively. Tricin and quercetin treatment significantly reduced total COX activity and PGE_2 levels compared with the OxLDL treated group. Data are expressed as mean ± SEM. "a" indicates significant differences compared with the control group, whereas "b" indicates significant differences compared with the OxLDL treated group.

C. Effect of Tricin on MPO Activity and MDA Levels in OxLDL-stimulated RAW 264.7 cells

OxLDL stimulation significantly increased MPO and MDA activity in RAW 264.7 macrophages indicating enhanced inflammatory response, oxidative stress, and lipid peroxidation. Pretreatment with 15 µM tricin markedly attenuated MPO activity and significantly reduced MDA levels compared to that of OxLDL treated group. Similarly, quercetin treatment also decreased MPO activity and MDA levels when compared with the OxLDL treated group.

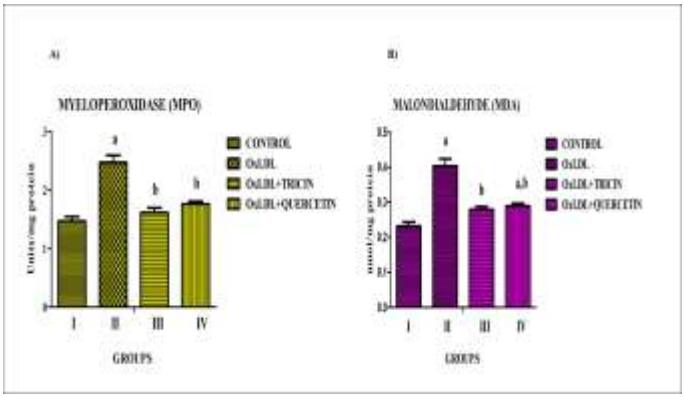

Figure 3 : A) Effect of tricin on myeloperoxidase (MPO) activity by spectrophotometry. B) Effect of tricin on malondialdehyde (MDA) levels by spectrophotometry. RAW 264.7 macrophages were pretreated with tricin (15 µM) or quercetin (25 µM) for 1 h prior to stimulation with oxidized low-density lipoprotein (OxLDL, 50 µg/ml) for 24 h. MPO and MDA activity was analyzed spectrophotometrically in cell lysates. Tricin treatment significantly reduced MPO and MDA activity compared with the OxLDL-treated group. Data are expressed as mean ± SEM. "a" indicates significant differences compared with the control group, whereas "b" indicates significant differences compared with the OxLDL treated group.

D. Effect of Tricin on Superoxide dismutase (SOD) Activity in OxLDL-stimulated RAW 264.7 cells

OxLDL stimulation significantly reduced SOD activity in RAW 264.7 cells indicating elevated oxidative stress and disruption of the cellular antioxidant defense system. Pretreatment with 15 µM tricin markedly restored SOD activity compared with the OxLDL-treated group. Similar restoration of SOD activity was also observed in quercetin treated cells.

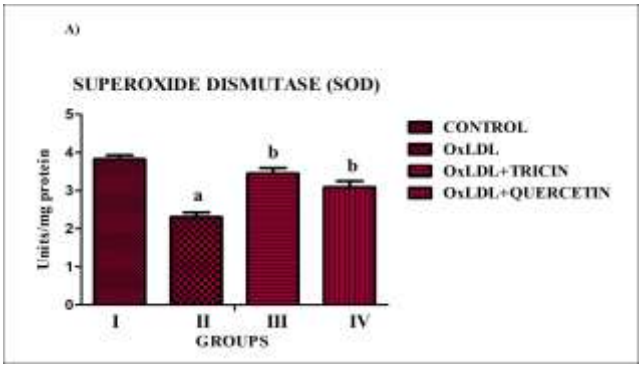

Figure 4 : A) Effect of tricin on superoxide dismutase (SOD) activity in OxLDL stimulated RAW 264.7 cells by spectrophotometry. RAW 264.7 macrophages were pretreated with tricin (15 µM) or quercetin (25 µM) for 1 h prior to stimulation with oxidized low density lipoprotein (OxLDL, 50 µg/ml) for 24 h. SOD activity was analyzed spectrophotometrically in cell lysates. Tricin treatment significantly increased SOD activity compared with the OxLDL treated group. Quercetin treated group also elevated SOD activity. Data are expressed as mean ± SEM. "a" indicates significant differences compared with the control group, whereas "b" indicates significant differences compared with the OxLDL treated group.

E. Effect of Tricin on TNF-α and IL-1β Levels in OxLDL-stimulated RAW 264.7 cells

OxLDL stimulation significantly increased TNF-α and IL-1β levels in RAW 264.7 cells indicating activation of inflammatory signaling pathways, macrophage activation, amplification of and progression of oxidative inflammatory injury. Pretreatment with 15 µM tricin markedly reduced TNF-α and IL-1β levels. Similar reductions in TNF-α and IL-1β levels were observed in quercetin treated cells compared with the OxLDL treated group.

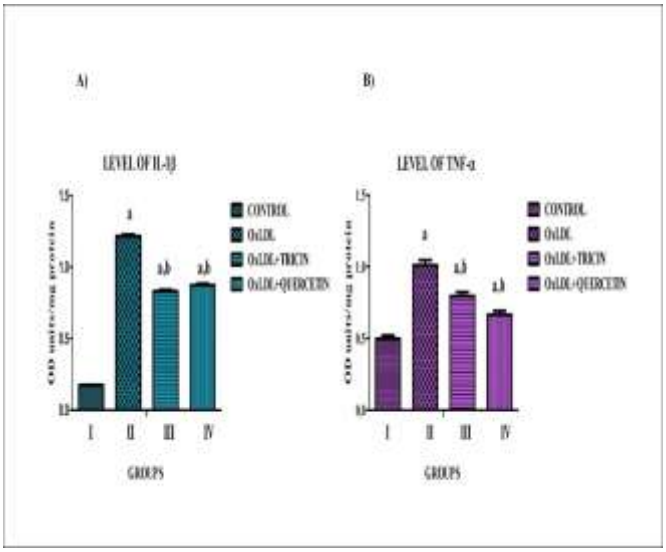

Figure 5 : A) Effect of tricin on tumor necrosis factor-alpha (TNF-α) by ELISA. B) interleukin-1 beta (IL-1β) levels by ELISA. RAW 264.7 macrophages were pretreated with tricin (15 µM) or quercetin (25 µM) for 1 h prior to stimulation with oxidized low density lipoprotein (OxLDL, 50 µg/ml) for 24 h. TNF-α and IL-1β levels were measured in culture supernatants. Tricin and quercetin treatment significantly reduced TNF-α and IL-1β levels compared with the OxLDL treated group. Data are expressed as mean ± SEM. "a" indicates significant differences compared with the control group, whereas "b" indicates significant differences compared with the OxLDL treated group.

F. Effect of Tricin on IL-10 and TGF-β levels in OxLDL-stimulated RAW 264.7 cells

OxLDL stimulation significantly reduced IL-10 and TGF-β levels in RAW 264.7 cells indicating impairment of the endogenous anti-inflammatory defence system. Pretreatment with 15µM tricin markedly increased IL-10 and TGF-β levels demonstrating its anti-inflammatory and

immunomodulatory potential maintaining immune homeostasis in macrophages. Similarly, quercetin treatment also restored IL-10 levels compared with the OxLDL treated group.

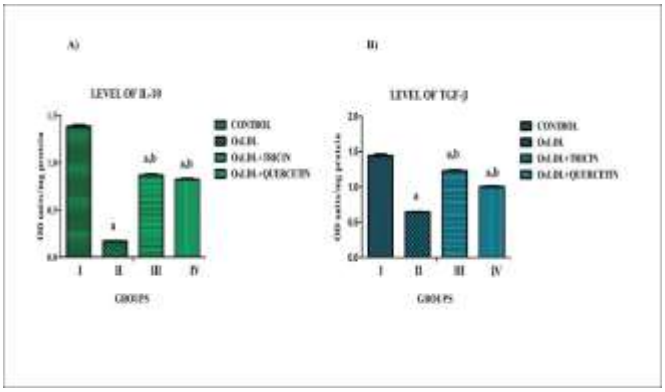

Figure 6 : A) Effect of tricin on interleukin-10 (IL-10) levels by ELISA. B) Effect of tricin on transforming growth factor beta (TGF-β) by ELISA. RAW 264.7 macrophages were pretreated with tricin (15 µM) or quercetin (25 µM) for 1 h prior to stimulation with oxidized low-density lipoprotein (OxLDL, 50 µg/ml) for 24 h. IL-10 and TGF-β levels were measured in culture supernatants. Tricin as well as quercetin treatment significantly increased IL-10 and TGF-β levels compared with the OxLDL treated group. Data are expressed as mean ± SEM. "a" indicates significant differences compared with the control group, whereas "b" indicates significant differences compared with the OxLDL treated group.

IV. DISCUSSION

OxLDL induced macrophage activation is considered a critical event in the progression of inflammation and atherosclerosis. The present study showed that tricin successfully reduced OxLDL-induced oxidative stress and inflammatory reactions in RAW 264.7 macrophages. DAPI staining revealed nuclear condensation and changed nuclear morphology, indicating cellular stress and inflammatory damage in OxLDL treated RAW 264.7 cells. On the other hand, tricin-treated cells showed less nuclear condensation and relatively intact nuclear morphology, indicating protection against OxLDL-induced cellular changes.

OxLDL stimulation significantly elevated total COX activity and PGE_2 levels in RAW 264.7 macrophages, indicating enhanced inflammatory mediator synthesis and activation of inflammatory pathways. Increased production of pro-inflammatory cytokines such as TNF-α and IL-1β may further contribute to enhanced COX activity and subsequent PGE_2 generation. Similar observations have been reported in OxLDL-induced macrophage inflammatory models (Zhang et al., 2015). Treatment with tricin markedly reduced total COX activity and PGE_2 production, demonstrating its potent anti-inflammatory activity. Previous studies have also shown that tricin suppresses inflammatory mediator production and modulates inflammatory signaling pathways in macrophages (Li et al., 2021; Shalini et al., 2015).

Oxidative stress plays a major role in OxLDL induced macrophage dysfunction and cellular injury. Elevated MPO activity observed in the OxLDL treated group reflects enhanced inflammatory oxidative injury and macrophage activation, whereas increased MDA levels indicate enhanced lipid peroxidation and membrane damage caused by excessive reactive oxygen species (ROS) generation. In contrast, decreased SOD activity suggests impairment of endogenous antioxidant defense mechanisms under OxLDL stimulation (Lara-Guzmán et al., 2018). Therefore, OxLDL induced inflammatory signaling may contribute to oxidative stress mediated cellular damage through increased ROS production and suppression of antioxidant defense systems.

Interestingly, tricin treatment significantly reduced MPO activity and MDA levels while restoring SOD activity, indicating strong antioxidant potential. The antioxidant effect of tricin may be associated with its free radical scavenging ability and its capacity to suppress oxidative stress-mediated inflammatory injury. Similar antioxidant and anti-inflammatory effects of flavonoids have been reported in OxLDL-stimulated macrophages, where these compounds attenuated oxidative stress, inflammatory cytokine production, and foam cell formation (Li et al., 2022).

Macrophages exposed to OxLDL generate excessive ROS and inflammatory cytokines, leading to amplification of inflammatory responses and oxidative injury (Poznyak et al., 2020). In the present study, OxLDL stimulation significantly increased TNF-α and IL-1β levels, indicating enhanced inflammatory activation and macrophage dysfunction. However, tricin treatment markedly suppressed these pro-inflammatory cytokines, further confirming its anti-inflammatory potential against OxLDL-induced inflammatory injury.

IL-10 is an important anti-inflammatory cytokine involved in suppression of inflammatory signaling and maintenance of immune homeostasis, whereas TGF-β plays a key role in regulating inflammatory responses and maintaining cellular homeostasis. In the present study, OxLDL exposure significantly decreased IL-10 and TGF-β levels, while tricin treatment restored the expression of these anti-inflammatory cytokines, suggesting modulation of the inflammatory microenvironment toward an anti-inflammatory state.

Although the precise molecular signaling pathways were not investigated in the present study, the protective effects of tricin may be associated with suppression of oxidative stress mediated inflammatory pathways. Further molecular investigations are required to elucidate the exact signaling mechanisms underlying the anti-inflammatory and antioxidant actions of tricin in OxLDL-stimulated RAW 264.7 cells.

V. CONCLUSION

The present study revealed that tricin preserved nuclear morphology and reduced cellular injury in RAW 264.7 macrophages, as evidenced by DAPI staining analysis. Tricin also exhibited significant anti-inflammatory and antioxidant activities by reducing inflammatory mediator production, oxidative stress, and lipid peroxidation while enhancing antioxidant defense mechanisms and anti-inflammatory cytokine levels. These findings suggested that tricin possesses potent cytoprotective, anti-inflammatory, and antioxidant properties and may serve as a promising natural therapeutic candidate for the prevention and management of inflammatory disorders. Further molecular and in vivo studies are required to elucidate the precise mechanisms underlying the protective effect of tricin.

VI. AUTHOR CONTRIBUTION

Salu Valsala Sasi Kumar : Data collection, Experiments, figure creation, article writing. **Haritha Rajan, Abhirami Sunitha, Amrutha Dileepkumar Sreejakumari** : Article writing, editing. **Helen Antony** : Conceptualization, Project administration, Editing and final drafting, Corresponding author.

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