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Full Length Original Research Paper

Quercetin alleviate oxidative stress and inflammation through up-regulation of antioxidant machinery and down-regulation of COX2 and NF-κB expression in collagen induced rheumatoid arthritis

Md. Meraj Ansari

Neha

Haider A. Khan*

Heavy metal and Clinical Toxicology Laboratory, Department of Medical Elementology and Toxicology, Hamdard University, Hamdard Nagar, New Delhi, India-110062

Corresponding Authors:
Haider Ali Khan
E-mail halitox@gmail.com

Abstract:
Rheumatoid arthritis (RA) is a chronic inflammatory disease that is characterized by persistent inflammation in the synovial membranes of joints, migration of activated phagocytes and other leukocytes into synovial and periarticular tissue, erosive damage of articular cartilage and bone. Quercetin (QU) is one of common polyphenolic flavonoid biosynthesized by plants and has been suggested to modulate inflammatory responses in various models. Therefore, we studied the ameliorative effect of QU on collagen induced arthritis (CIA) in wistar rat. The effect of treatment in the rats were monitored by clinical scoring, biochemical parameters, histopathological evaluation and immunohistochemical expression of proteins. Rat with treatment with QU significantly replenished lipidperoxidation (LPO) level, nitric oxide (NO) activity and articular Elastase (ELA) level. It also restored altered enzymatic and non enzymatic antioxidant system of tissue in arthritis rat. Histomorphometric and immunohistochemistry analysis revealed that collagen induced inflammatory cell infiltration ,as well as the elevated expression of NF-κB p65 and pro-inflammatory ptotein cyclooxygenase-2 observed in synovial tissue,were significantly inhibited by treatment with QU. The biochemical alterations were further supported by histopathological observations and immunohistochemical expression .we hypothesized that anti-inflammatory activity of QU may be related to predisposition to scavenge free radical, upregulate antioxidant machinery and down regulate immunohistochemical expression of cox2 and NF-κBp65 in arthritis rat.

Keywords: Quercetin, Flavonoids, rheumatoid arthritis, antioxidant.

Introduction

The pathology of RA is characterized by the proliferation of synovial cells , angiogenesis and pannus formation. Multiple cell types, including lymphocytes, dendritic cells, macrophages, and synovial fibroblasts, contribute to the chronic inflammatory responses of RA and comprise a major portion of the invasive pannus.[1] Reactive nitrogen species (RNS) and Reactive oxygen species (ROS) may perpetuate inflammation by facilitating the generation of chemotactic factors at the local site. The major RNS and ROS generated are the superoxide anion radical ($O_2^{\cdot-}$) the hydroxyl radical (OH) and nitric oxide radical (NO).[2]Production of Oxygen radical is comparatively high in the joints of patients affected with RA.[3, 4] Polymorphonuclearcells (PMNs) alter IgG by generating free radicals, which could in turn activate PMNs to produce additional superoxides.[5,6] NO is produced from L-arginine by three nitric oxide synthase (NOS) enzymes; endothelial NOS(eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS).Low physiological levels of NO are produced by constitutively expressed eNOS and nNOS, whereas iNOS is responsiblefor prolonged production of larger amounts of NO.[7] NO is a free radical that serves as an essential inflammatory messenger molecule.[8] Pro-inflammatory cytokines induce the

expression of iNOS in number of cells, including fibroblasts[9] may have either a toxic or a protective effect.[10,11] Chemokines induced by NO also contribute to disease development in arthritis. Decreased production of NO via suppressing or inhibiting iNOS reduces arthritic symptoms and affords protection.[12] Mast cells are involved in several of inflammatory and immune events. Mast cell derived mediators induce edema, destroy connective tissue, and are involved in lymphocyte chemotaxis and infiltration and in pathological fibrosis of RA joints. Moreover, mast cells are involved in angiogenesis during RA, and their proteolytic activity results in cartilage destruction and bone remodelling.[13]

Current treatments for RA either produce symptomatic relief (non-steroidal anti-inflammatory drugs; NSAIDs) or modify the disease process (disease-modifying anti-rheumatic drugs; DMARDs). Though effective, their use is also limited by their side effects including perforation and gastrointestinal ulcers, cardiovascular complications and opportunistic infections due to immunosuppressant.[14] Due to the long-term use, side effects are associated with these agents, patients with arthritis rely on other substitutes like use of complementary and alternative medicine (CAM) and according to reports CAM therapy is on rise as 60-90% dissatisfied patients are likely to seek option of CAM therapy.[15]

Quercetin is one of a group of over 4000 naturally available plant phenolics whose isolation and biological identification were first described by Szent-Gyorgyi in 1936. Flavonoids, regular constituents of the diet, were first identified as vitamin P, and, along with vitamin C were found to be important in the maintenance of capillary wall integrity and capillary resistance. The antioxidant molecules quercetin has been reported to modulate numerous aspects of cell function relevant to inflammatory arthritis.[3] At the molecular level, quercetin is known to inhibit NF-κB, a central transcription factor in inflammatory and proliferative diseases.[2,4,6] Quercetin inhibits inflammatory aspects of synovial cell function[2] , neutrophil activation[8] and the proliferation of different cancer cell types.[9] In this study we used collagen induced arthritis (CIA) rat model to evaluate the antioxidant, anti-inflammatory and anti-arthritis activity of quercetin.

Materials and Methods

Chemicals

Quercetin was purchased from SD fine limited, Mumbai ,Freund's adjuvant complete (CFA) was purchased from Sigma, USA, N-methoxysuccinyl-Ala-Ala-Pro-Val p-nitroanilide and Griess Reagent system were purchased from Sigma Chemical Co. (St Louis, MO, USA). Bovine serum albumin (BSA),Collagen type II from bovine nasal septum was purchased from Elastin Products Co, INC, Owensville, Missouri, USA. Thiobarbituric acid (TBA),epinephrine, glycine, poly-L-lycine, trichloroacetic acid (TCA), 5-5'dithio-bis-2-nitrobenzoicacid (DTNB), nitrobluetetrazolium (NBT), ethylene diamine tetra-acetic acid (EDTA), Poly-HRP plus ONE detection System (Thermo Scientific) , toluidine blue, Tris hydrochloride were purchased from SD Fine chemicals India. All other routine chemicals used in this investigation were of research grade.

Animals

Female Wistar rats (150-170g), 6-8 weeks old, were obtained from the Central Animal House Facility of Hamdard University (New Delhi, India). Rats were housed in an animal care facility under an ambient temperature of 25±2 °C with 12 hr light

/dark cycles after initial acclimatization for about 1 week. They had free access to standard rodent pellet diet and water ad libitum. Animals received humane care in accordance with the guideline of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA),Government of India, and prior permission was sought from the Institutional Animal Ethics Committee (IAEC NO:173/CPCSEA,28 January 2000).

Treatment Regimen

To study the effect of treatment with quercetin against collagen induced rheumatoid arthritis, 24 female Wistar rats were randomly allocated to 4 groups of 6 rats each.

Group I (Control). Rats received basal diet and corn oil orally (5 ml/kg b.wt. once daily, from 9th day).

Group II (CIA). Served as CIA induced arthritis group.

Group III (CIA+QU). Served as arthritis treated group (160mg/kg b.wt orally from onset of disease once daily, from 9th day)

Group IV (Only QU). Rats received basal diet+ quercetin (160 mg/kg b.wt. orally from 9th day) dissolved in corn oil every day from 9th day throughout the experiment (Figure 1). All the rats were anaesthetized with mild anesthesia and sacrificed by cervical dislocation after 21 day.

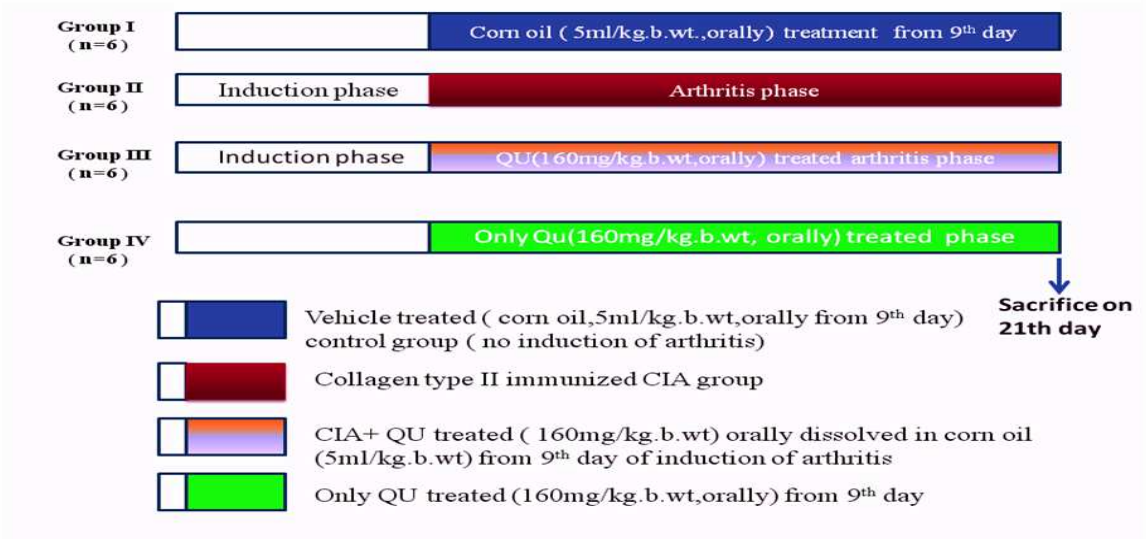

Figure 1: Schematic representation of the experimental design

Induction of collagen-induced arthritis (CIA)

Arthritis was induced in rats as described previously.[16] Collagen Type II from bovine nasal septum was dissolved in 0.05 M acetic acid at a concentration of 2 mg/ml was emulsified with an equal volume of Freund's adjuvant complete (CFA) containing 1 mg/ml *Mycobacterium tuberculosis* H37 RA and stored on ice before use. Rats were immunized intradermally at about 1.5 cm distal from the base of the tail.

Measurement of Clinical Severity of Arthritis

For examine the severity of disease paw thickness have been examined before and after the onset of the disease daily with a digital calliper (YAMAYO, Japan). Evaluation of joint inflammation was performed by an observer having no knowledge of the treatment protocol. The severity of the arthritis was examined after every third day by a clinical score measurement[17] from 0 to 4.

Preparation of Cell-Free Extract of the Knee Joints tissue

At the end of experiment animals were anesthetized and sacrifices by cervical dislocation. Arthritic and non arthritic joints were removed and cut into small pieces and homogenized in 5 volume of 50 mM Tris HCl buffer, pH 7.4 containing 0.1 M NaCl and 0.1% Triton X-100 and 1 vol. of fine glass powder by using a mortar and pestle. The crude extract then was sonicated for 20 sec. The homogenate was centrifuged at 3,000 × *g* for 5 minutes, and the resulting supernatant was stored at - 20°C until further analysis.

Articular Elastase (ELA)

ELA levels in the articular joints were evaluated as an index of polymorphonuclear leukocyte (PMNs) accumulation and activation in the inflamed tissue as described earlier.[18] Briefly, tissue samples were homogenized in a solution containing 20 mM potassium phosphate buffer pH 7.0 in a ratio of 1:10 (w/v) and centrifuged for 20 minutes at 10,000 x g at 4^0C. An aliquot of each sample was incubated for 24 hr at 37 ^{0}C with 0.1M Tris–Hcl buffer, (pH 8.0), containing 0.5M NaCl and 1mM N-methoxysuccinyl-Ala-Ala-Pro- Val p-nitroanilide, a high specific synthetic substrate for neutrophil elastase (ELA). The amount of p-nitroanilide liberated was measured spectrophotometrically at 405nm and was considered as neutrophil ELA activity. The ELA activity was converted and expressed as nanogram/gram of protein using molar extinction coefficient (9500) of substrate as per manufacturer recommendation.

Estimation of thiobarbituric acid reactive substances (TBARS)

The assay of TBARS was done according to earlier method [19] adapted to microplate reader by bringing the final volume to 150µl. In brief, tissue homogenate was prepared in 0.15M KC l (5% w/v homogenate) and aliquots of 30µl were incubated for 0°C and 37°C at 1 hour. Subsequently, 60 µl of 28% w/v TCA was added and the volume was made up to 150 µl by adding 60 µl of distilled water followed by centrifugation at 3000 x g for 10 minutes. The supernatant (125 µl) was taken and colour was developed by addition of 25 µl of 1% w/v TBA dissolved in 0.05 N NaOH and kept in boiling water bath for 15 minutes and the absorbance was read at 532nm in a plate reader (Bio-Rad, U.S.A.). The result was expressed in nanomoles TBARS formed/hour/gram tissue using a molar extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$.

Assay for reduced glutathione (GSH)

The GSH content in joint tissue was determined by the method [20] in which 1.0 ml of PMS fraction (10%) was mixed with 1.0 ml of sulphosalicylic acid (4%). The samples were incubated at 4°C for at least 1 hour and then subjected to centrifugation at 1200 × g for 15 minutes at 4°C. The assay mixture contained 0.4 ml filtered aliquot, 2.2 ml joint buffer (0.1 M,pH 7.4), and 0.4 ml DTNB (10 mM) in a total volume of 3.0 ml. The yellow colour developed was read immediately at 412 nm on spectrophotometer .The GSH content was calculated as micromole DTNB conjugate formed/gram tissue using molar extinction co-efficient of $13.6 \times 10\ M^{-1}\ cm^{-1}$.

Assay for Superoxide dismutases (SOD) activity

Manganese superoxide dismutase (MnSOD) activities were measured according to the method.[21] The assay was based on the ability of SOD to inhibit the auto-oxidation of epinephrine at alkaline pH. The mitochondrial suspension (0.2ml) was treated with 0.8ml of 50mmol/ l glycine buffer (pH 10.4) and 0.020 ml epinephrine. MnSOD activity was measured kinetically at

480nm. The activity was measured indirectly by the oxidized product of epinephrine. MnSOD activity was expressed as nanomoles of (-) epinephrine protected from oxidation per minute per milligram protein by using a molar extinction coefficient of 4020M^{-1} cm^{-1}.

Assay for catalase activity

Catalase activity was assayed by as described earlier method .[22] The reaction mixture consisted of 1.95 ml joint buffer (0.1 M, pH 7.4), 1.0 ml hydrogen peroxide (0.019 M), and 0.05 ml 10% PMS in a final volume of 3 ml. Changes in absorbance were recorded kinetically by spectrophotometer at 240 nm. Catalase activity was calculated as nanomoles H_2O_2 consumed per minute per milligram protein by using a molar extinction coefficient of 39.6M^{-1} cm^{-1}.

Nitric oxide (NO): Griess Reaction

Joint tissue from the sacrificed animals were washed with PBS (pH 7.4) and placed on ice as described by earlier method .[23]Briefly a 50 µl sample was added with 100 µl of Griess reagent and reaction mixture was incubated for about 5-10 minutes at room temperature and protects it from light, the optical density was measured at 540nm in microplate reader according to the manufacturer's protocol. Calculations were done after generating a standard curve from sodium nitrite in the same buffer which is used for preparation of homogenate.

Histological examinations

Rats were sacrificed on 21th the day by anesthetized and sacrificed by cervical dislocation. Knee joints were removed and fixed in 4% paraformaldehyde. After decalcification in 5% formic acid, the samples were processed for paraffin embedding .[24]Tissue sections (5 µm thick) were stained with haematoxylin–eosin and observed under an Olympus microscope (Olympus U-TV 0.63×C; SN 9L01588 T2, Tokyo, Japan (Made in Philippines)).

Mast Cell Staining

For detection of mast cells, Rats were anesthetized and sacrificed by cervical dislocationon on 21th day. Knee joints were removed and fixed in 4% paraformaldehyde. After decalcification in 5% formic acid, the samples were processed for paraffin embedding. [25] The joint sections of 5 µm were cut from formalin-fixed, paraffin-embedded tissue blocks and mounted on Poly-L-Lysine coated microscopic slides. Paraffinized sections were dewaxed in xylene and rehydrated through graded series of ethanol to water. The sections were stained with 0.1% toluidine blue (pH 2.3) in 1% sodium chloride solution for 5 minutes. The slides were then washed three times in distilled water and dehydrated quickly in alcohol, clear in xylene and mounted by using mounting media. The slides were then evaluated under the light microscope (Olympus U-TV 0.63×C; SN 9L01588 T2, Tokyo, Japan (Made in Philippines)). Staining with toluidine blue permits the identification of mast cells because mast cell granules stain metachromatically, resulting in deep purplish-blue granular cytoplasmic staining.

Immunohistochemistry

Sections of paraformaldehyde-fixed, paraffin-embedded joints were obtained on poly-L-lysine coated slides. Primary antibodies: Anti-rat NF-kB-p65 rabbit antibody (dilution 1:100, Biolegend); Anti-rat COX-2 polyclonal antibody (dilution 1:200, Santa Cruz Biotechnology, Inc.). The samples were processed according to the manufacturer's protocol recommended for the NF-κB p65 and COX-2 immunohistochemistry with slight modifications.

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Protein content

Protein was determined by Bradford method [26] using bovine serum albumin (BSA) as a standard.

Statistical analysis

Results are expressed as mean ± SEM. Statistical analysis of the data was done by applying the analysis of variance (ANOVA), followed by Turkey's test for all parameters. The $p < 0.05$ was considered significant.

Results

Effect of quercetin on clinical severity and paw swelling

Arthritis developed rapidly in rats immunized with collagen type II (CII). Clinical signs of the disease were erythema of one or more ankle joints, followed by involvement of the metatarsal and interphalangeal joints, first appeared in the hind paws between 8 and 9 days after CII immunization, with a 100% incidence by day 12±1 (Figure 2A). Quercetin treatment suppressed the progression of collagen induced arthritis. There was no evidence of either hind paw erythema or edema in the control group. Clinical severity of disease at the end of experiment shows in figure 2C. Our data suggested that oral quercetin administration to collagen-immunized rats reduced the progression of arthritis by inhibiting the increase in arthritis score (Figure 2B) and paw swelling compared to CIA rats.

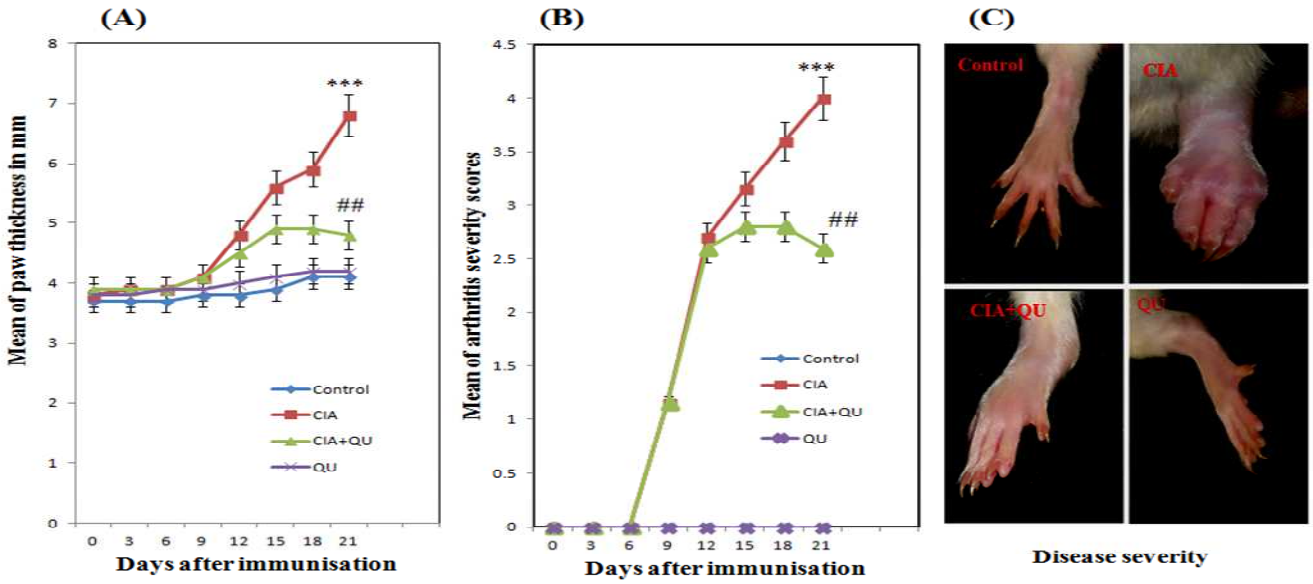

Figure 2: Effect of quercetin (QU) on time course of change in hind paw diameter (mm) (2A) ,mean clinical severity score (2B) and disease severity at the end of experiment rats immunized with collagen type II in figure 2C.. Significant differences indicated by ***$p < 0.001$ as compared to control and significant differences indicated by ##$p < 0.01$ as compared to CIA groups.

Effects of quercetin supplementation on elastase activity (ELA) in joints tissue of rats

The activity of ELA (Figure 3A) was increase significantly (*$p < 0.05$) in CIA group (Group II) as compared to control group (Group I). Treatment with quercetin showed a significant decrease in the activity of ELA in CIA+Qu group (Group III) (##$p < 0.01$) when compared with CIA group (Group II). Group IV showed no significant

changes in ELA activity as compared to control group.

Effects of quercetin supplementation on the lipid peroxidation in joints tissue of rats

The level of lipid peroxidation products (TBARS) (Figure 3B) was enhanced significantly (***p < 0.001) in CIA group (Group II) as compared to control group (Group I). Treatment with quercetin showed a significant decrease in the level of TBARS in CIA+Qu group (Group III) (#p < 0.05) when compared with CIA group (Group II). Group IV exhibited no significant changes in the level of TBARS as compared to control group.

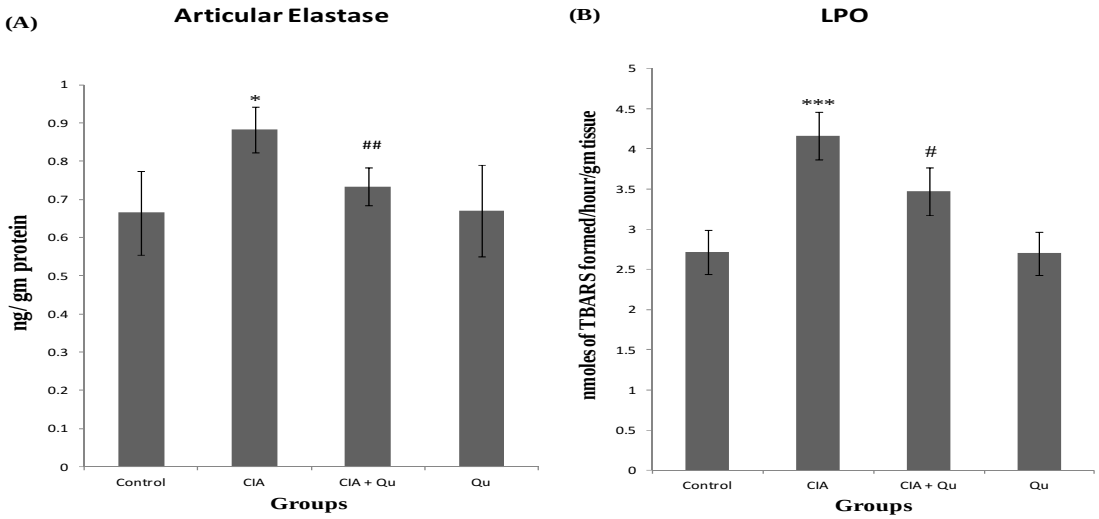

Figure 3: Effect of quercetin treatment on (A) ELA activity (B) lipid peroxidation in joints of rats. Each value is represented as mean±SEM (n=6). Significant differences indicated by *p < 0.05, ***p < 0.001 as compared to control and significant differences indicated by ##p < 0.01, #p < 0.05, as compared to CIA groups.

Effects of quercetin supplementation on GSH level in joints tissue of rats

The level of GSH (Figure 4A) was depleted significantly (**p < 0.01) in CIA group (Group II) as compared to control group (Group I). Treatment with quercetin showed a significant increase in the level of GSH in CIA+Qu group (Group III) (#p < 0.05) when compared with CIA group (Group II). Group IV exhibited no significant changes in the level of GSH as compared to control group.

Effects of quercetin treatment on the level of nitric oxide (NO) in joints tissue of rats

The level of NO (Figure 4B) was enhanced significantly (***p < 0.001) in CIA group (Group II) as compared to control group (Group I). Treatment with quercetin showed a significant decrease in the level of nitric oxide (NO) in CIA+Qu group (Group III) (#p < 0.05) when compared with CIA group (Group II). Group IV showed no significant changes in the level of TBARS as compared to control group.

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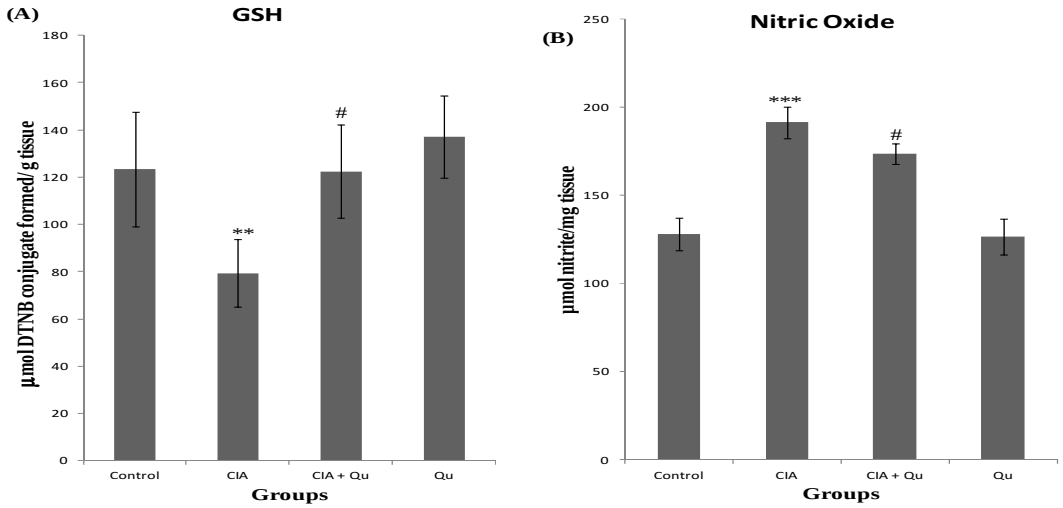

Figure 4: Effect of quercetin treatment on (A) GSH content and (B) NO level in joints tissue of rats. Each value is represented as mean± SEM (n=6). Significant differences were indicated by **p< 0.01, ***p< 0.001 as compared to control and #p < 0.05 as compared to CIA group.

Effects of quercetin treatment on the catalase activity in joints tissue of rats

The activity of catalase (Figure 5B) was reduced significantly (*p < 0.05) in CIA group (Group II) as compared to control group (Group I). Treatment with quercetin showed a significant increase in the activity of catalase enzyme in CIA+Qu group (Group III) (#p < 0.05) when compared with CIA group (Group II). Group IV showed no significant changes in catalase activity as compared to control group.

Effects of quercetin treatment on the superoxide dismutase (SOD) activity in joints tissue of rats

The activity of SOD (Figure 5A) was reduced significantly (*p < 0.05) in CIA group (Group II) as compared to control group (Group I). Treatment with quercetin showed a significant increase in the activity of SOD enzyme in CIA+Qu group (Group III) (#p < 0.05) when compared with CIA group (Group II). Group IV showed no significant changes in SOD activity as compared to control group.

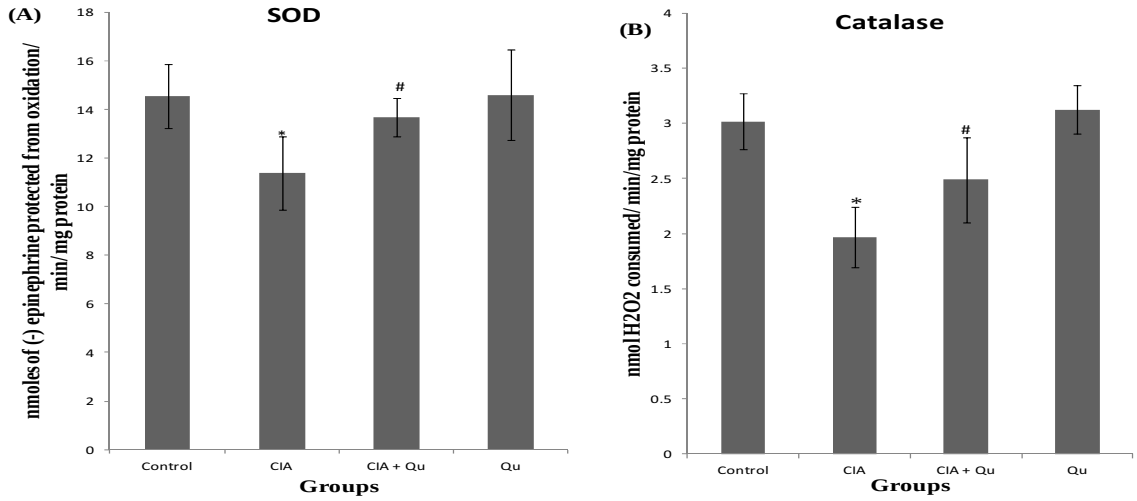

Figure 5: Effect of quercetin treatment on (A) SOD activity (B) Catalase activity in joints tissue of rats. Each value is represented as mean±SEM (n=6). Significant differences indicated by *p < 0.05 as compared to control and #p < 0.05 as compared to CIA group.

Effects of quercetin against collagen-induced histopathological alterations in the joints of rats

In corroboration with the biochemical alterations, the histological findings (Figure 6) showed intense infiltration of inflammatory cells in the histological section of joints of CIA group (Group II). Bone suffered resorption and pannus formation, whereas synovial hyperplasia was consistent finding. Treatment with quercetin markedly attenuated collagen induced histopathological changes.

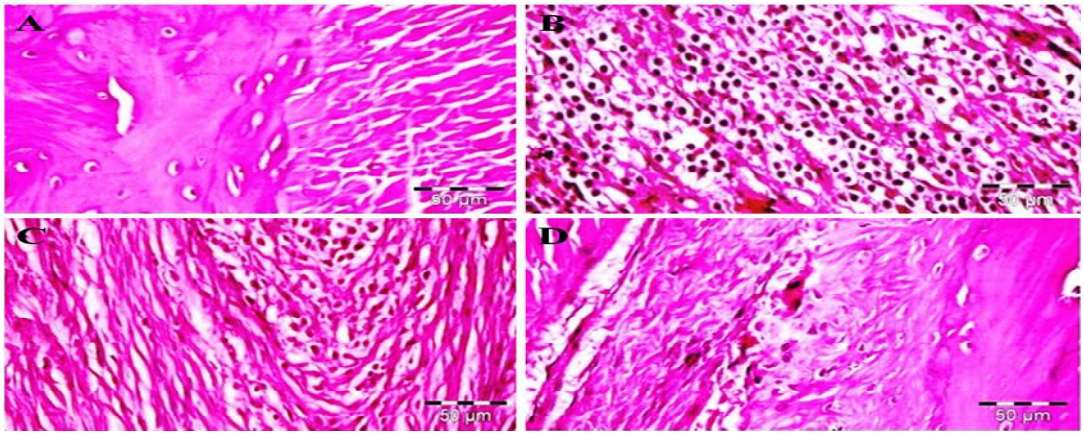

Figure 6: **Photomicrographs depicting Hematoxylin-Eosine staining**. (A) control group showing no infiltration of nutrophil cell in synovium, (B) arthritis group showing massive nutophil cell infiltration in synovium, (C) quercetin treated arthritis group showing less neutrophil cell infiltration in synovium with compare to arthritis group and (D) there is no neutrophil cell infiltration in synovium in only quercetin group. Magnified view (40x magnifications) .scale bar = 50µm

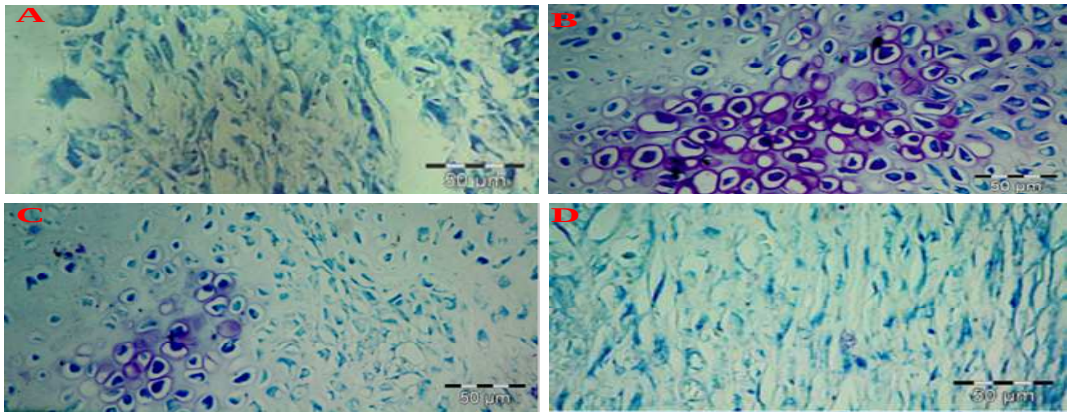

Figure 7: **Representative Photomicrographs depicting mast cells staining (Toludine blue staining).** (A) Control group showing no infiltration of mast cell in synovium, (B) arthritis group showing massive mast cell infiltration in synovium in purplish-blue staining, (C) quercetin treated arthritis group showing less mast cell infiltration in synovium with compare to arthritis group and (D) there is no mast cell infiltration in synovium in only quercetin group. Magnified view (40x magnifications) .scale bar = 50µm

Effect of quercetin on NF-κB expression in rat joint tissue

The nuclear transcription factor (NF-κB) play important role in inflammatory disease. Cells which have NF-κBp65 expression in synovial lining are thought to be macrophase like synoviocytes. For immunohistochemical analyses, brown colour indicates specific immunostaining of NF-κB p65. Quercetin treated CIA group (Figure 8) shows down-regulate NF-κB p65 expression. A moderate NF-κB p65 expression in CIA group was observed, whereas control group and only quercetin treated group showed very low or no expression of NF-κB p65 in joint tissues sections.

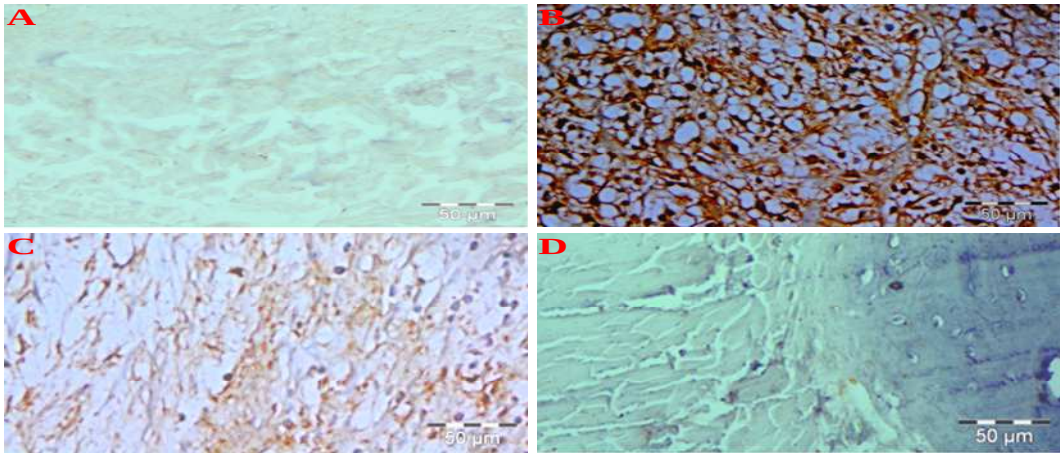

Figure 8. Representative photomicrograph of NF-κB p65 expression in rat joint sections. For immunohistochemical analyses, brown colour indicates specific immunopositve staining of NF-κB p65.(A) NF-κB p65 expression in control group showing very less or no immunopositive staining,(B) NF-κB p65 expression in CIA group showing intense immunopositive staining,(C) NF-κB p65 expression in quercetin treated CIA group showing reduced immunopositive staining, (D) NF-κB p65 expression in only quercetin treated group showing very less or no immunopositive staining similar to control group. Original magnification 40x. Scale bar = 50 µm.

Effect of quercetin on COX2 expression in rat joint tissue

COX2 is dominant source of prostaglandins formation in inflammation and in inflammation, PGE_2 is involved in all processes leading to the classic signs of inflammation e.g. redness, swelling, and pain. For immunohistochemical analyses, brown colour indicates specific immunostaining of COX2.The immunohistochemical evaluation showed intense expression of COX2 in CIA group (figure 9). Quercetin treatment down-regulate the COX2 expression. Control and only quercetin treated group show very low or no expression of COX2 expression in rat joint tissue sections.

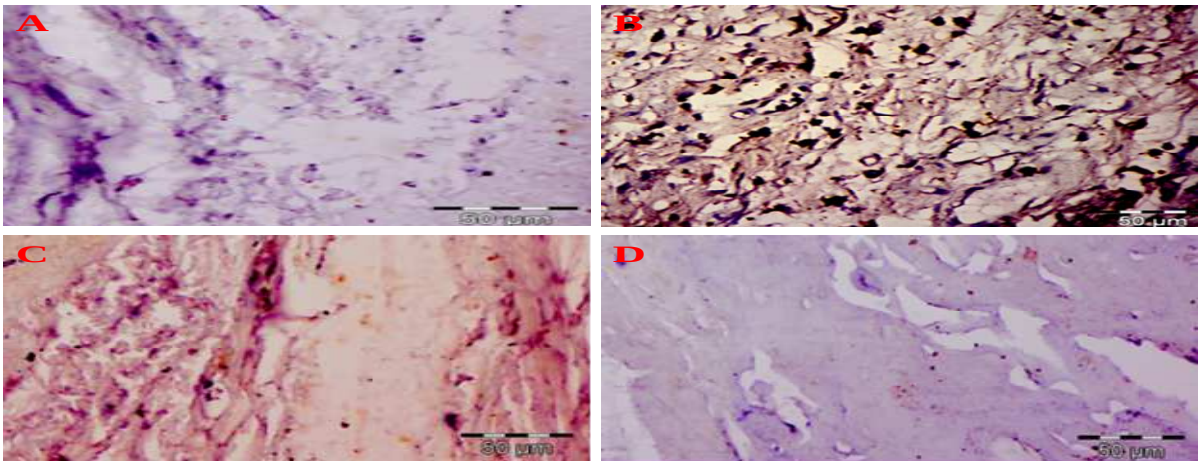

Figure 9. Representative photomicrograph of COX-2 expression in rat joint sections. For immunohistochemical analyses, brown colour indicates specific immunopositive staining of COX-2.(A) COX-2 expression in control group showing very less or no immunopositive staining,(B) COX-2 expression in CIA group showing intense immunopositive staining,(C) COX-2 expression in quercetin treated CIA group showing reduced immunopositive staining, (D) COX-2 expression in only quercetin treated group showing very less or no immunopositive staining similar to control group. Original magnification 40x. Scale bar = 50 µm.

Discussion

Many plants polyphenolic compounds, including a large class of bioflavonoids, are known to offer health benefits to humans. Flavonoids represent a group of phytochemicals exhibiting a wide range of biological activities arising mainly from their antioxidant properties and ability to modulate several enzymes or cell receptors.[27,28,29,30] Flavonoids have been recognized to exert antibacterial and antiviral activity, anti-inflammatory, antiangiogenic and antiallergic effects, analgesic,hepatoprotective, cytostatic, apoptotic, estrogenic, and antiestrogenic properties.[31,32,33,34] The beneficial effects have been attributed to their antioxidant and anti-inflammatory properties. The current study shows that anti-inflammatory and anti-arthritis activity of quercetin may be related to predisposition to scavenge free radical, upregulate antioxidant machinery and down regulate immunohistochemical expression of COX2 and NF-κBp65 in arthritis rat.

Elastase is a primary serine granule proteinase with broad substrate specificity includes proteoglycan, elastin, and collagen. Tremendous increase in the elastase burden in certain diseases due to the influx of large numbers of activated polymorphonuclear neutrophils (PMNs).[35] We evaluated elastase activity is directly proportional to the accumulation and activation of PMNs in the inflamed tissue. The inflammation so caused by the infiltrating cells leads to the release of nitrogen species and reactive oxygen species.[36,] We suggest that the decrease in elastase activity might be due to the inhibition of lipid peroxidation and the consequent decrease in the reduction of chemotactic peroxide.[38]

The large amount of TBARS found is consistent with the occurrence of damage mediated by free radicals. Oxidative stress is an imbalance between the antioxidant and prooxidants . In normal condition of aerobic metabolism free radical production occurs in the body. Disturbances in this normal redox state can cause toxic effects through the production of free radicals and peroxides.[36] In order to protect tissues from oxidative injuries,the body possesses natural antioxidant enzymatic systems such as superoxide dismutase and catalase enzymes. It has been reported that in arthritis decreases serum or synovial SOD and catalase activities together with other endogenous antioxidant systems.[39] In this study, it is suggested that quercetin which is a potent antioxidant, reduced paw inflammation significantly in quercetin treated animals by scavenging free radicals, which are thought to initiate cellular damage in cartilage in experimental animals.[40]

A reduction in GSH may impair H_2O_2 clearance and endorse OH·formation, thus increasing of the free radical loads that result in the disruption of cell homeostasis. As all antioxidant defences are interrelated [41],disruption of the microenvironment by a single factor can shift the entire balance and lead to a catastrophe. Quercetin treatment increased the activities of antioxidant enzymes significantly in the CIA group. Our results clearly indicate that the protective role of quercetin was mediated via its antioxidant effect through the suppression of lipid peroxidation and up-regulation of the antioxidant defence system. Nitric oxide (NO) is an important signalling molecule, produced as part of the inflammatory response from activated cells and macrophages .[42,43]In the present study, increased NO level have been detected in arthritic group similar with those

previously reported in synovial fluids of patients with rheumatoid arthritis.[37] Quercetin treatment suppressed NO production.

Mast cells are involved in several of inflammatory and immune events. Mast cell derived mediators induce edema, destroy connective tissue, and are involved in lymphocyte chemotaxis and infiltration and in pathological fibrosis of RA joints. Moreover, mast cell are involved in angiogenesis during RA, and their proteolytic activity results in cartilage destruction and bone remodelling [13] .In present study, increased mast cell infiltration detected in arthritis rat but quercetin treatment suppressed mast cell infiltration in arthritis rat.

The antioxidant molecule quercetin has been reported to modulate numerous aspects of cell function relevant to inflammatory arthritis. [44,45] At the molecular level, quercetin is known to inhibit NF-κB, a central transcription factor in inflammatory and proliferative diseases.[46,47,48,49] Quercetin inhibits inflammatory aspects of synovial cell function[46], neutrophil activation and the proliferation of different cancer cell types.[50,51] In present study, arthritis rat show down-regulation of NF-κB p65 after treatment with quercetin.

Prostaglandins (PGs) play a key role in the generation of the inflammatory response. During inflammatory processes, large amounts of the proinflammatory mediator PGE2 are generated, which affect the immune system by suppressing the proliferation of T and B cells, as well as cytokine synthesis.[52] Blockade of these molecules resulted in a reduction of disease severity and bone resorption.[53,54,55,56] PG production is determined by the differential expression of cyclooxygenase isoenzymes (COXs) within cells present at sites of inflammation. COX2 is dominant source of prostaglandins formation in inflammation and PGE_2 is involved in all processes leading to the classic signs of inflammation e.g. redness, swelling, and pain. Our result demonstrated that in control and only quercetin treated group shows very less or no expression of COX2 where as arthritis group shows higher expression of COX2 but quercetin treated arthritis group shows down-regulation of COX2 expression.

Hence, it is plausible to suggest that part of the beneficial anti-inflammatory and cartilage/bone protective effects of quercetin may be mediated through the inhibition of proinflammatory cytokines. The biochemical alterations were further supported by histopathological observations of the joints. The higher number of infiltrating cells, extensive bone degradation and synovial hyperplasia which are hallmarks of RA was found in CIA. Treatment with quercetin was able to prevent the histological findings to normal.

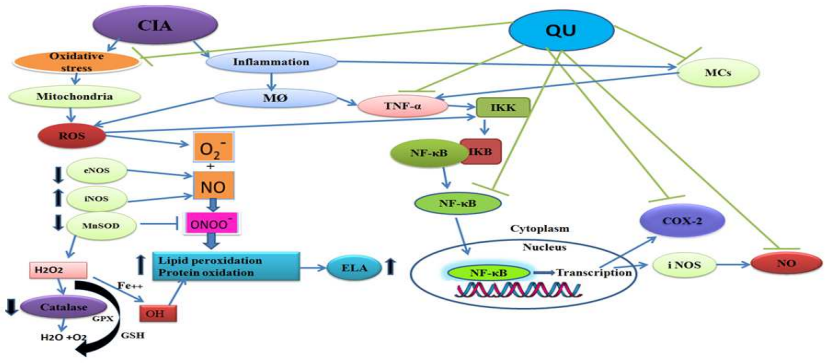

Figure 10: Targets of action of QU against collagen induced rheumatoid arthritis in Wistar rats

Collagen induced rheumatoid arthritis showed oxidative stress, increase inflammatory mediators, up-regulate pro-inflammatory cytokines, infiltration of neutrophil and mast cells in synovium. QU treatment restore catalase, SOD and GSH level whereas reduces LPO, ELA and NO level .QU treatment reduces oxidative stress by restoring enzymatic and non enzymatic antioxidant, attenuate cellular infiltration, reduces inflammatory mediators and down-regulate pro-inflammatory cytokines expression of NF-κB and COX-2 in synovium.CIA= Collagen induced arthritis; QU= Quercetine; ROS=Reactive oxygen species; MnSOD= Manganese Superoxide dismutase; GPx= Glutathione peroxidase; LPO=Lipid peroxidation; GSH= Reduced glutathione; NO= Nitric oxide; NF-κB=Nuclear transcription factor kappa B;IKK= I Kappa B kinase; IKB= I Kappa B; COX-2=Cycloxygenase-2;ELA=Articular Elastase Activity; TNF-a = Tumor Necrosis Factor-alpha; eNOS= endothelial Nitric Oxide Synthase; iNOS= inducible Nitric Oxide Synthase; MØ=Macrophase; MCs=Mast cells.

In conclusion (Figure 10), the major findings of the present study were that quercetin acts as antioxidant and anti-inflammatory by suppressing of nitric oxide production, the accumulation of lipid peroxidation products, upregulate the activity of antioxidant enzymes, down regulate COX2 and NF-κB p65 expression, eliminated the accumulation and activation of polymorphonuclear cell. We believe that our results will contribute to the clinical applications in the treatment of rheumatoid arthritis. More work directed toward understanding molecular and immunological aspects of the disease is required.

Conflict of interest

The authors declare that they have no conflict of interest.

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