The Therapeutic Potential of Plant Flavonoids on Rheumatoid Arthritis

Samuel D. Hughes[1], Natkunam Ketheesan[2] & Nagaraja Haleagrahara[3,*]

[1,2,3]Biomedicine, College of Public Health, Medical & Veterinary Sciences, and [2, 3]Australian Institute of Tropical Health and Medicine (AITHM), James Cook University, Townsville, Australia

[*]Address correspondence to: Nagaraja Haleagrahara, Biomedicine, College of Public Health, Medical & Veterinary Sciences, James Cook University, Townsville, Australia. Email: haleagrahara.nagaraja@jcu.edu.au

Abstract

Rheumatoid arthritis (RA) is an autoimmune condition that mainly affects peripheral joints. Although immunosuppressive drugs and non-steroidal anti-inflammatory drugs (NSAIDs) are used to treat this condition, these drugs have severe side effects. Flavonoids are the most abundant phenolic compounds which exhibit antioxidant, anti-inflammatory and immunomodulatory properties. Many bioactive flavonoids have powerful anti-inflammatory effects. However, a very few have reached clinical use. Dietary flavonoids have been reported to control joint inflammation and alleviate arthritis symptoms in both human RA and animal models of arthritis. There is little scientific evidence about their mechanism of actions in RA. We review the therapeutic effects of different groups of flavonoids belonging to the most common and abundant groups on RA. In particular, the probable mechanisms of major flavonoids on

cells and chemical messengers involved in the inflammatory signalling components of RA are discussed in detail.

Keywords

Cytokines; inflammation; arthritis; collagen-induced arthritis, immunosuppression

Abbreviations

AIA Adjuvant-induced arthritis

AP-1 Activating protein-1

CCL Chemokine (C-C motif) ligand

CIA Collagen-induced arthritis

COX Cyclooxygenase

CXCL Chemokine (C-X-C motif) ligand

CXCR CXC chemokine receptor

EGCG Epigallocatechin-3-gallate

IL Interleukin

LO Lipoxygenase

MCP-1 Monocyte chemoattractant protein-1

MMP Matrix metalloproteinase

mRNA Messenger ribonucleic acid

NF-κB Nuclear factor-κB

NO Nitric oxide

RA Rheumatoid arthritis

RA-FLS Human rheumatoid arthritis fibroblast-like synoviocytes

TNF Tumour necrosis factor

INTRODUCTION

Rheumatoid arthritis

Rheumatoid arthritis (RA) is a systemic autoimmune disease. It is the most commonly occurring form of inflammatory arthritis. It is a disease affecting the mobile joints, progressively disabling over time and thus, inflicts significant suffering for patients and care givers. RA is characterised by a deforming symmetrical polyarthritis varying in extent and severity (Khurana and Berney, 2005; Lee and Weinblatt, 2001). Systemic features may also be observed during the course of the disease including, vasculitis, pulmonary and serositis inflammation. In 2010, an estimated 0.5-1% of adults in the developed world were affected by RA. Rheumatoid arthritis is more prevalent in most European populations (1%) than in the Asian and African ancestry (< 0.5%). Disease incidence is less common in countries such as South Africa, Nigeria, Indonesia, Pakistan, China, the Philippines and Argentina, compared to Western people (Kalla & Tikly 2003). Moreover, a three times higher frequency of RA was reported in women than in men (Scott and Wolfe, 2010). Life expectancy of patients with RA decreases by 20 years into disease, varying depending on the therapy. The WHO reports that 80% of those suffering from RA are severely disabled. This causes loss of a substantial portion of their basic functions. Not only does this affect the livelihood of patients with RA but also poses a social and economic burden to the community. Even though arthritis can occur at any age, the incidence of RA is more common in those aged between 40-70 years (Khurana and Berney, 2005; Symmons et al., 1986).

Typical features of RA are symmetrical inflammation in the small joints of the hands, wrists, and feet (Gordon, 1998). Though any synovial joint may be involved at any point of the

disease RA often begins subtly with symptoms of joint pain and stiffness associated with swelling over the joint area. The joints most commonly involved first are the metacarpophalangeal joints (MCP), proximal interphalangeal joints (PIP), wrists and metatarsophalangeal joints. A typical manifestation of RA is known as Boutonniere's deformity; a swelling of the PIP and MCP joints with associated loosening of ligaments (Lee and Weinblatt, 2001; Khurana and Berney, 2005).

Pathogenesis and treatment of RA

The initiating cause of RA is still unknown, but through many recent developments into the disease, there have been significant progress in the understanding the pathogenesis of RA. It is clear that dysregulated immune system plays a major role in the propagation of this disease. An unknown trigger stimulate the activation of autoreactive CD4+ T-cells and B-cells (Chaiamnuay, 2005). It has been speculated that APCs are responsible for this, initiating an autoimmune response to collagen type II found in the synovium by presenting this antigen to T-cells (Fournier, 2005). These T-cells (known as collagen-specific T cells or CII-T) undergo clonal expansion and stimulate various other cells in the joint to produce cytokines and other inflammatory mediators (Lee and Weinblatt, 2001). These particularly play a major role in the development of RA. Cytokines such as TNF-α and IL-1β are produced by macrophages and synoviocytes (Figure 1). Both these cytokines act synergistically in the production of inflammatory mediators such as matrix metalloproteinases (MMP) and the expression of various cell adhesion molecules (CAM) (Dayer and Burger, 1999). MMPs are known to cause remodelling and destruction of the extracellular matrix leading to degradation of cartilage and

bone destruction (Yasuda, 2006). On the other hand, CAMs are responsible for the recruitment of inflammatory cells into the synovium (Tarrant and Patel, 2006). High levels of cytokines circulating the synovium are speculated to activate the translocation of a transcription factor nuclear factor-κB (NF-κB) into nuclei (Romas et al., 2002). An increasing number of studies have described the involvement of this factor in RA as it has been shown to regulate the genes encoding pro-inflammatory cytokines involved in RA. Thus, there is a cascade of events which contribute to a vicious cycle of inflammation (Romas et al., 2002; Gossye et al., 2009).

Early treatment for RA has a significant beneficial effect. Several drugs exist for the treatment of rheumatoid arthritis which includes non-steroidal anti-inflammatories (NSAIDs), glucocorticoids, disease modifying anti-rheumatic drugs (DMARDs) and biological DMARDs. Non-steroidal anti-inflammatories target the cyclooxygenase (COX) pathway; reducing prostaglandin (PG) biosynthesis (Crofford, 2013; Smolen et al., 2014). The inhibition of PG synthesis underlies the ability of these molecules to provide symptomatic relief in RA. Glucocorticoids interfere with the generation of immune cell and reduce most leukocytes, except for B cells (Ferreira, 2016). A wide variety of molecules come under the umbrella of DMARDs including methotrexate, sulfasalazine, leflunomide, tofacitinib, cyclosporin A, chloroquine and hydroxychloroquine (Her and Kavanaugh, 2015; Smolen et al., 2014). These DMARDs are immunosuppressive in nature and, therefore, increase the risk of infection. (Nandi et al., 2008). Renal, gastric, allergic, nervous, hematologic, cardiovascular and hepatic side effects and delayed bone healing has been reported with NSAID therapy. The cardiovascular side effects and delayed bone healing are more problematic; given the association of these complications in patients with RA (Crofford, 2013; Roubille, et al., 2015). A large number of conventional and

biologic therapies are currently being used to modify disease response in RA. These include anti-TNF and anti-IL-1 therapies (Lee and Weinblatt, 2001). These biological response modifiers are used in combination with DMARD therapy in clinical practice. Currently TNF-α, IL-6, and IL-1 are targeted by biological DMARDs which consists of monoclonal antibodies, direct cytokine inhibitors, or receptor antagonists (Nigrei et. al., 2016; Singh et. al., 2016). Additionally, T and B lymphocyte activity is also targeted by biological DMARDs (Negrei et al., 2016). Recent research into the immune and inflammatory pathways in RA has lead to therapies that target pro-inflammatory mediators like IL-1, IL-6 and its receptors, IL-17 and GM-CSF. Although promising, these therapies are expensive and do not confer complete protection (Nandi et al., 2008; Roubille, et al., 2015). Side effects such as increased risk of infection, vasculitis, arterial wall and cellular inflammation have been reported (Bernelot Moens et al. 2016; de La Forest Divonne et al., 2016; Gutiérrez-González 2016; Ishiguro et al., 2016). Hence, new approaches that reprogram the immune dysregulation in RA are necessary. Many natural compounds exhibit anti-inflammatory and immunomodulatory properties and have the potential for treating inflammatory diseases. In recent years there is a greater interest in natural compounds, such as dietary supplement and herbal remedies to reduce pain and inflammation. Many of these compounds work by inhibiting the inflammatory mediators. Flavonoids are one such group of compounds that have long been reported to possess anti-inflammatory, anti-atherogenic, and anti-osteoporotic activities. Flavonoids represent a highly diverse class of secondary metabolites. These compounds are under evaluation for development as therapies for RA (Guardia et al., 2001; Serafini. et al., 2010). Bioflavonoids are naturally ingested as part of human diet, and there is growing interest in the pharmacological potential of these compounds as possible modulators

of inflammation in rheumatoid arthritis. A number of plant flavonoids and related polyphenols have been studied in recent years in animal models of rheumatoid arthritis (Ansari et. al., 2014; Imada et al., 2008; Li et al., 2015; Morinobu et al., 2008).

What are flavonoids?

In recent years, numerous dietary compounds present in vegetables and fruits have been isolated and evaluated for their therapeutic potentials. Some of these important compounds are flavonoids and non-flavonoid polyphenols. Flavonoids are a class of secondary plant metabolites (de Villiers et al., 2015). There are more than 6,000 flavonoids, which act as physiological and metabolic regulators in plants (Saija et al., 1995). Flavonoids are commonly isolated from plants; however, they are also present in dietary sources (Burda, and Oleszek, 2001; Peterson and Dwyer, 1998). These sources range from beverages, such as tea, wine or beer, to grains, legumes, berries, herbs, fruits, and vegetables (Peterson and Dwyer, 1998). The widespread distribution of flavonoids and the relatively low toxicity compared to other active plant compounds mean that humans may safely ingest significant quantities of flavonoids in their diet. These polyphenolic compounds vary in the degree of oxidation and saturation of the central ring; some of the general structures of these flavonoids are shown in Figure 2. These polyphenolic compounds contain 2-phenylbenzopyron, which is the heterocyclic aromatic skeleton of the compound. Flavonoids are synthesized by the phenylpropanoid metabolic pathway in which the amino acid phenylalanine is used to produce 4-coumaroyl-CoA, yielding the backbone of flavonoids (de Villiers et al., 2015; Jackson et al., 2006). Along this pathway, six flavonoid subgroups with distinct structural patterns are formed, they are; flavonols, flavones, isoflavones, flavonones, flavanols and

anthocyanidins (Figure 2; Table 1). The structural variations in the different classes of flavonoids explain the observed differences in the bioactivity of these related compounds.

Flavonoids are more commonly known for their antioxidant and anti-radical properties (Cao et al., 1997; Burda, and Oleszek, 2001; Saija et al., 1995; Torel et al., 1986). However, flavonoids can also exert an anti-inflammatory effect by inhibiting the production of pro-inflammatory cytokines, nitric oxide (NO), eicosanoids, and interfere with NF-κB and activating protein-1 (AP-1) signalling (Li et al., 2014; Serafini et al., 2010). In this review, we present an overview of the anti-inflammatory, immunosuppressive, and anti-arthritic properties of quercetins, tea flavonoids, hesperetins, and a few other flavonoids like apigenin, kaempferol, nobiletin, myricetin, leuteolin.

Quercetin

Quercetin (3,3',4',5,7-pentahydroxyflavone) is a flavonol, commonly found in broccoli, tea, onions, apples, and green leafy vegetables. This antioxidative flavonoid (Figure 3) is widely distributed in the human diet. Additionally, quercetin is one of a group of over 5000 naturally available plant phenolic compounds. Quercetin and its conjugates have been shown to possess potent anti-inflammatory effects in several disease models. Quercetin has also been reported to influence several aspects of cell function relevant to RA.

Effect on joint state

Ex vivo articular cartilage explants was protected against degradation by collagenase with quercetin treatment (Natarajan et al. 2015). Matsuno et al. (2009) have shown that co-

administration of quercetin, as quercetin 3-4"(*O*-α-glucosyl)$_{1-6}$-*O*-β-glucoside, with glucosamine and chondroitin, did not alter clinical measurements of synovial fluid properties in RA patients. Arthritis associated weight loss in adjuvant-induced arthritis (AIA) and collagen-induced arthritis (CIA) animals was diminished with quercetin and rutin(a quercetin glycoside) therapy (Kauss et al. 2008; Mamani-Matsuda et al. 2006). Clinical scores in several models of RA such as CIA, AIA, and adjuvant-carrageenan induced arthritis, were improved with administration of quercetin or rutin (Ansari et al. 2014; Guardia et al. 2001; Kauss et al. 2008; Mamani-Matsuda et al. 2006). However, only rutin was observed to remain active during the chronic phase of the disease; reducing clinical scores in rats with adjuvant-carrageenan induced arthritis (Guardia et al. 2001). Quercetin and rutin reduced paw edema in animals with CIA, AIA, or adjuvant-carrageenan induced arthritis (Ansari et al. 2014; Guardia et al. 2001; Jeyadevi et al. 2013; Rotelli 2003). Changes in joint histopathology in CIA or AIA models was also attenuated with quercetin or rutin treatment (Ansari et al. 2014; Choi et al. 2009; Jeyadevi et al. 2013; Kauss et al. 2008); in some cases resulting in cartilage regeneration (Jeyadevi et al. 2013).

Quercetin inhibited the proliferation of rabbit synoviocytes *in vitro* with an IC_{50} of 45 μM. Gene expression of MMP-1 and -3 in inflammation stimulated chondrocytes was also reduced by quercetin treatment (Jackson et al. 2006). However, other report showed no inhibition of MMP-1 by quercetin in pure enzyme assays (Lee & Kim 2010). The proliferation of *in vitro* RA synovial fibroblasts, induced by IL-1β, was inhibited by quercetin (Sung et al. 2012). Expression and production of MMP-1, MMP-3, COX-2 and PGE_2 in IL-1β stimulated RA synovial fibroblasts was inhibited by quercetin therapy. Additionally, quercetin also inhibited the expression of NF-κB in IL-1β stimulated RA synovial fibroblasts (Sung et al., 2012).

Effect on immune cells

Quercetin reduced the *in vitro* viability of THP-1 macrophages and B lymphocytes at doses greater than 25μM and 50μM, respectively. Activation of neutrophils was antagonised by quercetin *in vitro* (Drummond et al. 2013). There was inhibition of reactive oxygen species generation but no inhibition of phagocytic function or cytotoxicity, in neutrophils isolated from RA patients with quercetin treatment (Santos et al. 2014). Activation and accumulation of polymorphonuclear leukocytes, measured by articular elastase activity, was diminished in CIA rats treated with quercetin (Ansari et al. 2014). Expression of several proinflammatory genes and production of TNF-α, NO, and IL-1β and IL-6 was inhibited by rutoside treatment *in vitro*. Kauss et al (2008) showed that the production of TNF-α and NO was reduced in macrophages isolated from rutoside treated AIA rats. Quercetin reduced nitrites and TNF-α from *in vitro* macrophages isolated from AIA rats. Expression of inducible NO synthase mRNA and production of nitrites was also reduced in activated macrophages treated with quercetin (Mamani-Matsuda et al. 2006).

Effect of cytokines and inflammatory pathways

Quercetin inhibited the genetic expression and secretion of TNF-α, IL-1β, -6 and -8 mRNA in stimulated human mast cells *in vitro*. Additionally, NF-κB in stimulated human mast cells was downregulated by quercetin treatment *in vitro* (Min et al. 2007). Choi et al (2009) reported that the mice with CIA that were supplemented with 5% quercetin in their diet, expressed decreased TNF-α and IL-1β mRNA in their tissues compared to controls. Serum monocyte chemoattractant protein-1 (MCP-1), NO, and PGE_2 was significantly reduced in CIA mice treated with quercetin.

This was confirmed by Gardi et al (2015), who have shown that plasma IL-1β and MCP-1 was significantly reduced with quercetin in AIA rats. Rutoside decreased serum TNF-α, IL-1β and MCP-1 concentrations in rats with AIA (Kauss et al., 2008). Secretion of TNF-α, IL-1β and MCP-1 was reduced in *ex vivo* activated macrophages treated with quercetin (Mamani-Matsuda et al. 2006). Enzymes involved in inflammation, such as hyaluronidase, 15-lipoxygenase (15-LO), COX-1 and -2, were inhibited by quercetin, and synthesis of NO in macrophages was also attenuated by quercetin with an IC_{50} of 62.4 μM (Lee & Kim 2010). Peripheral 12- and 15-LO activity, in addition to NF-κB activation, was reduced in AIA rats treated with quercetin (Gardi et al. 2015). Expression of NF-κB and COX-2 in the joints of CIA rats, as well as NO production in the joints, was reduced with quercetin treatment (Ansari et al. 2014). Thus, several recent *in vivo* and *in vitro* studies have shown a significant antiinflammatory and antiarthritic role of flavonoid quercetin. Quercetins antiinflammatory role may be mediated mainly through the inhibition of proinflammatory cytokines like TNF-α and IL-1β. Quercetin also showed bone/cartilage protective effects by inhibiting the level and expression of different MMPs.

Tea flavonoids

Tea leaves contain a large amount (more than 35% of the dry substance) of polyphenols, mainly flavonoids. The major class of flavonoids present in tea both black and green tea are flavanols class of flavonoids, which include catechin, epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin gallate (Figure 3).

Effects on joint state

Natarajan et al., (2015) have recently shown that tea flavonoid catechin and epigallocatechin gallate protected articular cartilage explants against degradation by collagenase. Glycosaminoglycan loss in enzymatically digested explants and *in vitro* TNF-α stimulated break down of cartilage proteoglycans was also attenuated by epigallocatechin gallate treatment (Adcocks et al. 2002). Epicatechin and epicatechin gallate (Figure 3) inhibited cartilage breakdown mediated by IL-1α *in vitro*. Proteoglycan breakdown was enhanced in human RA cartilage explants treated with TNF-α and IL-1β, and epicatechin gallate and epigallocatechin gallate significantly antagonised this effect. Adcocks et al. (2002) reported that the breakdown of type II collagen, mediated by IL-1α, was ameliorated by epicatechin gallate, epigallocatechin, and epigallocatechin gallate. However, intra-articular administration of epigallocatechin gallate inhibited cartilage degradation, but not paw volume, in CIA rats (Natarajan et al., 2015). Scanning electron microscopy of the bone of rats with AIA showed a breakdown in the collagen fibril network and pore formation, which was rescued with theaflavin treatment. Moreover, bone joint destruction seen in AIA rats was partially reversed with theaflavin treatment (Datta et al., 2014). A combination of dexamethasone, a potent synthetic steroid, and epigallocatechin resulted in reduced paw swelling in AIA rats; monotherapy with either agent produced a similar effect, however, to a lesser extent. Combination therapy decreased serum markers of inflammation which was absent in epigallocatechin monotherapy (Roy et al., 2013). This study also reported that the monotherapy with epigallocatechin or combination therapy with dexamethasone and epigallocatechin increased bone mineral density in AIA animals. Additionally, bone erosion and inflammation was greatly reduced with combination therapy of dexamethasone and epigallocatechin. There was a significant improvements in histopathological markers of AIA,

such as synovial proliferation, diminished joint space, bone and cartilage breakdown, bone erosion, fibrin exudation, pannus formation and bone matrix invasion, occurred with dexamethasone, epigallocatechin or combination treatment (Roy et al., 2013).

Epigallocatechin-3-gallate (EGCG) improved clinical scores, and inflammation in obese CIA mice. Arthritis severity, paw edema, clinical scores, ankle circumferences, and histopathological changes were reduced with EGCG in CIA and AIA rats (Ahmed et al. 2008; Byun et al. 2014; Lin et al. 2008; Liu et al. 2012). Increased clinical scores and foot swelling seen in collagen type II antibody induced arthritis was antagonised with (-)-epigallocatechin-3-gallate treatment. Histopathological scores and osteoclast numbers were reduced in antibody-induced arthritis animals treated with (-)-epigallocatechin-3-gallate (Morinobu et al. 2008). Paw edema and haematological abnormalities, such as an elevated white blood cell count, in AIA rats was abolished with methotrexate/epigallocatechin-3-gallate therapy. Additionally radiological and histopathological markers of arthritis were also reduced in methotrexate/(-)-epigallocatechin-3-gallate combination therapy treated AIA rats (Roy et al., 2012).

Osteoclasts differentiation was inhibited by theaflavin-3,3'-digallate, epigallocatechin gallate and EGCG *in vitro*. Theaflavin-3, 3'-digallate and (-)-epigallocatechin gallate dose-dependently reduced osteoclast formation, MMP-9 mRNA, MMP-2 and MMP-9 activity in osteoclast precursor cells (Morinobu et al. 2008; Oka et al. 2012). Epigallocatechin-3-gallate reduced the number of osteoclasts in ankles of AIA rats, and bone resorption was attenuated by this tea flavonoid treatment on osteoclasts. Study by Lin et al (2008) has shown that EGCG inhibited the *in vitro* production of MCP-1 mRNA in oncostatin M stimulated, human bone

marrow-derived osteoblasts. In another study, theaflavin increased serum osteocalcin, a bone formation marker levels in AIA rats (Datta et al., 2014).

Genetic expression and synthesis of IL-6 by RA synovial fibroblasts, induced by IL-1β, was reduced by EGCG. Furthermore, protein levels and activity of MMP-2 in IL-6 stimulated RA synovial fibroblasts and joints of AIA rats was attenuated with epigallocatechin-3-gallate treatment *in vitro* (Ahmed et al. 2008). Levels of MMP-1 and -3, both mRNA and protein, and activation of AP-1 were reduced in TNF-α stimulated human RA synovial fibroblasts treated with EGCG (Yun et al. 2008). This flavonoid also inhibited the expression and production of TNF–α and MMP-13 in human chondrocytes treated with advanced glycation end products. Activation of NF-κB in human chondrocytes was also inhibited by epigallocatechin gallate in another study by Rasheed et al. (2009). Expression, production, and activity of MMP-1, MMP-13, and TIMP-1 in human chondrocytes stimulated with IL-1β, was inhibited by epigallocatechin gallate treatment. There was also the inhibition of NF-κB activation in human chondrocytes stimulated with IL-1β (Ahmed et al. 2004).

Effects on immune cells

According to Datta et al (2014), a greater percentage of white blood cells were arrested at the G_0/G_1 phase of the cell cycle in theaflavin-treated AIA rats compared to indomethacin-treated animals. EGCG reduced BAFF-mediated B-cell proliferation and enhanced IGF-1 mediated B-cell apoptosis *in vitro*. Infiltration of AIA rat ankle joints by macrophages was reduced with EGCG treatment (Lin et al. 2008).

Effects on cytokines and inflammatory pathways

Immunolocalisation of MCP-1 was attenuated in the joints of AIA rats treated with EGCG (Lin et al. 2008). The number of cells staining positive for TNF-α, IL-1β, IL-6 or IL-17 were reduced in obese CIA animals treated with EGCG (Byun et al. 2014). Additionally, production of CCL5, MCP-1, CXCL5, and CXCL1 in RA synovial fibroblasts, mediated by IL-1β, was antagonised with EGCG treatment (Ahmed et al. 2006). The number of splenocytes containing mRNA for IL-17 and IL-21 was reduced in EGCG treated, obese CIA animals (Byun et al. 2014). Theaflavin decreased serum IL-12 and C-reactive protein levels in AIA rats. Additionally, concentrations of IL-6 and TNF-α were reduced in the synovial fluid of theaflavin-treated AIA rats (Datta et al. 2014). Enzymes involved in inflammation, such as hyaluronidase and 15-LO, were weakly to moderately inhibited by catechin in pure enzyme assays. Additionally, catechin was also shown to be a selective, albeit poor, COX-2 inhibitor (Lee & Kim 2010). Activation and DNA translocation of NF-κB in IL-1β stimulated RA synovial fibroblasts was attenuated with EGCG treatment (Ahmed et al. 2006). Immunohistochemical staining revealed that increases in TNF-α and IL-6, mediated by AIA, were reduced with dexamethasone and epigallocatechin co-administration (Roy et al., 2013). Methotrexate and EGCG co-administration reduced immunopositivity of TNF-α and IL-6 in the joints of AIA rats (Roy et al., 2012).

Tea flavonoids exhibited inhibitory effects against several MMPs. Theaflavin reduced serum levels of MMP-1 in AIA rats (Datta et al., 2014). Epicatechin gallate inhibited MMP-2

and MMP-9 with an IC_{50} of 95 and 28 μM, respectively. Epigallocatechin gallate was a more potent inhibitor of MMP-2 and MMP-9 with IC_{50} values of 6 and 0.3 μM, respectively. Epicatechin gallate and epigallocatechin gallate also inhibited the activity of MMP-12 by 60% (Demeule et al. 2000). EGCG antagonised the production of MMP-2 in RA synovial fibroblasts, mediated by IL-1β, CCL5, CXCL-1 or -5 (Ahmed et al. 2006; Riegsecker et al. 2013). EGCG is the most commonly used tea flavonoid in the animal models of arthritis studies, and this tea flavonoid has shown a significant antiarthritic effects in rat and mouse models of arthritis. Most of the studies have confirmed a significant decrease in TNF-α, IL-1β, IL-6 and MCP-1 levels. *In vitro* studies using tea flavonoids have also shown positive results against inflammatory cytokines and chemokines expressions.

Hesperetins

Hesperidin, is a natural flavanone glycoside composed of hesperetin (aglycone) (Figure 3) and rutinose, and is found richly in citrus fruits. *In vivo* and *in vitro* studies have attributed a significant antioxidant and anti-inflammatory role for hesperidin derivatives.

Effects on joint state

Collagen induced arthritis mice treated with α-glucosylhesperidin exhibited reduced clinical scores, joint damage, synovial cell proliferation, inflammatory cell infiltration and pannus formation (Kometani et al., 2008). Administration of 7,3'-dimethoxyhesperetin (Figure 4, Structure 2) reduced paw edema and arthritis scores in AIA rats (Li et al., 2012; R. Li et al., 2013). Synovial hyperplasia, inflammatory cell infiltration, vascular proliferation, and bone or

cartilage loss were attenuated in 7,3'-dimethoxyhesperetin treated AIA rats (Li et al., 2012). Apoptosis of fibroblast-like synoviocytes and synovial cells, isolated from AIA rats, was enhanced with 7,3'-dimethoxyhesperetin (Li, et al., 2010; Li et al., 2013). The proliferation of fibroblast-like synoviocytes, isolated from AIA rats, was attenuated with 7,3'-dimethoxyhesperetin (Li, et al., 2010).

In a study by Rovenský et al. (2009) using a combination of detralex, a mixture of diosmin and hesperidin, and methotrexate in AIA rats, improved paw edema, bone mineral density and clinical scores. Several other studies have shown a reduction in arthritis scores in AIA rats treated with hesperidin (Ansari et al., 2014; Li et al., 2008; Rotelli 2003; Umar et al., 2013; Yang, et al., 2010). Additionally, hesperidin reduced both paw swelling and arthritic clinical scores in rats with adjuvant-carrageenan induced arthritis (Guardia et al., 2001). Bone destruction, vascular proliferation, synovial hyperplasia and inflammatory cell infiltration were attenuated in hesperidin treated AIA animals. Pathological changes in joint structure, splenic histology, and inflammation scoring were reduced in hesperidin treated AIA rats. The proliferation of synoviocytes taken from AIA rats was inhibited with hesperidin. A few other studies have also confirmed hesperidin induced apoptosis in synovial fibroblasts, obtained from RA patients, stimulated with TNF-α (Li et al., 2010; Li et al., 2008; Ahmed et al., 2015; (Kokotkiewicz et al. 2013).

Effects on immune cells

Cartilage elastase activity, a measure of neutrophil activation and infiltration, was reduced in CIA rats treated with hesperidin (Umar et al. 2013). Production of IL-2 by AIA rat

splenocytes and proliferation of AIA rat T lymphocytes was enhanced and the production of IL-1, IL-6, and TNF-α by peritoneal macrophages was attenuated with hesperidin in AIA rats (Li et al. 2008).

Effects on cytokines and inflammatory pathways

A study by Kometani et al. (2008) showed a reduction in plasma C-reactive protein in RA patients treated with α-glucosyl hesperidin (Kometani et al. 2008). Production of NO, measured by serum nitrite/nitrate, was decreased in AIA rats treated with methotrexate/detralex combination therapy (Rovensky et al. 2009). Li et al. (2010) showed a reduced TNF-α and IL-1β mRNA and protein whilst increasing IL-10 mRNA and protein in AIA rat synoviocytes. Serum levels of TNF-α and IL-10 were reduced and increased, respectively, in AIA rats treated with hesperidin (Ahmed et al. 2015). Expression and production of IL-6 in AIA rats was reduced with 7, 3'-dimethoxyhesperetin treatment. In another study, serum levels of IL-1β and TNF-α protein were reduced in AIA rats treated with 7, 3'-dimethoxyhesperetin. Moreover, hesperetin significantly reduced the production of MMP-3 and IL-6 in human synovial cells stimulated with IL-1β (Choi and Li 2010; Li et al. 2012; Li et al. 2013).

Other flavonoids

Effects on joint state

The onset and severity of arthritis were reduced in apigenin (4', 5,7-trihydroxyflavone) (Figure 4) treated CIA mice. Histopathology confirmed that apigenin exerted an anti-arthritic effect in AIA rats; reducing inflammation scores, inflammatory cell infiltration, cartilage

damage, synovial edema and cellular damage in the hind paw (Li et al. 2015; Chang et al. 2015). Flavonoid astilbin decreased footpad swelling, clinical scores and disease incidence in CIA mice (Cai et al. 2003; Chang et al. 2015). Histopathological changes, such as inflammatory cell infiltration, fluid accumulation in in the joint cavity, synovial hyperplasia, and hyperemia and edema of the synovial membrane, were reduced in CIA mice treated with astilbin. A few other studies have shown that the flavonoid baicalein (Figure 4) treatment improved paw edema, weight changes and arthritis scores in AIA rats (Butenko et al. 1993; Cai et al. 2003; Kubo et al. 1984). Other studies have reported that treatment with baicalin, a glucuronide of baicalein, improved symptomology and pro-inflammatory cytokine mRNA in AIA or CIA rodents (Butenko et al. 1993; Kubo et al. 1984; Yang et al. 2013). Baicalin also inhibited pathophysiology and progression of arthritis in CIA rats. Daidzein reduced the severity of RA symptoms and incidence of arthritis in CIA rats. Additionally, histopathological changes induced by CIA were reduced with daidzein treatment (Mohammad Shahi et al 2011). Genistein, (4',5, 7-trihydroxyisoflavone, found in soy beans) improved clinical scores, arthritis incidence, and symptom severity in CIA rats and mice. Additionally, genistein reduced paw volumes and radiological changes in both sham operated and ovariectomised CIA rats. Histopathological changes seen in CIA animals such as synovial hyperplasia, inflammatory cell infiltration, synovitis, and cartilage-bone destruction were improved with genistein treatment (Li et al. 2013; Mohammad Shahi et al 2011; Verdrengh et al. 2003; Wang et al. 2008). Enhanced synovial cell growth, induced by IL-1β and TNF-α, was reduced with genistein treatment *in vitro* (Zhang et al. 2012). Luteolin (3',4',5,7-tetrahydroxylflavone) (Figure 4) improved clinical scores, paw edema, the severity of arthritis and weight loss in AIA rats and CIA mice (Zhang et al. 2012; Shi

et al. 2015); this effect was synergistic with palmitoylethanolamide, an endogenous fatty acid amide. Additionally radiological and histopathological changes in CIA were improved with luteolin/palmitoylethanolamide compared to monotherapy with either agent. Moreover, greater improvements in locomotor activity and pain sensitivity were reported in CIA animals treated with luteolin/palmitoylethanolamide combination therapy (Impellizzeri et al. 2013). Paw edema, body weight losses and joint damage in AIA rats were ameliorated with morin treatment (Rotelli 2003); the effect was synergistic with indomethacin, a NSAID. An improvement in several histopathological markers was also reported with morin and morin plus indomethacin treatment in AIA rats. In female CIA rats, treatment with morin was comparable to dexamethasone in terms of reducing paw edema, joint damage, clinical and histopathological scores (Sultana and Rasool 2015; Zeng et al. 2015). Myricetin (Figure 4) improved clinical scores, decreased paw thickness in CIA mice. Histopathological changes associated with CIA were also reduced with myricetin treatment (Zeng et al. 2015; Yuan et al. 2015). Additionally, myricetin antagonised the production of IL-6 and MMP-1 in IL-1β stimulated synovial cells (Yuan et al., 2015; Lee et al. 2007). In another study, Hiermann et al. (1998) reported that the glycosides of myricetin reduced paw edema induced by either carrageenan or AIA in rats. Naringin and its aglycone, naringenin, reduced paw edema and clinical scores in AIA and CIA mice. Additionally, histopathological changes in AIA or CIA mice were ameliorated by either naringin or naringenin. Expression of TNF-α mRNA was reduced in the knee joints of naringin treated CIA mice. The expression of IL-1β, IL-17 and MCP-1 mRNA was reduced in the ankle tissues of naringin treated AIA mice (Ahmad et al. 2014; Kawaguchi et al. 2011; Li et al. 2015). Footpad swelling, incidence and clinical scores were reduced in CIA mice treated with nobiletin. Histopathological markers of

CIA such as inflammation, pannus formation, bone, and cartilage destruction, were reduced by nobiletin in these animals (Imada et al. 2008; Murakami et al. 2007). Clinical scores and histopathology were improved in CIA rats treated with kaempferol (3,4', 5,7-tetrahydroxyflavone) (Figure 4) (Lin et al. 2015).

Apigenin treatment resulted in inhibition of RANKL-mediated osteoclastogenesis from mononuclear cells and bone resorption by primary osteoclasts and enhanced apoptosis of mature osteoclasts. Levels of IL-6, MCP-1, and MCP-3, released from TNF-α stimulated osteoblasts were decreased with apigenin. Production of CXCL-9 and CXCL-10 from IFN-γ stimulated osteoblasts was reduced with apigenin treatment (Bandyopadhyay et al. 2006). Kaempferol inhibited IL-1β stimulated osteoclast differentiation from bone marrow derived precursors, in a dose dependent manner. The IL-1β mediated survival of osteoclast precursor cells was also attenuated by kaempferol accoding to Lee et al. (2014). *In vitro* osteoblast activity was stimulated by luteolin or orientin. Furthermore, reductions in IL-6, TNF-α, osteoprotegrin, osteopontin and sclerostin proteins were seen in luteolin or orientin treated osteoblasts. Luteolin treated CIA rat synovial fibroblasts also showed a reduction in the expression of IL-6, IL-8 and IL-15 mRNA (Hou et al. 2009). Additionally, mineralisation of Saos2 cells was increased with orientin or luteolin treatment (Nash et al. 2015). Transforming growth factor-β mRNA was reduced in CIA rat synovial fibroblasts treated with luteolin (Hou et al. 2009). Myricetin induced differentiation of osteoblasts and enhanced mineralisation and collagen type I levels of osteoblasts *in vitro* (Kuo, 2005; Hsu et al. 2007). Nobiletin (Figure 4) inhibited osteoclastogenesis and TRAP activity *in vitro* (Murakami et al. 2009). Expression of proMMP-1

mRNA and production of proMMP-1 and proMMP-3 was decreased in IL-1α stimulated human synovial fibroblasts treated with nobiletin. Conversely, the production of TIMP-1 was increased with IL-1α stimulated human synovial fibroblasts treated with nobiletin. Additionally, production of COX-2 mRNA and protein and PGE_2 was reduced in IL-1α stimulated human synovial fibroblasts treated with nobiletin (Lin et al. 2003).

Apigenin reduced the proliferation of, and induced apoptosis in, RA-FLS *in vitro*. Apoptosis of RA-FLS was induced with apigenin treatment *in vitro*; this effect was synergistic with TNF-related apoptosis-inducing ligand (TRAIL) (Shin et al. 2009; Sun et al. 2011). However, TRAIL mediated induction of RA-FLS proliferation was reduced with apigenin treatment *in vitro* (Sun et al. 2011). Baicalein reduced the production of TNF-α and IL-1β from RA-FLS, and splenocyte adhesion to IL-17 stimulated synoviocytes *in vitro* (Wang et al. 2014; Yang et al. 2013). *In vitro* proliferation of RA-FLS, stimulated by IL-1β, was attenuated with baicalein treatment (Chen et al. 2013). Genistein inhibited proliferation of RA-FLS and induction of MMP-2 and MMP-9 in these cells, mediated by IL-1β or TNF-α *in vitro* (Zhang et al. 2012). Inhibition of IL-1β mediated proliferation of, and induction of apoptosis in, human RA synovial fibroblasts occurred with kaempferol treatment *in vitro* (Yoon et al. 2013). Luteolin inhibited the proliferation of synovial fibroblasts isolated from CIA rats; with or without the stimulation of TNF-α. The secretion of MMP-1 and MMP-3 from CIA rat synovial fibroblasts, with or without TNF-α stimulation, was also inhibited by luteolin treatment *in vitro* (Hou et al. 2008). A recent study by Lou et al (2015) has shown a greater induction of apoptosis in IL-1β stimulated rat fibroblast-like synoviocytes treated with a combination of luteolin and chlorogenic acid, another

phytochemical, compared to monotherapy with either agent. Expression of NF-κB p100 in rat fibroblast-like synoviocytes, stimulated with IL-1β, was also enhanced. Conversely, expression of NF-κB p50 was reduced with luteolin/chlorogenic acid combination therapy or monotherapy with either agent (Lou et al. 2015). Nobiletin inhibited IL-1α stimulated expression of proMMP-9 mRNA and proMMP-1, -3 and -9 protein from rabbit synoviocytes *in vitro.* Additionally, production of PGE_2 was attenuated in IL-1α stimulated rabbit synoviocytes treated with this flavonoid (Ito et al.1999)

Effects on immune cells

Apigenin did not impair B lymphocyte viability but inhibited human macrophage viability at doses greater than 1 μM *in vitro* (Drummond et al. 2013). A study by Lin et al (2015) using flavonoid kaempferol has shown an enhanced Treg suppressive function *in vitro*. Additionally, expression of Foxp3 in Treg cells was enhanced with kaempferol treatment. The mRNA expression of *Ctla4, Il10, and Foxp3* was also increased in $CD4^+CD25^+$ T cells isolated from kaempferol-treated, CIA rat lymph nodes. An increased level of FOXP3 protein was reported in $CD4^+$ and $CD25^+$ spleen cells isolated from kaempferol-treated, CIA rats (Lin et al. 2015). In another recent study Shi et al. (2015) have shown a reduction in inflammatory cells in the joints of luteolin treated AIA rats. Naringin increased the Th2 response whilst downregulating the Th1 response in the blood of AIA mice. Naringin increased the number of Treg cells and $CD4^+CD25^+$ Treg cells whilst decreasing the number of $CD4^+$ and $CD25^+$ T cells (Ahmad et al. 2014). Cytokine production and proliferation of T cells, mediated by activated dendritic cells, was reduced with naringenin, and the production of IL-17^+ $CD4^+$ and IFN-γ^+

$CD4^+$ T cells was reduced in CIA mice treated with naringenin treatment (Li et al., 2015). Dendritic cell maturation and cytokine secretion were reduced in bone marrow derived dendritic cells treated with apigenin. Additionally, expression of chemokine receptor 4 (CXCR4) in, and migration of, immune stimulated bone marrow derived dendritic cells was inhibited by apigenin treatment. Dendritic cell maturation during the acute phase of CIA was reduced with apigenin treatment (Li et al. 2015). Moreover, in apigenin-treated CIA mice, dendritic cell percentages and the number of Langerhans cells in draining lymph nodes were reduced (Li et al. 2015). Neutrophil and mast cell infiltration into the joints of CIA mice was greatly reduced by luteolin/palmitoylethanolamide therapy (Impellizzeri et al. 2013).

Effects on cytokines and inflammatory pathways

Serum concentrations of TNF-α, IL-1β and IL-6 were reduced in AIA rats or CIA mice treated with apigenin (Chang et al. 2015; Li et al. 2015). Moreover, production of TNF-α, IL-1β and IL-6 by lymph nodes isolated from CIA mice, was attenuated with apigenin treatment. Dendritic cell expression of CXCR4 in the periphery was reduced in CIA mice treated with apigenin (Li et al. 2015). Upregulated mRNA expression of TNF-α, IL-1β, IL-6, IL-17, and MCP-1, and increased serum protein levels of TNF-α, IL-1β, and MCP-1, were decreased in morin or morin plus indomethacin treated AIA rats (Sultana and Rasool. 2015). Baicalin reduced serum levels of TNF-α and IL-1β in CIA rats (Wang et al. 2014). Expression of ICAM-1, VCAM-1, TNF-α and IL-6 in IL-17 stimulated synoviocytes was reduced with baicalin treatment (Yang et al. 2013). Increased serum concentrations of TNF-α and IL-6 observed in CIA rats was attenuated by genistein or daidzein treatment (Mohannad Shahi et al. 2011). *In*

vitro production of IFN-γ was reduced from splenocytes isolated from sham operated or ovariectomised CIA rats treated with genistein. Decreased T-bet mRNA was observed in the splenocytes of genistein treated CIA animals which were correlated to IL-4 levels (Wang et al. 2008). Concanavalin A stimulated production of IFN-γ was inhibited by kaempferol-3-*O*-β-D-glucopyranoside *in vitro* . Additionally, kaempferol-3-*O*-β-D-glucopyranoside inhibited concanavalin A stimulated production of TNF-α *in vitro* (Dang et al. 2008). Plasma concentrations of TNF-α, IL-1β, IL-6 and IL-17 were reduced in AIA rats treated with luteolin (Shi et al. 2015). Levels of MIP-1α, MIP-2, IL-1β, IL-6, and TNF-α protein were greatly reduced in CIA mice treated with luteolin/palmitoylethanolamide (Impellizzeri, 2013). Zeng et al. reported that the concentrations of TNF-α and IL-6 in the serum was reduced with flavonoid morin treatment in female CIA rats (Zeng et al. 2015). *Ex vivo* production of IL-4 was reduced from splenocytes isolated from sham operated CIA rats treated with genistein. Increased GATA-3 mRNA was observed in the splenocytes of genistein treated CIA animals which were correlated to IL-4 levels (Wang et al. 2008). Morin treatment increased IL-10 levels in the sera of female CIA rats (Zeng et al. 2015). Expression of IL-4, Foxp3 and GATA-3 mRNA was enhanced in the ankle tissues of naringin treated AIA mice (Ahmad et al. 2014).

Apigenin reduced NF-κB protein expression in the joints of AIA animals (Chang et al. 2015). Leukotriene synthesis in macrophages was inhibited by baicalein with an IC_{50} of 9.5 μM *in vitro* (Butenko et al. 1993). Treatment of RA-FLS with IL-1β or macrophage migration inhibitory factor (MIF) increased NF-κB DNA binding; this effect was decreased with baicalein administration. However, co-administration of MIF with IL-1β abolished the positive effects of

baicalein on NF-κB DNA binding (Chen et al. 2013). Wang et al. (2014) have shown that the activation of NF-κB in CIA rat synovium and rheumatoid arthritis fibroblast-like synoviocytes was attenuated by baicalein. Increased protein and mRNA expression of MMP-1, -3, and COX-2, and the increased production of PGE_2 in IL-1β stimulated human RA synovial fibroblasts was attenuated by kaempferol treatment. Moreover, increased NF-κB activity in human RA synovial fibroblasts, mediated by IL-1β, was lessened by kaempferol (Yoon et al. 2013). Expression of COX-2 and NF-κB, and serum PGE_2 was reduced in AIA rats treated with morin or morin plus indomethacin (Sultana, and Rasool, 2015). Myricetin-3-O-β-D-glucuronide inhibited 5-LO activity in human polymorphonuclear leukocytes, in addition to isolated COX-1 and COX-2 activity, with low micromolar IC_{50} values. Prostaglandin synthesis in perfused rabbit ear was also inhibited by myricetin-3-O-β-D-glucuronide treatment (Hiermann et al. 1998). Binding of NF-κB in activated dendritic cells was reduced with naringenin (Li et al. 2015). *In vitro* activation of NF-κB in macrophages was reduced with nobiletin treatment (Murakami et al. 2007).

Astilbin attenuated MMP-2 and MMP-9 activity in spleens isolated from CIA mice. Proliferation of, and NO production by, concanavalin A stimulated spleen cells from CIA mice was reduced with astilbin treatment. Furthermore, survival and concanavalin A stimulated proliferation of spleen cells from naïve mice was maintained with astilbin treatment (Cai et al. 2003). Splenic enlargement and $CD4^{+,}$ IL17 and Th17 splenocytes numbers were diminished in AIA mice by baicalein treatment (Yang et al. 2013). The proliferation of splenocytes was reduced in sham-operated, and ovariectomised CIA rats treated with genistein (Wang et al. 2008). Proliferation of lymph node cells from CIA rats was inhibited by kaempferol-3-*O*-β-D-

glucopyranoside *in vitro* (Dang et al. 2009). Morin treatment inhibited angiogenesis in both an in vitro setting and in female CIA rats (Zeng et al. 2015). Thus several flavonoids such as apigenin, luteolin, kaempferol, nobiletin, morin, baicalein, and naringin inhibited pro-inflammatory pathways, enhanced anti-inflammatory mechanisms, and improved clinical signs in cell lines and animal models of RA. There was also matrix degradation inhibition and reduced Nf-kB and MMP expression after treatment with these flavonoids in several recent studies (Figure 5) (Agarwal 1982; Darwish et al. 2014; Lee et al. 2009; Lee et al. 2012; Li et al. 2015; Kubo et al. 1984; Wang & Zhong 2015).

Conclusions

Flavonoids are a family of compounds including flavonols, flavanones, flavanols, isoflavones, and anthocyanins. Flavonoids have attracted increased attention in recent years due to their disease preventing effects. Quercetin, as quercetin 3-4" (*O*-α-glucosyl) 1-6-*O*-β-glucoside, and hesperetin, as α–glucosylhesperidin, has been trialled in humans. No effect was seen in RA patients treated with quercetin, however, some effect was seen in a small trial with hesperetin. The beneficial effect of α-glucosyl hesperidin in RA patients warrants further investigation in a larger trial, or as a lead compound for further development. The reviewed flavonoids affected joint state in several animal and cellular models; inhibiting cellular proliferation, cartilage destruction, edema, inflammatory cell infiltration, pannus formation and bone loss whilst modulating cytokine and MMP function (Figure 5). The inhibition of bone loss might be due to the inhibition of osteoclastic activity and function, and the promotion of osteoblastic function and bone mineralisation. Immune cells were also affected by the reviewed

flavonoids. Inhibition of cytokine production by immune cells was a shared mechanism of these flavonoids, however, reduced cell viability, activity, and maturation were also observed. In addition to the inhibition of inflammatory mediators, production of inflammatory cytokines at the genetic and protein levels was antagonised by many flavonoids. Several mechanisms of action have been proposed to explain the anti-inflammatory and immunomodulatory actions of flavonoids. Modulation of cellular activities of macrophages, and neutrophils, modulation of pro-inflammatory enzyme activities, modulation of the recruitment and production of pro-inflammatory cytokines and altered pro-inflammatory gene expression are some of the important mechanisms by which these flavonoids provide anti-arthritis effects in RA. A vast number of *in vitro* and laboratory animal studies and some *in vivo* studies show flavonoids inducing immunomodulatory mechanisms against inflammatory mediators and protecting against inflammation-induced joint destruction. However, there are not many clinical research on flavonoids and their anti-inflammatory effects in RA. Moreover, there is not enough data concerning the bioavailability of flavonoids in humans. Additionally most of these flavonoids have not been tested against all the pathological features of RA. Natural product based therapy could be a promising preventative/treatment for RA. Flavonoids could have a more effective and less toxic therapeutic potential for the treatment of RA. New anti-inflammatory therapies are inevitably required for RA treatment. Flavonoids could contribute to future disease modifying anti-rheumatic strategy for RA. Further research with the aim of determining the disease modifying effects of these flavonoids should include human trials and interpretation of their anti-inflammatory mechanism of actions.

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Table 1. Subgroups of flavonoids

Subgroup	**Examples**
Flavones	Apigenin, Baicalein, Diosmin, Luteolin, Techtochrysin, Nobiletin.
Isoflavones	Daidzin, Genistein
Flavonols	Kaempferol, Myricetin, Myricitrin, Quercetin, Quercitrin, Rutin.
Flavonones	Hesperidin, Hesperetin, Naringin, Naringenin.
Flavanols	Catechin, Gallocatechin, Epicatechin, Epigallocatechin, Theaflavin, Theaflavin-3-gallate.
Anthocyanidins	Delphinidin

Table 2. Flavonoids in modulating Rheumatoid arthritis pathogenesis

RA Pathogenesis	**Flavonoids**
Physical changes (Arthritis clinical scores, body weight changes)	Quercetin, Rutin, Epigallocatechin-3-gallate, (-)-Epigallocatechin-3-gallate,α glucosyl hesperidin, 7,3'-dimethoxyhesperetin, Hesperidin, Apigenin, Astilbin, Baicalein, Baicalin, Genistein, Daidzein, Luteolin, Morin, Myricetin, Naringin, Naringenin, Nobiletin, Kaempferol, Nepitrin, Fisetin, Galangin, Isorhamnetin.
Paw edema	Quercetin, Rutin, Epigallocatechin), Epigallocatechin-3-gallate, (-)-Epigallocatechin-3-gallate, (-)-Epigallocatechin-3-gallate, 7,3'-dimethoxyhesperetin, Apigenin, Baicalein, Baicalin,Genistein, Luteolin, Morin, Myricetin glycosides, Naringin, Nobiletin, Nepitrin, Chrysin, Galangin, Wogonin
Synovial fibroblast function	Quercetin, 7,3'-dimethoxyhesperetin, Hesperidin, Kaempferol, Luteolin, Tangeretin

Histological changes	Quercetin, Rutin, Epigallocatechin, (-)-Epigallocatechin-3-gallate), 7,3'-dimethoxyhesperetin, Hesperidin, Apigenin, Astilbin, Baicalin, Daidzein, Genistein, Luteolin, Morin, Myricetin, Naringin, Naringenin, Nobiletin, Kaempferol, Fisetin, Galangin
Cartilage breakdown	Catechin, Epigallocatechin gallate, Epicatechin, Epicatechin gallate,Theaflavin
Synoviocyte proliferation	Quercetin, 7,3'-dimethoxyhesperetin, Hesperidin, Genistein, Apigenin, Baicalein, Fisetin
Osteoclast function	Theaflavin-3,3'-digallate, Epigallocatehin, Epigallocatechin-3-gallate, Apigenin, Kaempferol, Nobiletin, Galangin
Osteoblast function	Luteolin, Orientin, Myricetin
Immune cell viability and function	Quercetin, Theaflavin, Epigallocatechin-3-gallate, Hesperidin, Baicalin, Apigenin, Naringenin, Naringin
Increased pro-inflammatory cytokines	Rutoside, Quercetin, Epigallocatechin-3-gallate, Theaflavin, Epigallocatechin,

	Hesperidin, α glucosyl hesperidin, 7,3'-dimethoxyhesperetin, Hesperetin, Baicalin, Myricetin, Naringin, Apigenin, Luteolin, Orientin, Baicalein, Morin, Genistein, Daidzein, Kaempferol, Fisetin, Galangin, Isorhamnetin
Increased pro-inflammatory pathways	Quercetin, Epigallocatechin, Epigallocatechin-3-gallate, Epigallocatechin gallate, Baicalin, Luteolin, Apigenin, Baicalein, Kaempferol, Naringenin, Nobiletin, Tangeretin
Increased pro-inflammatory pathways	Quercetin, Epigallocatechin, Epigallocatechin-3-gallate, Epigallocatechin gallate, Baicalin, Luteolin, Apigenin, Baicalein, Kaempferol, Naringenin, Nobiletin, Tangeretin
Decreased anti-inflammatory molecules	Hesperidin, Nobiletin, Kaempferol, Genistein, Morin, Naringin, Isorhamnetin
Treg function	Kaempferol, Naringin

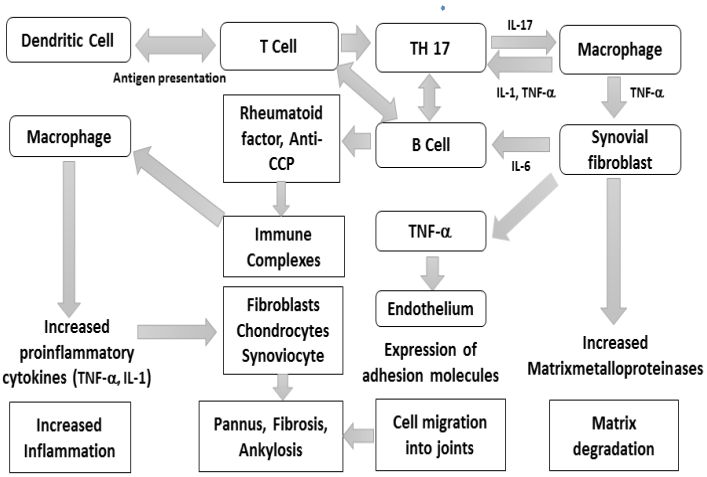

Figure 1. Pathogenesis of rheumatoid arthritis. Dendritic cell, T-cell, B-cell, TH-17 cell, synovial fibroblast, macrophage, endothelium and chondrocyte are the important cells in the pathogenesis of RA (IL=Interleukin; TNF=tumor necrosis factor; Anti CCP= Anti-cyclic citrullined peptide)

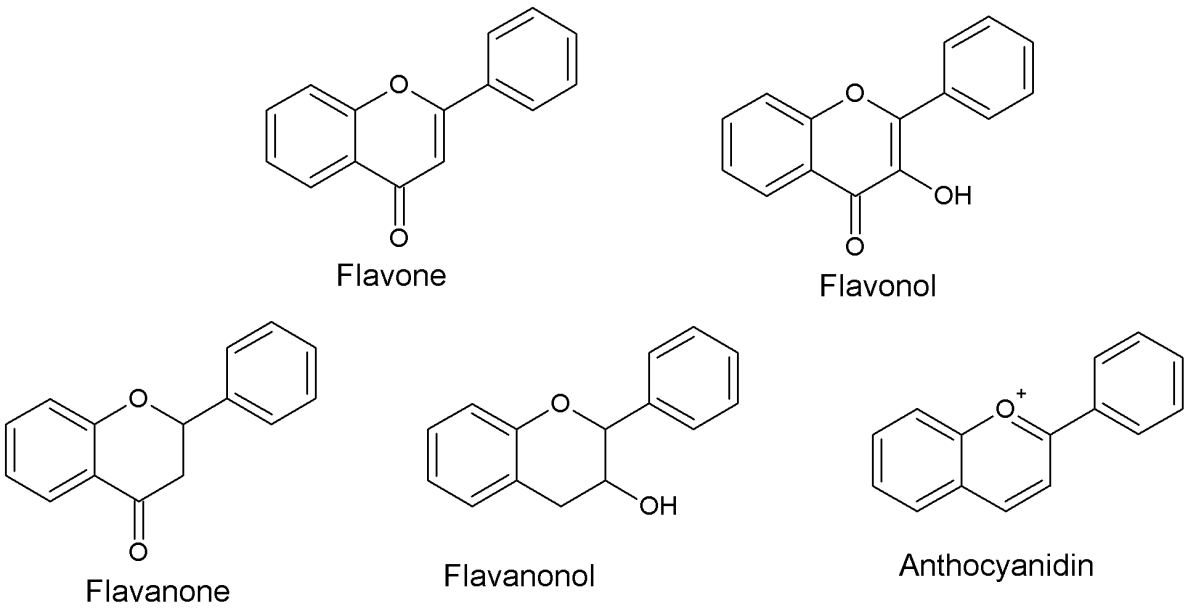

Figure 2. General backbones of the flavonoids reviewed.

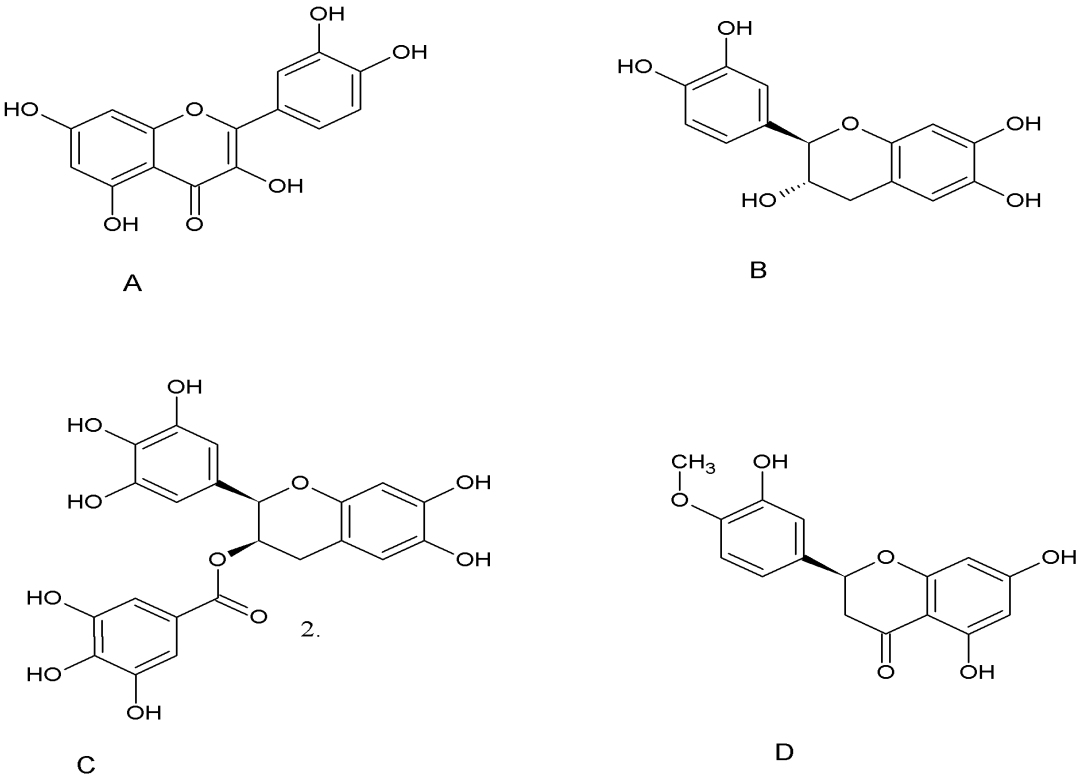

Figure 3. Structures of flavonoids; A- Quercetin; B – Catechin; C - (-)-Epigallocatechin-3-gallate; D – Hesperetin

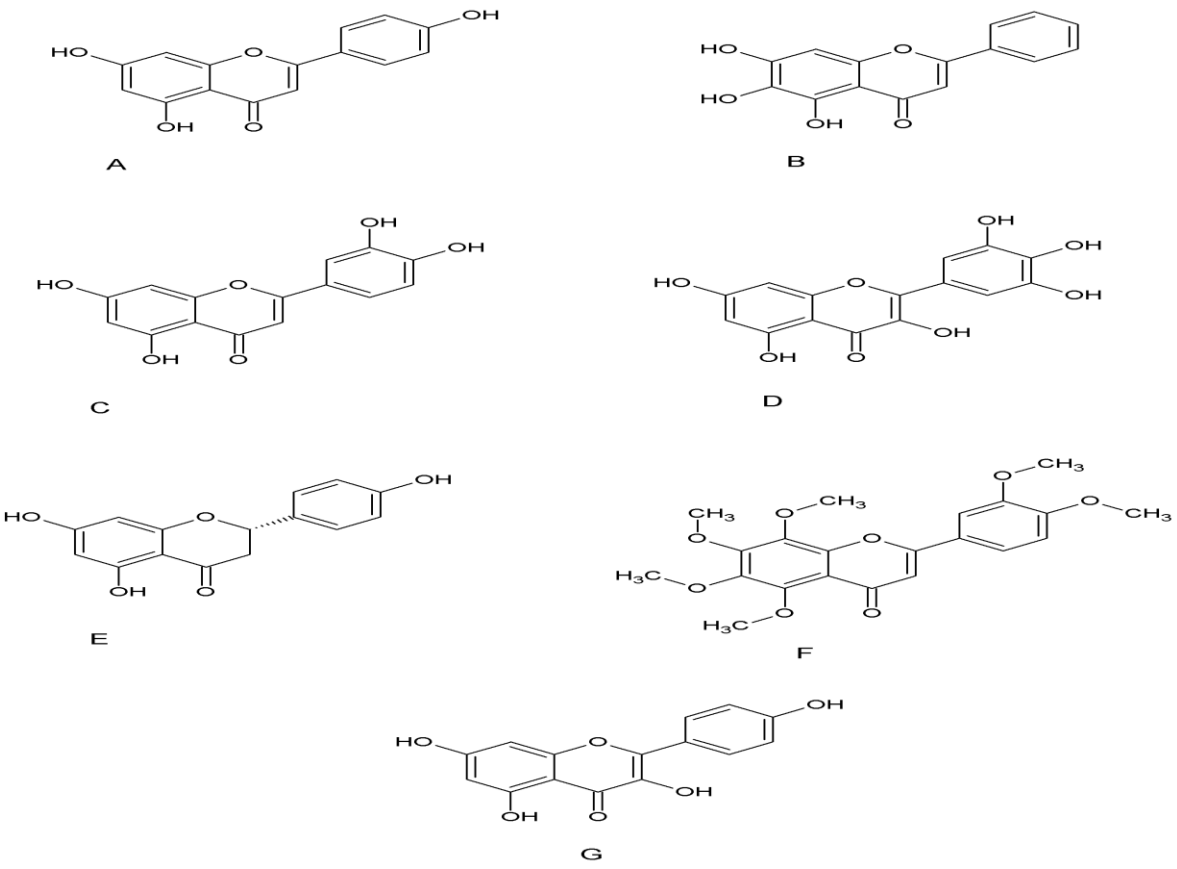

Figure 4: The structures of other flavonoids with antiarthritic properties. A- Apigenin; B – Baicalein; C - Luteolin; D – Myricetin; E – Naringenin; F – Nobiletin; G – Kaempferol

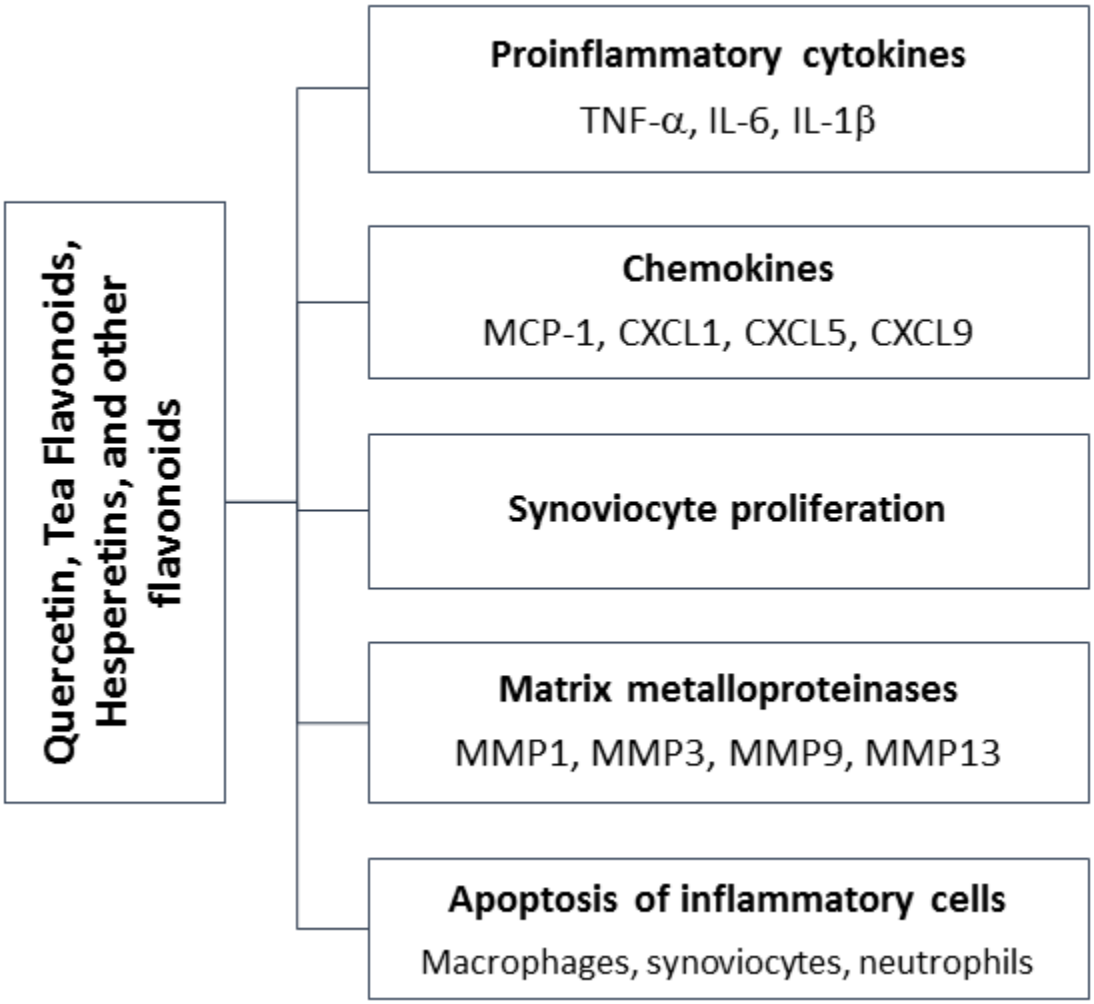

Figure 5. Common immunomodulatory effects of flavonoids in RA. Querecetin, tea flavonoids, hesperetins, and other flavonoids inhibit the proinflammatory cytokines, chemokines, matrix metalloproteinases and synovial proliferation. However, they stimulate the apoptosis of inflammatory cells.