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Research Article

Biological Activity and Therapeutic Potential of Quercetin for Inflammatory Bowel Disease

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Abstract

Rates of inflammatory bowel disease (IBD) are increasing globally. IBD is an idiopathic chronic inflammatory disorder of the gastrointestinal tract characterized by acute disease flares and remission. IBD has a multifactorial pathogenesis whereby environmental and genetic factors contribute to disease onset. IBD is a serious clinical disorder that significantly impairs the patient's quality of life, and is associated with increased morbidity, mortality, and cancer risk. IBD is characterized by the loss of tolerance to luminal antigens resulting in a persistent and excessive immune response. There is currently an unmet need for novel treatment options for patients with IBD. In recent years, flavonoids, a class of polyphenols widely distributed in fruits and vegetables gained significant interest as potential therapeutic agents for inflammatory disorders including IBD. Many flavonoids regulate cellular transcription factors critical in antioxidant and anti-inflammatory pathways within the innate and adaptive immune response. Among these flavonoids, quercetin and its related glycosides have demonstrated remarkable effects capable of attenuating inflammation in experimental models of IBD. To date, many mechanisms underlying quercetin's biological activity have been elucidated; wherein quercetin is reported to downregulate NFκB, MAPK, STAT and AP-1, and upregulate Nrf2. Thus, the aim of the review is to present the evidence surrounding quercetins biological activity, and its potential as a novel dietary intervention for IBD.

Keywords: Inflammatory bowel disease; Flavonoids; Quercetin; Inflammation

Abbreviations

AP- alkaline phosphatase; AP-1- Activator protein 1; Arg-1-Arginase-1; CD-Crohn's disease; COX-2-Cyclooxygenase-2; DSS- Dextran sulfate sodium ERK- Extracellular signal kinases; FIZZ-1- Resistin-like molecule alpha; GI- Gastrointestinal tract; GCLM-Glutamate-cysteine ligase modifier subunit; GLCL- Glutamate-cystine ligase; GM-CSF-Granulocyte-macrophage colony stimulating factor; HO-1- Hemeoxygenase-1; IBD-Inflammatory Bowel Disease; ICAM-1- Intracellular adhesion molecule-1; IFN-γ- Interferon gamma; IL- interleukin; LPS-Lipopolysaccharide; JNK- Jun N-terminal kinase; LSP1-Lymphocyte specific protein-1; iNOS- Nitric oxide synthase; MAPK - Mitogen activated protein kinase; MPO- Myeloperoxidase; NFκB- Nuclear factor kappa light-chain-enhancer of activated B cells; NO- Nitric oxide; Nrf2- Nuclear factor erythroid 2-related factor 2; NQO1-NAD(P)H quinone dehydrogenase 1; PPARγ- Peroxisome proliferator-activated receptor gamma; ROS-Reactive oxygen species; SRXN1- Sulfiredoxin-1; STAT-Signal transducer and activator of transcription; TLR-Toll-like receptor; TNBS-trinitrobenzene sulfonic acid; TNF-α- Tumor necrosis factor alpha; UC-Ulcerative colitis; VCAM-1-Vascular cell adhesion molecule; YM-1-Chintinase-like protein 3

1. Introduction

The rate of inflammatory bowel disease (IBD) is increasing worldwide [1]. IBD prevalence is greatest in Europe, followed by the United States, with accelerated incidence in newly industrialized countries such as Africa, Asia, and South America [1,2]. In the US alone, 1.6 million people have been diagnosed with IBD, with 70,000 new cases each year [3]. IBD affects men and women equally and is commonly diagnosed between 15 to 35, although increasing incidence among children is a growing concern [1,3]. IBD is a term used to describe an idiopathic chronic relapsing inflammatory disorder of the gastrointestinal tract (GI) [4,5]. IBD is characterized by periods of acute inflammation and remission, and is associated with decreased quality of life, high morbidity, and colorectal cancer risk [6-8]. IBD is characterized by upregulated synthesis and release of many pro-inflammatory mediators such as reactive oxygen species (ROS), nitric oxide (NO), cytokines, eicosanoids, and platelet activating factors [9]. When these pro-inflammatory signals are sustained for a significant duration, intestinal injury ensues [9]. Excessive concentrations of inflammatory mediators can lead to edema, ulceration, and carcinogenesis of the colonic tissue [10]. Symptoms of IBD are a direct result of the inflammatory process and vary in severity, but include weight loss, diarrhea, constipation, bloody stool, and abdominal pain among others [5]. IBD commonly manifests in two main forms known as Crohn's disease (CD) and ulcerative colitis (UC) [2,11]. CD and UC differ in clinical features and histopathology [12]. UC is characterized by inflammation restricted to the colon and affects the superficial layer of the mucosa. In contrast, CD commonly occurs in the ileum of the small intestine and affects all layers of the intestinal wall [12]. Unlike UC, CD can occur anywhere from the esophagus to the rectum [12]. The chronic inflammation characterizing IBD likely results from deregulation of cellular pathways involved in the innate and adaptive immune response whereby tolerance to luminal antigens are lost [13,14]. What causes perturbation of the immune response is poorly understood, although its known that IBD onset is multifactorial whereby a combination of elements including genetic predisposition, environmental factors such as diet, as well as microbial factors underlie disease pathogenies [4,13]. Numerous IBD risk loci have been identified suggesting that IBD is

characterized by a collection of individual pathophysiology's which manifest similarly in the clinic [15]. Current treatments aim to control the excessive immune response by suppressing the immune system to induce or maintain remission [10]. Current pharmacologic agents include immunosuppressing corticosteroids and 5-aminosalicylic acid which act to inhibit transcription factors involved in the regulation of pro-inflammatory genes including AP-1 and NFκB [14]. In addition, 5-aminosalicylic acid disrupts the metabolism of aracadonic acid to pro-inflammatory lipid mediators and acts as an ROS scavenger [10,15]. However, many of these therapeutics have serious side-effects, and a high percentage of IBD patients are unresponsive to current treatments and require surgical interventions [14]. Therefore, there is an unmet need for alternative treatments with reduced side effects. Interestingly, many IBD patients report diet as a triggering factor in the relapse of disease, and epidemiological studies have highlighted the role of diet in disease development [18]. In recent years, food to gene interactions have been elucidated shifting interest to numerous dietary bioactive compounds as novel pharmacological agents for IBD [14-20]. Many essential nutrients and polyphenols found in fruits and vegetables have been reported to regulate cellular transcription factors critical in inflammatory resolution within the innate and adaptive immune response [12-20]. Among these polyphenols, quercetin and its related glycosides have gained considerable interest in treating chronic inflammatory disorders [20]. Therefore, this review aims to evaluate the emerging therapeutic potential of flavonoids, specifically quercetin and its related glycosides in the treatment of IBD. This review will begin with a brief summary of intestinal inflammation and resolution as well as how this is perturbed in IBD. Next, quercetin and its derivatives will be introduced, and their potential mechanisms supporting their use as pharmacological agents will be summarized through discussions surrounding in-vitro and in-vivo studies. Lastly, this review will conclude with an integrative discussion regarding the therapeutic potential of quercetin in IBD treatment, current limitations, and next steps for future research.

1.1 Dysregulation of immune function

In order to understand the potential application of food derived bioactive constituents in IBD, it is important to discuss the inflammatory process and thus, potential cellular targets. Here, the inflammatory process and what is known about the dysregulation of this process in IBD will be described. This understanding will be used to highlight the critical immune cells of the innate and adaptive immune system in addition to the inflammatory markers that can be mediated therapeutically to resolve inflammation. The luminal space of the GI contains food derived antigens, toxins, as well as trillions of commensal microorganisms [21]. Thus, the hosts innate and adaptive immune system must work in concert to maintain intestinal homeostasis with the complex gut ecosystem [21]. Immune defence begins with the physical and chemical barriers of the gut which act to exclude internal access of the intestinal microbes [21]. The enterocytes have a critical role in maintaining this interface, and as such, are highly regulated and possess high rates of apoptosis and repair [21]. [Interestingly, many of the reported IBD risk loci are involved in regulating the intestinal epithelium [22]. This suggests that some IBD patients may have a dysfunctional epithelial barrier and increased tight junction permeability enhancing exposure to luminal antigens and microorganisms [22]. In addition to acting as physical barrier, enterocytes secrete a wide range of cytokines and

chemokines that recruit and regulate immune cells including neutrophils, macrophages, and T-cells [22]. When the physical and chemical barriers of the host are damaged or insufficient against pathogens, the inflammatory process is initiated through the release of damage associated molecular patterns and pathogen associated molecular patterns [14,21]. These cellular signals favour the infiltration and activation of leukocytes from the innate immune system which work concomitantly with lymphocytes of the adaptive immune system to maintain host health and gut homeostasis [14,21]. The innate and adaptive immune cells act to neutralize invaders, clear dying cells and debris, and repair damaged tissue [14]. In healthy individuals the inflammatory response is a tightly regulated self-limiting acute process whereby the pathogen or damaged cells are eliminated, inflammation is resolved, and the tissues are returned to homeostasis [14]. There are two leading hypotheses for the chronic inflammation that characterizes IBD [23]. First, loss of tolerance toward microbiota derived luminal antigens triggers intestinal inflammation, which is chronically maintained due to continuous and unavoidable exposure [23]. Second, perturbation of the immunological mechanisms involved in the resolution of inflammation leads to a persistent and excessive inflammatory response [14]. Interestingly, macrophages are believed to play an essential role in both tolerance and inflammatory resolution [14]. In recent years, the cellular pathways involved in luminal tolerance and the active process of inflammatory resolution have been elucidated [14]. Following infection or tissue damage, apoptotic cells are cleared by macrophages via efferocytosis; triggering a functional shift towards an anti-inflammatory M2 phenotype producing cytokines, growth factors, and anti-inflammatory lipid mediators derived from omega 3 fatty acids [14,23]. This shift is essential for inflammatory resolution and the return to gut homeostasis [14,23]. The molecular mechanisms underlying the initiation of the pro-resolving functional shift in macrophages is still poorly understood, but some research groups suggest PPARγ activation is an important transcriptional regulator for the induction of the anti-inflammatory program [24,25]. Of note, following efferocytosis, macrophages highly express IL12B, LSP1 and SEPTIN1 among other genes, and interestingly, these genes have been implicated in IBD susceptibility [14]. These findings suggest that defects in efferocytosis may underlie IBD pathogenesis in some individuals [14]. The discovery that inflammatory resolution is an active process highlights the flaws in current pharmacological approaches in IBD, which have aimed to dilute pro-inflammatory signals through immunosuppression rather than initiate pro-resolving inflammatory pathways [23]. Immunosuppression limits inflammation by disrupting important mediators in the cellular immune cascade, wherein cytokines including TNF-α and IFN-γ are common targets [14,23]. Although, immunosuppressive therapies have numerous side effects and neglect to restore pro-resolving function, highlighting the unmet need for novel therapeutic approaches [14]. In addition to the role of macrophages as key cells in inflammatory resolution, pro-resolving macrophages promote tolerance to harmless commensal microorganisms and dietary antigens within the adaptive immune response [22]. Pro-resolving macrophages favour the expansion of antigen-specific CD4+CD25+ regulatory T (Treg) cells through the production of IL-10 [22]. Interestingly, Treg cell populations are significantly decreased in IBD patients compared to healthy individuals [22]. T-cells are key players in the adaptive immune response. They work concomitantly with other cells from the innate immune system to

secrete a range of pro-inflammatory cytokines such as IFNγ, TNF-α, IL-4, IL-17, among others, which potentiate the immune response [22]. T-cells have the potential to differentiate into T helper subtypes; a process mediated by the effector cytokines produced by antigen presenting cells of the innate immune system including macrophages [22]. Among the effector cytokines, IL-4 promotes Th2 differentiation, IL-12 and IFNγ generates Th1 cell types, IL-6, IL-1β, IL-17A and TGF-β promote Th17 cell differentiation, whereas TGF-β and IL-10 promote Treg cell subtype [15,16]. Of note, numerous IBD risk loci have been identified within the cellular pathway's involved T-cell differentiation, which may underlie disease pathogenesis in some individuals [15]. Given the critical role of macrophages in inflammatory resolution and luminal tolerance, there is strong evidence that targeted therapies enforcing pro-resolving phenotypes in macrophages may be a novel approach to IBD therapy [14].

1.2 Dietary interventions for IBD

Given the reported significance of diet in IBD pathogenesis, it's important to note that rates are highest in countries consuming a Western diet, therefore, characteristics of this diet likely underly disease susceptibility [18,26]. Common features of the Western diet include low fruit, vegetable, and omega 3 intake, and high omega 6 and refined sugar intake [26,27]. Thus, the Western diet is often rich in calories, but low in nutrient and polyphenol bioavailability [26]. These dietary habits have been associated with disruption of pro-resolving inflammatory pathways and alterations of the microbiota which may lead to IBD in genetically susceptible hosts [26]. A growing body of evidence suggest that polyphenols including flavonoids can mediate cellular pathways favouring resolution of inflammation and thus have pharmacological potential as a novel treatment for IBD [12,19,28].

1.3 Flavonoids

Flavonoids are a large family of hydroxylated polyphenolic compounds found in fruits, vegetables, nuts, seeds, and plants with over 7000 naturally occurring constituents [20,29]. Flavonoids are characterized by a C6-C3-C6 structure containing 2 aromatic rings linked by a 3-carbon chain [30]. Flavonoids are further classified into seven groups based on varying features of their aglycon rings [20]. The flavonoid family is made up of flavones, flavanones, flavanols, flavanonols, isoflavones, catechins, and anthocyanidins [20]. Naturally, flavonoids are commonly conjugated to sugars, and when bound, they are referred to as glycosides. Glycosides are hydrolyzed in the small intestines by brush boarder enzymes, or by the microbiota in the large intestine to form aglycones prior to absorption [20]. Flavonoid intake in the human diet is variable with estimates ranging from 50 mg/day to greater than 800mg/day, wherein these values are highly dependent on dietary intake of fruits, vegetables, and tea [9,31]. Many research groups have reported that flavonoids possess anti-inflammatory and antioxidant properties in-vitro and in-vivo and thus, have pharmacological potential for IBD [28].

1.4 Flavonols

Among the flavonoid family, flavonols are reported to have many therapeutic biological activities [22]. Flavonols are characterized by a 3-hydroxyflavone structural backbone, wherein the most well investigated constituents include quercetin (3,3',4',5,7-pentahydroxyflavone) and its glycosylated derivatives rutin (quercetin 3-rutinoside) and quercitrin (quercetin 3-rhamnoside). Quercetin and its related glycosides are the most commonly

occurring flavonoids in the diet accounting for up to 75% of flavonoid intake [31]. Quercetin and its derivatives are found in various foods including buckwheat, parsley, apples, onion, tomatoes, apricots, tea, among many others [31,32]. Following absorption, several tissues are involved in quercetin metabolism including the small intestine, large intestine, kidneys, and liver giving rise to glucuronidated, sulfated, and methylated forms of quercetin; furthermore, free quercetin is found in the plasma [31].Quercetin and its glycosylated derivatives are reported to reduce inflammation in-vitro and have attenuated IBD in several animal models [9,22,32,33]. Interestingly, the average intake of flavonoids in the United States, wherein IBD rates are the highest globally next to Europe, are significantly less than other parts of the world at an average intake of 9.75mg/day [32]. Given that quercetin and its derivatives often account for up to 75% of flavonoid intake in the average balanced diet. The glycosylated derivate quercitrin was first reported to have anti-inflammatory activity as early as 1976 [34]. After inducing enterocolitis in rats with intravenous administration of antigens with Freunds adjuvant, Galsanov et al. administered 25 or 100 mg/kg of glycosylated quercitrin for 10 days. Through histological observation, both treatment groups receiving quercitrin had decreased lymphocyte and monocyte infiltration of the intestinal mucosa, reduced immunomorphological changes associated with inflammation, and restoration of vascular permeability compared to untreated rats. These results strongly supported quercitrin's potential as a therapeutic agent for intestinal inflammation [34]. Since then, numerous studies have been published investigating the mechanisms underlying the anti-inflammatory effects of quercetin and its glycosylated derivatives [22,28]. Interestingly, in-vitro and in-vivo studies have reported inconsistent results dependant on the form of quercetin and method of administration [22,28].

1.5 Anti-inflammatory activity of quercetin, quercitrin, and rutin in-vitro

Hämäläinen et al. conducted a study with murine J774 macrophages stimulated with bacterial endotoxin, lipopolysaccharide (LPS), and treated at 100uM with 36 different flavonoids including quercetin, quercitrin, and rutin. Nitric oxide (NO) production was measured, and the mechanisms underlying alterations in NO production were elucidated [35]. When comparing quercetin and its related glycosides, inhibition of NO was greatest in response to the aglycone quercetin at 89.7%, followed by its glycosylated derivatives quercitrin at 19.1%, and rutin at 8.7%. Furthermore, the effects of quercetin were mediated through significant reduction of inducible nitric oxide synthase (iNOS) mRNA and protein expression [35]. Decreased iNOS expression was a result of decreased activation by nuclear translocation of NFκB and signal transducer and activator of transcription (STAT-1) [35]. Similar results regarding quercetin's anti-inflammatory and antioxidant activity were reported in many other in-vitro models independent of treatments before, or after murine macrophage activation with IFNγ or LPS. Concentrations of quercetin ranging from 10μM to 100μM had a dose dependant and inverse relationship with inflammatory markers via suppression of iNOS, lipoxygenase (LOX), and cyclooxygenase (COX- 2) enzymes, and pro-inflammatory transcription factors NFκB, AP-1, and STAT-1 and increased expression of heme oxygenase (HO-1) [36-45]. Furthermore, the anti-inflammatory effects of quercetin have been reported in several other cell lines including glial cells, fibroblasts, microvascular endothelial cells, among others [46]. Recently, it was demonstrated that when rat intestinal

intravascular epithelial cells were pre-treated with 80μM of quercetin and stimulated with LPS expression of pro-inflammatory intracellular adhesion molecule (ICAM-1) and vascular cell adhesion molecule (VCAM-1) were reduced, corresponding with decreased toll-like receptor 4 (TLR-4), NFκB, extracellular signal kinases (ERK), Jun N-terminal Kinase (JNK), and STAT activity [10]. Interestingly, a study conducted in H9C2 rat myoblasts reported that supplementation with 100μg/ml of quercetin significantly increased expression of PPARγ and decreased AP-1 expression [47]. Although this study was conducted in myoblasts, it provides evidence that quercetin can activate PPARγ which has been reported as an important regulator inducing the pro-resolving M2 macrophage phenotype [25]. Therefore, future studies should aim to investigate PPARγ activation in macrophages treated with quercetin. Similar results reported in human cell lines [46,48-50]. Min et al. pre-treated human mast cells with 30μM of quercetin and stimulated them with phorbol 12-myristate 13-acetate and calcium ionophore A23187. Quercetin significantly decreased expression of pro-inflammatory cytokines including TNFα, IL-1β, IL-6 and IL-8 by supressing activation of NFκB and P38 MAPK, but not ERK or JNK [50]. This contrasted a report by Kimata et al. whereby pre-treatment with quercetin decreased inflammatory markers in human mast cells by supressing NFκB, ERK, and JNK, but not P38 MAPK activation [49]. Although this discrepancy is likely to arise in the difference in stimulatory agents wherein Kimata et al. stimulated human mast cells with immunoglobulin E (IgE), highlighting the complexity of environmental stimuli on cellular inflammatory regulation. The in-vitro studies discussed herein are summarized in Table 1.

Reference	Cell line	Treatments	Results	Conclusions
35	Murine macrophage j774	Posttreatment with 10μM or 100μM of quercetin, quercitrin and rutin in cells stimulated with LPS	% NO inhibition at 100μm: 89.7% for quercetin; 19.1% for quercitrin; 8.7% rutin; Quercetin decreased iNOS protein, by reducing NFkB (80%) and STAT1 (91%) activity	Quercetin had the greatest affect in macrophages stimulated with LPS
36	Murine macrophage j774	Pre-treatment with 30μM of quercetin, then stimulation with LPS	Decreased protein expression of iNOS, and IL-1β, and reduced IL-1β and NO concentrations.	Quercetin pre-treatment reduced pro-inflammatory markers in macrophages stimulated with LPS
37	Murine macrophage RAW 264.7	Posttreatment: 5μM, 10μM, 20μM or 40μM of rutin, quercetin, and quercetin pentaacetate; Cells were then treated - 12 hours with LPS (100ng/ml)	Quercetin, rutin, and quercetin pentaacetate significantly reduced NO, PGE2, iNOS, and COX2 mRNA expression, but did not affect enzyme activity.	Quercetin was anti-inflammatory in chronically stimulated macrophages
38	BV-2 murine microglia cells	Quercetin, but not quercetin-3'-sulfate significantly inhibited NO production and iNOS expression by inhibiting NFkB, STAT-1, AP-1	Quercetin pre- treatment had the greatest anti- inflammatory effect in chronically activated microglial cells	Quercetin pre-treatment had the greatest anti-inflammatory effect in chronically activated microglial

		activity, and induced HO-1		cells
39	Murine macrophage RAW 264.7	Cells were pretreated with 20μM of quercetin 30 mins before LPS	Quercetin decreased INOS and COX2 expression, reducing NO and PGE2 synthesis. Reduced TLR4/MyD88/PI3K complexes and JNK1/2 and p38MAPK activation; Reduced FκB, NFα, IL-1β, IL-6, GM-CSF; Increased HO-1 and IL-10;	Quercetin pre-treatment possessed anti-inflammatory effect in LPS stimulated macrophages.
41	Murine macrophage RAW 264.7	Cells were pretreated with 5- 20μM of quercetin for 24 hours, then treated with LPS for 16 hours.	Suppression of NO, IL- 6, and TNFα, and reduced expression of COX_2 and iNOS.	Quercetin pre-treatment possessed anti- inflammatory effects in macrophages.
42	RBL-2H3 and Caco-2 cell lines	RBL-2H3 cells were pretreated with 5 or 10 μM of quercetin, then sensitized with DNP-specific IgE. Caco-2 cells were pretreated with 10 or 20 μM of quercetin then stimulated with either IL-4, or IgE- complex either IL-4, or IgE-complex	Inhibition of TNF-α and IL-4 in antigen stimulated RBL-2H3 cells, with greatest effects at 10μM. 20μM of quercetin inhibited the IL-4, induced activation of p38 MAPK in Caco-2 cells, decreased ERK phosphorylation, IL-8 and MIP-3a.	Quercetin pre-treatment possessed anti- inflammatory effects in basophilic leukemia cell lines, and colorectal adenocarcinoma cells.
43	Murine macrophage RAW 264.7	Cells were pre- treated with 1-100 μM quercetin, then stimulated with either LPS and/or IFN-γ. In addition, cells were post-treated with 50μM of quercetin.	Quercetin pre- treatment or post- treatment inhibited LPS, IFN-γ, and LPS+IFN-γ induced production of NO in a dose dependant manor. Pre-treatment reduced iNOS expression. Quercetin pre-treatment inhibited NFkB, ERK1/2, p38, JNK/SPAK, and STAT-1 phosphorylation Quercetin pre- treatment inhibited NFkB, ERK1/2, p38, JNK/SPAK, and STAT-1 phosphorylation	Quercetin pre-treatment possessed anti- inflammatory effects in macrophages stimulated with LPS and IFN-γ.
44	Murine macrophage j774A.1	Cells were pre- treated with quercetin (0.5- 50uM) then stimulated with LPS.	Pre-treatment inhibited NO, PGE2 production and iNOS and COX2 expression in a dose dependant manor.	Quercetin possessed potent anti-inflammatory effects in macrophages stimulated with LPS.
45	Murine macrophage RAW 264.7 and primary peritoneal macrophage+ in-vivo analysis in balb/c mice.	Cells were stimulated with LPS in the presence of absence of rutin or quercetin (20uM, 40uM, or 80uM); mice were injected with 6mg/kg of rutin and 3mg/kg of quercetin, with or without l-NAME, then injected with (10mg/kg)	Quercetin, but not rutin inhibited NO production and iNOS expression in RAW264.7 and peritoneal macrophages; Quercetin inhibited PGE2 production via reduction in COX-2 expression.	Quercetin was anti-inflammatory in vitro and intraperitoneal injection of both rutin and quercetin in LPS stimulated mice demonstrated therapeutic activity

		LPS without l-NAME, then injected with (10mg/kg) LPS		
10	Rat intestinal microvascular endothelial cells (RIMVECs)	Cells were pre- treated with 20, 40, or 80 uM of quercetin then stimulated with LPS.	Quercetin reduced the expression of ICAM-, VCAM-1, and TLR4, decreased IkB-α degradation and NFκB-p65 phosphorylation; ERK, JNK, and STAT phosphorylation were inhibited.	Quercetin pre-treatment possessed anti- inflammatory effects in RIMVECs stimulated with LPS.
48	Human umbilical cord blood-derived cultured mast cells (hCBMCs)	Pre-incubation with 0.01, 0.1, 1, 10 or 100uM quercetin prior to stimulation with anti-IgE	Quercetin reduced Il-6, IL-8, and TNFα protein; Tryptase and histamine release were reduced, in addition with PKCθ phosphorylation	Quercetin pre-treatment was anti-inflammatory in a model of human allergic inflammation
49	Human cultured mast cells	HCMCs were sensitized with IgE, then treated with quercetin at 1, 3, 10, 30, or 100 µM, before stimulation with anti-human IgE antibody.	Quercetin inhibited the release of PGD2, histamine and GM-CSF. Quercetin supressed activation of ERK and JNK, inhibited Ca influx and PKC activation.	Quercetin was a potent inhibitor of mast cell activation in a model of allergic inflammation.
50	Human mast cell line HMC-1	Cells were pre- treated with either 3 or 30 µM of quercetin, then stimulated with phorbol 12- myristate 13 acetate and Ca ionophore A23187.	30 µM of quercetin reduced TNF-a, IL-1B, IL-6, and IL-8 mRNA. Treatment increased IKBα, reducing NFκB activation. p38 MAPK activity was reduced.	Quercetin pre-treatment possessed anti- inflammatory effects in human mast cells.
51	Primary lymphocytes from IBD patients and controls	Cells were stimulated with hydrogen peroxide or 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) in the presence or absence of 0, 100, 200, or 250 ug/ml of quercetin	Quercetin dose dependently reduced H2O2 and IQ induced DNA damage in IBD patient derived lymphocytes	Quercetin reduced oxidative stress in lymphocytes from IBD patients.

Table 1: Summary of cell culture studies

In-vitro results strongly support quercetin as a therapeutic approach to IBD by reducing the expression of pro-inflammatory enzymes including COX, LOX, and iNOS, by affecting the regulatory transcription factors NFκB, STAT-1 and AP-1 [36-45], although some studies have reported contradictory results [22]. There exist several experimental models of IBD that share similarities to humans with IBD including chemically induced models via acetic acid, dextran sulphate sodium (DSS), and trinitrobenzene sulfonic acid (TNBS), and genetically engineered rodents such as HLA-B27 rats and IL-10 knock-out mice [52]. In addition, there are T-cell transfer models, and infectious models of colitis including C. Rodentium, among others which are used to investigate the efficacy of various potential therapeutics for IBD [52]. Kwon et al. reported that when mice with DSS-induced colitis were treated with 6mg/day of quercetin, or 6mg/day of rutin orally for 2-weeks, only rutin had anti-colitis

effects. Rutin significantly reduced colon shortening by 73%, attenuated weight loss by 54%, and improved food intake by 61% [52]. Furthermore, pro-inflammatory IL-1β protein levels were significantly supressed by 58% [52]. These results strongly support rutin as a therapeutic protocol for IBD, whereas quercetin had virtually no effect, contrasting the aforementioned studies [52]. Galvez et al. showed earlier that prophylactic administration of rutin at 25mg/kg or 100mg/kg could improve injury and glutathione depletion in rats with acetic acid induced colitis [53]. In addition, it has been reported that oral supplementation of rutin at 28.5mg/kg and 57mg/kg per day exhibited anti-colitis activity in a mouse T-cell transfer model [54]. Furthermore, oral administration of rutin at 10mg/kg for 6 days significantly reduced inflammation of the colon, decreased neutrophil infiltration, measured by myeloperoxidase activity (MPO) activity, and had similar effectiveness as sulfasalazine at 30mg/kg in TNBS-induced colitis [55]. When mice were treated with 1mg/kg of quercetin, or 1mg/kg of quercitrin following induction of colitis with DSS, only quercitrin significantly reduced the inflammatory processes, as evidenced by decreased disease activity index scores, decreased MPO activity, decreased TNFα and IL-1β concentrations, and decreased iNOS expression and NFκB activity [9]. These results were replicated with pre-treatment of 1mg/kg or 5mg/kg of quercitrin in rats with DSS induced colitis [56,57]. Overall, many preclinical studies in varying animal models of IBD have demonstrated paradoxical results wherein quercetins glycosides, but not quercetin ameliorated IBD [9, 32, 53-56, 58, 59]. In contrast, a recent study reported that oral quercetin administered at 10mg/kg for 7-weeks following induction of colitis using a CD4+ naïve T-cell dependant model in Rag1-/- mice significantly reduced chronic intestinal inflammation [40]. This was attributed to the reduction in the percentage of CD4+T cells, and the percent of IFNγ and TNF-α-positive CD4+T cells in the colonic lamina propria and mesenteric lymph nodes [40]. In addition, concentrations of IFNγ, TNF-α, IL-17A, IL-23, IL-12, and IL-6 were significantly reduced, and pro-resolving IL-10 expression was enhanced [40]. Furthermore, anti-inflammatory effects on macrophages were measured, reporting a significant decrease in macrophage infiltration for treated mice, and increased expression of M2 anti-inflammatory signatures including YM-1, FIZZ-1, Arg1, TGF-β and IL-10 expression [40]. In addition, Ju et al. reported a concomitant increase in antioxidant substrates including GLCL, GCLM, HO-1, SRXN1, and NQO1 and Nrf2 expression [40]. Interestingly, quercetin treatment was reported to beneficially and significantly alter the enteric microbiota corresponding with a decrease in endotoxin (LPS) levels in the colonic lumen of treated mice [40]. Ju et al. demonstrated that quercetin supplementation significantly limited experimental IBD by modifying tissue cytokine milieu, and inducing anti-inflammatory macrophage polarization and bacterial killing, thus supporting the findings from in-vitro models. While PPARγ activity levels were not measured by Ju et al. an in-vivo study conducted in rat myoblasts suggests quercitrin supplementation activates this transcription factor, which may elucidate the mechanism underlying quercetin supplementation enforcing the pro-resolving M2 phenotype [25,47]. Similar results were found in a C. Rodentium-induced colitis mouse model, whereby pre-treatment with 30mg/kg of quercetin significantly improved histopathology scores, reduced pro-inflammatory cytokines, increased IL-10 and restored microbial balance [60]. The in-vivo results discussed herein are summarized in Table 2. Interestingly, many research groups investigating quercetin supplementation at doses

ranging from 0.05% to 0.8% of dietary intake, or 10mg/kg to 160mg/kg reported a significant decrease in inflammatory cytokines, ROS, NO, and increased anti-inflammatory markers including IL-10 [61-72]. However, these animal models mimicked inflammatory disease states outside of those classically used in IBD such as obesity, arthritis, experimental autoimmune myocarditis, among others. Yet, these reports remain relevant as they support the therapeutic potential for oral supplementation of quercetin in IBD by improving immune function and reducing inflammation in-vivo [31].

Reference	Models	Study protocols	Results	Conclusions
47	Rat H9C2 rat myoblast cells	Cells were treated with 100ug/ml of quercetin and angiotensin II for 48 hours.	Quercetin dose dependently inhibited cellular hypertrophy increasing PPARγ and decreasing AP- 1 expression.	Quercetin attenuated angiotensin II induced H9C2 cell hypertrophy
32	DSS-induced colitis in pathogen free ICR mice	Mice pre-treated with either 6mg/kg/day, 0.6mg/kg/day. Or 60ug/kg/day of quercetin or rutin for 2 weeks. Three days after induction of colitis mice were fed 6mg/kg/day quercetin or rutin or rutin.	Rutin, but not quercetin improved histological scores and reduced IL-1B, IL-6, GM-CSF and COX-2 mRNA. Therapeutic treatment with rutin, but not quercetin reduced colorectum shortening and suppressed IL-1B expression shortening and suppressed IL-1B expression	Rutin possessed potent anti-inflammatory affects when administered preceding, or prior to the induction of colitis.
53	Acetic acid induced colitis in female Wistar rats	Mice were pre-treated with rutin at dosages of 10, 25 or 100mg/kg 24 hours after inducing colitis.	25mg/kg or 100mg/kg reduced colonic damage. 25mg/kg increased GSH but did not alter MPO and alkaline phosphatase activity.	Rutin reduced colonic damage and enhanced antioxidant availability.
54	CD4+ and CD62L+ T cell transfer model in female Rag-/- mice Murine T-cells and splenocytes were used for ex-vivo analysis.	Following induction of colitis, mice were treated 12 days with 57mg/kg/day of rutin	Rutin reduced the disease activity index, weight loss, and MPO, and AP activity 36% and 54%, respectively. COX2, IFNγ, TNFα, CXCL1, S100A8, IL-1β, IL- 6, IL-17, and plasma IFNγ, TNFα were reduced. Rutin reduced pSTAT-4 and p- IkBα, INFγ, and IL1β expression in T-cells and splenocytes TNFα were reduced. Rutin reduced pSTAT-4 and p-IkBα, INFγ, and IL1β expression in T-cells and splenocytes	Rutin possessed anti-inflammatory effects and inhibited T-cell activation.
55	TNBS- induced colitis in male Sprague- Dawley rats HCT116, SW620, and HT-29 Human colonic epithelial	1 day after the induction of colitis, Rutin (10mg/kg), or sulfasalazine (30mg/kg) were administered for 6 days. Human colonic	Rutin reduced the colonic damage score and lowered MPO activity similarly to sulfasalazine. From the ex-vivo analysis, quercetin dose dependently reduced NFκB activity and expression, and IL1β expression	Rutin acted as a quercetin delivery system and possessed anti-inflammatory

	cells were used for ex-vivo analysis	epithelial cell lines were stimulated with TNFα in the presence or absence of quercetin (10-100μM)		effects.
9	DSS induced colitis in female Wistar rats. Bone marrow- derived macrophage (BMDM) were used for ex-vivo analysis.	Colitis was induced, and rats were treated with 1mg/kg of quercetin or quercitrin for 10 days and compared to controls. BMDM cells were pre-treated with varying doses of quercetin (1uM, 10uM, 50uM), or quercetrin (1uM, 5uM, 50uM) then stimulated with LPS.	Quercitrin reduced the disease activity index, MPO activity, and NFκB expression. Quercetin, dose dependently inhibited M-CSF induced proliferation, and reduced LPS stimulated NFκB, iNOS, TNFα, and IL1β expression in BMDM cells.	The intestinal microbiota hydrolyzed quercetrin to quercetin, which acted locally to reduce colonic damage.
57	DSS induced colitis in male Wistar rats	Following induction of colitis, mice were treated with quercitrin at 1 or 5mg/kg for 10 days and compared to controls	Quercetin dose dependently reduced macroscopic and microscopic damage, and reduced serum TNFα and bacterial translocation to the liver, spleen, mesenteric lymph nodess, and serum; Colonic MPO activity was reduced.	Quercitrin possessed anti-inflammatory effects.
58	TNBS induced colitis in female Wistar rats	Rats were pre-treated with (5, 10, 25, 50, or 100mg/kg) of rutin 24 hours before TNBS treatment then sacrificed after 2 days. Colitis was induced, and rats were treated with 10mg/kg or 25mg/kg of rutin for 1 or 2 weeks.	Pre-treatment with 10mg/kg or 25mg/kg increased food intake and reduced macroscopic colonic damage. Furthermore, 5, 10, and 25mg/kg increased GSH levels, and 25mg/kg reduced MPO and AP activity. As a therapeutic, 10 mg/kg and 25mg/kg significantly reduced colonic damage, MPO activity, and increased GSH. Furthermore, 2 weeks of treatment at 25mg/kg reduced AP activity and LTB4 synthesis.	Rutin possessed potent anti-inflammatory and anti-oxidant capacity when administered preceding or prior to the induction of colitis.
59	TNBS induced colitis in female Wistar rats	Rats were treated with varying doses of quercetrin 2 hours before, or 24 hours after the induction of colitis	Pre-treatment with 1 mg/kg or 5mg/kg significantly reduced macroscopical colonic damage, MPO activity AP activity, and reduced LTB4 levels. In addition, GSH increased and colonic fluid transport was improved.	Quercetrin possessed anti-inflammatory and antioxidant capacity when administered preceding or prior to the induction of colitis.
40	CD4+ CD62L+ T cell transfer mediated colitis	Following induction of colitis, mice were treated with 10mg/kg	Quercetin supplementation reduced histological damage. CD4+T cells and TNFα, INFγ, positive CD4+T cells	Quercetin modulated fecal

	in Rag-/- mice Ex-vivo analysis was performed in bone marrow derived macrophages. DSS induced colitis in C57BL/6 mice	once every 3 days for 7 weeks. Hmox1 siRNA or control scrambled siRNA (Src)-transfected BMDMs were treated with 100uM quercetin and stimulated with LPS and IFNγ Quercetin or PBS treated BMDM cells were transferred into mice 4 days after DSS treatment.	were reduced, and the expression of CD69, CD40L, and 4-1BB were inhibited. Colonic TNFα, INFγ, IL-17A, and IL-6 expression were decreased and IL- 10, Yml, Fizzl, Arg1, TGF-β, Nrf2 and HO-1 were increased. Quercetin treatment induced the M2 phenotype in control BMDM- Src cells, and this effect was lost in Hmox1 siRNA BMDM cells. Quercetrin treated BMDM-Src BMDMs protected mice from colitis, and reduced the expression of TNFα, INFγ, IL-17 and fecal E.coli content.	bacterial content and possessed potent anti-inflammatory and antioxidant affects via Nrf2/HO-1 pathway.
60	C.rodentium induced colitis in pathogen- free female C57BL/6 mice	Pathogen free mice were pre-treated for 2 weeks with 30mg/kg quercetin prior to induction of colitis.	Quercetin Pre- treatment improved histopathology scores, increased IL-10, and reduced IL-17, IL-6 and TNFα levels. Bacterial populations of Bacteroides, Bifidobacterium, Lactobacillus, and Clostridia, were enhanced, but Fusobacterium and Enterococcus were suppressed.	Quercetin pre-treatment possessed anti-inflammatory effects and mediated gut microbial composition.
73	TNBS- induced colitis in male Wistar rats	3 days after the induction of colitis, chitosan/nutriose coated nanovesicles containing 9mg/kg of quercetin (n=6) were administered intragastrically by gavage once a day for 3 days and compared to controls.	Quercetin supplementation significantly reduced colonic damage assessed macroscopically, and inhibited MPO activity by 90%	Quercetin possessed therapeutic effects when administered via coated vesicles.
74		Mice were treated 2 hours before, or 10 hours after the induction of colitis with microcapsules containing 1, 10, or 100mg/kg of quercetin and euthanized 24 hours following induction and compared to controls.	Quercetin treatment dose dependently reduced macroscopic and microscopic damage and MPO activity. Additionally, expression of IL-10, glutathione, FRAP and ABTS were increased, and IL-33 and IL1β were decreased	Quercetin possessed therapeutic effects when administered in a microcapsule .

Table 2: Summary of animal studies

1.6 Rational for conflicting in-vivo and in-vitro results

Conflicting results in-vitro and in-vivo have been attributed to differences in the absorption bioavailability of flavonoids [28]. It is generally

considered that aglycones like quercetin are rapidly absorbed in the upper intestinal tract, reducing the likelihood that effective concentrations are met in the inflamed tissue of the lower GI [9]. On the contrary, flavonoid glycosides like quercitrin and rutin are resistant to acidic hydrolysis in the stomach and arrive intact to the small intestine [31]. Glycosides are poorly absorbed in the small intestine and thus, reach the large intestine intact and in high amounts [9,31]. Therefore, large concentrations of rutin and quercitrin reach the colon, where the fecal microbiota hydrolyze the glycosides releasing quercetin in sufficient amounts for local anti-inflammatory effects [9].Therefore, it's likely that higher concentrations of quercetin are required to reach pharmacological dosages capable of producing anti-inflammatory effects in the colon [9]. As such, differences in dosage may explain the results between two distinct animal models, wherein 10mg/kg and 30mg/kg of quercetin reduced colitis but less than 10mg/kg were reported as ineffective [9,32,34,40,60]. Taken together, glycosylated derivatives can be considered a pro-drug enabling transport and delivery of biologically active quercetin in the colon [31]. This theory is consistent with experimental IBD models wherein chitosan/nutriose-coated vesicles as well as pectin/casein polymer microcapsules were used to prevent absorption and deliver quercetin to the colonic region, reporting a significant reduction in disease activity in DSS and TSBN animal models at dosages of 9mg/kg and 100mg/kg respectively [73-74]. As such, future studies should aim to compare glycosylated derivatives including rutin and quercitrin with higher dosages of quercetin, or with coated vesicles to determine the most effective vehicle for achieving pharmacological efficacy of quercetin in-vivo.

1.7 Human clinical trials

Despite the growing body of evidence that quercetin possesses therapeutic potential for IBD, no human clinical trials using IBD patients have been published. There have been a small number of clinical trials conducted in healthy athletes, middle-aged individuals, and elderly adults that aimed to determine the effects of quercetin on inflammation and immune function summarized in Table 3 [31].

Reference	Participants	Study protocol	Results	Conclusion(s)
76	Healthy adult individuals	In a double- blind randomized control trial runners were supplemented 1000mg/day of quercetin for 3 weeks before, during, and 2 weeks after an endurance race and compared to placebo.	There was no significant difference between supplement and control with respect to natural killer cells, granulocyte respiratory burst activity, salivary IgA output, neutrophil and monocyte cell counts, and post-race illness rates.	Quercetin had no effect on illness rates, leukocyte subset counts, or granulocyte respiratory burst activity and salivary IgA healthy adults.
75	Healthy elderly individuals 60-70	In a double-blind randomized placebo-controlled trial, participants were supplemented with 25 mg of resveratrol, pterostilbene, quercetin, δ-tocotrienol, nicotinic acid, morin hydrate twice daily (NS-7), or 25mg/kg of the later without morin hydrate,	Supplementation with NS-7 and NS-6 significantly reduced NO (39%,24%), C-reactive protein (19%,21%), γ- glutamyl-transferase activity (9%,18%) and uric acid (6%, 12%)	Quercetin supplementation was well tolerated and demonstrated anti-inflammatory effects in healthy elderly adults.

		and 50mg of quercetin (NS-6) twice daily for 4-weeks.		
74	Healthy adult individuals aged 18-85	In a randomized double-blind placebo- controlled trial, individuals received wither 500mg/day (n- 334) or 1000mg/day (n=333) of quercetin for 12 weeks and compared to placebo (n=356) on upper respiratory tract infection (UTRI) rates	There was no significant effect on supplementation and UTRI rates. However, in individuals over 40, 1000mg/day of quercetin resulted in a significant 33% reduction in the severity of URTI and total number of sick days.	Quercetin supplementation was well tolerated and demonstrated a therapeutic effect in older individuals with UTRIs.
78	Healthy professional cyclists	In a randomized double-blind placebo- controlled trial, individuals received 1000mg (n=20) of quercetin daily for 3 weeks preceding, during, and a 2-weeks after a 3-day period of intense cycling exercise.	There were no significant differences in pre- and post-exercise changes in natural killer cell activity, PHA-stimulated lymphocyte proliferation, polymorphonuclear oxidative burst activity, and salivary IgA output. However, quercetin supplementation significantly reduced post-exercise UTRI rates	Quercetin supplementation did not mediate several measures of immune function but reduced the UTRI infection rate in the 2-week period following intense exercise.
79	Healthy professional cyclists	In a double-blind, randomized, placebo-controlled trial participants were supplemented with 1000mg quercetin + 1000mg Vit C + 40mg niacinamide+80ug folic acid (QUR)(n=13), or 400mg quercetin + 120mg EGCG + 220mg DHA +180mg EPA (Q- EGCG) (n=14) 2 weeks before, during, and 1 week after 3 days of intense cycling exercise.	Plasma quercetin content was greatest in the Q-EGCG group, and had the most significant positive effects on CRP, IL-6, granulocyte colony- stimulating factor, and monocyte chemoattractant protein content following exercise.	Quercetin supplementation significantly reduced markers of exercise induced inflammation, with greatest effects seen in Q-EGCG which resulted in increased quercetin bioavailability noted by increased serum quercetin levels.
80	Patients hospitalize d with ST-segment elevation myocardial infraction	In a randomized trial, quercetin was administered 30 minutes after admission intravenously in addition to standard treatment (n=93) for 5 days and compared to controls (n=105)	Quercetin significantly reduced myocardial necrosis (26.4%), improved left ventricular function, and reduced LOX activity, serum diene conjugates, and leucotriene C4.	Quercetin supplementation had therapeutic effects in patients hospitalized for myocardial infraction through the inhibition of lipid peroxidation and leucotriene C4 concentrations.
77	Healthy female subjects ages 30-79	In a double-blind randomized placebo-controlled trial, participants were supplemented with 500mg (n=38) or 1000mg (n=40) of quercetin twice daily for 12 weeks and were compared to placebo (n=42).	Supplementation significantly increased plasma quercetin levels, but had no effect on blood leukocyte subsets, plasma IL-6 or TNFα, natural killer cell activity, granulocyte oxidative burst activity, or phagocytosis.	Quercetin supplementation had no influence on several measures of inflammation in healthy female community dwelling adults.

Table 3: Summary of human clinical trials

However, many of these studies had the notable limitation that quercetin administration was often paired with other vitamins, which may confound conclusions about the sole efficacy of quercetins [75,77]. In addition, most studies have been conducted in athletes and healthy persons, thus these trials are evaluating the acute effects of supplementation on immune function, rather than in chronic inflammation wherein immune regulation is perturbed [31,38]. Of note, all trials conducted in healthy individuals and athletes reported no adverse effects, even at dosages as high as 2000mg/day [74-79]. Elderly individuals commonly suffer from compromised immune function associated with aging, and thus, some comparisons can be drawn to individuals with IBD [75]. Qureshi et al. reported that elderly persons had significantly higher NO levels compared to younger adults, and 4-week supplementation with quercetin at 50mg/day or 100mg/day decreased NO, as well as inflammatory marker C-reactive protein, and γ-glutamyl transferase activity. Although, it is difficult to draw conclusions from this study given that supplements also contained resveratrol, pterostilbene, morin hydrate, δ-tocotrienol, riboflavin, and nicotinic acid [75]. Currently, it is incompletely understood whether the beneficial effects of quercetin are due to local action, or if they are derived from a systemic effect following absorption and metabolism [22]. Some human clinical trials that have administered quercetin intravenously report positive outcomes [80]. In a randomized trial, Parkhomenko et al. administered quercetin intravenously in 93 individuals hospitalized with myocardial infraction. Intravenous supplementation of quercetin was repeated for 5 days in addition to standard treatment and compared to controls (n=105) [80]. They reported that quercetin irreversibly blocked the LOX pathway involved in pro-inflammatory lipid mediator synthesis, decreased serum concentration of leukotriene B4, and acted as an antioxidant evidenced by decreased diene conjugates [80]. Therefore, the results from this study support previous reports in-vitro and in animal models wherein quercetin possessed anti-inflammatory and antioxidant activity [80]. In summary, current human clinical trials have had significant limitations, focusing primarily on the effects of quercetin in attenuating acute inflammation, rather than chronic inflammation in healthy individuals as an exercise aid [31]. Importantly, these studies suggest that oral doses at 500mg/kg and 1000mg/kg were well tolerated and free from adverse events in healthy individuals [74-79]. Clinical trials administering quercetin intravenously showed promising anti-inflammatory and antioxidant effects and warrant further investigation in states of chronic inflammation including IBD [80]. Taken together, these findings suggest quercetin is a safe and tolerable therapeutic that may have pharmacologic applications for IBD.

1.8 Quercetin as a dietary intervention for IBD

Numerous in-vivo and in-vitro models have clearly demonstrated the quercetin has biological functions involved in regulating the innate and adaptive immune response promoting immune homeostasis by modulating inflammatory signalling, reducing inflammatory molecule production, activation, and recruitment [22]. Importantly, several studies have implicated excessive oxidative stress from ROS and reactive nitrogen species including NO in IBD

severity and etiology [22]. ROS and NO can lead to cytotoxicity within cells, leading to additional pro-inflammatory cytokine release, perpetuating the immune response and preventing resolution [22]. Key enzymes involved in the production of reactive oxygen species are MPO and iNOS [22]. Numerous studies within this review reported decreased expression and activity of iNOS and MPO in response to quercetin supplementation in experimental models [37-40]. These findings strongly support quercetin supplementation as a potential therapeutic aid in IBD [40]. Furthermore, quercetin enhances endogenous cellular antioxidant pathways involved in mediating cellular stress from ROS and NOS [81]. This was demonstrated both in-vitro and in-vivo, whereby quercetin supplementation increased HO-1 expression [45]. HO-1 is regulated by several transcriptional factors including Nrf2, and inhibition of HO-1 in animal models potentiates colonic damage and inflammation [81]. Ju et al. reported that quercetin supplementation increased Nrf2 expression, which likely explains the increased HO-1 expression in response to supplementation [40]. In addition to regulating HO-1, Nrf2 is an important regulator of numerous endogenous antioxidant pathways, thus, increased expression in response to quercetin may help attenuate oxidative stress in IBD [40]. In addition, pro-inflammatory lipid mediators derived from arachidonic acid including prostaglandins such as PGE2 and leukotrienes such as LTB4 are found in high concentrations in the inflamed intestinal mucosa [22]. Prostaglandins and leukotrienes contribute to the inflammatory process perpetuating the excessive immune response [22]. Numerous experimental models demonstrated that quercetin supplementation reduced the activity of COX2 and LOX, which are critical enzymes in prostaglandin and leukotrienes synthesis respectively [22]. Interestingly, quercetin supplementation was shown to significantly reduce COX and LOX expression in numerous experimental models within this review [43,44]. Furthermore, cytokines, chemokines, and adhesion molecules play a critical role in IBD pathogenesis as these cellular signals recruit and activate immune cells within the innate and adaptive immune system and perpetuate the immune response [22]. IBD is associated with increased synthesis and release of proinflammatory cytokines including IFNγ, TNFα, IL-17A, IL- 6, GM-CSF, and IL-1β, chemokines, such as IL-8, MIP-2 and MCP-1, and adhesion molecules, such as ICAM-1and VCAM-1 [28]. Several studies have indicated quercetin supplementation decreased concentrations of IFNγ, TNFα, IL-6, IL-1β, IL-8, ICAM-1, and VCAM-1 with a concomitant increase in concentrations of IL-10 and TGFβ [28,40] . Overall, this shift in cytokine milieu reduces immune cell infiltration and activation, resulting in adaptations in the adaptive immune cells via increased populations of Treg cells, and decreased populations of Th1, Th2, and Th17 [22,40]. Several cellular pathways have been identified in-vitro and in-vivo that underly quercetin's biological activity. Some of the proposed anti-inflammatory mechanisms are related to the inhibition of NFκB, MAPK, AP-1, and STAT activation [36-45]. NFκB is ubiquitously expressed, and inactive in the cytoplasm bound to IκB [22]. Phosphorylation of IκB and subsequent release of NFκB occurs in response to various stimuli including proinflammatory cytokines, oxidative stress, or LPS via TLR activation [22]. NFκB is then free to translocate to the nucleus where it upregulates several pro-inflammatory genes [22]. Bian, Comalada and Hämäläinen et al. reported that quercetin supplementation decreased NFκB through inhibition of TLR4/ NFκB signalling pathway, and through reduced IκB phosphorylation preventing translocation to the nucleus. Furthermore, MAPK

increases the expression of genes involved in cytokine production, apoptosis and migration [22]. The MAPK signalling pathway involves a canonical three-tiered kinase cascade involving ERK, JNK, and P38. Several studies within this review have reported quercetin supplementation inhibited ERK, JNK and well as P38 significantly impairing MAPK activity [49,50]. In addition, the JAK/STAT pathway is activated in response to a wide range of extracellular cytokines in order to orchestrate a cellular response [22]. Ligand binding facilitates subunit dimerization and association with JAK tyrosine kinases allowing STAT proteins to dissociate from the receptor [22]. Dissociated STAT proteins translocate to the nucleus and increase the expression of pro-inflammatory and apoptotic genes [28]. Importantly, many experimental models within this review demonstrated that quercetin supplementation inhibits JAK/STAT signalling, reducing levels of activated STAT-1 and STAT-3 [35, 40]. Furthermore, AP-1 is another important transcription factor involved in the inflammatory response increasing the expression of inflammatory mediators including cytokines [47]. AP-1 is activated by a wide range of stimuli including bacterial and viral infections, oxidative stress, and growth factors [47]. Numerous studies within this review have indicated that quercetin reduced AP-1 expression, highlighting an additional mechanism underlying quercetins anti-inflammatory activity [27,38, 39, 47]. Lastly, the recent report by Ju et al. clearly demonstrated that quercetin supplementation increased pro-resolving macrophage markers Yml, Fizzl, Arg1, TGF-β, and IL- 10 in an experimental model of colitis. Interestingly, this was accompanied by an overall reduction in CD4+ T helper cell concentrations [40]. Thus, this study provides evidence supporting the hypothesis that pro-resolving macrophages are central to inflammatory resolution and tolerance to luminal antigens [40]. Interestingly an in-vitro study conducted by Yan et al. in myoblasts demonstrated quercetin supplementation increased PPARγ activity, which is involved in regulating the pro-resolving macrophage phenotype [25]. Future studies should aim to investigate quercetins ability to activate PPARγ in macrophages to elucidate the mechanisms underlying Ju et al. results. Despite these promising reports, some of the inconsistencies between in-vitro and in-vivo models have created debate regarding the efficacy of quercetin as a dietary intervention for IBD [46]. These inconsistences have been attributed to differences in aglycone and glycoside absorption and low bioavailability; whereby quercetin supplementation is only effective in oral doses greater than 10mg/kg in-vivo, or when protected in novel delivery systems to enhance colonic concentration [9,32,34,40,60]. Intravenous administration of quercetin was safe and efficacious in attenuating inflammation and reducing reactive species in patients with ST-segment elevation myocardial infraction [80]. Therefore, a pilot study in IBD patients to determine clinical dosing and effective mode of delivery of quercetin is warranted. Of note, many of the studies discussed in this review have reported that rutin and quercitrin can be considered pro-drugs, delivering therapeutic dosages of quercetin in the colon; whereby doses as low as 1mg/kg ameliorated IBD in experimental models [9]. Importantly, rutin and quercitrin are commonly found in a variety of plant foods, suggesting that a diet rich in fruit and vegetables may be efficacious in delivering therapeutic dosages of quercetin to the inflamed tissue, among other potentially beneficial flavonoids and nutrients [18]. In fact, recent reviews suggest that several other classes of flavonoids can attenuate gastrointestinal inflammation in-vitro and in-vivo [28]. Of note, dietary constituents belonging to the varying classes of flavonoids are reported to

have similar cellular mechanisms underlying their anti-inflammatory effects, shared by quercetin [28]. Although others are reported to have unique cellular mechanisms that have not been reported in trials with quercetin, suggesting that foods or supplements rich in numerous flavonoid classes may have synergistic effects in IBD treatment [20,28]. Although clinical trials in many of these flavonoids are scarce, some have reported significant results wherein supplementation induced and maintained remission in IBD patients [82-84]. Thus, there is a growing body of evidence supporting the role of flavonoids, including quercetin, as therapeutic dietary interventions for the treatment of IBD.

2. Conclusion

IBD is a serious idiopathic chronic inflammatory disorder of the GI affecting millions of people worldwide. IBD rates are increasing and are greatest among populations accustom to a western diet: high in calories but low in nutrients and polyphenols. To date there is no effective treatment to cure IBD, thus, there is an unmet need for novel therapeutic agents with minimal adverse effects. Flavonoids are a large family of polyphenols rich in many fruits and vegetables. Among these flavonoids, quercetin has demonstrated potent anti-inflammatory and antioxidant activity that could be an effective alternative for IBD treatment by promoting inflammatory resolution and immune homeostasis. The studies discussed in this review have provided strong evidence that quercetin and its glycosylated derivatives improve immune function by regulating pro-inflammatory transcription factors including NFκB, AP-1, NrF2, and STAT-1. Quercetins biological activity is reported to reduce the synthesis and secretion of pro-inflammatory markers within the innate and adaptive immune cells, induce a phenotypic shift to the pro-resolving macrophage phenotype, quench free radicals, enhance host antioxidant pathways, and positively influence the intestinal microbiota. Thus, the studies summarized in this review support quercitrin and its derivatives for consideration as a safe and novel treatment for IBD, highlighting the need for future human clinical trials.

References

1. M'koma AE. Inflammatory bowel disease: an expanding global health problem. Clinical Medicine Insights: Gastroenterology 6 (2013) S12731.

2. Ng SC, Shi HY, Hamidi N, et al. (2017). Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. The Lancet 390 (10114): 2769-2778.

3. Chrons & Colitis Foundation of America. The facts about inflammatory bowel diseases (2019).

4. Hoter A, Naim HY. The Functions and Therapeutic Potential of Heat Shock Proteins in Inflammatory Bowel Disease- An Update. International journal of molecular sciences 20 (2019): 5331.

5. Neuman MG. Immune dysfunction in inflammatory bowel disease. Translational research: the journal of laboratory and clinical medicine 149 (2007): 173-186.

6. Axelrad JE, Lichtiger S, Yajnik V. Inflammatory bowel disease and cancer: The role of inflammation, immunosuppression, and cancer treatment. World journal of gastroenterology 22 (2016): 47-94.

7. Kappelman MD, Rifas-Shiman SL, Porter CQ, et al. Direct health care costs of Crohn's disease and ulcerative colitis in US children and adults. Gastroenterology 135 (2008): 1907-1913.

8. Iacucci M, De Silva S, Ghosh S. Mesalazine in inflammatory bowel disease: a trendy topic once again?. Canadian Journal of Gastroenterology and Hepatology 24 (2010): 127-133.

9. Comalada M, Camuesco D, Sierra S. In vivo quercitrin anti-inflammatory effect involves release of quercetin, which inhibits inflammation through down-regulation of the NF-κB pathway. European journal of immunology 35 (2005): 584-592.

10. Bian Y, Liu P, Zhong J. Quercetin attenuates adhesion molecule expression in intestinal microvascular endothelial cells by modulating multiple pathways. Digestive Diseases and Sciences 63 (2018): 3297-3304.

11. Triantafillidis JK, Merikas E, Georgopoulos F. Current and emerging drugs for the treatment of inflammatory bowel disease. Drug design, development and therapy 5 (2011): 185.

12. Bora S, Cantorna MT. The role of UVR and vitamin D on T cells and inflammatory bowel disease. Photochemical & Photobiological Sciences 16 (2017): 347-353.

13. Grenier L, Hu P. Computational drug repurposing for inflammatory bowel disease using genetic information. Computational and structural biotechnology journal 17 (2019): 127-135.

14. Na YR, Stakenborg M, Seok SH. Macrophages in intestinal inflammation and resolution: a potential therapeutic target in IBD. Nature Reviews Gastroenterology & Hepatology 1 (2019).

15. Chen ML, Sundrud MS (2016) Cytokine networks and T-cell subsets in inflammatory bowel diseases. Inflammatory bowel diseases 22: 1157-1167.

16. Neuman MG. Immune dysfunction in inflammatory bowel disease. Translational research : the journal of laboratory and clinical medicine 149 (2007): 173-186.

17. Punchard NA, Greenfield SM, Thompson RPH. Mechanism of action of 5-arninosalicylic acid. Mediators of inflammation 1 (1992): 151-165.

18. Marion-Letellier R, Savoye G, Ghosh S. IBD: in food we trust. Journal of Crohn's and Colitis 10 (2016): 1351-1361.

19. Ferguson LR, Philpott M, Barnett MP Nutrigenomics: integrating genomic approaches into nutrition research. In Molecular Diagnostics. Academic Press.

20. Pan L, Sinden MR, Kennedy AH, et al. Bioactive constituents of Helianthus tuberosus (Jerusalem artichoke). Phytochemistry Letters 2 (2009): 15-18.

21. Chassaing B, Kumar M, Baker MT, et al. Mammalian gut immunity. Biomedical journal 37 (2014): 246.

22. Vezza T, Rodríguez-Nogales A, Algieri F, et al. Flavonoids in inflammatory bowel disease: a review. Nutrients 8 (2016): 211.

23. Onali S, Favale A, Fantini MC. The Resolution of Intestinal Inflammation: The Peace-Keeper's Perspective. Cells 8 (2019): 344.

24. Elliott MR, Koster KM, Murphy PS. Efferocytosis signaling in the regulation of macrophage inflammatory responses. The Journal of Immunology 198 (2017): 1387-1394.

25. Tugal D, Liao X, Jain MK. Transcriptional control of macrophage polarization. Arteriosclerosis, thrombosis, and vascular biology: 33 (2013): 1135-1144.

26. Ye Y, Pang Z, Chen W, et al. The epidemiology and risk factors of inflammatory bowel disease. International journal of clinical and experimental medicine 8 (2015): 22529.

27. Haskey N, Gibson DL. An examination of diet for the maintenance of remission in inflammatory bowel disease. Nutrients 9 (2017): 259.

28. Salaritabar A, Darvishi B, Hadjiakhoondi F, et al. Therapeutic potential of flavonoids in inflammatory bowel disease: A comprehensive review. World journal of gastroenterology 23 (2017): 5097.

29. Thilakarathna SH, Rupasinghe HP. Flavonoid bioavailability and attempts for bioavailability enhancement. Nutrients 5 (2013): 3367-3387.

30. Leyva-López N, Gutierrez-Grijalva EP, Ambriz-Perez DL, et al. Flavonoids as cytokine modulators: a possible therapy for inflammation-related diseases. International journal of molecular sciences 17 (2016): 921.

31. Li Y, Yao J, Han C, et al. Quercetin, inflammation and immunity. Nutrients 8 (2016): 167.

32. Kwon KH, Murakami A, Tanaka T et al. Dietary rutin, but not its aglycone quercetin, ameliorates dextran sulfate sodium-induced experimental colitis in mice: attenuation of pro-inflammatory gene expression. Biochemical pharmacology 69 (2005): 395-406.

33. Guazelli CF, Fattori V, Colombo BB, et al. Quercetin-loaded microcapsules ameliorate experimental colitis in mice by anti-inflammatory and antioxidant mechanisms. Journal of Natural Products 76 (2013): 200-208.

34. Galsanov SB, Turova AD, Klimenko ED. Effect of quercitrin on structural changes in the large and small intestine in experimental enterocolitis. Bulletin of Experimental Biology and Medicine 81 (1976): 775-777.

35. Hämäläinen M, Nieminen R, Vuorela P, et al. Anti-inflammatory effects of flavonoids: genistein, kaempferol, quercetin, and daidzein inhibit STAT-1 and NF-κB activations, whereas flavone, isorhamnetin, naringenin, and pelargonidin inhibit only NF-κB activation along with their inhibitory effect on iNOS expression and NO production in activated macrophages. Mediators of inflammation (2007).

36. Blonska M, Bronikowska J, Pietsz G, et al. Effects of ethanol extract of propolis (EEP) and its flavones on inducible gene expression in J774A. 1 macrophages. Journal of Ethnopharmacology 91 (2004): 25-30.

37. Chen YC, Shen SC, Lee WR. Et al. Inhibition of nitric oxide synthase inhibitors and lipopolysaccharide induced inducible (2001). NOS and cyclooxygenase-2 gene expressions by rutin, quercetin, and quercetin pentaacetate in RAW 264.7 macrophages. Journal of cellular biochemistry 82 (2014) 537-548.

38. Chen JC, Ho FM, Chao PDL, et al. Inhibition of iNOS gene expression by quercetin is mediated by the inhibition of IκB kinase, nuclear factor-kappa B and STAT1, and depends on heme oxygenase-1 induction in mouse BV-2 microglia. European journal of pharmacology 521 (2005): 9-20.

39. Endale M, Park SC, Kim S, et al. Quercetin disrupts tyrosine-phosphorylated phosphatidylinositol 3-kinase and myeloid differentiation factor-88 association, and inhibits MAPK/AP-1 and IKK/NF-κB-induced inflammatory mediators production in RAW 264.7 cells. Immunobiology 218 (2013): 1452-1467.

40. Ju S, Ge Y, Li P, et al. Dietary quercetin ameliorates experimental colitis in mouse by remodeling the function of colonic macrophages via a heme oxygenase-1-dependent pathway. Cell Cycle 17 (2018): 53-63.

41. Jung WJ, Sung MK. Effects of major dietary antioxidants on inflammatory markers of RAW 264.7 macrophages. Biofactors 21 (2004): 113-117.

42. Lee EJ, Ji GE, Sung MK. Quercetin and kaempferol suppress immunoglobulin E-mediated allergic inflammation in RBL-2H3 and Caco-2 cells. Inflammation Research 59 (2010): 847-854.

43. Mu MM, Chakravortty D, Sugiyama T, et al. The inhibitory action of quercetin on lipopolysaccharide-induced nitric oxide production in RAW 264.7 macrophage cells. Journal of endotoxin research 7 (2001): 431-438.

44. Raso GM, Meli R, Di Carlo G, et al. Inhibition of inducible nitric oxide synthase and cyclooxygenase-2 expression by flavonoids in macrophage J774A. 1. Life sciences 68 (2001): 921-931.

45. Shen SC, Lee WR, Lin HY, et al. In vitro and in vivo inhibitory activities of rutin, wogonin, and quercetin on lipopolysaccharide-induced nitric oxide and prostaglandin E2 production. European journal of pharmacology 446 (2002): 187-194.

46. Yoshida T. Concise Commentary: Quercetin Flavonoid of the Month or IBD Therapy? Digestive diseases and sciences 63 (2018): 3305-3306.

47. Yan L, Zhang JD, Wang B, et al. Quercetin inhibits left ventricular hypertrophy in spontaneously hypertensive rats and inhibits angiotensin II-induced H9C2 cells hypertrophy by enhancing PPAR-γ expression and suppressing AP-1 activity. PLoS One 8 (2013): e72548.

48. Kempuraj D, Madhappan B, Christodoulou S, et al. Flavonols inhibit proinflammatory mediator release, intracellular calcium ion levels and protein kinase C theta phosphorylation in human mast cells. British journal of pharmacology 145 (2005): 934-944.

49. Kimata M, Shichijo M, Miura T, et al. Effects of luteolin, quercetin and baicalein on immunoglobulin E-mediated mediator release from human cultured mast cells. Clinical and experimental allergy: journal of the British Society for Allergy and Clinical Immunology 30 (2000): 501-508.

50. Min YD, Choi CH, Bark H, et al. Quercetin inhibits expression of inflammatory cytokines through attenuation of NF-κB and p38 MAPK in HMC-1 human mast cell line. Inflammation Research 56 (2007): 210-215.

51. Najafzadeh M, Reynolds PD, Baumgartner A, et al. Flavonoids inhibit the genotoxicity of hydrogen peroxide (H2O2) and of the food mutagen 2-amino-3-methylimadazo [4, 5-f]-quinoline (IQ) in lymphocytes from patients with inflammatory bowel disease (IBD). Mutagenesis 24 (2009): 405-411.

52. Kiesler P, Fuss IJ, Strober W. Experimental models of inflammatory bowel diseases. Cellular and molecular gastroenterology and hepatology 1 (2015): 154-170.

53. Galvez J, Cruz T, Crespo E, et al. Rutoside as mucosal protective in acetic acid-induced rat colitis. Planta medica 63 (1997): 409-414.

54. Mascaraque C, Aranda C, Ocón B, et al. Rutin has intestinal antiinflammatory effects in the CD4+ CD62L+ T cell transfer model of colitis. Pharmacological research 90 (2014): 48-57.

55. Kim H, Kong H, Choi B, et al. Metabolic and pharmacological properties of rutin, a dietary quercetin glycoside, for treatment of inflammatory bowel disease. Pharmaceutical research 22 (2005): 1499-1509.

56. Camuesco D, Comalada M, Rodríguez-Cabezas ME, et al. The intestinal anti-inflammatory effect of quercitrin is associated with an inhibition in iNOS expression. British journal of pharmacology 143 (2004): 908-918.

57. Dönder Y, Arikan TB, Baykan M, et al. Effects of quercitrin on bacterial translocation in a rat model of experimental colitis. Asian journal of surgery 41 (2018): 543-550.

58. Cruz T, Galvez J, Ocete MA, et al. Oral administration of rutoside can ameliorate inflammatory bowel disease in rats. Life sciences 62 (1998): 687-695.

59. De Medina FS, Gálvez J, Romero JA, et al. Effect of quercitrin on acute and chronic

experimental colitis in the rat. Journal of Pharmacology and Experimental Therapeutics 278 (1996): 771-779.

60. Lin R, Piao M, Song Y. Dietary Quercetin Increases Colonic Microbial Diversity and Attenuates Colitis Severity in Citrobacter rodentium-Infected Mice. Frontiers in microbiology 10 (2019).

61. Dong YS, Wang JL, Feng DY, et al. Protective effect of quercetin against oxidative stress and brain edema in an experimental rat model of subarachnoid hemorrhage. International journal of medical sciences 11 (2014): 282.

62. Jung JH, Kang JI, Kim HS. Effect of quercetin on impaired immune function in mice exposed to irradiation. Nutrition research and practice 6 (2012): 301-307.

63. Kobori M, Takahashi Y, Sakurai M, et al. Quercetin suppresses immune cell accumulation and improves mitochondrial gene expression in adipose tissue of diet-induced obese mice. Molecular nutrition & food research 60 (2016): 300-312.

64. Liu H, Zhang L, Lu S. Evaluation of antioxidant and immunity activities of quercetin in isoproterenol-treated rats. Molecules 17 (2012): 4281-4291.

65. Mamani-Matsuda M, Kauss T, Al-Kharrat A, et al. Therapeutic and preventive properties of quercetin in experimental arthritis correlate with decreased macrophage inflammatory mediators. Biochemical pharmacology 72 (2006): 1304-1310.

66. Milenković M, Arsenović-Ranin N, Stojić-Vukanić Z, et al. Quercetin ameliorates experimental autoimmune myocarditis in rats. Journal of Pharmacy & Pharmaceutical Sciences 13 (2010): 311-319.

67. Morikawa K, Nonaka M, Narahara M, et al. Inhibitory effect of quercetin on carrageenan-induced inflammation in rats. Life Sciences 74 (2003): 709-721.

68. Muthian G, Bright JJ. Quercetin, a flavonoid phytoestrogen, ameliorates experimental allergic encephalomyelitis by blocking IL-12 signaling through JAK-STAT pathway in T lymphocyte. Journal of clinical immunology 24 (2004): 542-552.

69. Rivera L, Morón R, Sánchez M, et al. Quercetin ameliorates metabolic syndrome and improves the inflammatory status in obese Zucker rats. Obesity 16 (2008): 2081-2087.

70. Schültke E, Kendall E, Kamencic H, et al. Quercetin promotes functional recovery following acute spinal cord injury. Journal of neurotrauma 20 (2003): 583-591.

71. Schültke E, Kamencic H, Skihar VM, et al. Quercetin in an animal model of spinal cord compression injury: correlation of treatment duration with recovery of motor function. Spinal cord 48 (2010): 112.

72. Stewart LK, Soileau JL, Ribnicky D, et al. Quercetin transiently increases energy expenditure but persistently decreases circulating markers of inflammation in C57BL/6J mice fed a high-fat diet. Metabolism 57 (2008): S39-S46.

73. Castangia I, Nácher A, Caddeo C, et al. Therapeutic efficacy of quercetin enzyme-responsive nanovesicles for the treatment of experimental colitis in rats. Acta biomaterialia 13 (2015): 216-227.

74. Heinz SA, Henson DA, Austin MD, et al. Quercetin supplementation and upper respiratory tract infection: A randomized community clinical trial. Pharmacological research 62 (2010): 237-242.

75. Qureshi AA, Khan DA, Mahjabeen W, et al. Suppression of nitric oxide production and cardiovascular risk factors in healthy seniors and hypercholesterolemic subjects by a combination of polyphenols and vitamins. Journal of clinical & experimental cardiology 8 (2012).

76. Henson D, Nieman D, Davis JM, et al. Post-160-km race illness rates and decreases in

granulocyte respiratory burst and salivary IgA output are not countered by quercetin ingestion. International journal of sports medicine 29 (2008): 856-863.

77. Heinz SA, Henson DA, Nieman DC, et al. A 12-week supplementation with quercetin does not affect natural killer cell activity, granulocyte oxidative burst activity or granulocyte phagocytosis in female human subjects. British journal of nutrition 104 (2010): 849-857.

78. Nieman DC, Henson DA, Gross SJ, et al. Quercetin reduces illness but not immune perturbations after intensive exercise. Medicine & Science in Sports & Exercise 39 (2007): 1561-1569.

79. Nieman DC, Henson DA, Maxwell KR, et al. Effects of quercetin and EGCG on mitochondrial biogenesis and immunity. Medicine & Science in Sports & Exercise 41 (2009): 1467-1475.

80. Parkhomenko A, Kozhukhov S, Gurjeva O. Clinical efficacy of intravenous lipoxygenase inhibitor quercetin in patients with acute ST-segment elevation myocardial infarction: results of a prospective randomized open-label trial. In Seminars in Cardiology 11 (2005): 154-158.

81. Takagi T, Naito Y, Mizuhima K, et al. Heme oxygenase-1 prevents murine intestinal inflammation. Journal of clinical biochemistry and nutrition 17 (2018): 128-133.

82. Dryden GW, Lam A, Beatty K, et al. A Pilot Study to Evaluate the Safety and Efficacy of an Oral Dose of (−)-Epigallocatechin-3-Gallate- Rich Polyphenon E in patients with mild to moderate Ulcerative Colitis. Inflammatory bowel diseases 19 (2013): 1904-1912.

83. Koláček M, Muchová J, Dvořáková M, et al. Effect of natural polyphenols (Pycnogenol) on oxidative stress markers in children suffering from Crohn's disease- a pilot study. Free radical research 47 (2013): 624-634.

84. Rastegarpanah M, Malekzadeh R, Vahedi H, et al. A randomized, double blinded, placebo-controlled clinical trial of silymarin in ulcerative colitis. Chinese journal of integrative medicine 21 (2015): 902-906.