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Anti-inflammatory effects of flavonoids evaluated in murine models: a descriptive review

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Several chronic diseases are directly related to inflammatory states and lead to tissue damage and failure. Millions of people die worldwide from inflammation-associated pathologies such as cardiovascular diseases, diabetes and obesity. Inadequate dietary habits contribute to a deterioration of these diseases. Diet anti-inflammatory models should be studied in more detail. Fruit and vegetable polyphenols have shown anti-inflammatory benefits. This article is aimed at evaluating the inflammatory response in different murine models administered polyphenol rich diets, with a primary focus on flavonoids extracted from plants and evaluated in several inflammatory induced conditions. Carrageenan as an inflammatory agent was used to induce inflammation and several anti-inflammatory polyphenols were monitored.

KEYWORDS: polyphenols / inflammation / murine models / carrageenan

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Several systemic inflammatory stages associated with chronic pathologies contribute to a high risk of atherosclerosis and early life cardiovascular disease [Mason and Libby 2015]. Heart failure accounts for several millions of deaths each year. Around 26 million patients live with heart failure and cardiovascular diseases [Ponikowski *et al.* 2014]. Survival rates are worse than those related to bowel, breast and prostate cancer, also promoted by Inflammation actively [Hartman and Frishman 2014, Ponikowski *et al.* 2014].

Chronic inflammation has negative effects on the body. It is related to cancer, diabetes, arthritis, Alzheimer's disease as well as lung, autoimmune and cardiovascular diseases. This type of inflammation contributes to oxidative stress involved in several stages of neoplasm development [Lai *et al*. 2011]. In the case of these diseases clinical management relies on nonsteroidal anti-inflammatory drugs than yield undesirable side-effects [Pérez *et al*. 2013]. In order to develop alternative therapies a thorough evaluation of natural products with anti-inflammatory effects is required.

The therapeutic use of plants has become more popular in recent years in Asian countries such as Japan, China and India, as well as some South American countries such as Colombia, which flora comprises more than 40,000 species [Coelho *et al*. 2016, Murillo *et al*. 2008]. At present, in order to develop new drugs pharmaceutical companies are seeking to identify bioactive compounds in plants with known ethnopharmacological use [Atanasov *et al.* 2015].

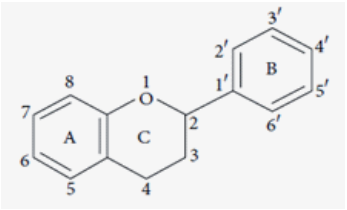

Fig. 1. Main structure of flavonoids [Kumar and Pandey 2013].

One group of extensively studied phytochemicals comprises polyphenolic compounds, with flavonoids as the mayor sub-class. Figure 1 presents the general chemical structure for this type of polyphenols [Aherne and O'Brien 2002]. Flavonoids participate in growth, reproduction and protection against pathogens and environmental damage. Studies reveal anti-inflammatory benefits from dietary flavonoids [Aherne and O'Brien 2002, Cassidy *et al*. 2015, Parhiz *et al*. 2015].

Types of inflammation and flavonoid action

Bowel Inflammation

Several pathologies are characterised by chronic inflammation in different segments of the intestine. Epithelial damage and increased mucosal permeability allow

the passage of the luminal content and dendritic cells as mediators for the migration of immune cells to the inflammatory zone [Sartor 2006]. Inflammation deteriorates with the production of molecules involved in exacerbating inflammatory responses, e.g. several interleukins (IL), tumour necrosis factor alpha (TNF-α), eicosanoids such as leukotriene B4 as well as reactive oxygen species and nitrogen metabolites [Ballester *et al*. 2006, Sartor 2006].

Studies reveal that flavonoids improve the efficacy of treatment in chronic inflammatory diseases [Comalada *et al*. 2005, Campolo *et al*. 2013, Rubió *et al*. 2013, Zhang and Tsao 2016]. A study on Wistar rats to reduce colitis with the administration of sodium dextran sulphate confirmed the inhibition of macrophage cell proliferation [Comalada *et al*. 2005]. Quercetin prevented macrophages from activating to a pro-inflammatory conformation, reducing reactive nitrogen species as well as TNF-α, IL-1, IL-6, and IL-1β [Comalada *et al*. 2005, Impellizzeri *et al.* 2016].

A study conducted by Ballester [2006] relates antioxidant activity with inflammatory mechanisms of flavonoids acting on nuclear Kappa B factor (FN-κB). FN-κB is an inflammatory agent, a modulator of gene transcription that promotes the synthesis of pro-inflammatory cytokines. Considering that reactive oxygen species work as transcription factors in the expression of FN-κB, flavonoids work as non-specific inhibitors [Ballester *et al*., 2006, Impellizzeri *et al*. 2016].

Collagen-Induced Arthritis

Collagen-induced arthritis (CIA) is a model for rheumatoid arthritis in studies conducted on rodents and primates through immunisation with type II collagen. Animals develop inflammation mediated by autoimmunity, marked by the stimulation of collagen specific T cells [Rosloniec *et al*. 2001]. These cells can produce damage distinguished by intense synovitis that corresponds to the clinical onset of arthritis and cartilage erosion [Rosloniec *et al.* 2001].

The CIA model is used to identify autoimmune mechanisms, including the role of individual cell types in the onset and progression of the disease, and consequently to design and test new therapies. It focuses on reducing the production of TNF-α as a dominant inflammatory mediator [Brand *et al*. 2007]. This methodology has been used in flavonoid studies to determine improvement in individuals with chronic inflammation.

Flavonoids and their relationship to TNF-α are relevant, because this cytokine plays a critical role in inflammation development and progression [Kumazawa *et al*. 2006, Kawaguchi *et al.* 2011]. Kawaguchi reports that animals given naringenin, a flavonoid derived from grapefruit, show a limitation of joint knee damage and infiltration of inflammatory cells. It also promotes a lowered TNF-α and mRNA expression, showing the flavonoid's ability to counteract negative effects on chronic inflammation and inhibiting proinflammatory cytokines [Kawaguchi *et al*. 2011]. Naringenin and quercetin are two common flavonoids (Fig. 2).

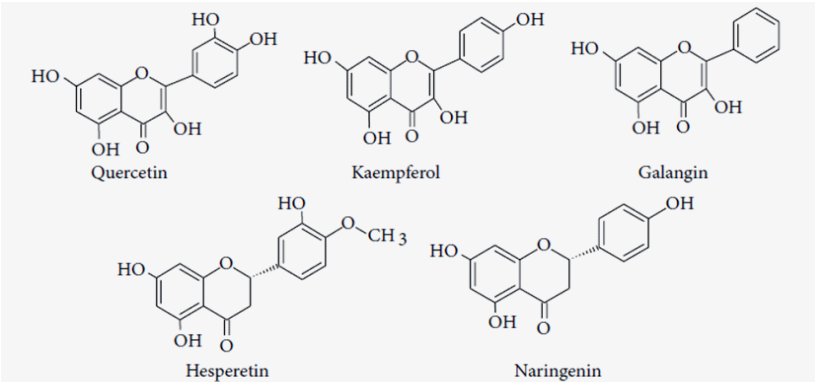

Fig. 2. Examples of commonly studied flavonoids [Kumar and Pandey 2013].

Obesity

Obesity is an imbalance in food intake and energy expenditure; it results in increased adipose tissue accumulation generating hypertrophy and hyperplasia [Drew 2012, Siriwardhana *et al*. 2013]. This tissue is recognised as an endocrine acting organ, capable of producing approx. 50 proteins, such as TNFα, IL-1, IL-6, leptin and adiponectin [Siriwardhana *et al.* 2013]. Adipocytes regulate physiological and pathological signalling pathways [Bulló *et al*. 2007, Siriwardhana *et al.* 2013, Drew 2012].

Therapeutic treatment focuses on the action of polyphenols and flavonoids, which improve lipid catabolism and glucose transport [Jung *et al*. 2006, Huong *et al*. 2006]. Polyphenols behave as activators of peroxisome proliferator-activated receptor-alpha (PPAR-α), regulating genes in charge of the oxidation of liver fatty acids [Fukuchi *et al.* 2008]. This action improves serum insulin, glucose and leptin levels, avoiding insulin resistance and problems related to the metabolic syndrome [Fukuchi *et al.* 2008].

An experiment with rats supplemented with naringenin showed that blood and liver lipid levels were reduced [Cho *et al*. 2011]. This ability is attributed to the positive regulation of the expression of hepatic PPARα protein, acting on the enzyme responsible for reducing oxidation rates in fatty acids and decreasing adipocyte triglyceride levels [Cho *et al*. 2011, Siriwardhana *et al*. 2013]

Another study, in which quercetin was administered to mice, yielded lower expression levels of transcription factors and enzymes related to cell differentiation, such as CEBPB alpha protein and PPARγ [Ahn *et al.* 2008]. Quercetin activates adenosine monophosphate protein kinase (AMPK), which inhibits pre-adipocyte differentiation, activates β-oxidation and lowers fatty acid levels [Ahn *et al*. 2008]. There is evidence confirming the anti-inflammatory effects on adipocytes and macrophages mediated by suppressing the activation of NF-κB induced by TNF-α [Siriwardhana *et al*. 2013].

Murine models of inflammation and flavonoid usage

Cotton pellet induced granuloma

This technique, initially described by Meier *et al*. [1950], was applied to induce proliferative inflammation in mice [Rotelli *et al.* 2003], . A sterilised cotton pellet of 10 mg was introduced in the scapular region or in the armpit folds to yield an inflammatory response. Neutrophils and monocytes were produced so that granulomatous and fibrous tissue was formed generated around the foreign body [Martínez Ruiz *et al.* 2004].

Cotton pellet induced granuloma may be compared with control groups where other anti-inflammatory substances are administered [Matiz *et al*. 2011]. This model is used to determine the anti-inflammatory action of flavonoids derived from vegetables, fruit, leaves and seeds [Martínez Ruiz *et al.* 2004]. Flavonoids in *Pipper ossanum* show a similar anti-inflammatory effect in granuloma formation in rats as that of a commercially available drug such as piroxicam [Martínez Ruiz *et al.* 2004].

Application of this technique using various flavonoids confirms that all the substances reduced granuloma size. A flavonol, morin, yielded the best percentage inhibition [Rotelli *et al.* 2003]. The anti-inflammatory effect is attributed to the flavonoid's ability to act on the arachidonic acid metabolism related to the cyclooxygenase and 5-lipoxygenase pathways [Matiz *et al.* 2011]. The above suggests that flavonoids inhibit mediators involved in this particular inflammation route [Matiz *et al.* 2011].

O-tetradecanoilforbo-13-acetate (TPA) induced atrial edema

TPA-induced atrial edema is used to produce acute inflammation in mice; it is based on the auricular application of a phorbol ester solution. The most commonly used compound is O-tetradecanoylforbol-13-acetate (TPA) contained in croton oil (*Croton tiglium L.*) [Gorzalczany *et al*. 2009]. It produces inflammation by binding to and activating protein kinase C (PKC), through an interaction with diacylglycerol. PKC is a Ca^{2+} and phospholipid dependent protein kinase, leading to cellular secretion, expression and proliferation [Gorzalczany *et al*. 2009].

Leukocytes aggregate and adhere to endothelial cells, generating migration and degranulation of mast cells [Yueqin 2007]. Maximum edema arises after 8 hours; it disappears after 12 to 14 hours. Vasodilation and erythema persists for 48 hours [Yueqin 2007]. The application of flavonoid-rich extracts of *Heterotheca subaxillaris var. Latifolia* inhibited up to 90% of total inflammation. This result is attributed to the ability of flavonoids to inhibit PKC [Gorzalczany *et al*. 2009].

Studies on the anti-inflammatory effect of drugs in the TPA model show that the most efficient inhibition is provided by medicaments acting on both cyclooxygenases (COX) and lipoxygenases (LOX) [Franco *et al.* 2007], as the mechanism appears to produce arachidonic acid interacting with PCK [Matiz *et al.* 2011]. Therefore, the flavonoids quercetin and kaempferol act well on the arachidonic acid cascade, as may be seen based on the conditions applied to this TPA model [Peters *et al*. 2015].

Carrageenan induced leg edema

The leg edema model is used to determine anti-inflammatory effects [D'Acquisto *et al.* 1999]. Enciso and Arroyo used this methodology in Holtzmann rats to determine the anti-inflammatory effects of flavonoids from *Jungia rugosa Less* leaves, observing no difference in edema inhibition compared to the anti-inflammatory drugs dexamethasone and ibuprofen [Enciso and Arroyo 2011]. Another study showed no differences between diclofenac and a flavonoid extract from *Satureja brevicalix* leaves [Aguilar 2014]. Other flavonoids from plants also showed anti-inflammatory action.

Table 1 presents flavonoid sources inhibiting carrageenan-induced inflammation. Various carrageenan concentrations and doses are relevant to positive anti-inflammatory effects. In addition to the flavonoid source, it is necessary to consider chemical structural differences due to the antagonist inhibitory effects related to anti-inflammatory and antioxidant activity [Kim *et al*. 1998]. The results indicating no anti-inflammatory effects could have been caused by early stage inflammation, whe histamine, serotonin and quinines are released. Flavonoids are incapable of inhibiting their activity at this point on the inflammation route [Matiz *et al*. 2011].

Anti-inflammatory flavonoids relate to arachidonic acid metabolism inhibiting COX and LOX [Alcaraz and Hoult 1985, Kim *et al*. 1998]. Flavonoid inhibition of the inflammatory pathway depends on the chemical structure. Highly hydroxylated flavonoids act more favourably on compounds related to LOXs, while less hydroxylated substituents inhibit compounds in the COX pathway [Alcaraz and Hoult 1985]. Based on this mechanism, quercetin blocks COX and LOX activity, while amentoflavone strongly and selectively inhibits COX [Kim *et al*. 1998].

Carrageenan-induced inflammation increases acute-phase proteins such as C-reactive protein (CRP). The synthesis of TNF-α, IL-1b and IL-6 contributes to the spread of inflammation [Vazquez *et al*. 2015]. Consequently, it is proposed that flavonoids inhibit the dual formation of prostaglandin E2 (PGE2) and leukotriene B4 (LTB4), affecting arachidonic acid metabolism, inhibiting IL-1 and IL-6 synthesis, and affecting CRP production [Enciso and Arroyo 2011].

Results and discussion

Flavonoid intake offers anti-mutagenic and antioxidant effects. These compounds are important dietary factors in cancer prevention, altering the cell cycle and protecting organisms against uncontrolled cell proliferation [Romagnolo and Selmin 2012]. Flavonoids have the ability to prevent cardiac diseases and they also have antioxidant functions, inhibiting oxidation of low-density lipoproteins and controlling atherogenic plaques [Siti *et al*. 2015].

Studies on flavonoids in murine models identify antioxidative and anti-inflammatory action and provide rapid and reliable results [Martínez Ruiz *et al.* 2004]. Flavonoids operate efficiently, controlling acute inflammation, comparable to results of dexamethasone and piroxicam derivatives [Martínez Ruiz *et al*. 2004]. They show

positive results in chronic inflammatory intestinal disease, reducing damage to the mucosa, and controlling pathogen passage through the epithelium. Flavonoids block mediators of inflammation such as IL-6, IFN-γ and TNF-α, leukotriene B4 and platelet activating factors, along with reactive metabolites derived from oxygen and nitrogen [Ballester *et al*. 2006]. Flavonoids in the diet prevent the appearance of inflammation in acute and semi-chronic phases [Ballester *et al*. 2006].

Carrageenan induced inflammation was lower in the presence of flavonoids from different plants, competing with anti-inflammatory drugs. As it shown in Table 1, carrageenan concentrations and doses should be considered. High carrageenan percentages can mask real anti-inflammatory effects from the flavonoid extracts [Enciso and Arroyo 2011, Aguilar 2014, Gonzalez *et al*. 2011]. Other flavonoids also showed anti-inflammatory action, as presented in Table 1. Flavonoid type, chemical structure and interaction with specific inflammation markers are relevant to determine efficient anti-inflammatory results [Kim *et al*. 1998].

Previous inflammation studies determined a flavonoid activity in reactions with COX and LOX synthesis [Alcaraz and Hoult, 1985; Enciso and Arroyo, 2011]. Flavonoids participate in various points of sequential reactions, such as the arachidonic acid pathway. More specific inflammatory agents are monitored and evaluated in the presence of flavonoids [Vazquez et al., 2015; Enciso and Arroyo, 2011]. Plant-derived and dietary flavonoids have been used to study the anti-inflammatory action on TNF-α, IL-1b, IL-6, PGE2, LTB4 and CRP [Vazquez *et al.* 2015] [Gorzalczany *et al*. 2009, Enciso and Arroyo 2011, Siriwardhana *et al*. 2013].

Table 1. Flavonoid-rich extracts tested on murine leg edema model induced by carrageenan at different concentrations

Flavonoid source, extract	Carrageenan (%)	Animal weight (g)	Dose (mg/Kg)	Effects	Reference
Satureja brevicalix, Leaves	0.1	180-250	50, 100 and 200	Less inflammation vs. control group from 100 mg/kg	Aguilar [2014]
Jungia Rugosa, Leaves	1	220	25, 50 and 100	Less inflammation vs. control group at 5 and 7 hours	Enciso and Arroyo [2011]
Garcinia Kola, Seeds	1	86-100	50	Edema inhibition in 6 hours	Tchimene *et al.* [2015]
Caesalpinia pulcherrima L, Leaves and Flowers	2	200-250	50, 100 and 200	No effect	Matiz *et al.* [2011]
Myrcianthes leucoxila and *Calea runifolia*, Leaves	3	100-320	250	No effect	González et al. [2011]

More studies should be conducted based on the anti-inflammatory and antioxidant action of flavonoids commonly found in fruits and vegetables. Tropical and Amazonian fruit varieties are potential sources of new flavonoids. The daily diets of humans

and animals include some of them. We are currently evaluating three varieties of *Passifloras* and their anti-inflammatory properties. Dietary inclusion of these products should be encouraged at an early age and studies must point out and highlight their beneficial effects.

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