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
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].

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).
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].
Anti-inflammatory effects of flavonoids evaluated in murine models

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-tetradecanoylforbo-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 Ca2+ 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, where 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].

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

<table>
<thead>
<tr>
<th>Flavonoid source, extract</th>
<th>Carrageenan (%)</th>
<th>Animal weight (g)</th>
<th>Dose (mg/Kg)</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Satureja brevicollis, Leaves</td>
<td>0.1</td>
<td>180-250</td>
<td>50, 100</td>
<td>Less inflammation vs. control group from 100 mg/kg</td>
<td>Aguilar [2014]</td>
</tr>
<tr>
<td>Jungia Rugosa, Leaves</td>
<td>1</td>
<td>220</td>
<td>50</td>
<td>Edema inhibition in 6 hours</td>
<td>Tchimene et al. [2015]</td>
</tr>
<tr>
<td>Garcinia Kola, Seeds</td>
<td>0.1</td>
<td>300-350</td>
<td>50, 100</td>
<td>No effect</td>
<td>Matiz et al. [2011]</td>
</tr>
<tr>
<td>Caesalpinia pulcherrima L., Leaves and Flowers</td>
<td>2</td>
<td>200-250</td>
<td>50, 100</td>
<td>No effect</td>
<td>Gonzalez, et al. [2011]</td>
</tr>
<tr>
<td>Myrcianthes leucoxila and Calea runifolia, Leaves</td>
<td>3</td>
<td>100-320</td>
<td>250</td>
<td>No effect</td>
<td>González et al. [2011]</td>
</tr>
</tbody>
</table>
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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