

# Impact of polyphenols in phagocyte functions

This article was published in the following Dove Press journal:  
*Journal of Inflammation Research*

Leandro Rodrigues da  
Cunha

Maria Imaculada

Muniz-Junqueira

Tatiana Karla dos

Santos Borges

Laboratory of Cellular Immunology,  
Pathology, Faculty of Medicine, University  
of Brasília, Brasília, Brazil

**Abstract:** Polyphenols are a broad group of substances with potential health benefits found in plant species. Several of these compounds are capable of influencing the activation of intracellular signaling pathways, such as NF- $\kappa$ B, MAPK and JAK-STAT, responsible for the production of various inflammatory mediators such as tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) and interleukin 1 beta (IL-1 $\beta$ ) and 12 (IL-12), enzymes involved in the production of reactive species such as inducible nitric oxide synthase (iNOS) and superoxide dehydrogenase (SOD), as well as enzymes involved in the production of eicosanoids, such as cyclooxygenase (COX) and lipoxygenase (LO). There is increased interest in the use of polyphenol-rich foods because of their immunomodulatory effect; however, the mechanisms used during macrophage responses are extremely complex and little is known about the effects of polyphenols on these cells. As such, this review summarizes the current view of polyphenol influences on macrophages.

**Keywords:** polyphenols, inflammation, macrophage activation, cytokine modulation

## Introduction

Polyphenols are the largest group of phytochemicals present in fruits and beverages, such as tea and red wine. Certain polyphenols, such as quercetin, are found in all plant-derived products, while others are specific in certain foods (flavanones in citrus fruits, isoflavones in soy and phlorizin in apples). In most cases, foods contain complex mixtures of polyphenols.<sup>1</sup> In plants, they are secondary metabolites and are generally involved in protection against a variety of physical, chemical or biological stress, including ultraviolet radiation and pathogens.<sup>2</sup>

In recent years, scientific interest has increased regarding potential health benefits from polyphenol consumption because of their anti-inflammatory properties. Most polyphenol actions occur by interfering in the enzymatic reactions of tyrosine and serine-threonine kinases, which are involved in cellular activation and cytokine production.<sup>3</sup> For instance, Dugo et al (2017), evaluating TPH-1 cells in vitro treated with cocoa polyphenolic extract, demonstrated reduction of inflammatory response in pro-inflammatory macrophages (M1 macrophages), promoting the secretion of anti-inflammatory cytokines tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) and interleukin 12 (IL-12) inducing a phenotypic switch to alternative anti-inflammatory state M2.<sup>4</sup> Another study has shown that pomegranate has dose-dependent anti-inflammatory properties, decreasing TNF- $\alpha$  and interleukin 6 (IL-6) production by macrophages in response to interferon gamma (IFN- $\gamma$ ) and lipopolysaccharide (LPS) stimulation; this same study showed that mice, supplemented with dietary pomegranate juice, which are rich in ellagic acid and gallic acid substantially inhibited the M2 to M1 macrophage phenotypic shift favoring anti-inflammatory M2 phenotype.<sup>5</sup> The polyphenols found in plum suppress in vitro nitric oxide (NO)

Correspondence: Tatiana Karla dos  
Santos Borges  
Laboratory of Cellular Immunology,  
Pathology, Faculty of Medicine, University  
of Brasília, Campus Darcy Ribeiro,  
Brasília, Distrito Federal 70.910.900,  
Brazil  
Email: tatianakarlalab@gmail.com

and cyclooxygenase-2 (COX-2) production in RAW 264.7 macrophages, as well as, malondialdehyde in lypho-stimulated macrophages.<sup>6,7</sup> Polyphenols metabolites, such as 3-glucoside/arabinoside/galactoside-based polymers, consisting of delphinidin, petunidine, peonidine, malvidin, cyanidine extracted from blueberries can inhibit the expression of IL-1, IL-6 and IL-12 in LPS-induced RAW 264.7 macrophages, modulating important anti-inflammatory responses.<sup>8</sup>

The mechanisms behind the immunomodulatory properties of polyphenol-rich foods have been the subject of several studies. These mechanisms might alter innate immune functions that are important to initiate anti-pathogen defense and stimulate the subsequent specific adaptive immune response. Macrophages have the ability to clear pathogens and apoptotic cells by recognizing antigens or damage-associated molecular patterns, producing chemical mediators in response and instructing other immune cells. However, they are also involved in inflammatory processes and degenerative diseases where their normal response becomes hyperactive or when they are continuously stimulated. The mechanisms used during macrophage responses are complex and little is known about the effects of polyphenols on these cells. This article presents a current view of the influence of polyphenols on macrophage functions.

## Impact on pathogen-associated molecular pattern (PAMP) receptors

Inflammation is triggered when innate immune cells detect infection or tissue damage. Surveillance mechanisms involve pattern recognition receptors (PRRs) on the cell surface and in the cytoplasm of the macrophages. Most PRRs respond to pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), triggering the activation of intracellular transcription factors nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B), activator protein 1 (AP-1), cAMP response-element binding protein (CREB), CCAAT-enhancer-binding proteins (c/EBP) and interferon regulatory factors (IRF). PAMPs are molecular structures derived from carbohydrates, lipids or proteins present in bacteria, viruses, fungi or other parasites, which, though they can be common to more than one pathogen, they are not found in normal human cells.<sup>9</sup> There are a variety of PAMPs receptors, including the toll family (TLRs) that recognize both molecular structures of bacteria, including teichoic acid from gram-positive, and LPS from gram-

negative bacteria, as they can recognize fungal mannose or viral nucleic acid.

Several studies support the hypothesis that polyphenols might regulate immune responses by suppressing toll-like receptor signaling. Epigallocatechin gallate-3, a polyphenol found in green tea, was shown to reduce in vitro TLR4 expression, after treatment with lipopolysaccharide, on the surface of murine macrophages and bone marrow dendritic cells.<sup>10,11</sup> In addition, Byun et al (2012) showed that the 67 laminin receptor protein (67LR) acts as a cell surface receptor for gallate-3 epigallocatechin, playing a vital role in mediating inflammation by positively regulating the expression of toll-interacting protein (Tollip), a regulator of TLR4 signaling through 67LR. Green tea polyphenols may also decrease in vitro TLR4 protein expression levels in LPS-activated melanoma murine cells, inhibiting proliferation, migration, and invasion of melanoma cells.<sup>12</sup>

Trans-3,5,4-trihydroxystilbene (resveratrol), a polyphenol found in red grapes and other plant sources, has potent anti-inflammatory properties through negatively controlling in vitro microglial inflammation triggered by LPS stimulation.<sup>13</sup> A study with microglial BV2-cells showed that resveratrol interfered in the oligomerization of TLR4 and, consequently, downregulated signaling cascades triggered by NF- $\kappa$ B and members of the signal transducer and activator of transcription (STAT) protein family, which are involved in pro-inflammatory mediator production.<sup>14</sup>

The polyphenol curcumin, widely used in Indian food, also suppresses the expression of inflammatory mediators by inhibiting the NF- $\kappa$ B pathway. Primary rat vascular smooth muscle cells stimulated with LPS (1  $\mu$ g/L) and curcumin (5, 10 or 30  $\mu$ mol/L) have shown reduced neuronal apoptosis through a mechanism related to the TLR4/myeloid differentiation primary response 88 (MyD88)/NF- $\kappa$ B pathway.<sup>15</sup> Mice receiving 200 mg/kg body weight of curcumin dissolved in pyrogen-free phosphate-buffered saline (PBS) showed hepatic protection by decreasing intrahepatic expression of genes encoding pro-inflammatory molecules, such as, TNF- $\alpha$  and IFN- $\gamma$ , and by inhibiting pro-inflammatory intracellular signaling initiated by TLR2, TLR4 and TLR-9.<sup>16</sup>

Polyphenols also influence initial signaling steps conducted by other recognition receptors. For example, curcumin and parthenolide alter the oligomerization of nucleotide-binding oligomerization domain containing protein 2 (NOD2), which detects a portion of a bacterial peptide, downregulating NF- $\kappa$ B proinflammatory

signaling.<sup>17</sup> In addition, acting on nucleotide fragments such as poly (dA:dT), quercetin (2, 5 and 10  $\mu$ M) decreased IL-18 secretion in human primary epidermal keratinocytes from neonatal foreskin, HEK293, and human keratinocyte cell line HaCaT, as it inhibits caspase 1-dependent-activation by interfering with interferon inducible protein AIM2 (absent in melanoma 2) signals and pro-IL-18 gene transcription by Janus kinase 2 (JAK2)/STAT1.<sup>18</sup>

However, if these effects described could be applicable for humans, should be better evaluated, because, the polyphenols concentration used in the in vitro tests and metabolites taken into consideration in these reports are not that normally used by humans.

## Impact on adhesion mechanism

Leukocyte trafficking to lymphoid organs or other tissues is initiated by chemoattractant stimuli and an adhesion step.<sup>19</sup> These mechanisms are particularly important when the immune system is responding to a pathogen in an injured tissue. Monocyte recruitment to inflammatory sites involves chemokines, adhesins and their receptors on the endothelium and leukocytes. Normally, early tethering and rolling interactions between endothelium and monocytes are mediated by proteins from the selectin ligands family and their receptors.<sup>20</sup> Mediators such as platelet-activating factor (PAF), thrombin, histamine, TNF- $\alpha$  and IL-1 $\beta$  stimulate the expression of selectins on the endothelial cell membrane.<sup>21</sup> Meanwhile, chemokines, produced at the injured site enter blood vessels associated with proteoglycans and are deposited at high concentrations on the endothelial cell surface. The chemokines activate the monocyte membrane and stimulate stable adhesion by interacting with integrins, such as intercellular cell adhesion molecules (ICAMs). Finally, transmigration and migration to inter-endothelial spaces occur based on the chemokine concentration gradient, which is more abundant at injured or inflamed sites.<sup>22</sup> There are few studies describing the effect of polyphenols on chemokine regulatory functions, but it was observed accelerated wound closure in rats by local treatment with verbascoside. In addition, in vitro treatment with quercetin and verbascoside of human keratinocytes obtained from skin biopsies of healthy volunteers decreased the chemokines IL-8, monocyte chemoattractant protein 1 (MCP-1) and IP-10 in supernatant of the cultures.<sup>23</sup> In another study carried out in HaCaT cells, a keratinocyte cell line from adult human skin, downregulation of chemokines IL-8 and interferon

gamma-induced protein 10 (IP-10) was observed in the presence of verbascoside (10 and 50  $\mu$ mol/L).<sup>24</sup>

In pathogenic conditions, proinflammatory cytokines stimulate the increase of adhesion expression and often this promotes an exacerbated response in the endothelium and in the adjacent tissue.<sup>25</sup> This response promotes monocyte/macrophage adhesion and activation on the microvasculature, which is an important pathogenic mechanism during atherosclerosis. Soluble forms of ICAM-1 are related to the pathogenesis of ischemic stroke<sup>26</sup> and inflammatory events in the cardiac vascular endothelium.<sup>27,28</sup> A randomized clinical trial study demonstrated that red wine, that is rich in phenolic compounds including resveratrol, reduced serum ICAM-1 and IL-6 concentrations and negatively regulated vascular cell adhesion protein 1 (VCAM-1) and E-selectin expression on leukocyte cell surfaces;<sup>29</sup> resveratrol metabolites including trans-3,5,4-trimethoxystilbene (TMS) inhibits human acute monocytic leukemia THP-1 cells adhesion to TNF- $\alpha$ -activated human umbilical vein endothelial cells (HUVECs) in vitro. These cells pretreated with resveratrol and TMS showed reduced TNF- $\alpha$  induced ICAM expression. In addition, it was shown that TMS can act by inhibiting the NF- $\kappa$ B pathway on HUVECs.<sup>30</sup> It was emphasized that cardiovascular risk might be prevented by following a Mediterranean-style diet rich in polyphenols present in red wine, nuts and olive oil because they decreased serum VCAM-1, ICAM-1 and IL-6 observed in a randomized trial.<sup>31</sup>

The isolated polyphenols present in apples suppress the reactive oxygen species (ROS)/mitogen-activated protein kinase (MAPK)/NF- $\kappa$ B signaling pathway, and consequently, downregulate chemokine (C-C motif) ligand 2 (CCL-2), ICAM-1 and VCAM-1 expression, important molecules related to atheroma plaque formation on rat aortic endothelial cells.<sup>32</sup> Further human randomized clinical trial suggests that polyphenolic compounds found in tea, red wine, cocoa, olive oil and blueberries improve cardiovascular protection by disrupting TNF- $\alpha$  signaling in the vascular wall.<sup>29</sup>

## Impact on the production of reactive species of nitrogen and oxygen

After phagocytosis, pathogens within the phagolysosomes are degraded to epitopes by lysosomal enzymes and by the action of reactive oxygen and nitrogen species produced at

this site. The production of reactive species is particularly important in eliminating or controlling intracellular infections, such as those caused by mycobacteria or leishmaniasis.<sup>33</sup> The release of reactive oxygen and nitric species, such as hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and NO, to the extracellular fluid stimulates paracrine production of inflammatory mediators, local neuronal transmission and vasodilatation.<sup>34</sup> If overproduced, these reactive species become involved in necrosis, apoptosis and tissue aggression. On the other hand, low NO bioavailability alters neuron signaling and endothelial relaxation.

Polyphenols influence the production of reactive species in different ways. Studies prove the importance of tannic acid as a chelating antioxidant and free radical scavenger. Tannic acid, the simplest form of hydrolysable tannin found in various foods such as grapes, lentils, chocolate, red wine, beer, coffee, black tea and green tea<sup>35,36</sup> complexes with metal ions and these act as free radical scavengers.<sup>37</sup> Green tea and tannic acid polyphenols were effective in inhibiting NO generation induced by 12-O-tetradecanoyl phorbol 13-acetate (TPA) in rat hepatocytes.<sup>38</sup>

Quercetin and resveratrol inhibited inducible nitric oxide synthase (iNOS) in murine macrophages in a dose-dependent manner and consequently decreased NO production;<sup>39</sup> while epigallocatechin gallate (EGCG) present in green tea eliminated NO in murine tumor cell lines.<sup>40</sup> In addition, increased plasma NO levels have been associated with a reduction in systolic and diastolic blood pressure levels, adding to the evidence that polyphenols might protect the cardiovascular system because they improve endothelial function by increasing NO production, resulting in vasodilation.<sup>41,42</sup>

The antioxidant effect of polyphenols on the protection of cell integrity and ROS suppression is well documented in the literature.<sup>43</sup> Polyphenols might decrease endothelial lesions via the MAPK/extracellular signal-regulated kinase (ERK) pathway, an important intracellular signaling pathway that promotes cell growth and proliferation in many mammalian cell types.<sup>44</sup> For example, quercetin and myricetin have been demonstrated to have antioxidant properties that protect against DNA injury. This process might provide for improved cancer coadjuvant treatment.<sup>45,46</sup>

High concentrations of resveratrol decreased NO production without influencing iNOS expression in LPS-activated RAW 264.7 cells;<sup>39</sup> however, higher plasma concentration of resveratrol is required to increase iNOS expression and to negatively regulate NF- $\kappa$ B.<sup>4</sup> Furthermore, resveratrol and curcumin, at final concentration ranging

from 10<sup>-4</sup> to 10<sup>-6</sup> M, increased the expression of endothelial nitric oxide synthase (eNOS) and NO production on equine neutrophils by inhibiting the expression of NADPH oxidase.<sup>48</sup> Other polyphenols, such as epigallocatechin-3-gallate (EGCG), the major constituent of green tea, also caused an increase in the activity of eNOS in bovine aortic endothelial cells.<sup>41</sup> In addition, rats fed with diets rich in either dealcoholated red wine, quercetin or catechin induced endothelium-dependent vasorelaxation in rat aorta in a resting state through the enhancement of nitric oxide production.<sup>47</sup>

In humans, a meta-analysis of 13 randomized controlled trials showed that the regular green tea consumption reduces blood pressure.<sup>49</sup> Similarly, in rats treated with curcumin at a dose of 50 or 100 mg/kg/day, there was an increased eNOS expression in the vascular tissue<sup>50</sup> and also reduced oxidative DNA damage in rats treated therapeutically with 100 mg/kg curcumin by gavage.<sup>51</sup>

Intracellular mechanisms to protect cell organelles from damage by the excessive formation of free radicals include neutralization by antioxidants (glutathione, carotenoids) and enzymes, such as superoxide dismutase, glutathione peroxidase and catalase.<sup>52</sup> In a study with diabetic subjects, the serum activity of glutathione peroxidase and superoxide dismutase increased significantly in the group that was given polyphenol supplements from *Sambucus nigra*.<sup>53</sup> Uto-Kondo et al showed that tea polyphenols might provide anti-atherosclerotic effects by inhibiting the oxidation of LDL, by increasing the endothelium-bound superoxide dismutase.<sup>54</sup>

In summary, polyphenols can protect against oxidation by a combination of several mechanisms involving their structure, phenolic ring characteristics, acidity of the medium, nature of the oxidizable substrate, the physical state of the system and the presence of pro-oxidants and synergists. The effectiveness of antioxidants also depends strongly on their concentrations at the reaction site, emphasizing that they act in different regions of the body depending on availability and demand. All these parameters need to be evaluated to better understanding of the efficacy of polyphenols as antioxidant agents.<sup>55</sup>

## Impact on production of cytokines

The response of phagocytes after pathogen recognition or any tissue change requires a series of communications between these cells and the external environment in order to stimulate subsequent cellular responses and present an efficient adaptive response.<sup>56</sup> For example, local actions

include: a) the attraction of neutrophils to response sites by IL-8;<sup>57</sup> b) expression of adhesins, reduction of tight-junctions on endothelial cells and vasodilation of vascular endothelium promoted by IL-1 $\beta$  and TNF- $\alpha$ ;<sup>58</sup> c) IL-12 enhancement of Natural Killer cells cytotoxicity and T cell activation during antigen recognition;<sup>59</sup> d) induction of new macrophage phenotypes, changing to the M1 form by INF- $\gamma$  or to M2 by IL-10;<sup>60</sup> and e) expression of costimulatory and major histocompatibility complex (MHC) molecules for induction of the adaptive response.<sup>61</sup>

There are several reports showing the modulation of cytokine production by polyphenols. For example, in human whole blood cultures stimulated with concanavalin A and treated with kaempferol, the IFN- $\gamma$  concentration was significantly lower than in cultures stimulated but not treated.<sup>63</sup> Other in vitro studies showed that curcumin reduced IL-8, macrophage inflammatory protein 1  $\alpha$  (MIP-1 $\alpha$ ), MCP-1, IL-1 $\beta$  and TNF- $\alpha$  production in monocytes and human alveolar macrophages<sup>64</sup> or IL-18 production in LPS-stimulated murine macrophage cell line RAW264.<sup>65</sup>

Luteolin is a polyphenolic flavonoid present in celery, green peppers, broccoli, carrots, olive oil and several other food sources.<sup>62</sup> Funaro et al (2016) evaluated the influence of luteolin and tangeretin in LPS-stimulated RAW 264.7 macrophages, and these compounds suppressed the overexpression of proinflammatory mediators, such as IL-1 $\beta$ , IL-6 and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), synergistically decreasing mRNA expression of iNOS and COX-2.<sup>66</sup> In LPS-stimulated murine microglial cells, luteolin downregulates NO, PGE<sub>2</sub>, TNF- $\alpha$  and IL-1 $\beta$ .<sup>67</sup>

The anti-inflammatory effects are potentiated when polyphenols are associated with other bioactive compounds. For example, chicory acid found in basil can enhance the anti-inflammatory effect of luteolin by enhancing Akt phosphorylation and decreasing the production of reactive species that activate the NF- $\kappa$ B pathway.<sup>68</sup> The association of chlorogenic acid and luteolin has a significant effect on the reduction of IL-1 $\beta$ -induced RSC-364 cell apoptosis. In addition, in a study using human keratinocytes, luteolin was able to inhibit the production of inflammatory mediators such as IL-6, IL-8 and TNF $\alpha$ . This mechanism possibly occurred by the inhibitory action of luteolin on the activation of NF- $\kappa$ B.<sup>69</sup>

Other polyphenols appear to have beneficial effects in in vitro and in vivo models. A study in rats has shown that quercetin and resveratrol decrease the expression of IL-1 $\alpha$  and TNF- $\alpha$  in LPS-treated microglia,<sup>70</sup> and protect glial

cells against the action of ROS.<sup>71</sup> Another study examined the anti-inflammatory properties of quercetin in human fetal brain cultures, where quercetin inhibited IL-1 $\beta$ , IL-6, IL-8, IFN- $\gamma$  and free radical production in astrocyte cultures.<sup>72</sup> The polyphenols present in blueberry have anti-inflammatory activity modulating the proinflammatory cytokines IL-1 $\beta$ , IL-6 and IL-12 on LPS-induced RAW264.7 macrophages.<sup>8</sup>

IL-1 $\beta$  is released in response to cell injury, pathogen antigens and other inflammatory cytokines. In the central nervous system (CNS), IL-1 $\beta$  is responsible for increasing the production of PGE<sub>2</sub> and COX-2 in neuronal and glial cells<sup>73</sup> and stimulates the production of TNF- $\alpha$  and IL-6 by microglia and astrocytes.<sup>74</sup> Systemically, IL-1 $\beta$  induces fever, suppresses appetite and stimulates muscle proteolysis.<sup>75</sup> It is also associated with a decrease in NK cells and the release of IL-6 to the bloodstream.<sup>76</sup> EGCG from green tea is a potent inhibitor of IL-1 $\beta$  signal transduction in vitro.<sup>77,78</sup> Polyphenols might interfere in cytokine production of human aortic endothelial cells by altering NF- $\kappa$ B signaling, as observed with avenanthramide present in oats.<sup>79</sup>

IL-6 is synthesized by mononuclear phagocytes in response to many DAMPs released during tissue aggression, trauma and burns,<sup>80,81</sup> and also in response to IL-1 $\beta$  and TNF- $\alpha$ . Some neurological and psychiatric reports have described its elevation in pathological conditions, such as autistic spectrum,<sup>82</sup> Alzheimer's disease<sup>83</sup> and depression.<sup>84</sup> The use of polyphenols might attenuate these conditions by decreasing IL-6 production from microglial cells. Consistent with this hypothesis, luteolin and tangeretin decreased the synthesis of IL-1 $\beta$  and IL-6 by RAW 264.7 macrophages, previously stimulated with LPS.<sup>66</sup> Similarly, Weng et al observed IL-6 decreased in cultures of keratinocytes seeded and treated with TNF- $\alpha$  and luteolin.<sup>69</sup> Other polyphenolic compounds, such as apigenin and quercetin have positive anti-inflammatory activity in human macrophages by suppressing IL-6 expression.<sup>85</sup> However, its effect on the disease should be better evaluated in randomized trials.

IL-12 is an important cytokine released by monocytes and macrophages. It plays an important role in cell-mediated immunity, especially in response to intracellular infections, acting on T cells.<sup>86</sup> Its main actions are to stimulate type 1 T helper (TH1) cell responses and CD8 T cell cytotoxicity.<sup>87</sup> Once activated, TH1 cells are potent producers of IFN- $\gamma$ . In turn, IFN- $\gamma$  stimulates the response of phagocytes, including macrophages, by increasing the



production of lysosomal enzymes and microbicidal reactive species, such as NO and H<sub>2</sub>O<sub>2</sub> and by increasing antigen presentation.<sup>88</sup> Some studies demonstrate that polyphenols might impair inflammation by interfering in IL-12 production. For instance, topical application of green and white tea extracts provides protection from solar-simulated ultraviolet light in human skin by showing protective effects against UVB-induced immunosuppression via IL-12 production.<sup>89</sup> In a human study, serum levels of IL-12 decreased in patients with controlled blueberry consumption. In fact, polyphenols might act by balancing the immune response by controlling the exacerbated cytokine response and by stimulating the appropriate immune response. Different polyphenols from green tea and red wine modulate the immune system by releasing IL-12 and consequently promoting macrophage, NK and cytotoxic cell responses toward various pathogens, such as bacteria, viruses and parasites.<sup>90</sup> Besides, few clinical trials showed changes on inflammatory response, such as, by evaluating biomarkers, as IL-6, after regular consumption of red wine by healthy human.<sup>29,91</sup>

## Impact on NF-κB and other signaling pathways

Many intracellular pathways are activated in macrophages during an immune response. These pathways are mainly regulated by the NF-κB, STAT, MAPK families, and any intervention in one of the proteins involved in the signaling cascades interferes significantly with the cellular activation state, or its mechanism of action, or cytokine production, or induces cell apoptosis. Considering that proinflammatory actions of macrophages are under transcriptional control of these signaling pathways, the role of polyphenols on macrophage intracellular activation has been extensively studied.

Many genes that encode proinflammatory mediators and proteins involved in the cell cycle, cell differentiation, apoptosis and oncogenesis are under NF-κB regulation. The NF-κB family has 5 members – RelA or p65, RelB or p68, cRel, NF-κB1 or p100 and NF-κB2 or p105. These factors form homo or heterodimers that share a Rel homology domain (RHD) necessary to bind the κB elements to DNA. In basal state, NF-κB signaling pathways are blocked by a family of inhibitors of NF-κB (IκB). A wide range of stimuli, including proinflammatory cytokines IL-1β and TNF-α, bacterial LPS, carcinogens, tumor promoters and UV radiation stimulate NF-κB activation by promoting IκB proteasomal

degradation or proteolytically processing of p105–p52.<sup>92</sup> This allows the release and dimerization of NF-κB proteins in the cytoplasm and permits nuclear translocation of the dimers.<sup>93</sup>

Considering that proinflammatory actions of macrophages are under transcriptional control of NF-κB, the role of polyphenols in this pathway has been extensively studied. Some polyphenols might exert their anti-inflammatory effect by interfering in the phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway. Akt activates inhibitory-κB kinase alpha (IKKα) that phosphorylates IκB proteins, resulting in NF-κB dimerization.<sup>94</sup> Other polyphenols, such as curcumin negatively regulate NF-κB and inhibit IκB kinase, thereby suppressing proliferation and inducing apoptosis of these cells.<sup>95</sup>

The MAPK subfamily consists of serine/threonine-specific protein kinases that respond to extracellular stimuli and regulate various cellular activities, such as gene expression, mitosis, differentiation, cell survival and apoptosis. The starting point for this pathway is the binding of a ligand to a transmembrane protein, a tyrosine kinase receptor (TKR). The resulting signaling cascade culminates with ERK translocation to the nucleus, where it activates Elk, Ets and Myc transcription factors.<sup>96</sup> JNK is a member of the MAPK family and controls essential processes such as inflammation, cell differentiation and apoptosis.<sup>97,98</sup> Mature macrophages have a selective requirement of JNK for their survival, proliferation and differentiation.<sup>99,100</sup> Polyphenols might influence such mechanisms, as some studies have shown that myricetin acts in suppressing the phosphorylation of MAPK family members, p38 and JNK, preventing apoptosis in human astrocytoma U373MG cells.<sup>101</sup> In rats, curcumin also blocks JNK-related signaling and reduces activation of MAPK p38 on a chronic experimental colitis model.<sup>102</sup>

Activation of NF-κB is essential for the survival of macrophages. Blocking the intracellular activation of NF-κB induces IκBα-induced apoptosis independent of caspase 3 by reducing mitochondrial function. Thus, activation of NF-κB preserves the viability of macrophages, maintaining mitochondrial homeostasis.<sup>103</sup> Mice bone marrow-derived macrophages pretreated *in vitro* with tannic acid blocked the cleavage of caspase-1 and inhibited IL-1β secretion, suppressing the activation of NF-κB signaling by inhibiting nuclear localization of NF-κB/P65, suggesting that tannic acid inhibited the activation of the inflammasome NLRP3.<sup>104</sup>

EGCG inhibited the CoCl<sub>2</sub>-induced apoptosis of PC12 cells through the mitochondria-mediated apoptosis pathway involved in modulating the Bcl-2 family.<sup>105</sup>

In vitro treatment with epicatechin or kaempferol acted in protecting mouse striatal neurons from LDL-oxidation by inhibiting the activation of c-Jun N-terminal kinases (JNK), c-Jun and caspase-3.<sup>106</sup> Another known anti-inflammatory activity related to inhibiting the expression of caspase-3 induced by resveratrol-promoted IL-1 $\beta$  was demonstrated in an in vitro model conducted in human articular chondrocytes.<sup>107</sup>

Other polyphenols found in *Lonicera japonica*, a native plant of Asia, alter these signaling pathways by inhibiting eicosanoids and free radical species production in RAW 264.7 macrophages as they downregulate COX-2 and iNOS activities or negatively interfering in the production of cytokines such as TNF- $\alpha$ , IL-1 $\beta$  and IL-6.<sup>108</sup> This study also demonstrated that in vitro treatment with polyphenol components isolated from Korea *L. japonica* inhibited the expression of p38 protein, but not JNK and ERK, in LPS-stimulated RAW 264.7 mouse macrophages.

Another important signaling pathway, IKK-NF- $\kappa$ B regulates the activity of important transcription factors such as NF- $\kappa$ B (p50/p65) and AP-1 (c-Fos/c-Jun), which after activation can induce the expression of numerous genes encoding inflammatory mediators in macrophages.<sup>109,110</sup> Polyphenols found in ethanol extract from Chinese propolis, containing abundant flavonoids, including rutin, myricetin, quercetin, kaempferol, apigenin, pinocembrin, chrysin and galangin are able to inhibit the phosphorylation of I $\kappa$ B $\alpha$  and AP-1 in LPS-stimulated RAW 264.7 by regulating the expression of the iNOS, IL-1 $\beta$  and IL-6 mRNA in a time- and dose-dependent manner.<sup>111</sup> In the same way, resveratrol in LPS-treated human intestinal cells can block the phosphorylation of the p65 subunit of NF- $\kappa$ B, inhibiting its translocation to the nucleus and its action on genes by inhibition of phosphorylation and degradation of I $\kappa$ B.<sup>112</sup> In general, polyphenols reduce the degradation of IKK by promoting retention of the p65 subunit in the cytosol.<sup>112</sup>

Atopic dermatitis-like skin lesions were induced in NC/Nga mice using cream containing *Dermatophagoides farinae* extract and oral treatment with tannic acid inhibited serum IL-4 and IF- $\gamma$ , while TNF $\alpha$ , high mobility group (HMG), B1 protein, receptor of advanced glycation end product (RAGE), extracellular signal-regulated kinase (ERK)1/2, NF- $\kappa$ B, cyclooxygenase (COX) 2, IL-1 $\beta$ , and increased the protein expression of peroxisome

proliferator-activated receptor (PPAR $\gamma$ ) in skin sample showed by western blot. These results suggest that skin inflammation can be mediated by NF- $\kappa$ B signaling and tannic acid might be a potential therapeutic agent for skin dermatitis treatment, which may possibly act by induction of the PPAR $\gamma$  protein.<sup>113</sup>

The effect of polyphenols on the intracellular pathways of macrophages is still controversial. In fact, considering the complexity of intracellular networks that control functions of the cells, it is expected that the diversity of polyphenols found in nature will have distinct effects especially concerning the dose and the metabolite used. Furthermore, the way these observations were done, by in vitro assays or by ingestion of in nature, the polyphenols may determine different results. In addition, they can also act differently on different cells and organs or tissues. Overall, the actions of polyphenols on intracellular mechanisms need further studies, mainly to be able to use this knowledge to improve human health.

## Impact on the phenotypes of macrophages

Macrophages have the ability to assume inflammatory or suppressive states and have repairing properties during resolution phases of inflammation.<sup>114</sup> This differentiation allows the organism to effectively combat antigens and re-establish itself after infection. In experimental systems, the classical phenotype of inflammatory macrophages is called M1 and is often induced by IFN- $\gamma$ .<sup>115</sup> M1 acts in the first phase of the response to tissue injury and in the inflammatory response against pathogens. This subtype is able to recognize pathogens and DAMPs efficiently, either eliminating them or stimulating a specific adaptive response by releasing cytokines, such as TNF- $\alpha$ , IL1 $\beta$  and IL-12 and IL-23.<sup>116</sup>

The M2 phenotype can be induced by IL-4 and IL-13. This macrophage blocks M1 response and induces pro-resolution molecule production, such as IL-10 and TGF- $\beta$  that suppress inflammation and contribute to tissue repair, remodeling, angiogenesis and homeostasis recover.<sup>117,118</sup>

It is considered that due to their anti-inflammatory action, polyphenols might alter the phenotype of macrophages, favoring an M2 anti-inflammatory state. For example, a polyphenolic extract of cocoa suppresses inflammation on THP1 cell line macrophages and stimulates their polarization to activated M2 macrophages.<sup>4</sup> A study of ellagic and gallic acids, polyphenols found on pomegranate, demonstrated, that these bioactive

compounds directly suppress the inflammatory responses of murine peritoneal macrophages and promote macrophage phenotype switching from M1 to M2.<sup>5</sup> Resveratrol also stimulates phenotype switching to M2 in human monocyte cell culture and neutralize pro-atherogenic signaling in the subgroups of M1 macrophages within atherosclerotic plaques.<sup>118</sup> Finally, in vitro treatment with EGCG regulates immune system cells, especially promoting the M2 polarization of macrophages in cartilage and bone tissue.<sup>119</sup>

## Impact on mechanisms of autophagy and apoptosis

The elimination of apoptotic cells is driven by a phagocyte mechanism.<sup>120</sup> Cell death by apoptosis contributes to cell turnover in most tissues. Removal of apoptotic cells maintains tissue integrity under healthy conditions and contributes to maintaining an anti-inflammatory state.<sup>121</sup> Apoptotic cells might also release IL-10<sup>122</sup> that acts simultaneously with TGF- $\beta$  to induce a suppressive immune response by promoting regulatory T cell formation.<sup>123</sup>

Polyphenols found in foods have the ability to inhibit genes involved in cell proliferation and inducing apoptosis.<sup>68</sup> Evidence suggests that cellular and molecular mechanisms responsible for the induction of apoptosis by polyphenols depend on the concentration of polyphenols, cell type, cell age and stage of the degenerative process.<sup>124</sup>

Studies have shown that plant extracts rich in polyphenols, and isolated polyphenols as single compounds and combinations might exert pro-apoptotic effects by selectively attacking cancer cells. For example, in vitro treatment of breast cancer cells with different concentrations of pomegranate extract induced cell death by apoptosis through activation of the caspase-3 pathway. Polyphenolic extract prepared from a pomegranate juice extract containing the ellagitannins punicalagin A, punicalagin B, anthocyanins delphinidin 3-glucoside and cyanidin-3-glucoside was shown in human mammary carcinoma cell lines to induce suppression of mRNA and specific protein transcription factors Sp1, Sp3 and Sp4, which are involved in cell cancer apoptosis.<sup>125</sup> It is important to emphasize that the predominant and therapeutically relevant compounds of pomegranate extract are ellagic acid and ellagitannins; both ellagic acid and ellagitannins are produced after microbial metabolism leading to dibenzopyranones known as urolithin A (3,8-dihydroxy-6H-dibenzopyran-6-one) and its monohydroxylated analog

known as urolithin B.<sup>126</sup> All these urolithin phenotypes could show differences in the human gut microbiota and should be considered in intervention trials dealing with health benefits of ellagitannins or ellagic acid.

Hsieh and Wu (2009) have shown that the combination of EGCG, quercetin and genistein decreased expression of the androgen receptor, tumor suppressor p53 and quinone reductase type 1 detoxification enzyme in human prostate cancer cells.<sup>127</sup> In vivo, tumors from nude mice injected with pancreatic cancer cells and treated with curcumin (1 g/kg) showed significant reductions in volume by inhibition of NF- $\kappa$ B-regulated gene products, such as cyclin D1, c-Myc, Bcl-2, Bcl-xL, COX-2, matrix metalloproteinase (MMP) and vascular endothelial growth factor (VEGF).<sup>128</sup>

Quercetin is present in high concentrations in fruits and vegetables, such as apples, strawberries, onions, potatoes, broccoli, soybeans, peanuts and red wine.<sup>129</sup> This polyphenol showed antioxidant and cytoprotective effects that prevented endothelial apoptosis caused by oxidizing agents in primary hippocampal cultures, significantly attenuating  $\beta$ -amyloid peptide-induced cytotoxicity, protein oxidation, lipid peroxidation and apoptosis.<sup>130</sup> Flow cytometry analyses demonstrate tannic acid increased the rate of early apoptosis both in in vitro prostate cancer PC-3 cells by 25.8% and in LNCaP cells by 20.9%, suggesting that tannic acid may be a promising candidate for combined therapy with great effectiveness to reduce the occurrence of prostate cancer.<sup>131</sup>

## Conclusion

Polyphenols found in a wide variety of foods such as fruits, vegetables, teas, olive oil and nuts showed anti-inflammatory and antioxidant properties that interfere at several steps during intracellular signaling, mainly in NF- $\kappa$ B inflammatory pathways. Some dietary polyphenols also induce apoptosis promoting cell renewal and suppressing the growth of cancer cells. All these effects reported in this review have been extensively studied and data show that polyphenols may have the potential to ameliorate inflammatory and degenerative diseases. However, it is important to note that these beneficial properties depend on the amount consumed and their bioavailability and those observations in vitro could not be the same that will be observed in vivo. Further, bioavailability, formulation and doses are crucial points to be made clear previously if we will be able to use these bioactive plants compounds in the clinical practice to improve human health.



To date, most research has been conducted in vitro or in animal models, and other clinical trials are necessary since in vitro results do not often coincide with the findings of in vivo studies. However, in vitro studies are necessary for the better understanding of the mechanism of action of these compounds that are not possible to know by only in vivo studies.

To the better understanding of the mechanisms of action of polyphenols, it is still necessary to establish daily consumption recommendations considering that a sufficient dose for its effect, it is necessary these foods to be ingested every day and that, unlike minerals and vitamins, the active component is not stored or temporarily retained in the body.

In addition, even though much research was already done about polyphenols present in each food, guidelines and daily recommendation are missing, furthermore, it is necessary to determine whether these compounds can exert their effects complexed in food.

Studies in vitro and in animals have used levels of the polyphenols to a much higher value than those commonly found in human diets. The safety and benefits therefore should be evaluated by quantitative research to extend the benefits of polyphenols described in this review to human health and to prevent pathology.

## Disclosure

The authors report no conflicts of interest in this work.

## References

- Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L. Polyphenols: food sources and bioavailability. *Am J Clin Nutr*. 2004;79(5):727–747. doi:10.1093/ajcn/79.5.727
- Kennedy DO. Polyphenols and the human brain: plant “secondary metabolite” ecologic roles and endogenous signaling functions drive benefits. *Adv Nutr*. 2014;5(5):515–533.
- Das J, Ramani R, Suraju MO. Polyphenol compounds and PKC signaling. *Biochim Biophys Acta Gen Subj*. 2016;1860(10):2107–2121. doi:10.1016/j.bbagen.2016.06.022
- Dugo L, Belluomo MG, Fanali C, et al. Effect of cocoa polyphenolic extract on macrophage polarization from proinflammatory M1 to anti-inflammatory M2 state. *Oxid Med Cell Longev*. 2017;2017:6293740. doi:10.1155/2017/6293740
- Aharoni S, Lati Y, Aviram M, Fuhrman B. Pomegranate juice polyphenols induce a phenotypic switch in macrophage polarization favoring a M2 anti-inflammatory state. *BioFactors*. 2015;41(1):44–51. doi:10.1002/biof.1199
- Bu SY, Lerner M, Stoecker BJ, et al. Dried plum polyphenols inhibit osteoclastogenesis by downregulating NFATc1 and inflammatory mediators. *Calcif Tissue Int*. 2008;82(6):475–488. doi:10.1007/s00223-008-9139-0
- Hooshmand S, Kumar A, Zhang JY, Johnson SA, Chai SC, Arjmandi BH. Evidence for anti-inflammatory and antioxidative properties of dried plum polyphenols in macrophage RAW 264.7 cells. *Food Funct*. 2015;6(5):1719–1725. doi:10.1039/c5fo00173k
- Cheng A, Yan H, Han C, Wang W, Tian Y, Chen X. Polyphenols from blueberries modulate inflammation cytokines in LPS-induced RAW264.7 macrophages. *Int J Biol Macromol*. 2014;69:382–387. doi:10.1016/j.ijbiomac.2014.05.071
- Newton K, Dixit VM. Signaling in innate immunity and inflammation. *Cold Spring Harb Perspect Biol*. 2012;4(3). doi:10.1101/cshperspect.a006049
- Kumazoe M, Nakamura Y, Yamashita M, et al. Green tea polyphenol epigallocatechin-3-gallate suppresses toll-like receptor 4 expression via up-regulation of E3 ubiquitin-protein ligase RNF216. *J Biol Chem*. 2017;292(10):4077–4088. doi:10.1074/jbc.M116.755959
- Byun EB, Choi HG, Sung NY, Byun EH. Green tea polyphenol epigallocatechin-3-gallate inhibits TLR4 signaling through the 67-kDa laminin receptor on lipopolysaccharide-stimulated dendritic cells. *Biochem Biophys Res Commun*. 2012;426(4):480–485. doi:10.1016/j.bbrc.2012.08.096
- Chen X, Chang L, Qu Y, Liang J, Jin W, Xia X. Tea polyphenols inhibit the proliferation, migration, and invasion of melanoma cells through the down-regulation of TLR4. *Int J Immunopathol Pharmacol*. 2018;32:039463201773953. doi:10.1177/0394632017739531
- Lu X, Ma L, Ruan L, et al. Resveratrol differentially modulates inflammatory responses of microglia and astrocytes. *J Neuroinflammation*. 2010;7(1):46. doi:10.1186/1742-2094-7-59
- Capiralla H, Vingtdoux V, Zhao H, et al. Resveratrol mitigates lipopolysaccharide- and A $\beta$ -mediated microglial inflammation by inhibiting the TLR4/NF- $\kappa$ B/STAT signaling cascade. *J Neurochem*. 2012;120(3):461–472. doi:10.1111/j.1471-4159.2011.07594.x
- Meng Z, Yan C, Deng Q, Gao D, Niu X. Curcumin inhibits LPS-induced inflammation in rat vascular smooth muscle cells in vitro via ROS-relative TLR4-MAPK/NF- $\kappa$ B pathways. *Acta Pharmacol Sin*. 2013;34(7):901–911. doi:10.1038/aps.2013.24
- Tu C, Han B, Yao Q, Zhang Y, Liu H, Zhang S. Curcumin attenuates concanavalin A-induced liver injury in mice by inhibition of toll-like receptor (TLR) 2, TLR4 and TLR9 expression. *Int Immunopharmacol*. 2012;12(1):151–157. doi:10.1016/j.intimp.2011.11.005
- Huang S, Zhao L, Kim K, Lee DS, Hwang DH. Inhibition of Nod2 signaling and target gene expression by curcumin. *Mol Pharmacol*. 2008;74(1):274. doi:10.1124/mol.108.046169
- Lee K-M, Kang JH, Yun M, Lee S-B. Quercetin inhibits the poly (dA: dT)-induced secretion of IL-18 via down-regulation of the expressions of AIM2 and pro-caspase-1 by inhibiting the JAK2/STAT1 pathway in IFN- $\gamma$ -primed human keratinocytes. *Biochem Biophys Res Commun*. 2018;503(1):116–122. doi:10.1016/j.bbrc.2018.05.191
- Nourshargh S, Alon R. Leukocyte migration into inflamed tissues. *Immunity*. 2014;41(5):694–707. doi:10.1016/j.immuni.2014.10.008
- Ingersoll MA, Platt AM, Potteaux S, Randolph GJ. Monocyte trafficking in acute and chronic inflammation. *Trends Immunol*. 2011;32(10):470–477. doi:10.1016/j.it.2011.05.001
- Muller WA. Getting leukocytes to the site of inflammation. *Vet Pathol*. 2013;50(1):7–22. doi:10.1177/0300985812469883
- Muller WA. Mechanisms of leukocyte transendothelial migration. *Annu Rev Pathol*. 2011;6:323. doi:10.1146/annurev-pathol-011110-130224
- Pastore S, Lulli D, Fidanza P, et al. Plant polyphenols regulate chemokine expression and tissue repair in human keratinocytes through interaction with cytoplasmic and nuclear components of epidermal growth factor receptor system. *Antioxid Redox Signal*. 2012;16(4):314–328. doi:10.1089/ars.2011.4053
- Pastore S, Potapovich A, Kostyuk V, et al. Plant polyphenols effectively protect HaCaT cells from ultraviolet C-triggered necrosis and suppress inflammatory chemokine expression. *Ann N Y Acad Sci*. 2009;1171(1):305–313. doi:10.1111/j.1749-6632.2009.04684.x

25. Maloney JP, Gao L. Proinflammatory cytokines increase vascular endothelial growth factor expression in alveolar epithelial cells. *Mediators Inflamm.* 2015;2015:1–7. doi:10.1155/2015/387842
26. Supanc V, Kes V, Biloglav Z, Demarin V. Role of cell adhesion molecules in acute ischemic stroke. *Ann Saudi Med.* 2011;31(4):365. doi:10.4103/0256-4947.83217
27. Demerath E, Towne B, Blangero J, Siervogel RM. The relationship of soluble ICAM-1, VCAM-1, P-selectin and E-selectin to cardiovascular disease risk factors in healthy men and women. *Ann Hum Biol.* 2001;28(6):664–678.
28. Tangney CC, Rasmussen HE. Polyphenols, inflammation, and cardiovascular disease. *Curr Atheroscler Rep.* 2013;15(5):324. doi:10.1007/s11883-013-0324-x
29. Chiva-Blanch G, Urpi-Sarda M, Llorach R, et al. Differential effects of polyphenols and alcohol of red wine on the expression of adhesion molecules and inflammatory cytokines related to atherosclerosis: a randomized clinical trial. *Am J Clin Nutr.* 2012;95(2):326–334. doi:10.3945/ajcn.111.022889
30. Deng Y-H, Alex D, Huang H-Q, et al. Inhibition of TNF- $\alpha$ -mediated endothelial cell-monocyte cell adhesion and adhesion molecules expression by the resveratrol derivative, trans-3,5,4'-trimethoxystilbene. *Phyther Res.* 2010;25(3):451–457.
31. Estruch R, Martínez-González MA, Corella D, et al. Effects of a mediterranean-style diet on cardiovascular risk factors: a randomized trial. *Ann Intern Med.* 2006;145(1):1–11. doi:10.7326/0003-4819-145-1-200607040-00004
32. Xu Z-R, Li J-Y, Dong X-W, et al. Apple polyphenols decrease atherosclerosis and hepatic steatosis in ApoE $^{-/-}$  mice through the ROS/MAPK/NF- $\kappa$ B pathway. *Nutrients.* 2015;7(8):7085–7105. doi:10.3390/nu7085324
33. Weiss G, Schaible UE. Macrophage defense mechanisms against intracellular bacteria. *Immunol Rev.* 2015;264(1):182–203. doi:10.1111/imr.12266
34. Takada Y, Mukhopadhyay A, Kundu GC, Mahabeshwar GH, Singh S, Aggarwal BB. Hydrogen peroxide activates NF- $\kappa$ B through tyrosine phosphorylation of I $\kappa$ B $\alpha$  and serine phosphorylation of p65. *J Biol Chem.* 2003;278(26):24233–24241. doi:10.1074/jbc.M212389200
35. Savolainen H. Tannin content of tea and coffee. *J Appl Toxicol.* 1992;12:191–192.
36. Kris-Etherton PM, Hecker KD, Bonanome A, et al. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *Am J Med.* 2002;113(Suppl. 9B):71S–88S. doi:10.1016/S0002-9343(01)00995-0
37. El-Sayed IH, Lotfy M, El-Khawaga O-AY, et al. Prominent free radicals scavenging activity of tannic acid in lead-induced oxidative stress in experimental mice. *Toxicol Ind Health.* 2006;22(4):157–163. doi:10.1191/0748233706th256oa
38. Srivastava RC, Husain MM, Hasan SK, Athar M. Green tea polyphenols and tannic acid as potent inhibitors of phorbol ester-induced nitric oxide generation in rat hepatocytes independent of their antioxidant properties. *Cancer Lett.* 2000;153(1–2):1–5.
39. Číž M, Pavelková M, Gallová L, et al. The influence of wine polyphenols on reactive oxygen and nitrogen species production by murine macrophages RAW 264.7. *Physiol Res.* 2008;57:393–402.
40. Crispo JAG, Ansell DR, Piche M, et al. Protective effects of polyphenolic compounds on oxidative stress-induced cytotoxicity in PC12 cells. *Can J Physiol Pharmacol.* 2010;88(4):429–438. doi:10.1139/y09-137
41. Lorenz M, Wessler S, Follmann E, et al. A constituent of green tea, epigallocatechin-3-gallate, activates endothelial nitric oxide synthase by a phosphatidylinositol-3-OH-kinase-, cAMP-dependent protein kinase-, and Akt-dependent pathway and leads to endothelial-dependent vasorelaxation. *J Biol Chem.* 2004;279(7):6190–6195. doi:10.1074/jbc.M309114200
42. Medina-Remón A, Tresserra-Rimbau A, Pons A, et al. Effects of total dietary polyphenols on plasma nitric oxide and blood pressure in a high cardiovascular risk cohort. The PREDIMED randomized trial. *Nutr Metab Cardiovasc Dis.* 2015;25(1):60–67. doi:10.1016/j.numecd.2014.09.001
43. Rodrigo R, Libuy M, Feliu F, Hasson D. Polyphenols in disease: from diet to supplements. *Curr Pharm Biotechnol.* 2014;15(4):304–317.
44. Huang S-M, Wu C-H, Yen G-C. Effects of flavonoids on the expression of the pro-inflammatory response in human monocytes induced by ligation of the receptor for AGEs. *Mol Nutr Food Res.* 2006;50(12):1129–1139. doi:10.1002/mnfr.200600075
45. Duthie SJ, Dobson VL. Dietary flavonoids protect human colonocyte DNA from oxidative attack in vitro. *Eur J Nutr.* 1999;38(1):28–34.
46. Cho D-I, Koo N-Y, Chung WJ, et al. Effects of resveratrol-related hydroxystilbenes on the nitric oxide production in macrophage cells: structural requirements and mechanism of action. *Life Sci.* 2002;71(17):2071–2082. doi:10.1016/S0024-3205(02)01971-9
47. Benito S, Lopez D, Sáiz MP, et al. A flavonoid-rich diet increases nitric oxide production in rat aorta. *Br J Pharmacol.* 2002;135(4):910–916. doi:10.1038/sj.bjp.0704534
48. Peng X, Zhou R, Wang B, et al. Effect of green tea consumption on blood pressure: a meta-analysis of 13 randomized controlled trials. *Sci Rep.* 2014;4:6251. doi:10.1038/srep06251
49. Derocchette S, Franck T, Mouithys-Mickalad A, et al. Curcumin and resveratrol act by different ways on NADPH oxidase activity and reactive oxygen species produced by equine neutrophils. *Chem Biol Interact.* 2013;206(2):186–193. doi:10.1016/j.cbi.2013.09.011
50. Boonla O, Kukongviriyapan U, Pakdeechote P, et al. Curcumin improves endothelial dysfunction and vascular remodeling in 2K-1C hypertensive rats by raising nitric oxide availability and reducing oxidative stress. *Nitric Oxide.* 2014;42:44–53. doi:10.1016/j.niox.2014.09.001
51. Ciftci G, Aksoy A, cenesiz S, et al. Therapeutic role of curcumin in oxidative DNA damage caused by formaldehyde. *Microsc Res Tech.* 2015;78(5):391–395. doi:10.1002/jemt.22485
52. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. *World Allergy Organ J.* 2012;5(1):9–19. doi:10.1097/WOX.0b013e3182439613
53. Ciociu M, Mirón A, Mares L, et al. The effects of sambucus nigra polyphenols on oxidative stress and metabolic disorders in experimental diabetes mellitus. *J Physiol Biochem.* 2009;65(3):297–304. doi:10.1007/BF03180582
54. Uto-Kondo H, Ayaori M, Kishimoto Y, et al. Consumption of polyphenol-rich juar tea increases endothelium-bound extracellular superoxide dismutase levels in men with metabolic syndrome: link with LDL oxidizability. *Int J Food Sci Nutr.* 2013;64(4):407–414. doi:10.3109/09637486.2012.759185
55. Losada-Barreiro S, Bravo-Díaz C. Free radicals and polyphenols: the redox chemistry of neurodegenerative diseases. *Eur J Med Chem.* 2017;133:379–402. doi:10.1016/j.ejmech.2017.03.061
56. Cui Y, Madeddu P. The role of chemokines, cytokines and adhesion molecules in stem cell trafficking and homing. *Curr Pharm Des.* 2011;17(30):3271–3279.
57. Selders GS, Fetz AE, Radic MZ, Bowlin GL. An overview of the role of neutrophils in innate immunity, inflammation and host-biomaterial integration. *Regen Biomater.* 2017;4(1):55. doi:10.1093/rb/rbw041
58. Zhang C. The role of inflammatory cytokines in endothelial dysfunction. *Basic Res Cardiol.* 2008;103(5):398. doi:10.1007/s00395-008-0733-0
59. Golden-Mason L, Rosen HR. Natural killer cells: multi-faceted players with key roles in hepatitis C immunity. *Immunol Rev.* 2013;255(1):68. doi:10.1111/imr.12090

60. Chuang Y, Knickel BK, Leonard JN. Regulation of the IL-10-driven macrophage phenotype under incoherent stimuli. *Immun. 2016*;22(8):647. doi:10.1177/1753425916668243
61. Matsuyama T, Kawai T, Izumi Y, Taubman MA. Expression of major histocompatibility complex class II and CD80 by gingival epithelial cells induces activation of CD4+ T cells in response to bacterial challenge. *Infect Immun. 2005*;73(2):1044–1051. doi:10.1128/IAI.73.2.1044-1051.2005
62. Dimitrios B. Sources of natural phenolic antioxidants. *Trends Food Sci Technol. 2006*;17(9):505–512. doi:10.1016/j.tifs.2006.04.004
63. Miles EA, Zoubouli P, Calder PC. Effects of polyphenols on human Th1 and Th2 cytokine production. *Clin Nutr. 2005*;24(5):780–784. doi:10.1016/j.clnu.2005.04.001
64. Abe Y, Hashimoto S, Horie T. Curcumin inhibition of inflammatory cytokine production by human peripheral blood monocytes and alveolar macrophages. *Pharmacol Res. 1999*;39(1):41–47. doi:10.1006/phrs.1998.0404
65. Yadav R, Jee B, Awasthi SK. Curcumin suppresses the production of pro-inflammatory cytokine interleukin-18 in lipopolysaccharide stimulated murine macrophage-like cells. *Indian J Clin Biochem. 2015*;30(1):109–112. doi:10.1007/s12291-014-0452-2
66. Funaro A, Wu X, Song M, et al. Enhanced anti-inflammatory activities by the combination of luteolin and tangeretin. *J Food Sci. 2016*;81(5):H1320–H1327. doi:10.1111/1750-3841.13300
67. Zhu L-H, Bi W, Qi R, Wang H, Lu D. Luteolin inhibits microglial inflammation and improves neuron survival against inflammation. *Int J Neurosci. 2011*;121(6):329–336. doi:10.3109/00207454.2011.569040
68. Park CM, Jin K-S, Lee Y-W, Song YS. Luteolin and chicoric acid synergistically inhibited inflammatory responses via inactivation of PI3K-Akt pathway and impairment of NF- $\kappa$ B translocation in LPS stimulated RAW 264.7 cells. *Eur J Pharmacol. 2011*;660(2–3):454–459. doi:10.1016/j.ejphar.2011.04.007
69. Weng Z, Patel AB, Vasiadi M, Therianou A, Theoharides TC. Luteolin inhibits human keratinocyte activation and decreases NF- $\kappa$ B induction that is increased in psoriatic skin. *PLoS One. 2014*;9(2):e90739. doi:10.1371/journal.pone.0090739
70. Bureau G, Longpré F, Martinoli M-G. Resveratrol and quercetin, two natural polyphenols, reduce apoptotic neuronal cell death induced by neuroinflammation. *J Neurosci Res. 2008*;86(2):403–410. doi:10.1002/jnr.21503
71. Chen T-J, Jeng J-Y, Lin C-W, Wu C-Y, Chen Y-C. Quercetin inhibition of ROS-dependent and -independent apoptosis in rat glioma C6 cells. *Toxicology. 2006*;223(1–2):113–126. doi:10.1016/j.tox.2006.03.007
72. Sharma A, Kaur M, Katnoria JK, Nagpal AK. Polyphenols in food: cancer prevention and apoptosis induction. *Curr Med Chem. 2014*;24:4740–1757.
73. Tanikawa M, Lee H-Y, Watanabe K, et al. Regulation of prostaglandin biosynthesis by interleukin-1 in cultured bovine endometrial cells. *J Endocrinol. 2008*;199(3):425–434. doi:10.1677/JOE-08-0237
74. Argaw AT, Zhang Y, Snyder BJ, et al. IL-1 $\beta$  regulates blood-brain barrier permeability via reactivation of the hypoxia-angiogenesis program. *J Immunol. 2006*;177(8):5574–5584. doi:10.4049/jimmunol.177.8.5574
75. Aribi M, Moulessehou S, Kendouci-Tani M, Benabadi A-B, Hichami A, Khan NA. Relationship between interleukin-1 $\beta$  and lipids in type 1 diabetic patients. *Med Sci Monit. 2007*;13(8):CR372–CR378.
76. Erta M, Quintana A, Hidalgo J. Interleukin-6, a major cytokine in the central nervous system. *Int J Biol Sci. 2012*;8(9):1254–1266. doi:10.7150/ijbs.4679
77. Wheeler DS, Catravas JD, Odoms K, Denenberg A, Malhotra V, Wong HR. Epigallocatechin-3-gallate, a green tea-derived polyphenol, inhibits IL-1 $\beta$ -dependent proinflammatory signal transduction in cultured respiratory epithelial cells. *J Nutr. 2004*;134(5):1039–1044. doi:10.1093/jn/134.5.1039
78. Ahmed S. Green tea polyphenol epigallocatechin-3-gallate (EGCG) differentially inhibits interleukin-1-induced expression of matrix metalloproteinase-1 and -13 in human chondrocytes. *J Pharmacol Exp Ther. 2003*;308(2):767–773. doi:10.1124/jpet.103.059220
79. Guo W, Wise ML, Collins FW, Meydani M. Avenanthramides, polyphenols from oats, inhibit IL-1 $\beta$ -induced NF- $\kappa$ B activation in endothelial cells. *Free Radic Biol Med. 2008*;44(3):415–429. doi:10.1016/j.freeradbiomed.2007.10.036
80. Hirano T. Interleukin 6 in autoimmune and inflammatory diseases: a personal memoir. *Proc Jpn Acad Ser B Phys Biol Sci. 2010*;86(7):717–730. doi:10.2183/pjab.86.717
81. Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. The pro- and anti-inflammatory properties of the cytokine interleukin-6. *Biochim Biophys Acta Mol Cell Res. 2011*;1813(5):878–888. doi:10.1016/j.bbamer.2011.01.034
82. Wei H, Alberts I, Li X. Brain IL-6 and autism. *Neuroscience. 2013*;252:320–325. doi:10.1016/j.neuroscience.2013.08.025
83. Mosher KI, Wyss-Coray T. Microglial dysfunction in brain aging and Alzheimer's disease. *Biochem Pharmacol. 2014*;88(4):594–604. doi:10.1016/j.bcp.2014.01.008
84. Voorhees JL, Tarr AJ, Wohleb ES, et al. Prolonged restraint stress increases IL-6, reduces IL-10, and causes persistent depressive-like behavior that is reversed by recombinant IL-10. *PLoS One. 2013*;8(3):e58488. doi:10.1371/journal.pone.0058488
85. Drummond EM, Harbourn N, Marete E, et al. Inhibition of pro-inflammatory biomarkers in THP1 macrophages by polyphenols derived from chamomile, meadowsweet and willow bark. *Phyther Res. 2013*;27(4):588–594. doi:10.1002/ptr.4753
86. Seder RA, Gazzinelli R, Sher A, Paul WE. Interleukin 12 acts directly on CD4+ T cells to enhance priming for interferon gamma production and diminishes interleukin 4 inhibition of such priming. *Proc Natl Acad Sci U S A. 1993*;90(21):10188–10192. doi:10.1073/pnas.90.21.10188
87. Gerosa F, Paganin C, Peritt D, et al. Interleukin-12 primes human CD4 and CD8 T cell clones for high production of both interferon-gamma and interleukin-10. *J Exp Med. 1996*;183(6):2559–2569. doi:10.1084/jem.183.6.2559
88. Ma X. TNF- $\alpha$  and IL-12: a balancing act in macrophage functioning. *Microbes Infect. 2001*;3(2):121–129.
89. Oyetakin-White P, Tribout H, Baron E. Protective mechanisms of green tea polyphenols in skin. *Oxid Med Cell Longev. 2012*;2012:560682. doi:10.1155/2012/560682
90. Cuevas A, Saavedra N, Salazar LA, Abdalla DSP. Modulation of immune function by polyphenols: possible contribution of epigenetic factors. *Nutrients. 2013*;5(7):2314. doi:10.3390/nu5072314
91. Djurovic S, Berge KE, Birkenes B, Braaten Ø, Retterstøl L. The effect of red wine on plasma leptin levels and vasoactive factors from adipose tissue: a randomized crossover trial. *Alcohol Alcohol. 2007*;42:525–528. doi:10.1093/alcal/agl083
92. Chen L-F, Fischle W, Verdin E, Greene WC. Duration of nuclear NF- $\kappa$ B action regulated by reversible acetylation. *Science (80-). 2001*;293(5535):1653–1657. doi:10.1126/science.1062374
93. O'Dea E, Hoffmann A. The regulatory logic of the NF- $\kappa$ B signaling system. *Cold Spring Harb Perspect Biol. 2010*;2(1):a000216–a000216.



94. Ruiz PA, Haller D. Functional diversity of flavonoids in the inhibition of the proinflammatory NF- $\kappa$ B, IRF, and Akt signaling pathways in murine intestinal epithelial cells. *J Nutr*. 2006;136(3):664–671. doi:10.1093/jn/136.3.664
95. Singh S, Khar A. Biological effects of curcumin and its role in cancer chemoprevention and therapy. *Anticancer Agents Med Chem*. 2006;6(3):259–270.
96. McCain J. The MAPK (ERK) pathway: investigational combinations for the treatment of BRAF-mutated metastatic melanoma. *Pharm Ther*. 2013;38(2):96.
97. Murphy LO, Blenis J. MAPK signal specificity: the right place at the right time. *Trends Biochem Sci*. 2006;31(5):268–275. doi:10.1016/j.tibs.2006.03.009
98. Lue H, Dewor M, Leng L, Bucala R, Bernhagen J. Activation of the JNK signalling pathway by macrophage migration inhibitory factor (MIF) and dependence on CXCR4 and CD74. *Cell Signal*. 2011;23(1):135. doi:10.1016/j.cellsig.2010.08.013
99. Arndt PG, Suzuki N, Avdi NJ, Malcolm KC, Worthen GS. Lipopolysaccharide-induced c-Jun NH<sub>2</sub>-terminal kinase activation in human neutrophils. *J Biol Chem*. 2004;279(12):10883–10891. doi:10.1074/jbc.M309901200
100. Himes SR, Sester DP, Ravasi T, Cronau SL, Sasmono T, Hume DA. The JNK are important for development and survival of macrophages. *J Immunol*. 2006;176(4):2219–2228. doi:10.4049/jimmunol.176.4.2219
101. Kim S-J, Jeong H-J, Lee K-M, et al. Epigallocatechin-3-gallate suppresses NF- $\kappa$ B activation and phosphorylation of p38 MAPK and JNK in human astrocytoma U373MG cells. *J Nutr Biochem*. 2007;18(9):587–596. doi:10.1016/j.jnutbio.2006.11.001
102. Camacho-Barquero L, Villegas I, Sánchez-Calvo JM, et al. Curcumin, a curcuma longa constituent, acts on MAPK p38 pathway modulating COX-2 and iNOS expression in chronic experimental colitis. *Int Immunopharmacol*. 2007;7(3):333–342. doi:10.1016/j.intimp.2006.11.006
103. Pagliari LJ, Perlman H, Liu H, Pope RM. Macrophages require constitutive NF-kappaB activation to maintain A1 expression and mitochondrial homeostasis. *Mol Cell Biol*. 2000;20(23):8855–8865. doi:10.1128/mcb.20.23.8855-8865.2000
104. Song D, Zhao J, Deng W, Liao Y, Hong X, Hou J. Tannic acid inhibits NLRP3 inflammasome-mediated IL-1 $\beta$  production via blocking NF- $\kappa$ B signaling in macrophages. *Biochem Biophys Res Commun*. 2018;503(4):3078–3085. doi:10.1016/j.bbrc.2018.08.096
105. Jung J-Y, Mo H-C, Yang K-H, et al. Inhibition by epigallocatechin gallate of CoCl<sub>2</sub>-induced apoptosis in rat PC12 cells. *Life Sci*. 2007;80(15):1355–1363. doi:10.1016/j.lfs.2006.11.033
106. Schroeter H, Spencer JP, Rice-Evans C, Williams RJ. Flavonoids protect neurons from oxidized low-density-lipoprotein-induced apoptosis involving c-Jun N-terminal kinase (JNK), c-Jun and caspase-3. *Biochem J*. 2001;358(Pt 3):547–557. doi:10.1042/0264-6021:3580547
107. Shakibaei M, John T, Seifarth C, Mobasher A. Resveratrol inhibits IL-1 $\beta$ -induced stimulation of caspase-3 and cleavage of PARP in human articular chondrocytes in vitro. *Ann N Y Acad Sci*. 2007;1095(1):554–563. doi:10.1196/annals.1397.060
108. Park K-I, Kang S-R, Park H-S, et al. Regulation of proinflammatory mediators via NF- $\kappa$ B and p38 MAPK-dependent mechanisms in RAW 264.7 macrophages by polyphenol components isolated from Korea *Lonicera japonica* THUNB. *Evid Based Complement Alternat Med*. 2012;2012:1–10.
109. Rao KM. MAP kinase activation in macrophages. *J Leukoc Biol*. 2001;69(1):3–10.
110. Acharyya S, Villalta SA, Bakkar N, et al. Interplay of IKK/NF-kappaB signaling in macrophages and myofibers promotes muscle degeneration in Duchenne muscular dystrophy. *J Clin Invest*. 2007;117(4):889–901. doi:10.1172/JCI30556
111. Wang K, Ping S, Huang S, et al. Molecular mechanisms underlying the in vitro anti-inflammatory effects of a flavonoid-rich ethanol extract from chinese propolis (poplar type). *Evid Based Complement Alternat Med*. 2013;2013:127672.
112. Cianciulli A, Calvello R, Cavallo P, Dragone T, Carofiglio V, Panaro MA. Modulation of NF- $\kappa$ B activation by resveratrol in LPS treated human intestinal cells results in downregulation of PGE2 production and COX-2 expression. *Toxicol Vitro*. 2012;26(7):1122–1128. doi:10.1016/j.tiv.2012.06.015
113. Karuppagounder V, Arumugam S, Thandavarayan RA, et al. Tannic acid modulates NFkB signaling pathway and skin inflammation in NC/Nga mice through PPAR $\gamma$  expression. *Cytokine*. 2015;76(2):206–213. doi:10.1016/j.cyt.2015.05.016
114. Fujiwara N, Kobayashi K. Macrophages in inflammation. *Curr Drug Targets Inflamm Allergy*. 2005;4(3):281–286.
115. Martinez FO, Gordon S. The M1 and M2 paradigm of macrophage activation: time for reassessment. *F1000Prime Rep*. 2014;6:13. doi:10.12703/P
116. Shapouri-Moghaddam A, Mohammadian S, Vazini H, et al. Macrophage plasticity, polarization, and function in health and disease. *J Cell Physiol*. 2018;233(9):6425–6440. doi:10.1002/jcp.26429
117. Tabas I, Bornfeldt KE. Macrophage phenotype and function in different stages of atherosclerosis. *Circ Res*. 2016;118(4):653–667. doi:10.1161/CIRCRESAHA.115.306256
118. Buttari B, Profumo E, Segoni L, et al. Resveratrol counteracts inflammation in human M1 and M2 macrophages upon challenge with 7-oxo-cholesterol: potential therapeutic implications in atherosclerosis. *Oxid Med Cell Longev*. 2014;2014:257543. doi:10.1155/2014/257543
119. Chu C, Liu L, Wang Y, et al. Macrophage phenotype in the epigallocatechin-3-gallate (EGCG)-modified collagen determines foreign body reaction. *J Tissue Eng Regen Med*. 2018;12(6):1499–1507. doi:10.1002/term.2687
120. Gregory CD, Devitt A. The macrophage and the apoptotic cell: an innate immune interaction viewed simplistically? *Immunology*. 2004;113(1):1–14. doi:10.1111/j.1365-2567.2004.01959.x
121. Szondy Z, Sarang Z, Kiss B, Garabuczi É, Köröskényi K. Anti-inflammatory mechanisms triggered by apoptotic cells during their clearance. *Front Immunol*. 2017;8:909. doi:10.3389/fimmu.2017.00909
122. Zhang Y, Kim H-J, Yamamoto S, Kang X, Ma X. Regulation of interleukin-10 gene expression in macrophages engulfing apoptotic cells. *J Interferon Cytokine Res*. 2010;30(3):113–122. doi:10.1089/jir.2010.0004
123. Chen W, Frank ME, Jin W, Wahl SM. TGF-beta released by apoptotic T cells contributes to an immunosuppressive milieu. *Immunity*. 2001;14(6):715–725.
124. D'Archivio M, Santangelo C, Scazzocchio B, et al. Modulatory effects of polyphenols on apoptosis induction: relevance for cancer prevention. *Int J Mol Sci*. 2008;9(3):213–228.
125. Banerjee N, Talcott S, Safe S, Mertens-Talcott SU. Cytotoxicity of pomegranate polyphenolics in breast cancer cells in vitro and vivo: potential role of miRNA-27a and miRNA-155 in cell survival and inflammation. *Breast Cancer Res Treat*. 2012;136(1):21–34. doi:10.1007/s10549-012-2224-0
126. Cerdá B, Espín JC, Parra S, Martínez P, Tomás-Barberán FA. The potent in vitro antioxidant ellagitannins from pomegranate juice are metabolized into bioavailable but poor antioxidant hydroxy-6H dibenzopyran-6-one derivatives by the colonic microflora in healthy humans. *Eur J Nutr*. 2004;43:205–220. doi:10.1007/s00394-004-0461-7
127. Hsieh T-C, Wu JM. Targeting CWR22Rv1 prostate cancer cell proliferation and gene expression by combinations of the phytochemicals EGCG, genistein and quercetin. *Anticancer Res*. 2009;29(10):4025–4032.

128. Kunnumakkara AB, Guha S, Krishnan S, Diagaradjane P, Gelovani J, Aggarwal BB. Curcumin potentiates antitumor activity of gemcitabine in an orthotopic model of pancreatic cancer through suppression of proliferation, angiogenesis, and inhibition of nuclear factor-B-regulated gene products. *Cancer Res.* 2007;67(8):3853–3861. doi:10.1158/0008-5472.CAN-06-4257
129. Nishimuro H, Ohnishi H, Sato M, et al. Estimated daily intake and seasonal food sources of quercetin in Japan. *Nutrients.* 2015;7(4):2345–2358. doi:10.3390/nu7042345
130. Choi Y-J, Kang J-S, Park JHY, Lee Y-J, Choi J-S, Kang Y-H. Polyphenolic flavonoids differ in their antiapoptotic efficacy in hydrogen peroxide-treated human vascular endothelial cells. *J Nutr.* 2003;133(4):985–991. doi:10.1093/jn/133.4.985
131. Karakurt S, Adali O. Tannic acid inhibits proliferation, migration, invasion of prostate cancer and modulates drug metabolizing and antioxidant enzymes. *Anticancer Agents Med Chem.* 2016;16(6):781–789.

## Journal of Inflammation Research

Dovepress

### Publish your work in this journal

The Journal of Inflammation Research is an international, peer-reviewed open-access journal that welcomes laboratory and clinical findings on the molecular basis, cell biology and pharmacology of inflammation including original research, reviews, symposium reports, hypothesis formation and commentaries on: acute/chronic inflammation; mediators of inflammation; cellular processes; molecular

mechanisms; pharmacology and novel anti-inflammatory drugs; clinical conditions involving inflammation. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/journal-of-inflammation-research-journal>